

LARVAL BIOLOGY AND ESTUARINE ECOLOGY OF THE NEMERTEAN EGG
PREDATOR *CARCINONEMERTES ERRANS* ON THE DUNGENESS CRAB,
CANCER MAGISTER

by

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DISSERTATION ABSTRACT

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Doctor of Philosophy

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Title: Larval Biology and Estuarine Ecology of the Nemertean Egg Predator
Carcinonemertes errans on the Dungeness Crab, *Cancer magister*

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The nemertean worm *Carcinonemertes errans* is an egg predator on the Dungeness crab, *Cancer magister*, an important fishery species along the west coast of North America. This study examined the estuarine distribution and larval biology of *C. errans*. Parasite prevalence and mean intensity of *C. errans* infecting *C. magister* varied along an estuarine gradient in the Coos Bay, Oregon. Crabs nearest the ocean carried the heaviest parasite loads, and larger crabs were more heavily infected with worms. Seasonal infection patterns were seen at some sites within the bay. Crabs from coastal waters carried significantly more worms than did crabs from the bay, suggesting that the estuary may be acting as a parasite refuge for estuarine crabs. In laboratory experiments, *C. errans* all died in salinities below 10 within 6 days, but *C. errans* showed some tolerance to salinities 20 and above. These results suggest that salinity alone does not likely account for the estuarine gradient of *C. errans* in Coos Bay.

Larvae of *C. errans* raised from hatching never settled in the laboratory. Competent larvae taken in plankton tows were morphologically distinct from larvae raised

in laboratory cultures and did settle successfully on *C. magister* under laboratory conditions. Initial settlement was reversible within a 24-hour window. After 48 hours, a non-reversible metamorphosis occurred wherein worms lost one pair of eyes and the propensity to swim. In field settlement experiments, *C. errans* was capable of infecting hosts from the water column and preferred to settle on crabs already infected with juvenile worms, although this preference was density dependent. In monthly plankton tows, larvae of *C. errans* were found only between August and November, suggesting a long larval life for this species. Larvae did not feed under laboratory conditions, nor did they absorb dissolved organics. When exposed to a natural angular light distribution, larvae of *C. errans* were rarely photopositive. Larvae were most sensitive to blue-green light. Low intensity light invoked a photonegative response. Larvae were geopositive at hatching but geonegative thereafter.

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CHAPTER I

GENERAL INTRODUCTION

Although far from being the most conspicuous organisms in any given habitat, parasites form a large proportion of the diversity of life on the earth (Smyth 1994). Using a rough and conservative estimate of known insect fauna, Price (1980) predicted that insect parasites alone represent nearly half of all known animal species. When other groups of parasites such as nematodes, flatworms, and protozoa are added to this number, it becomes clear that “parasitism as a way of life is more common than all other feeding strategies combined” (Price 1980). Considering this astounding diversity, it stands to reason that parasites should be important research subjects in evolution and ecology. Parasites provide interesting systems for studying reproductive strategies, organismal complexity, dispersal, population dynamics, coevolution, ecological niches, niche restriction, and community structuring (Rohde 1982). Although the definitions of parasite differ depending on author and situation, I will use the definition presented by Price (1980): “an organism living in or on another living organism, obtaining from it part or all of its organic nutriment, commonly exhibiting some degree of adaptive structural modification, and causing some degree of real damage to its host.”

Marine parasites are less familiar than many of their terrestrial counterparts, but they are equally diverse and prolific. The 1000 fish species occurring in the vicinity of Heron Island in the Great Barrier Reef are infected with at least 2,000 species of monogenean flukes alone, and the total number of fish parasite species in the region has been estimated at 20,000 (Rohde 1982). In addition to such common marine parasitic

groups as monogeneans, copepods, and rhizocephalan barnacles are the nemertean worms of the genus *Carcinonemertes* (Nemertea: Enopla: Hoplonemertea; Coe 1902).

Originally thought to be parasitic on the gills of their crab hosts, it wasn't until Wickham (1978) first observed these worms feeding on host eggs that the true nature of the host-parasite relationship was resolved. This discovery led some to argue that the term "parasite" should be replaced by "egg predator" when describing *Carcinonemertes*.

However, these worms spend their lives on one or a few host individuals, relying on these hosts for food as well as the completion of their life cycle. The harmful effects of *Carcinonemertes*, which cause a partial loss of host reproductive output, can also be modeled well as parasitic castration (Kuris and Lafferty 1992). Using Price's definition (1980), therefore, these worms are both egg predators and parasites of their decapod hosts.

Life history of *Carcinonemertes errans*

Carcinonemertes errans Wickham 1978 has received considerable attention in the literature due to its occurrence on the Dungeness crab *Cancer magister*¹ Dana 1852, an important fishery species off the west coast of North America from Alaska to central California (Wickham 1979a). Unlike *Carcinonemertes epialti*, the congener with which it shares much of its range, *C. errans* is considered to be species-specific in its host choice (Wickham 1978; Wickham and Kuris 1985). The validity of this proposition has recently been put in doubt, however. As part of an ongoing genetic study of the genus

¹ Based on morphological data, Schweitzer and Feldman (2000) rearranged the genus *Cancer* and placed the Dungeness crab into the genus *Metacarcinus*. This classification has not been widely accepted, however, and for this dissertation, I will follow the classification proposed in the new Light's Manual (Carlton 2007) and use the name *Cancer magister*.

Carcinonemertes, three specimens identified morphologically as *C. errans* from Alaska *C. magister* were found within a cluster of worms identified as *C. epialti* obtained from three other host species (J. Norenburg, pers. comm.). Although preliminary, these results suggest that *C. errans* and *C. epialti* may be one species, and that all sources referring to either of these species should be considered with that possibility in mind.

The major life events of *Carcinonemertes errans* are closely linked to those of its host, *Cancer magister*. Juvenile worms can be found on the exoskeleton of both male and female hosts, usually concentrated on and around limb joints, eye stalks, sternal regions, abdomen, and the copulatory appendages of males (Wickham 1979b). Although the juveniles can move about the external surfaces of the crab, they spend much of their time curled up on the arthrodistal membranes of the host (Wickham et al. 1984). Crow et al. (1982) suggested that juvenile worms subsist on their crab hosts when eggs are not available by absorbing dissolved organics leaking out of their crab hosts through these membranes (see also Roe et al., 1981).

During host molting, juveniles of *Carcinonemertes errans* migrate to the developing fissure and move onto the new exoskeleton of the crab as it crawls out of its old shell (Wickham et al., 1984). Worms also migrate from male crabs to females during host mating, which in *Cancer magister* involves a prolonged copulatory embrace (Wickham et al. 1984). This migration is vital to the life history of *C. errans* because maturation can only occur on an ovigerous female crab (Wickham 1979a). Within a day or two of host oviposition, *C. errans* individuals migrate to the host egg mass and begin to feed, potentially causing significant brood loss (Wickham 1979b). As they feed, worms grow larger, develop gonads, and mate (Wickham 1980). Females then lay egg

strings that wrap around the host eggs (Fig. 1.1). Worm embryos develop within these strings for 1-2 weeks until they hatch out as swimming larvae (Wickham 1980; Roe 1986). Following mating and egg deposition, adult worms begin to shrink and resemble juveniles again. It is not known if the same individuals can reproduce more than once or if they die following their first reproductive episode (Roe 1984). After hatching, the larvae are planktonic for an unknown amount of time, possibly several months (Wickham 1980), before reaching a competent stage and finding a new host to infect.

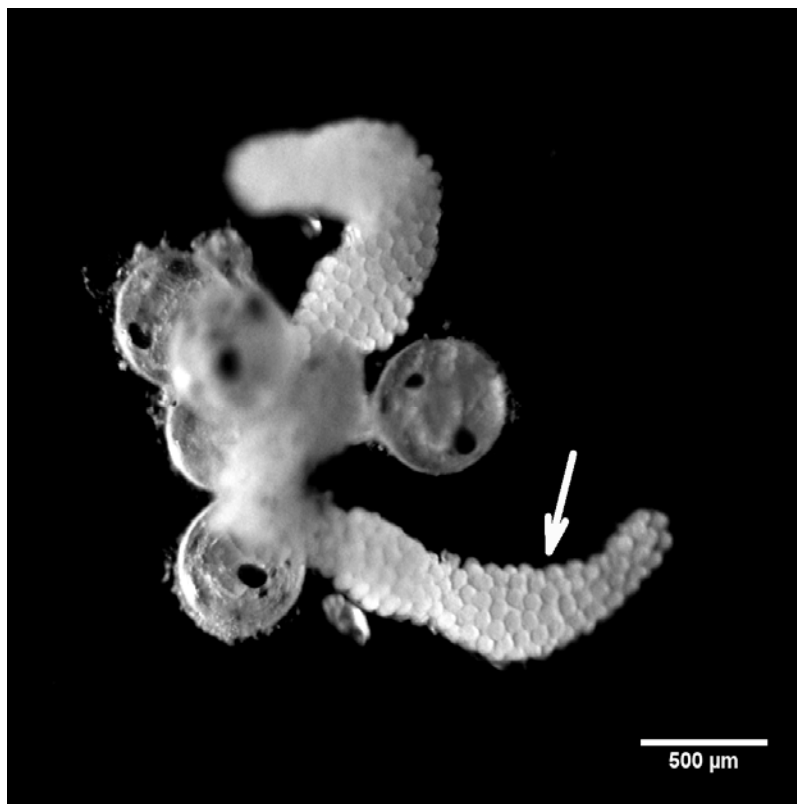


Fig. 1.1. Egg Strings of *Carcinonemertes errans*. Developing embryos of *C. errans* (arrow) are laid in strings wrapped around the much larger embryos of the host, *Cancer magister*.

Parasite refuges

An important ecological aspect of any host-parasite interaction is the geographical overlap of the two species' ranges (Price 1980). The range of host-specific parasites is necessarily limited to that of their hosts, but the reverse is not necessarily true. A parasite's distribution within a host's range may be limited by mode of transmission, availability of intermediate hosts, dispersal potential of parasite vectors, and physiological tolerances (Bush et al. 2001). In the case of *Carcinonemertes errans*, worms have been found on *Cancer magister* along the entire length of the host's range (Wickham 1980). However, samples have almost exclusively been taken from adults of coastal crab populations (Wickham 1979a; Wickham 1979b; Wickham 1980). *Cancer magister* is also a common inhabitant of estuaries (Cleaver 1949; Waldron 1958; Pauley et al. 1989; Armstrong et al. 2003). In the only study where the distribution of *C. errans* on *C. magister* within an estuary was examined, the authors reported a clear gradient in infection, with crabs farther up the estuary less often infected with *C. errans* (McCabe et al. 1987). Lower instances of parasite infections in some hosts living in estuaries have led several authors to suggest that these host populations may be experiencing "salinity refuges" from their parasites (Haskin and Ford 1982; Reisser and Forward 1991; Kvach 2004; Tolley et al. 2006). Could the movement into estuaries by some Dungeness crabs serve as a refuge from *C. errans*?

Temperature and salinity tolerance

Temperature supplies the energy to disrupt bonds between atoms and molecules. Although some bonds are more susceptible to breaking than others, all proteins are eventually denatured, nucleic acids are damaged, and the permeability of cell membranes

is altered at some threshold (Kinne 1970). While some organisms have the ability to regulate their body temperature, most marine animals conform to the temperature of their surroundings (Whittow 1970). Among these thermal conformers, there is large variation in the temperature range that different species can tolerate. The vast majority of marine organisms are considered stenothermic, meaning that they can tolerate a relatively narrow range of temperatures before entering into a temperature-induced coma and dying (Moore 1940). Some organisms, however, are considered eurythermic and can survive larger variations in temperature (Kinne 1970).

Salinity is a unitless measure of the dissolved ion concentrations in a body of water (UNESCO United Nations Educational Scientific and Cultural Organization 1985). While salinity does change the physical properties of the water itself (density, osmotic pressure, dissolved gases, radiation, surface tension, and sound transmission), it affects marine organisms directly by altering the ionic composition of the environment (Anger 2003). This alteration can lead to 1) net movement of water into or out of the organism, potentially damaging cells, and 2) disruption of favorable gradients of biologically essential ions, forcing the organism to expend extra energy to maintain ionic balance (Kinne 1971). In addition to the direct effects of osmotic imbalance, salinity stress can also affect an organism's metabolism by altering its ability to move, changing the salt and/or water contents of body and intracellular fluids, modifying internal ion ratios, and interfering with neuromuscular, hormonal, and enzymatic mechanisms (Kinne 1966). Some organisms are better able to cope with changes in salinity than others. Termed "euryhaline," these species can typically tolerate changes in salinity of 10-30 (Kinne

1971). Most marine organisms, however, can tolerate salinity ranges of 10 or less and are classified as stenohaline (Evans 2009).

When considering temperature or salinity as a potential environmental stressor, one must not only examine the extreme values, but also past and present patterns of variation. An organism may be able to tolerate a brief encounter with very high temperatures, for example, but may die when exposed over longer periods to a relatively modest increase (Kinne 1963). Within estuaries, the fluctuations in temperature and salinity can be much more extreme than most marine environments because of shallower water, proximity to land, and freshwater runoff (Kennish 1986). If a parasite is less eurythermic or euryhaline than its host, the possibility exists that host individuals entering an estuary could experience a refuge from their parasite created by differences in physiological tolerance.

Larval biology

The larval stage of marine invertebrates is vital in determining the distribution of the species (Crisp 1976). Although often viewed as passive particles, larvae are capable of exhibiting active responses to environmental stimuli that determine their vertical position in the water column (Young 1995). The two most important stimuli for larval orientation and vertical navigation are light and gravity (Young and Chia 1987; Forward 1988). A larva's response to these two stimuli will determine to a large extent where that larva will be carried and what habitats it will be exposed to (Crisp 1979). This becomes particularly important at the time of settlement when the larva ends its pelagic life and begins its existence as a benthic juvenile (Crisp 1974; Pawlik 1992; Hadfield and Paul

2001). Settlement is an important event for all planktonic larvae of marine invertebrates, but it may be especially so for parasitic larvae that must find an appropriate host or perish (Pawlik 1992). Because larval settlement is the exact moment at which the host and the parasite begin their relationship, this event is one of the most important aspects of host-parasite ecology and evolution. Parasites should potentially experience strong selection favoring the ability to locate an appropriate host as well as potential mates on that host, making larval settlement of a parasite an excellent opportunity to study both associative and gregarious settlement patterns (Chia 1978; Pawlik 1992; Boone et al. 2004).

Scope and objectives

My primary objectives in developing this dissertation project were to examine the interaction between *Carcinonemertes errans* and *Cancer magister* within an estuarine system and to describe several aspects of the parasite's larval biology with special emphasis on larval settlement.

Chapters II and III of this dissertation focus on the ecology of *Carcinonemertes errans* within the Coos Bay Estuary, Oregon. Chapter II describes a three-year field survey I conducted to track the infection of *Cancer magister* by *C. errans* within the estuary. I describe the distribution of the parasite based on location within the estuary and the size and sex of the host, as well as the seasonal variations observed in that distribution. I also compare these findings to *C. magister* sampled in Oregon coastal waters. In Chapter III, I focus on the physiological tolerances of *C. errans* across life stages. I conducted temperature and salinity tolerance experiments on both juvenile and larval worms in the laboratory and measured the survival of the animals when exposed to

various temperature-salinity combinations. I then compare these results to the conditions found within the Coos Bay Estuary and consider the possible existence of a salinity refuge for *C. magister*.

In Chapters IV and V, I present data dealing with the larval biology of *Carcinonemertes errans*. Chapter IV is focused on the processes surrounding larval settlement. I describe the first recorded instance of *Carcinonemertes* settlement under laboratory conditions, as well as the subsequent metamorphosis that signals the beginning of the juvenile stage on *Cancer magister*. I also describe a series of field experiments that examined patterns of larval settlement within the Coos Bay Estuary. Mode of infection by competent larvae and the effect of location in the bay on larval settlement patterns were tested using cages placed along the estuarine gradient. The possibility of gregarious settlement behavior in larvae of *C. errans* was tested using crabs that carried various numbers of juvenile worms. Finally, plankton tows were performed within the Coos Bay Estuary and offshore waters to determine the distribution of the competent larvae of *C. errans* both in time and space.

In Chapter V, I describe feeding trials performed with the larvae of *Carcinonemertes errans*, both with particulate food choices and with dissolved organic matter (DOM). I then discuss possible feeding mechanisms for uniformly ciliated planuliform larvae. Experiments to test the phototactic behavior of larvae from the time of hatching until one month old are then described. Trials examined the response of larvae to light of varying intensity and wavelength, as well as increased hydrostatic pressure under an experimental design made to mimic the natural light field of the ocean rather than the narrow-beam light stimulus commonly used in laboratory experiments. I

then compare these findings with light intensities found in natural conditions and discuss the possible ecological consequences of observed behaviors.

CHAPTER II

FINDING REFUGE: THE ESTUARINE DISTRIBUTION OF THE NEMERTEAN EGG PREDATOR *CARCINONEMERTES ERRANS* ON THE DUNGENESS CRAB, *CANCER MAGISTER*

INTRODUCTION

Estuaries have played an important role in human history as the natural crossroads of rivers and oceans. In addition to being major hubs of commerce, estuaries have functioned as a significant source of food for people (Lotze et al. 2006). Many kinds of estuarine fishes and shellfishes, such as crabs and oysters, are the targets of major fisheries in areas around the world. This dependence on estuaries has led to many studies examining the physical, chemical, and biological processes associated with these zones of rapid transition (reviewed in Kennedy 1982, Kennish 1986). While the estuarine distribution and ecology of macrofauna such as crabs and fish have garnered much attention, parasitic organisms remain largely overlooked, even though they often represent the majority of biodiversity in a given habitat (Price 1980, Rohde 1982). A recent study by Kuris et al. (2008) showed that the biomass of parasites in three estuaries exceeded that of top predators. It is therefore likely that parasitic organisms have great influence over important ecological factors such as species distribution and population size within the estuarine environment (Haskin & Ford 1982).

In many cases, transitions in temperature and salinity along an estuarine gradient have been shown to affect the ecology and distribution of organisms within the estuary (Kennish 1986). This is also true of some parasites (Barber et al. 1997, Kvach 2004,

Tolley et al. 2006). Additionally, characteristics of host-parasite populations may vary with the seasonal changes in temperature and salinity that accompany periods of high or low runoff in estuarine environments (Crosby & Roberts 1990). Lower instances of parasite infections in some hosts living in estuaries have led several authors to suggest that these host populations may be experiencing “salinity refuges” from their parasites (Reisser & Forward 1991, Tolley et al. 2006).

Most studies of salinity refuges have examined the relationships between the Atlantic oyster *Crassostrea virginica* and its parasites, the sporozoan *Haplosporidium nelsoni* and the apicomplexan protozoan *Perkinsus marinus* (Andrews 1964, Haskin et al. 1965, Haskin et al. 1966, Sprague et al. 1969, Haskin & Ford 1982, Crosby & Roberts 1990, Burreson & Ragone Calvo 1993, Chu et al. 1996, Barber et al. 1997). The data from these studies suggest that *C. virginica* may find refuge from its parasites by living in parts of estuaries where low salinities and high temperatures do not allow the parasite to either infect the oysters or proliferate within them. Another study showed that prevalence of the rhizocephalan barnacle *Loxothylacus panopaei* in the xanthid crab *Panopeus obesus* was reduced upstream during seasonally wet months, when salinity gradients were more fully expressed, leading the authors to conclude that estuaries may act as spatiotemporal refuges for successful reproduction by potential host crabs (Tolley et al. 2006). The refuge concept may also work in reverse. Childers et al. (1996) found that prevalence of the microsporidian parasite *Nadelspora canceri* in the crab *Cancer magister* was higher within crabs in estuaries than in oceanic crabs.

The Dungeness crab, *Cancer magister* Dana 1852, is an important commercial and sport fishery species that occurs along the Pacific coast of North America from

Alaska to California (Pauley et al. 1989). Although the majority of crabs are found offshore on sandy bottoms, many move into estuaries for part or all of their lives (Armstrong et al. 2003). Adult Dungeness crabs are known to tolerate salinities ranging from 11 to 35, though juveniles tolerate less saline conditions (Cleaver 1949, Robinson & Potts 1979). Could the movement into estuaries by some crabs serve as a refuge from parasitism?

Cancer magister is known to host the nemertean worm *Carcinonemertes errans* Wickham 1978 (Wickham 1979a). Worms of the genus *Carcinonemertes* (Nemertea: Enopla: Hoplonemertea) are egg predators of decapod crustaceans (Coe 1902, Humes 1942, Wickham 1978, Roe 1984). The descriptive term “egg predator” has been adopted by some authors instead of “parasite” because the worms feed on many host embryos during their lifetime, mimicking the feeding behavior of a predator more closely than that of a parasite (Kuris 1997). However, since these worms spend their life on one or a few host individuals and their biology is closely attuned to that of their hosts, they can easily be modeled as parasitic castrators, having an effect on host reproductive output (Kuris & Lafferty 1992).

The majority of studies involving the relationship between *Cancer magister* and *Carcinonemertes errans* have been carried out using oceanic populations of adult crabs, where parasite intensity can be in the tens of thousands on single host specimens (Wickham 1979b). The one study that examined the estuarine dynamics of this relationship found that the occurrence of *C. errans* on *C. magister* followed a salinity gradient in the river-dominated Columbia River Estuary (McCabe et al. 1987). Within this estuary, parasite prevalence was 6% compared to 79% in offshore waters.

Prevalence at the estuary mouth was intermediate (25%; McCabe et al. 1987). Although no rigorous studies have tested the salinity tolerance of *C. errans* (but see Chapter III), no worms were found on crabs where salinity reached 0 (McCabe et al. 1987). Scrocco and Fabianek (1970) found adult specimens of the Atlantic congener *Carcinonemertes carcinophila* to be tolerant to salinities above 10. Below that threshold, however, all worms died within two days. No studies have examined long-term changes in prevalence or intensity of *C. errans* within or between estuaries, although such data would be ideal for understanding these dynamic environments where conditions are highly dependent on both freshwater runoff and tidal influence and vary widely from one estuary to another.

In this study, I conducted a multi-year survey of the distribution of *Carcinonemertes errans* on *Cancer magister* along an estuarine gradient in a Pacific Northwest estuary and assessed the potential of such estuarine habitats to provide salinity refuges for Dungeness crabs.

MATERIALS AND METHODS

Study site

Coos Bay is a drowned river estuary 54 km² in area located along the southern coast of Oregon. Input from rivers and streams varies seasonally, from 150 m³ s⁻¹ during the rainy winter to <3 m³ s⁻¹ in the dry summer months (Roegner et al. 2007). The bay can be divided into four distinct salinity regimes: the euhaline regime (>30) which is located near the mouth of the bay, the polyhaline regime (18-30) which stretches from about river mile 5 to river mile 12, the mesohaline regime (5-18) which consists of most of the upper-bay sloughs, and the oligohaline regime (<5) which is riverine (Davidson

2006). Based on pilot trapping surveys, I chose seven sampling sites that spanned the distribution of adult *Cancer magister* within the estuary and were accessible by shore and boat (Fig. 2.1). Three of the sites (OIMB Boathouse, Clam Island, and Empire Docks) were located within the euhaline, or lower, region of the bay. The OIMB Boathouse is closest to the mouth of the bay (1.74 km away), Clam Island is 5.38 km from the mouth, and Empire Docks is 7.98 km upriver from the mouth (Fig. 2.1). The Jordan Cove and Highway sites lie between the polyhaline main bay and the mesohaline North Slough and Haynes Inlet, 14.41 km and 15.32 km from the mouth, respectively (Fig. 2.1). The final two sites were in the South Slough, a branch off the main bay. Collver Point is a marine to polyhaline site, approximately 5.1 km from the mouth of the bay. Valino Island is a polyhaline to mesohaline site 1.7 km up river from Collver Point and 6.82 km from the mouth of the bay (Table 2.1).

Estuarine distribution of *Carcinonemertes errans*

Dungeness crabs were captured year-round in the Coos Bay Estuary. Sampling occurred monthly between June 2008 and June 2011, and each site was sampled at least once quarterly. All trapping was performed using baited Fukui FT-100 multi-species marine traps (60 cm x 45 cm x 20 cm; Fig. 2.2). The 12 mm mesh size of these traps captured nearly all size classes of crabs. Bait was typically tuna, but squid, herring, and halibut were also occasionally used. To maximize the size range of crabs available for examination, trapping was conducted both intertidally and subtidally. Intertidal traps were set during a low tide, allowed to soak through an entire tidal cycle, and then

examined the next day. Subtidal traps were deployed by boat, allowed to soak 2-4 hours before and after a slack tide, then collected.

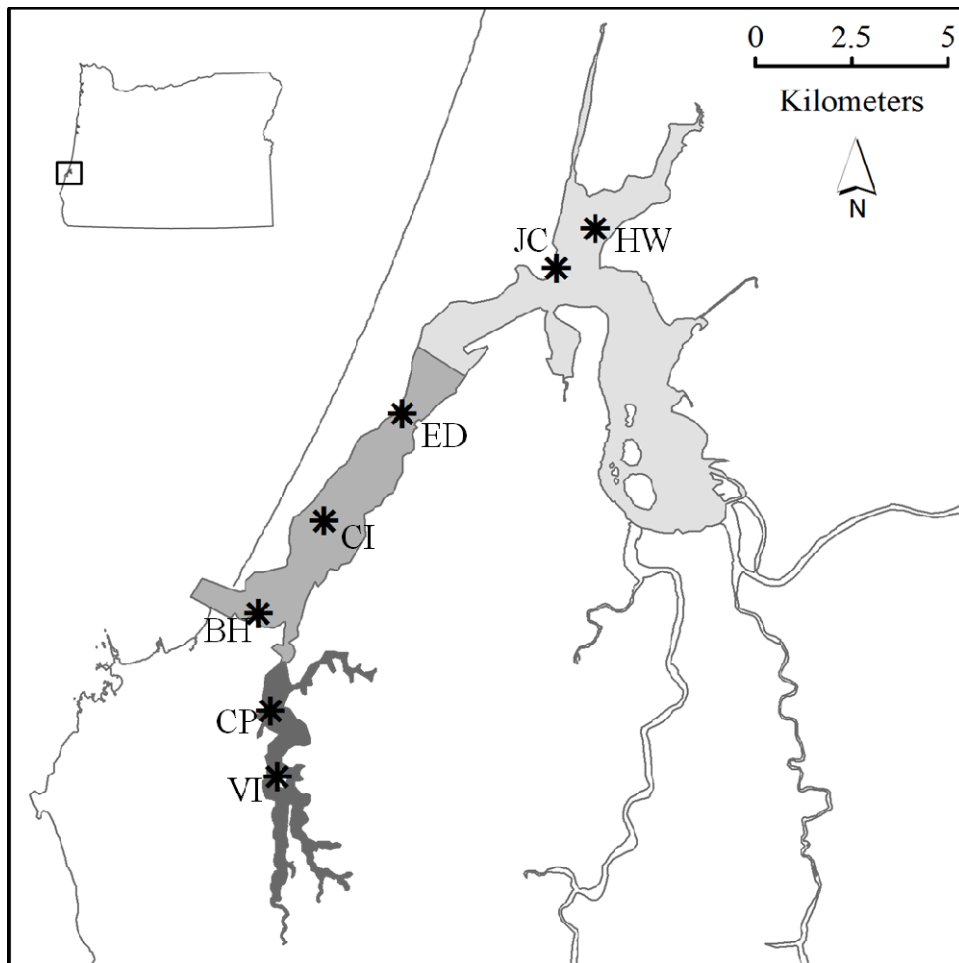


Fig. 2.1. Sites for trapping survey within the Coos Bay Estuary and the South Slough, Oregon. The three regions of the bay are represented by dark gray (South Slough), gray (Lower Bay), and light gray (Upper Bay). Site abbreviations: BH = OIMB Boathouse, CI = Clam Island, ED = Empire Docks, JC = Jordan Cove, HW = Highway, CP = Collver Point, VI = Valino Island.

Table 2.1. Description of trapping sites for survey of *Carcinonemertes errans* infecting *Cancer magister* in the Coos Bay and South Slough, Oregon. Salinity regions are defined as in Davidson (2006). Trapping depths represent averages of subtidal sampling.

Site	River km (distance from jetties)	Salinity Region	Trapping Depth
OIMB Boathouse	1.74 km	Euhaline (lower bay)	8 meters
Clam Island	5.38 km	Euhaline (lower bay)	6 meters
Empire Docks	7.98 km	Euhaline (lower bay)	6 meters
Jordan Cove	14.41 km	Mesohaline (upper bay)	3 meters
Highway	15.32 km	Mesohaline (upper bay)	3 meters
Collver Point	5.10 km	Euhaline (South Slough)	5 meters
Valino Island	6.82 km	Mesohaline (South Slough)	5 meters

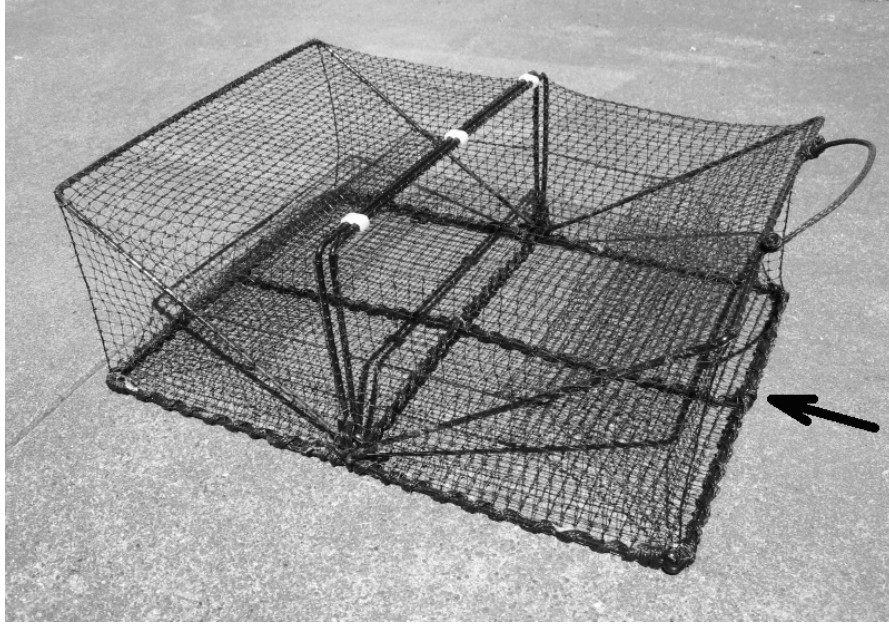


Fig. 2.2. Fukui FT-100 multi-species marine trap used to sample *Cancer magister*. Crabs enter the trap through side openings (arrow).

Short trapping durations were necessary to avoid substantial drifting (and subsequent loss) of traps left for more than a few hours. Temperature and salinity were measured at each sampling site at the time of trapping using a hand-held YSI meter (YSI Model 30 Salinity, Conductivity, and Temperature System). Crabs taken from offshore waters with the aid of commercial fishermen were also examined, mostly at the time of collection on the fishing vessel. The rest were examined at a fish processing plant, where I would examine a subset of crabs as they were offloaded from fishing vessels.

The carapace width (CW) of each captured crab was measured just anterior to the 10th lateral spine. The sex of the crab was also noted. Infections by *Carcinonemertes errans* were determined using two standard parasite metrics: parasite prevalence and parasite intensity (Margolis et al. 1982). To determine parasite prevalence, each crab was carefully examined for the presence of nemerteans. Most worms were found under the

abdomen and on the arthroal membranes of the walking legs. In very heavy infections, they were also present around the eye stalks, especially on female hosts (Wickham 1979b). If worms were found on a crab, the individual was given a prevalence score of 1. If no worms were found, the score was 0. Parasite intensity is determined by counting the individual worms on each infected crab. This was performed at the collection sites whenever possible. When an individual crab carried more worms than could be counted on site, the crab was taken to the laboratory at the Oregon Institute of Marine Biology. There the worms were removed from the crab using a water pick and counted with the aid of a dissecting microscope. Following enumeration of all worms, the mean intensity for crabs at each site was calculated. All crabs were returned to the bay following examination.

To determine if parasite prevalence or mean intensity varied seasonally, crabs were trapped at the same sites during different months of the year. Using rainfall, salinity, and water temperature data retrieved from data loggers maintained by the System-wide Monitoring Program of the South Slough National Estuarine Research Reserve (National Oceanic and Atmospheric Administration 2008), I divided the year into two seasons. The wet season went from November through April and was defined by 5+ inches of average rainfall per month and average salinities <30 at the Valino Island SWMP station, 6.82 km from the mouth of the bay. The dry season ran from May through October, months in which average rainfall was less than 5 inches per month and average monthly salinities >30 at the Valino Island station. The parasite prevalence and mean intensity of *Carcinonemertes errans* were compared for each site between the wet and dry season.

Statistical analysis

Despite relatively equal sampling effort, the number of crabs captured at each site varied widely in this survey. Thus, sample sizes were uneven and the variances in both prevalence and mean intensity were unequal. I therefore analyzed the data using non-parametric tests. Variation in parasite prevalence and mean intensity of *Carcinonemertes errans* within Coos Bay and offshore waters were compared by using site and crab size as factors in Kruskal-Wallis H tests (Sokal & Rohlf 1981). Individual differences between levels of a given factor were examined using post-hoc Mann-Whitney U tests or Dunn's tests, both of which allow for unequal sample sizes. Differences in parasite prevalence and intensity by season and by sex were examined using Mann-Whitney U tests and t-tests, respectively (Zar 2010).

RESULTS

Within the Coos Bay Estuary and nearshore waters, I captured 896 individual *Cancer magister*. Of these, 577 were infected with *Carcinonemertes errans*. The site-by-site breakdown of animals examined is shown in Table 2.2.

Parasite prevalence by site

Parasite prevalence of *Carcinonemertes errans* within the Coos Bay Estuary and offshore waters varied significantly with site (Kruskal-Wallis H test, $H = 453.07$, $p < 0.001$). All crabs collected offshore during the study were infected with *C. errans*. Within the estuary, three distinct groups emerged (Fig. 2.3). Like offshore crabs,

Table 2.2. Summary of all individual *Cancer magister* examined during the trapping survey. CW = crab carapace width. The wet season was defined as November-April, and the dry season was May-October.

Site	Total sampled	# infected	mean CW (cm)	median CW (cm)	# of males	# of females	# sampled in wet season	# sampled in dry season
Offshore	101	101	13.02	12.7	70	31	49	52
OIMB Boathouse	158	158	11.60	11.6	85	73	47	111
Clam Island	73	72	11.75	11.9	58	15	50	23
Empire Docks	85	84	11.35	11.3	61	24	47	38
Jordan Cove	35	6	8.06	8	20	15	0	35
Highway	82	8	7.50	7	39	43	21	61
Collver Point	58	27	11.87	11.95	47	11	0	58
Valino Island	304	121	10.16	10	238	66	48	256
Total	896	577	10.66	10.56	618	278	262	634

prevalence was at or near 100% at lower bay sites OIMB Boathouse (100% ± SE 0), Clam Island (98.63% ± SE 1.4%), and Empire Docks (98.82%, ± SE 1.2%; Fig. 2.3). A second group consisted of the two upper bay sites where prevalence ranged from 17.14% ± SE 6.4% (Jordan Cove) to 9.76% ± SE 3.3% (Highway). The two South Slough sites formed a third group, with intermediate parasite prevalence values of 46.55% ± SE 6.6% at Collver Point and 39.80% ± SE 2.8% at Valino Island.

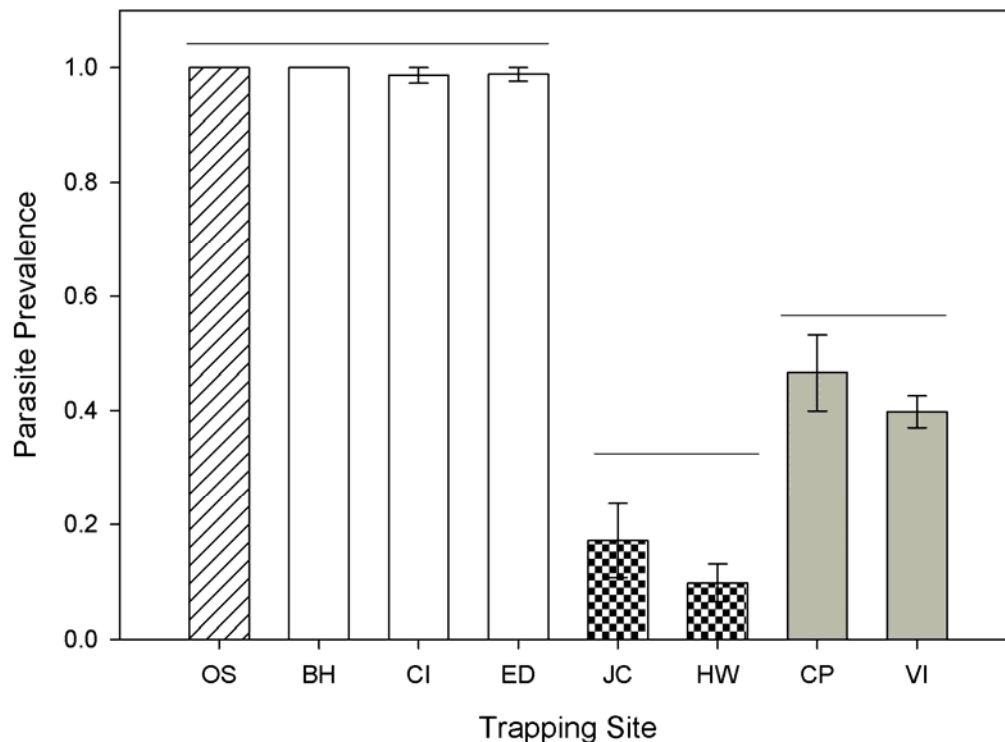


Fig. 2.3. Prevalence of *Carcinonemertes errans* on *Cancer magister* in offshore waters and within the Coos Bay Estuary. Error bars represent 1 standard error. Coloration within bars represent different regions within the bay (Stripes = offshore, white = lower bay, checkered = upper bay, gray = South Slough). Lines above bars represent significant differences determined by Mann-Whitney post-hoc tests. Abbreviations: OS = offshore, BH = OIMB Boathouse, CI = Clam Island, ED = Empire Docks, JC = Jordan Cove, HW = Highway, CP = Collver Point, VI = Valino Island.

Mean intensity by site

Mean intensity of *Carcinonemertes errans* varied significantly with site (Kruskal-Wallis H test, $H = 338.88$, $p < 0.001$). The mean intensity of worms infecting offshore crabs ($872.15 \pm \text{SE } 40.81$) was significantly higher than anything observed within the Coos Bay Estuary (Fig. 2.4a). An estuarine gradient in mean intensity of worms was observed. Crabs caught at the three lower bay sites had significantly higher mean intensities of infection (OIMB Boathouse = $129.61 \pm \text{SE } 15.12$, Clam Island = $101.49 \pm \text{SE } 15.65$, and Empire Docks = $102.85 \pm \text{SE } 22.82$), than the upper bay (Jordan Cove = $5.33 \pm \text{SE } 3.08$, Highway = $5.25 \pm \text{SE } 2.41$) and South Slough sites (Collver Point site = $46.48 \pm \text{SE } 27.69$, Valino Island = $12.81 \pm \text{SE } 3.94$; Fig. 2.4b). Though not significant, a gradient in worm infections was also apparent on crabs within the South Slough branch of the estuary, with a higher mean intensity in the more marine Collver Point than the more riverine Valino Island (Fig. 2.4b).

Parasite prevalence and mean intensity by size

A significant positive relationship between size and parasite prevalence was detected in bay crabs (Kruskal-Wallis $H = 185.93$, $p < 0.001$; Fig. 2.5). Dunn's post-hoc tests detected four groups: crabs < 8 cm CW, 8-10 cm CW, 10-14 cm CW, and > 12 cm CW (Fig. 2.5). Because all crabs taken offshore were infected, there was no relationship between size and prevalence for this group.

Mean intensity of *Carcinonemertes errans* on bay crabs varied significantly with size (Fig. 2.6). A regression analysis of carapace width against mean intensity detected a significantly positive relationship ($p < 0.001$, $r^2 = 0.137$; Fig. 2.6a).

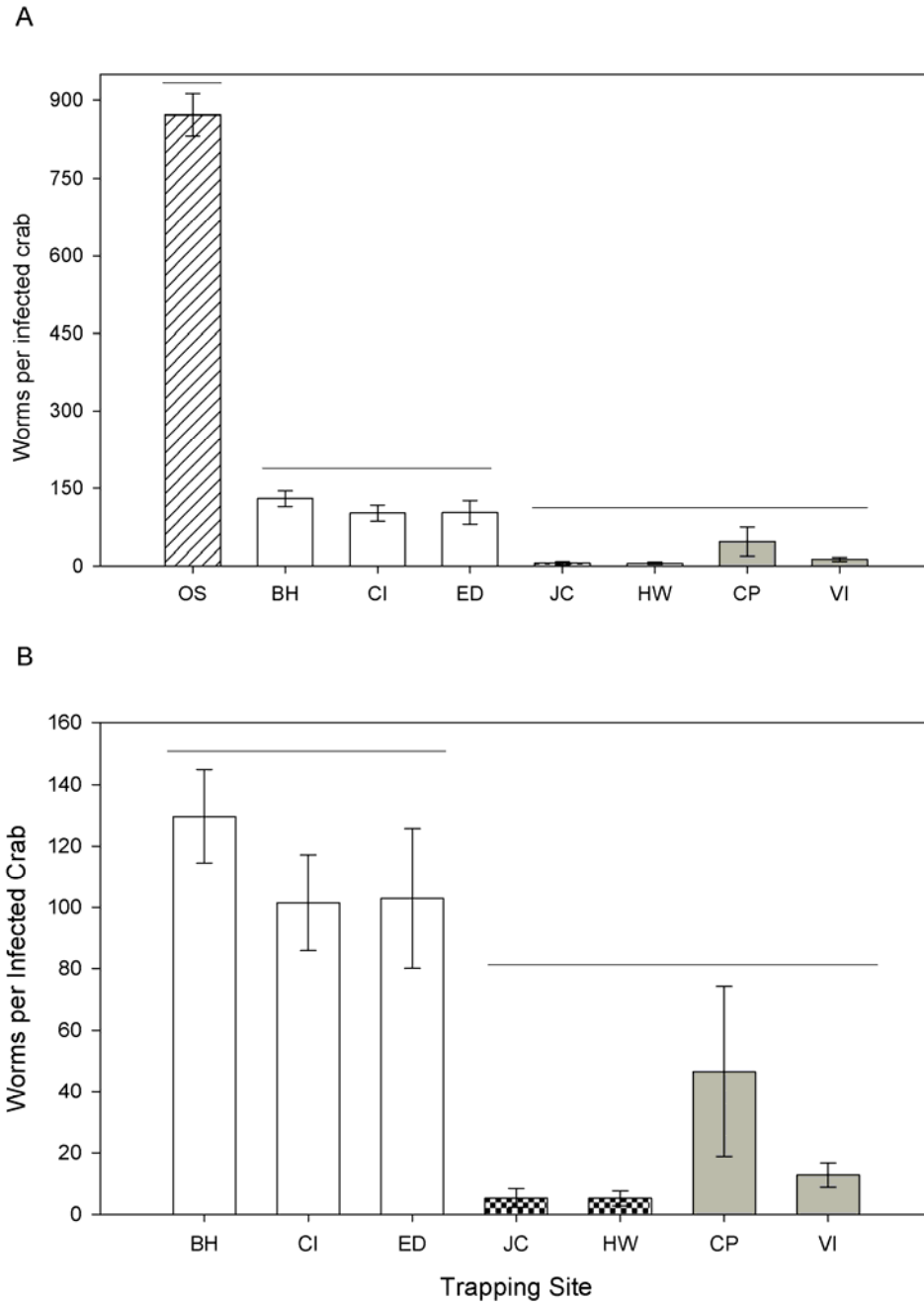


Fig. 2.4. (A) Mean intensity of *Carcinonemertes errans* on Dungeness crabs in offshore waters and within the Coos Bay Estuary and (B) mean intensity only within the Coos Bay. Error bars represent 1 standard error. Coloration within bars represent different regions within the bay (Stripes = offshore, white = lower bay, checkered = upper bay, gray = South Slough. Lines above bars represent significant results of Mann-Whitney post-hoc tests. Abbreviations: OS = offshore, BH = Boathouse, CI = Clam Island, ED = Empire Docks, JC = Jordan Cove, HW = Highway, CP = Collver Point, VI = Valino Island.

Likewise, there was a significant difference in mean intensity among crab size classes (Kruskal-Wallis $H = 75.691$, $p < 0.001$; Fig. 2.6b). Dunn's post-hoc tests found four significantly different groups: <10 cm CW, 8- 12 cm CW, 10-14 cm CW, and >14 cm CW (Fig. 2.6b). Similar patterns were observed among crabs sampled offshore, with a significant correlation between size and mean intensity ($p < 0.001$, $r^2 = 0.281$; Fig. 2.7a) and between size class and mean intensity (Kruskal-Wallis, $H = 38.002$, $p < 0.001$). Dunn's post-hoc tests determined a significant difference between crabs 8 to 11.9 cm CW and crabs larger than 12 cm CW (Fig. 2.7b).

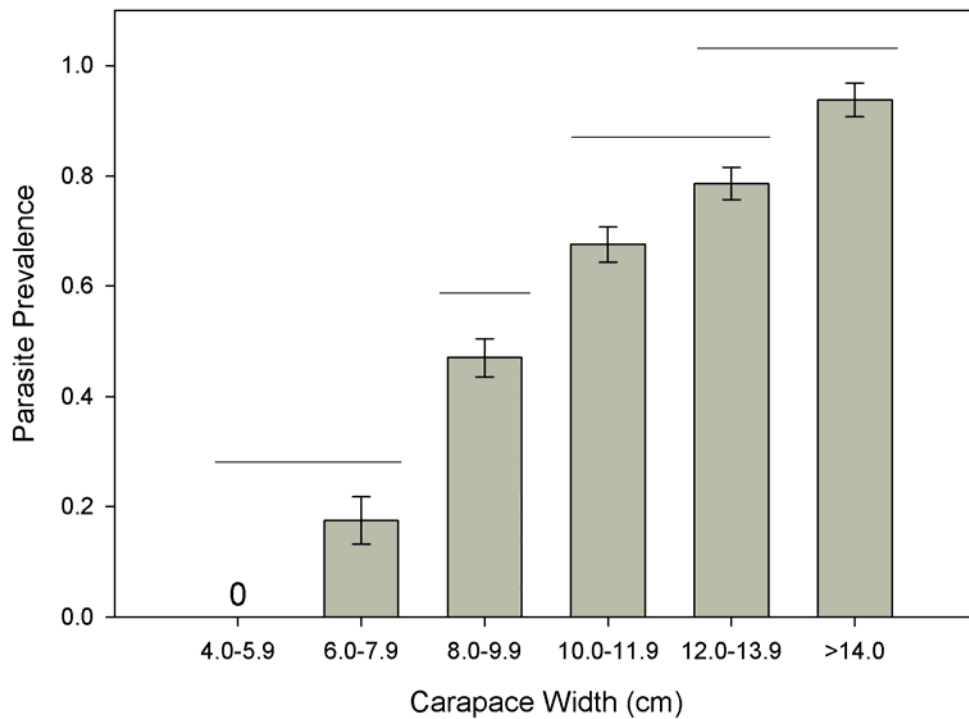


Fig. 2.5. Prevalence of *Carcinonemertes errans* on various size classes of *Cancer magister* from the Coos Bay Estuary. Error bars represent 1 standard error. Lines above bars represent significant results of Dunn's post-hoc tests.

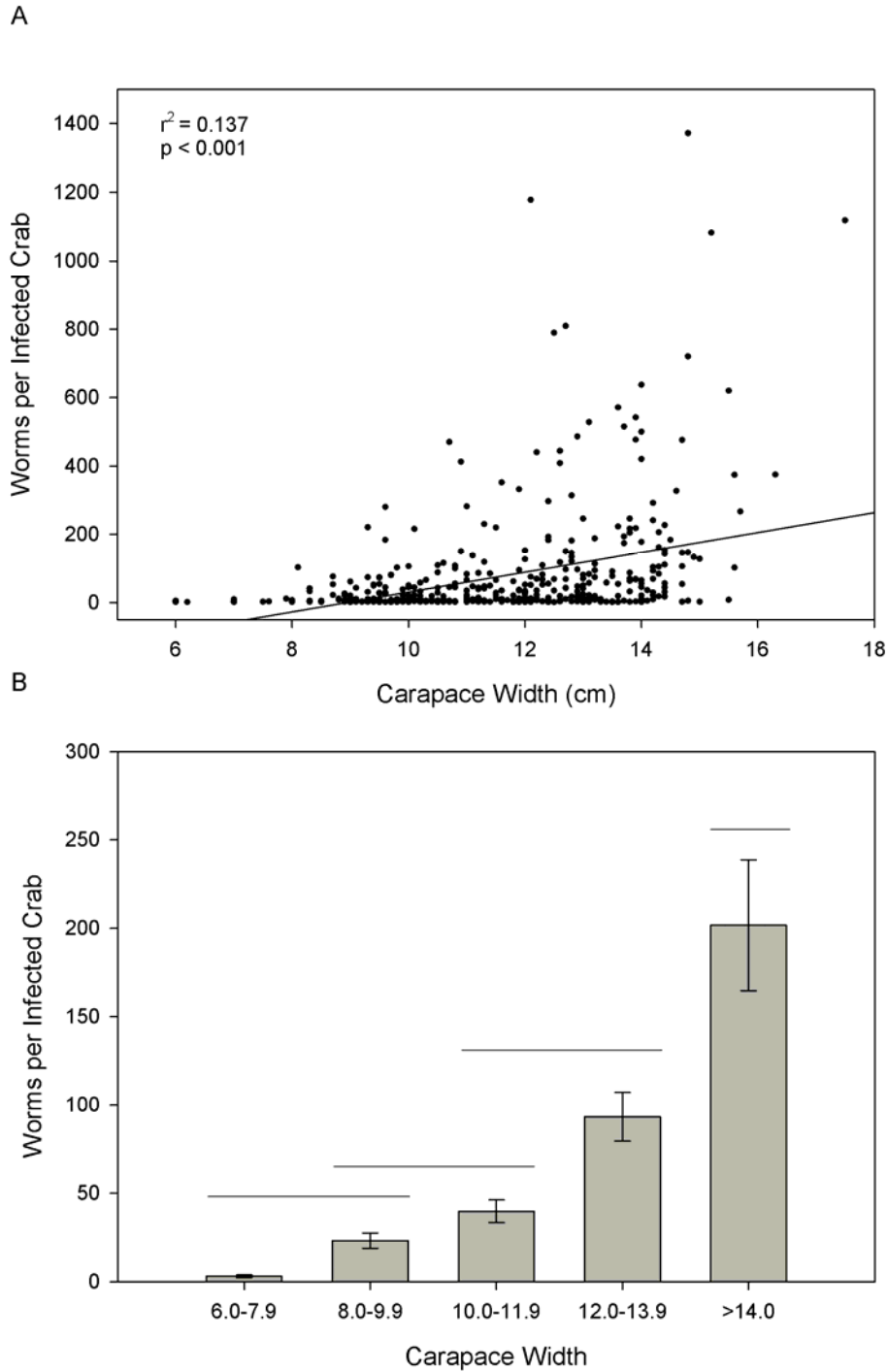


Fig. 2.6. Relationship between crab size and mean intensity of *Carcinonemertes errans* from the Coos Bay Estuary. (A) linear regression of crab carapace width (CW) vs. intensity, and (B) mean intensity of *C. errans* on different size classes of Dungeness crabs. Error bars represent 1 standard error. Lines above bars represent significant results of Dunn's post-hoc tests.

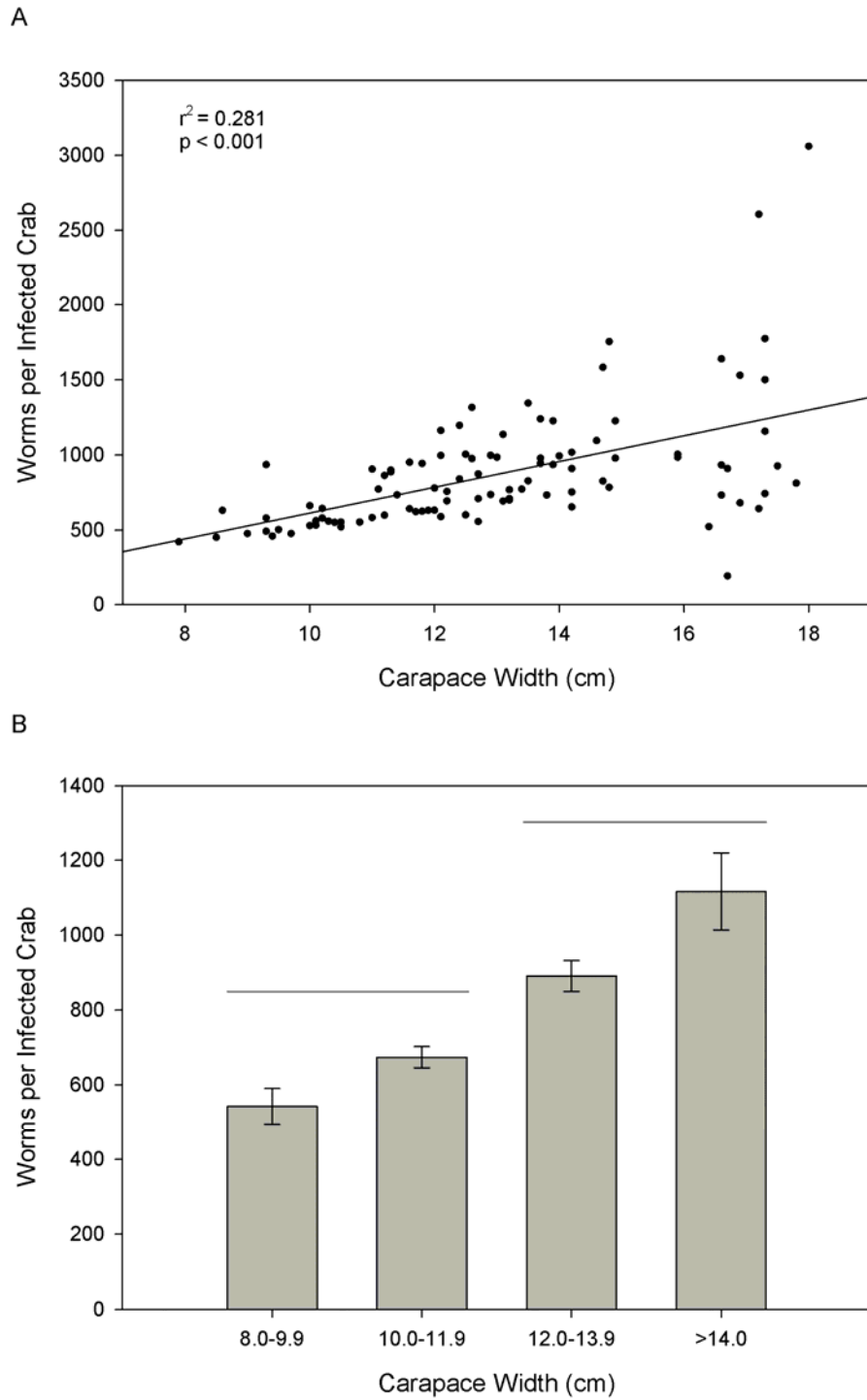


Fig. 2.7. Relationship between crab size and mean intensity of *Carcinonemertes errans* in offshore waters. (A) linear regression of crab carapace width (CW) vs. intensity, and (B) mean intensity of *C. errans* on different size classes of Dungeness crabs. Error bars represent 1 standard error. Lines above bars represent significant results of Dunn's post-hoc tests.

Parasite prevalence and mean intensity by sex

There was a significant difference in parasite prevalence between male and female crabs trapped in the Coos Bay Estuary (t-test, $p = 0.016$), with male crabs ($61.14\% \pm \text{SE } 2.1\%$) being more likely to carry *Carcinonemertes errans* than female crabs ($52.16\% \pm \text{SE } 3.1\%$). Male crabs were also significantly larger than females (t-test, $p < 0.001$), however, suggesting that the pattern has more to do with the size of the crab than its sex. No significant difference in mean intensity was found between male and female crabs in the estuary (t-test, $p = 0.832$).

Parasite prevalence and mean intensity by season

Parasite prevalence of *Carcinonemertes errans* was significantly higher in the wet season ($86.39\% \pm \text{SE } 2.4\%$) than in the dry season ($48.42\% \pm \text{SE } 2\%$) in the Coos Bay Estuary (Mann-Whitney U test, $p < 0.001$). When the sites that had sufficient numbers of crabs sampled during both seasons (all except Jordan Cove and Collver Point) were examined separately, it became clear that Valino Island was the site driving the seasonal pattern (Mann-Whitney U test, $p < 0.001$). None of the other sites showed significant differences between seasons (Fig. 2.8a).

A significant seasonal effect was also seen in the mean intensity of infection (Mann-Whitney U test, $p < 0.001$), with crabs carrying heavier parasite loads during the wet season ($107.91 \pm \text{SE } 13.74$) than the dry season ($51.03 \pm \text{SE } 7.85$). This time it was the OIMB Boathouse site that was driving the pattern (Mann-Whitney U test, $p < 0.001$), while all other sites, including Valino Island, showed no significant change between seasons (Fig. 2.8b). Interestingly, the same significant pattern in mean intensity

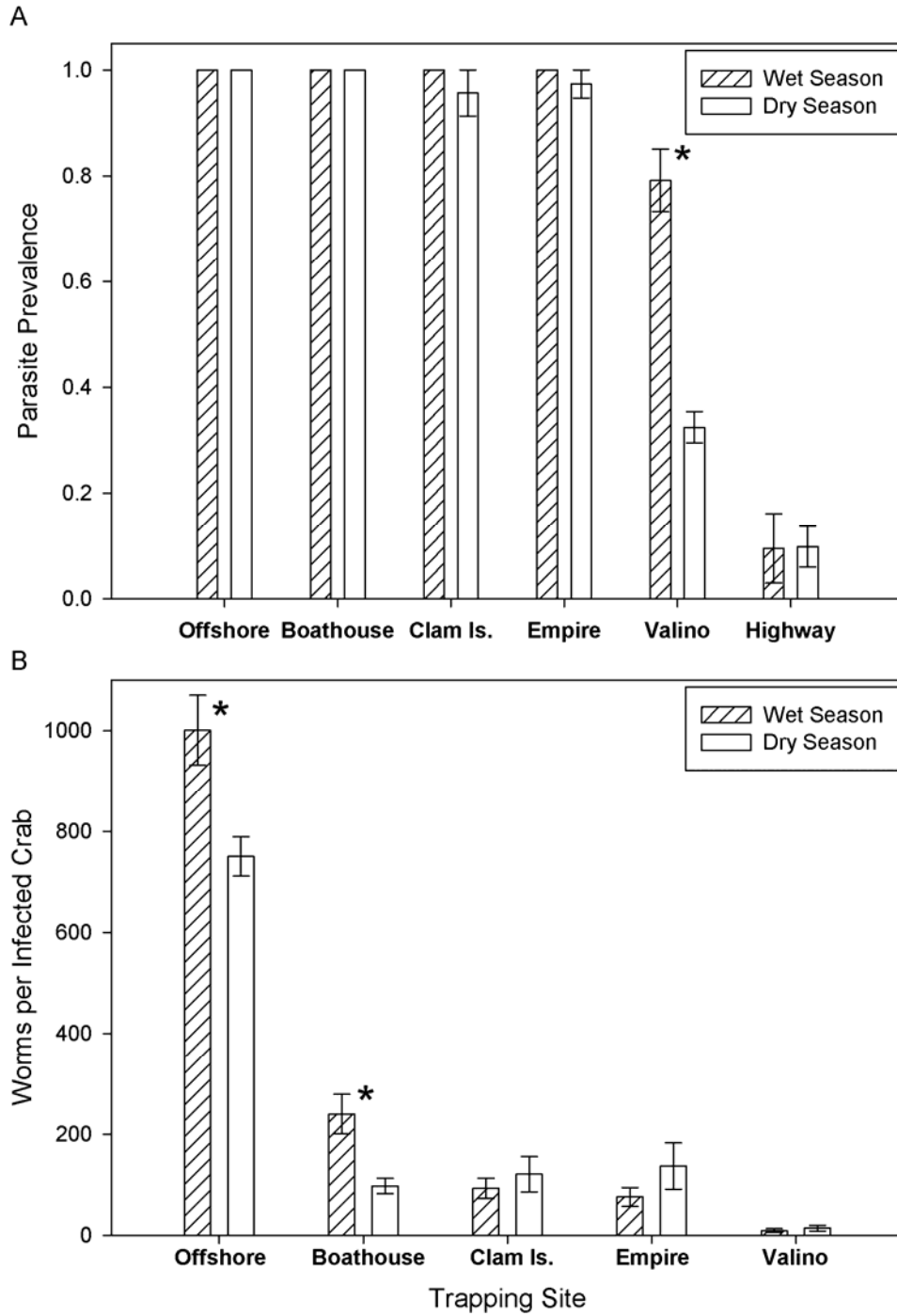


Fig. 2.8. Presence of *Carcinonemertes errans* by season and site. (A) Prevalence and (B) mean intensity of *C. errans* on *Cancer magister* from the Coos Bay Estuary and offshore waters. Error bars represent 1 standard error. *= significant result of Mann-Whitney post-hoc test between seasons.

between wet and dry seasons at the OIMB Boathouse was also observed for offshore crabs (Mann-Whitney U test, $p < 0.001$).

T-tests showed that crabs taken during the wet season at Valino Island ($t = 3.863$, $p < 0.001$), the OIMB Boathouse ($t = 4.154$, $p < 0.001$), and offshore ($t = 5.826$, $p < 0.001$) were significantly larger than crabs taken during the dry season. Size did not vary significantly with season at any of the sites where no seasonal pattern was detected (T-tests, $\alpha = 0.05$). To determine whether the size of the crabs caught was driving the seasonal differences in parasite intensity, I calculated the parasite density by dividing the mean intensity of each crab by its estimated surface area. These density values were then used in separate Mann-Whitney U tests to determine if density changed significantly with season within each site. The results are given in Fig. 2.9. For offshore crabs, parasite density did not vary significantly between seasons ($U = 1109$, $p = 0.264$), suggesting that size was significantly affecting the observed seasonal difference in parasite intensity (Fig. 2.9). The parasite density during the wet season at the OIMB Boathouse site, however, remained significantly higher than the density during the the dry season ($U = 0$, $p < 0.001$), suggesting that size alone did not explain the observed pattern (Fig. 2.9).

DISCUSSION

The distribution of *Carcinonemertes errans* followed a distinct estuarine gradient in the Coos Bay Estuary. When measured by parasite prevalence, no significant change in prevalence of *C. errans* occurred until one reached the upper bay and South Slough

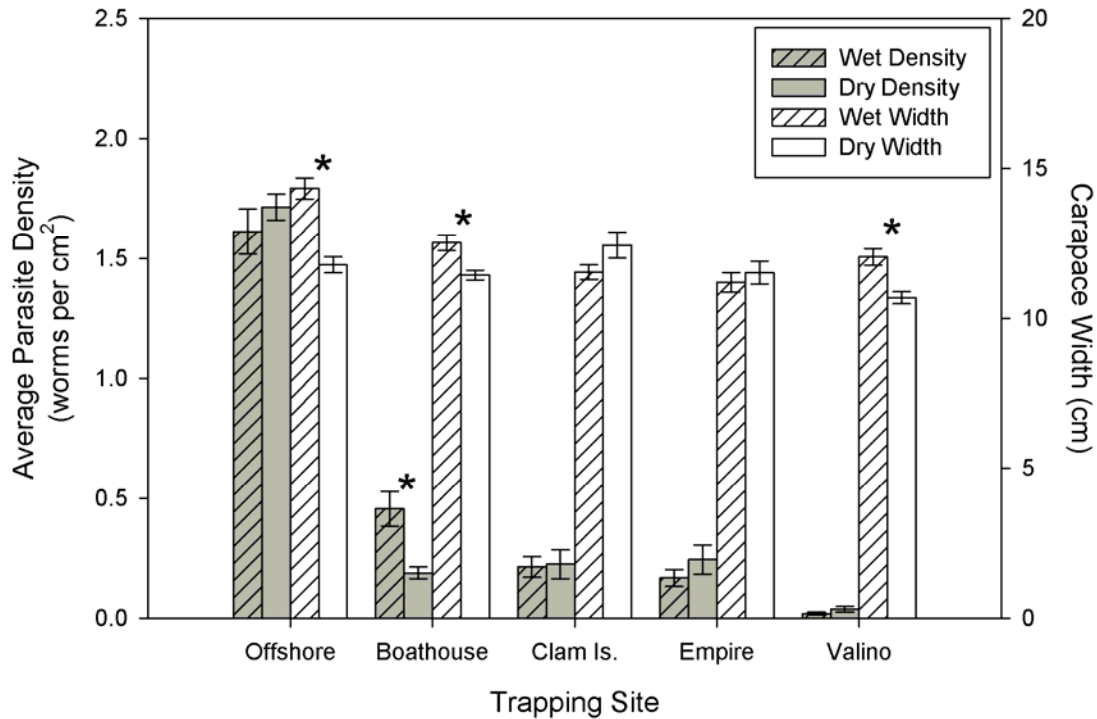


Fig. 2.9. Average parasite density and average carapace width for *Cancer magister* taken during the wet and dry seasons within the Coos Bay Estuary and offshore waters. Error bars represent 1 standard error. *=significant difference between seasons for either density or carapace width (Mann-Whitney U tests, $\alpha=0.05$).

sites, with crabs captured at lower bay sites just as likely to carry *C. errans* as crabs from offshore waters (Fig. 2.3). The pattern in mean intensity, however, showed a distinct, highly significant change in the number of worms per infected crab immediately upon entering the estuary (Fig. 2.4a). Once inside the bay, the pattern resembled that of parasite prevalence, with similar infection levels at the three lower bay sites that were much higher than those seen in upper bay sites or the South Slough (Fig. 2.4b). Although there appears to be a trend of intermediate mean intensity at the South Slough sites, low numbers of infected individuals there and at the two upper bay sites probably made statistical detection of this difference much more difficult. I propose two major

mechanisms for the observed distribution pattern: 1) The pattern is an artifact of the life history of *Cancer magister*, and 2) The pattern is a function of one or more parameters of the estuarine environment itself.

Dungeness crab life history artifact

Many of the Dungeness crabs found within the estuaries of the Pacific Northwest enter these habitats as megalopae on their return journey from offshore waters (Lough 1976). These megalopae ride the rising tide into the bay, some settling out early on, while others are carried far into the upper reaches of tidal waters. Once there, the megalopae molt into first instar juveniles and begin their benthic existence (Brown & Terwilliger 1992). During their first few years of life, juvenile Dungeness crabs tend to remain in relatively shallow waters, moving into the intertidal to forage, particularly at night (Holsman et al. 2006). This tendency to remain shallow and forage intertidally, coupled with a better ability to osmoregulate than adult crabs, keeps juveniles somewhat segregated from their larger, cannibalistic conspecifics. Living in higher temperature waters and having access to the large amount of potential food items allows juveniles within estuaries to grow at a significantly higher rate than the vast majority of their cohort that ended up settling on the coastal shelf (Gunderson et al. 1990).

As the juvenile crabs grow larger, they move into deeper water. This may be due to their increased ability to compete with large conspecifics, their decreased ability to tolerate low salinity water and large changes in salinity, or both (Brown & Terwilliger 1992). Particularly in the case of possible osmotic stress, this migration to deeper water may also coincide with a migration to more marine regions of the estuary. Eventually,

most crabs that settle in an estuary find their way out of the bay and into the ocean (Armstrong et al. 2003). This movement, however, is not unidirectional. Tagging studies have clearly shown that crabs tagged in coastal waters are recovered in bays (Clever 1949, Waldron 1958). Some crabs have been known to move repeatedly into and out of the same and different bays tens of kilometers apart (Waldron 1958).

Given this ontogenetic migration of Dungeness crabs within estuaries, one possible explanation for the observed estuarine gradients in parasite prevalence and mean intensity is that these patterns are simply an artifact of crab age and size. The mean size of crabs sampled at sites in the upper bay and South Slough was smaller than that of crabs caught in the lower bay and offshore (Table 2.2). During my three-year study, crab carapace width was consistently a significant predictor of parasite prevalence in the Coos Bay Estuary as well as mean intensity of *Carcinonemertes errans* both within the estuary and in coastal waters. A positive relationship between parasite prevalence (and intensity) and host size is common in many host-parasite systems, particularly parasitic castrators (Lim & Heyneman 1972, Baudoin 1975, Sorensen & Minchella 2001, Hechinger 2010). In the *Carcinonemertes-Cancer* system, the positive relationship between parasite load and host size could be due to a number of factors. First, there is a direct relationship between crab age and crab size (Pauley et al. 1989). Larger crabs have had a longer possible “exposure time” to the infective stage of *C. errans* and thus have a higher probability of both being infected by the worm and also carrying more worms (Baudoin 1975). Second, larger crabs may be more attractive to *C. errans* larvae. This could be a question of providing more of some chemical cue that the parasite could use to locate the host or possibly even providing a different kind of cue than smaller crabs do. Third,

larger crabs may be more likely to become infected because they have a larger area for infecting *C. errans* larvae to encounter (Crisp 1965). If larvae are not able to track hosts from a large distance and are contacting them by random chance, then a larger target would be a better target for encounter. Finally, the pattern might be a function of the molting cycle of *Cancer magister*. During the first few years of life, Dungeness crabs grow rapidly and molt frequently (Butler 1961). Once they reach maturity at about two years old, however, crabs average only one molt per year (Pauley et al. 1989). As *C. errans* inhabits the exoskeleton of its host, the possibility exists that some or all of the worms infecting an individual crab will be left behind on the old shell following a molting event (Kuris 1978). Even if this possibility is small, a higher frequency of molting could still lead to a higher number of worms being lost.

The hypothesis that the correlation of crab size and infection by *Carcinonemertes errans* is a function of crab size independent of “exposure time” and location is refuted by the field settlement experiments described in Chapter IV of this dissertation. Briefly, when crabs of different sizes were placed next to each other in cages and left for a known amount of time, the number of worms recruiting to larger crabs was not higher than the number recruiting on smaller individuals. This suggests that any possible settlement cue(s) emitted by crabs are the same regardless of size and that the surface area of the crab did not play a significant role. A second, independent measure that refutes this hypothesis is the result of the parasite density calculations (Table 2.3). The average density of *C. errans* on crabs offshore (1.664 ± 0.053 worms cm^{-2}) was much higher than that of lower bay crabs (0.217 ± 0.034 worms cm^{-2}), and parasite density still decreased as you moved up the estuary (Table 2.3).

Table 2.3. Mean parasite density calculations for each of the sites within the Coos Bay Estuary and offshore. Parasite density (worms/cm²) is equal to the number of worms on a crab (intensity) divided by the crab's estimated surface area.

Site	Mean Parasite Density	error
Offshore	1.664	0.053
OIMB Boathouse	0.195	0.027
Clam Island	0.217	0.035
Empire Docks	0.201	0.033
Jordan Cove	0.015	0.005
Highway	0.015	0.004
Collver Point	0.085	0.045
Valino Island	0.031	0.009

The molt-frequency hypothesis is also unlikely to play a major role in determining the distribution of *Carcinonemertes errans* in the Coos Bay because worms are known to transfer to their host's new exoskeleton with near 90% efficiency (Wickham et al. 1984). Although some worms may still be occasionally lost in the molting process, the size-infection pattern appears to be too large to be driven by a small proportion of worms being left behind during relatively infrequent molting events. The hypothesis that the pattern is a function of possible "exposure time" to parasite vectors cannot be refuted by any available data and is possibly supported by results of other settlement experiments described in Chapter IV, wherein crabs held in cages for longer periods of time tended to carry higher parasite loads than those caged for shorter periods.

Crab mobility is another possible explanation for the observed patterns in parasite distribution. Although many tagged crabs do seem to exhibit some level of fidelity to a given area, others are known to move in and out of coastal estuaries (Waldron 1958). There is also the problem of crabs moving from one area of the bay to another. My sampling regime was not designed to track crab movements whether within the bay or

between the bay and coastal waters. It is therefore highly probable that some proportion of crabs trapped at any given site on any given day represented crabs that had only recently moved to that site from elsewhere. Although such movements undoubtedly occurred between sampling, the clear gradient in mean intensity between ocean crabs and bay crabs and in both prevalence and mean intensity between different regions within the bay seems to indicate that mixing is happening mostly within the regions shown in Fig. 2.1 rather than between them. The Collver Point site is probably an exception to this, with possible influences coming from both the direction of the OIMB Boathouse and Valino Island. Clearly, the mechanism(s) driving the gradient are strong enough to overcome crab motility.

Finally, reproductive biology of both *Cancer magister* and *Carcinonemertes errans* could influence the observed estuarine gradient in parasite prevalence and intensity. Although large female crabs can often be found in estuarine habitats along the Oregon coast, most are located in coastal waters (pers. comm., S. Groth, Oregon Department of Fish and Wildlife). This means that a large proportion of ovigerous female crabs will spend their incubating time on the near-shore shelf. Juvenile *Carcinonemertes* worms feed, grow, mature, mate, and lay their egg strings within the egg masses of their crab hosts. About two weeks after being deposited, the embryos within the worm egg strings hatch out as small planuliform larvae, which then begin a planktonic existence of unknown duration before becoming competent to settle and infect a new host (see Chapter IV). Thus, the source for the majority of competent larvae of *C. errans* is the coastal ocean.

Some larvae of estuarine invertebrates, particularly crustaceans, are known to utilize tidal movements to reinvade estuaries (Forward & Rittschof 1994). As described above, the megalopae of Dungeness crabs appear to be capable of riding the tides deep into estuaries (Lough 1976). Whether the competent stage of *Carcinonemertes errans* is capable of using tidal currents in a similar fashion is unknown. Assuming that *C. errans* is not capable of behavior-related transport into estuaries and is only passively washed in and out during each tidal cycle, one would hypothesize a gradient in the number of larvae available as one moves from the ocean to the lower bay and from there to the upper bay and the South Slough. The pattern of flow within the Coos Bay Estuary reinforces this pattern. Water in the lower estuary is thoroughly flushed every tidal cycle. The water from the upper estuary, however, is only replaced every 2-3 high tides (pers. comm., S. Rumrill, South Slough National Estuarine Research Reserve). The same is true for the South Slough branch of the estuary (Rumrill 2006). Thus, although the Empire Docks site is slightly farther from the mouth of the bay than is the Valino Island site (4.96 km vs. 4.24 km), the volume of oceanic water that reaches the former is much higher than that which reaches the latter. This coincides perfectly with the observed distribution of juvenile worms on crabs. In Chapter IV of this dissertation, I describe the results of a one-year plankton tow survey within the Coos Bay Estuary. The larvae were most common at lower bay sites and much less common or absent in the upper bay and South Slough. The results of larval settlement experiments conducted along the estuarine gradient suggest that while competent larvae are present even in the upper bay, they are more common in the lower bay region.

Estuarine environment

Estuaries are natural mixing bowls. The environment that an organism encounters within an estuary can be vastly different than that of the subtidal zone of the coastal ocean (Kinne 1966). Whereas the environment of the coastal subtidal remains relatively static through time with regard to water properties such as temperature, salinity, dissolved oxygen, etc., estuaries can experience dramatic changes in these factors on tidal and seasonal timescales (Kennish 1986). If a given organism is not adapted to cope well with these fluxes and extremes, the estuary could represent a highly stressful environment. For a marine organism, these stresses would presumably become more acute as the organism moves away from the ocean-estuary interface and farther up the estuary (Chapter III). In the case of *Carcinonemertes errans* on crabs in the Coos Bay Estuary, possible environmental stressors unique to or magnified within the estuary could be acting in two ways to create the observed gradient. First, stressors could act directly on the larval stage, killing any unfortunate larvae that are brought into intolerable estuarine waters before they can infect their hosts (Anger 2003). Second, juvenile or adult worms already present on the exoskeleton of a host individual could be eliminated if the host moved into a part of the estuary where conditions were not tolerable to the worms.

Although the Coos Bay Estuary is not river-dominated like the Columbia River estuary in the study of McCabe et al. (1987), it can still experience low average salinities and occasional strong freshets during the wet season (November-April; Queen & Burt 1955). For example, during the 2008 sampling season, the salinity at Valino Island regularly dropped below 10 during the wet season and bottomed out at 3.1 on February 4 (National Oceanic and Atmospheric Administration 2008). In 2009 at the Empire Docks

site in the main bay, average salinities varied between 34.3 in the dry season and 27.8 in the wet season but could dip down below salinity 5 on occasion (Confederated Tribes 2010). Although no monitoring data exists for Jordan Cove or Highway sites, their position farther upriver from Empire Docks as well as their proximity to freshwater inputs at North Slough and Haynes Inlet suggest that both average and extreme salinities would be lower than that at Empire Docks.

Chapter III of this dissertation deals directly with the questions regarding the physiological tolerances of the different life stages of *Carcinonemertes errans* to temperature and salinity stress. One of the results of these studies was that both larval and juvenile worms have somewhat similar tolerances to salinity as do their crab hosts. This along with the seasonal data discussed below suggests that while physical factors such as salinity and temperature may play some role in creating and maintaining the observed gradients, they are probably not acting alone.

Seasonal changes

In their study examining the infection intensity of the protozoan *Perkinsus marinus* in an estuarine population of oysters (*Crassostrea virginica*), Crosby and Roberts (1990) found a significant difference between the infections observed during the hot dry summer when infections were highest and the cool wet winter when infections reached their lowest point. They concluded that the parasite may be controlled in part by low temperatures and salinities. Although I predicted that the same would be true of *Carcinonemertes errans* on Dungeness crabs in the Coos Bay Estuary, the data did not support this hypothesis (Figs. 2.8 & 2.9).

In five of the six sites where enough crabs were sampled during both seasons to compare prevalence (Fig. 2.8a), there was no observed difference between crabs caught during the wet season (November-April) and the dry season (May-October). At Valino Island, the one site where a significant difference between seasons was observed, prevalence during the wet season was more than double what it was during the dry season. Data from the Valino Island SWMP station (National Oceanic and Atmospheric Administration 2008) showed significantly lower salinities at Valino Island during the wet season months, suggesting that some mechanism(s) apart from salinity was driving the pattern. One possibility is a change in average crab size. Crabs caught at Valino Island during the wet season were significantly larger than those trapped during the dry season (Mann-Whitney U test, $p < 0.001$). This observed increase in average crab carapace width might be the function of molting, which typically occurs during late summer and fall for adult males and late fall and early winter for females. If one molting event is all that separated the crabs caught during the wet season from those caught during the dry season, however, one would expect to see similar instances of infection, which is not the case. Another alternative is that these larger crabs may be arriving at the Valino Island site from offshore or from more marine regions of the bay where they may have been exposed to more parasites and brought their higher prevalence rates with them. The factors driving this proposed migration of larger crabs into the lower salinity waters of the estuary is unknown, although anecdotal evidence among crab fishermen certainly supports the idea of a migration of oceanic crabs into the bay around November (pers. comm., M. Lane, commercial crab fisherman). It is also possible that smaller crabs from

upriver are moving through the Valino Island area during the dry season, driving down both the average size and parasite prevalence of crabs trapped there.

Along with the size and possible migration of crab hosts, the supply of competent larvae may also be a significant factor forcing the seasonal differences. Competent larvae of *Carcinonemertes errans* were most commonly captured in the plankton during the late summer and early fall (Chapter IV). Although no larvae were captured during tows at the Valino Island site, some were found at Collver Point (Chapter IV). The larvae disappear from the plankton in November, leading one to hypothesize that the peak in larval settlement should be during September and October. Although these two months are part of the dry season, data from Valino Island crabs captured during September and October show 80% prevalence, just like in the wet season months of November and December. This doesn't come through in the seasonal data because the September and October crabs are overwhelmed in numbers by crabs caught during June, July, and August when prevalences are much lower. Within the wet season itself, crabs show the highest prevalence of *C. errans* during November and December followed by a decline, possibly caused by worms dying from the physiological stresses of low salinity conditions. It is also possible that the larger crabs seen in the early wet season move out of the area as the season progresses.

The mean intensity of *Carcinonemertes errans* varied significantly with season as well, both in offshore crabs and in crabs within the estuary. The estuarine pattern, however, was completely driven by one site: OIMB Boathouse. Again, the data showed higher mean intensity values during the wet season than the dry, the opposite of what one might predict with a salinity-based gradient model. As with parasite prevalence at

Valino Island, at least part of the pattern seems to be explained by the size of the crabs; crabs sampled offshore and at OIMB Boathouse site were significantly larger during the wet season than the dry (Mann-Whitney U tests, $p < 0.001$). Correcting for size by calculating parasite density eliminated the significant size effect for offshore crabs, but not for crabs from OIMB Boathouse, suggesting that additional factor(s) may be at play. Possible candidates for this factor(s) are similar to those described for the prevalence pattern at Valino Island: 1) migration of crabs from areas of higher parasite infections (offshore in the case of OIMB Boathouse) during the wet season, 2) migration of crabs from areas of lower infection during the dry season, and 3) a peak of larval settlement at the end of the dry season and the beginning of the wet season followed by a slow die-off of juvenile worms due to natural mortality, physiological stress, or both. Unlike prevalence at the mesohaline Valino Island site, mean intensity at euhaline OIMB Boathouse did remain high throughout most of the wet season, suggesting that salinity stress may play a role at the former but not at the latter. By June, however, intensities were back to much lower levels. This makes it seem likely that seasonal migration patterns are occurring, with crabs moving in from the ocean during the fall and coming from the upper estuary during the summer.

CONCLUSION

The presence of a distinct estuarine gradient in both parasite prevalence and mean intensity of *Carcinonemertes errans* within the Coos Bay Estuary suggests that individual Dungeness crabs may indeed experience a spatiotemporal refuge from some or all of the effects of their nemertean egg predator by inhabiting an estuary rather than coastal

waters. This gradient, which varies seasonally, is potentially the product of one or more life history traits of *Cancer magister* and *C. errans*, the physical parameters of the estuary itself, or some combination of the two.

It is not clear if the same patterns exist in every estuary in the Pacific Northwest. However, the fact that similar patterns were observed in the two largest estuaries in Oregon (the Columbia and the Coos), each with quite different flow regimes, suggests that it may be widespread (McCabe et al. 1987). Preliminary results from the Alsea Bay estuary on the central Oregon coast also showed very few nemertean on crabs (unpublished data), but sample sizes were too low to make any conclusions as yet. It is also not yet clear to what extent crabs actually take advantage of the estuarine refuges that do exist. Although many crabs inhabit estuaries from Alaska to California, the majority of all Dungeness crabs, both juvenile and adult, inhabit the subtidal sandy substrate of the nearshore coastal shelf (Armstrong et al. 2003). It is therefore unlikely that estuarine refuges from *Carcinonemertes errans* play a significant role in the population dynamics of this important fishery species. However, for those crabs fortunate enough to have settled in an estuary and to have spent their early years there or migrated into an estuary later on, the resultant lower worm load would certainly result in more potential offspring surviving to hatching, and, possibly, higher fitness.

BRIDGE

In Chapter II, I examined the estuarine distribution of *Carcinonemertes errans* on its host *Cancer magister* and discovered a clear estuarine gradient in both parasite prevalence and mean intensity. The possible mechanisms creating this infection gradient

will now be examined. In the following chapter, I present experiments in which I tested the physiological tolerances of two life stages of *C. errans* to salinity and temperature combinations the nemerteans could experience within the Coos Bay Estuary.

CHAPTER III

PHYSIOLOGICAL TOLERANCES OF THE NEMERTEAN EGG PREDATOR *CARCINONEMERTES ERRANS* TO SALINITY AND TEMPERATURE STRESS

Introduction

All organisms must cope with the demands of their physical environment. The nature of the physical environment is not uniform in either time or space, however, and each environmental variation presents its inhabitants with a unique suite of potentially harmful stressors (Kinne, 1970). In marine systems, these environmental stressors may include light, pressure, availability of dissolved gasses, pH, temperature, and salinity (Rankin and Davenport, 1981). The importance of these last two factors in the biology of marine organisms has long been recognized, and studies of each have produced an extensive literature. Here I will summarize some of the effects of temperature and salinity on marine life as they pertain to the estuarine environment, but more extensive reviews are provided by Bullock (1955), Gunter (1957), Kinne (1963), Rose (1967), Whittow (1970), and Prosser (1991) for temperature and Schleiper (1955), Pearse and Gunter (1957), Robertson (1957), Shaw (1960), Potts and Parry (1964), Kinne (1966), Rankin and Davenport (1981), and Evans (2009) for salinity.

Estuaries are of particular interest when examining biotic responses to changes in temperature and salinity. An estuary's location at the junction of fresh and marine water as well as its semi-enclosed nature present excellent opportunities for examining life's ability to deal with physical stressors at many temporal and spatial scales (Kennish, 1986). Almost any location within an estuary will experience moderate to large

fluctuations in both temperature and salinity within a single tidal cycle (Kennedy, 1982). Even larger changes can be observed seasonally (Crosby and Roberts, 1990). Because they live in a constantly changing environment, organisms inhabiting estuarine (or brackish) waters are often both eurythermic and euryhaline. But even these species have their limits, and the distribution of a given species within an estuary is often determined by its physiological tolerances to physical factors such as temperature and salinity (Haskin and Ford, 1982).

Within an estuary, the extreme ranges and periodicities of physical stressors frequently interact and organisms must cope with multiple physical factors at once, often experiencing synergistic interactions as well. This fact has not escaped researchers, and the literature is full of studies where the responses of organisms to multiple environmental variables were examined simultaneously (for an extensive review, see Alderdice, 1972). In general, optimal intensities of other simultaneous environmental stressors are required for the maximum tolerance of any one stressor (Kinne, 1970).

Salinity and temperature affect organisms within many estuaries by creating clear horizontal gradients as one moves from the riverine part of the estuary to the marine end (Kennish, 1986). Some estuaries also experience vertical gradients in temperature and/or salinity, with cooler, more saline waters being found on the bottom while the fresher, warmer water floats on top. Other estuaries experience extensive mixing so that little or no distinct vertical gradient forms. This can also change seasonally, as greater freshwater input allows for less mixing, creating a stratified water column (Kennish, 1986). These variations in temperature and salinity can be lethal to marine organisms brought into the

estuary on the rising tide, potentially causing abnormal or delayed development of embryos and larvae (Morgan, 1995).

Although the constant demands of the estuarine environment are thought to keep the biodiversity of estuaries lower than that of both freshwater and marine systems, it is common for some marine organisms to enter estuaries. While some of these marine species exploit estuarine habitats only during certain life stages, others may invade throughout their lives. These invasions may be temporary or permanent and may vary from individual to individual within a population (Kennish, 1986).

Whenever a primarily marine organism does invade an estuarine habitat, it must cope with the new combination of stressors found there, particularly salinity. Coping strategies may include ionic regulation, volume regulation, intracellular regulation, or behavioral control (Rankin and Davenport, 1981). The physical factors of the estuary may also exert indirect ecological effects on invaders by modifying the species composition of the ecosystem in which they are now found. The invading species may need to cope with predators not found in its previous habitat and potentially find new sources of food. Invasion of the estuarine environment may also cause changes between the invader and its parasites. An obligate parasite, particularly an ectoparasite, is exposed to nearly the same environmental conditions as its host. If only a portion of the host's population invades an estuary, and the parasite proves to have a lower tolerance than the host to the conditions experienced there, the possibility exists that host individuals entering the estuary could experience a refuge from their parasite based on this difference in physiological tolerance (Tolley *et al.*, 2006). Such "salinity refuges" have been studied in several systems on the east coast of North America, including the Virginia

oyster *Crassostrea virginica* with its protozoan parasite *Haplosporidium nelsoni* (Haskin *et al.*, 1965; Haskin *et al.*, 1966; Haskin and Ford, 1982; Barber *et al.*, 1997) and the mud crab *Panopeus obesus* with its rhizocephalan parasite *Loxothylacus panopaei* (Tolley *et al.*, 2006).

Another excellent model relationship for testing this hypothesis exists in the estuaries of the Pacific Northwest of the United States and Canada. The Dungeness crab, *Cancer magister*, can be found in the coastal waters off the west coast of North America from Alaska to California. Although the majority of crabs inhabit nearshore sandy bottoms from the intertidal zone to at least 180 m depth, a significant number of crabs also inhabit estuarine systems (Pauley *et al.*, 1989). This is particularly true of younger crabs, which may invade the estuary as megalopae and experience a significant advantage in growth over those crabs that do not settle in the estuary (Armstrong *et al.*, 2003). Within estuaries, Dungeness crabs tend to inhabit the euhaline (salinity >30) and polyhaline (salinity 18-30) regions while only occurring in the mesohaline (salinity 5-18) and oligohaline (salinity <5) regions rarely or as very early instars (see Chapter II).

Throughout its range, *Cancer magister* is infected by the nemertean egg-predator *Carcinonemertes errans*. These ectoparasitic worms feed on their host's developing embryos while the female crab incubates them under her abdominal flap, potentially causing significant brood loss (Wickham, 1979). However, there is a clear difference in infection rate between crabs in the ocean and those in estuaries, both in parasite prevalence and mean intensity (Chapter II).

Although no rigorous studies have tested the salinity tolerance of *Carcinonemertes errans*, it was not found on any crabs where salinity reached 0 in a

Columbia River survey of Dungeness crabs (McCabe *et al.*, 1987). Wickham (1980) also noted that worms placed in distilled water died within minutes. No data exist for the tolerance of *C. errans* to varying temperatures. The following study tested the hypothesis that the distribution of *C. errans* on *Cancer magister* within Pacific Northwest estuaries is limited by low physiological tolerance to salinity and temperature stress.

Materials and Methods

Juvenile salinity and temperature tolerance

The physiological tolerance of juvenile *Carcinonemertes errans* to salinity and temperature stress was tested experimentally in the laboratory. Juvenile worms taken from crabs captured in the Coos Bay Estuary, Oregon, in the spring of 2009 were subjected to salinity treatments of 5, 10, 20, 25, and 30 and temperature treatments of 8, 12, 16 and 20 °C. These specific salinity and temperature treatments were chosen because they represent the range that worms could realistically encounter along the estuarine gradient of Coos Bay and the South Slough, the areas examined during my trapping survey (Figs. 3.1-3.6).

Three replicate water baths at each temperature treatment were placed in one recirculating sea table at the Oregon Institute of Marine Biology, Charleston, Oregon. The water temperature in the sea table was maintained at 8 °C by a chiller. Each water bath was kept at the appropriate treatment temperature using aquarium heaters and was tested for consistency twice a day for three days leading up to the beginning of the experiment and daily during the course of the experiment.

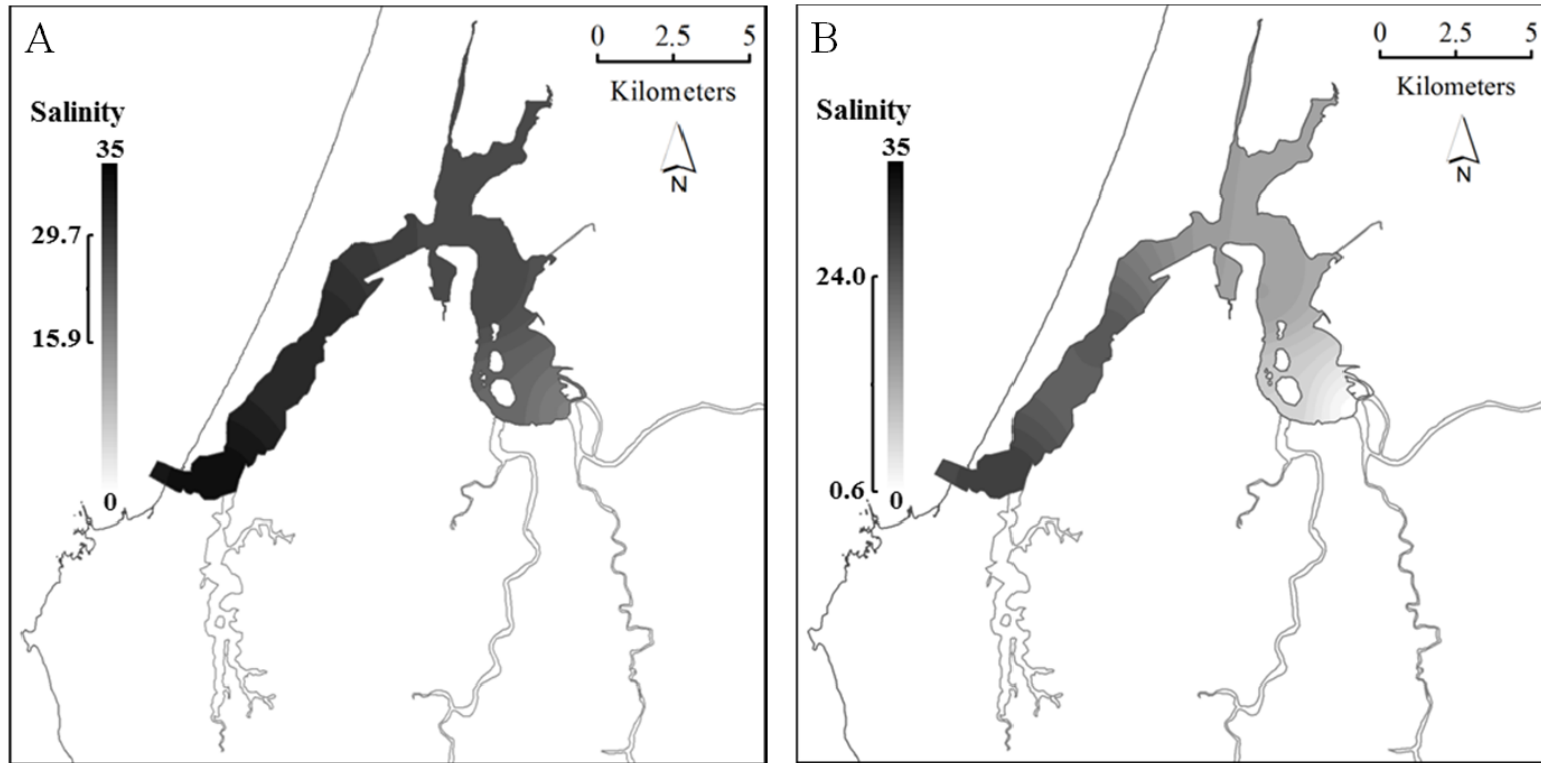


Figure 3.1. Average bottom salinities in the Coos Bay Estuary during (A) the dry season (May-October) and (B) the wet season (November-April). Bracketed values on scale bars represent the salinity range present in each map. Data for these figures was taken from monthly surveys performed by Queen and Burt (1955). All maps in this chapter were created using Spatial Analyst and 3D Analyst in ArcMap 10. Interpolation between points used IDW (Inverse Distance Weighted) methodology with a power of 2 and cell size of 10 m^2 . The analysis covered the entire region surrounding the bay but was masked using the bay outline polygon shape. Subsequently, this is not a precise hydrologic model, but still qualifies as a valid gradient analysis for this water body.

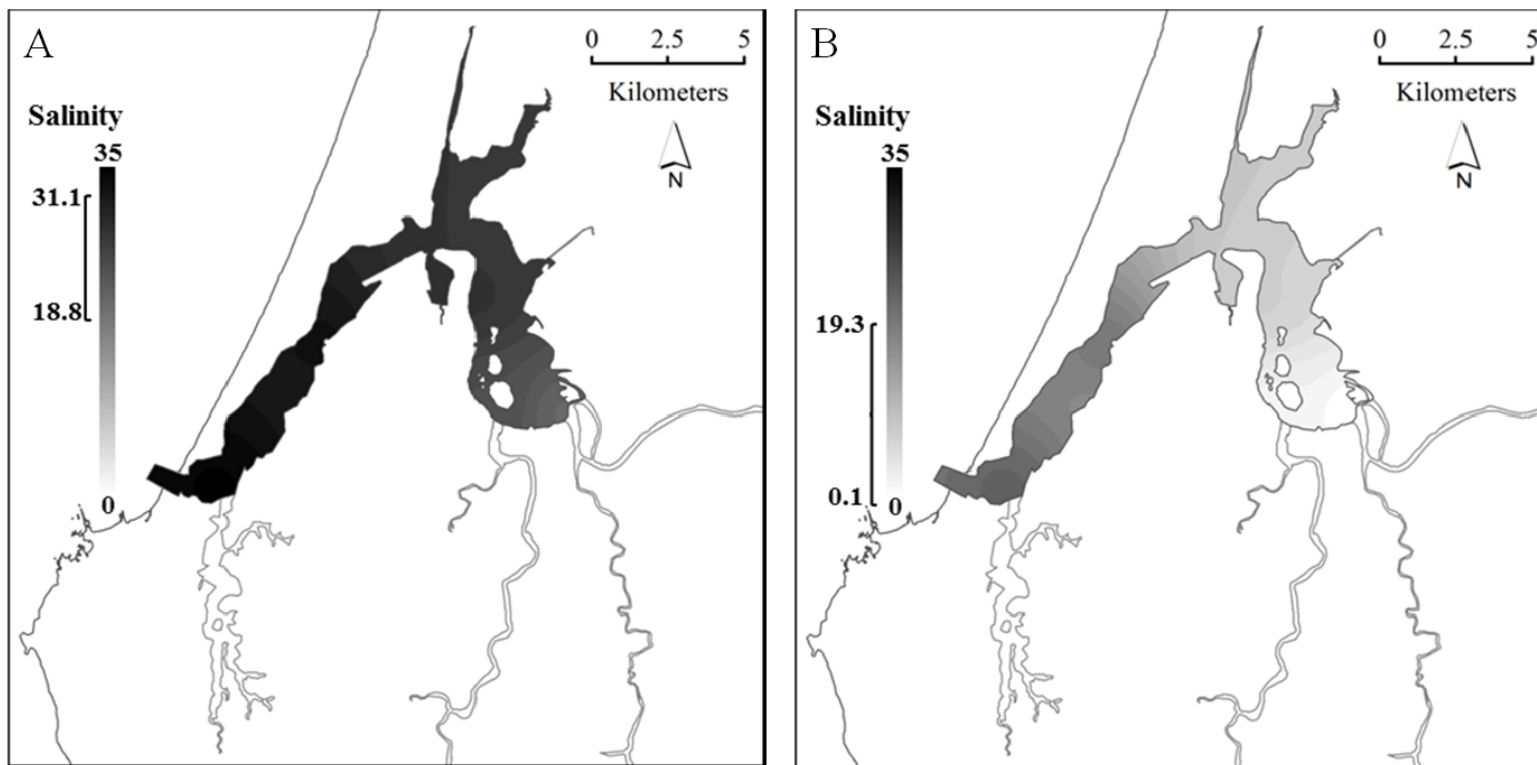


Figure 3.2. Bottom salinity extremes experienced in the Coos Bay Estuary include (A) average salinity at high slack tide in the dry season (May-October) and (B) average salinity at low slack tide in the wet season (November-April). Bracketed values on scale bars represent the salinity range present in each map. Data for these figures was taken from monthly surveys performed by Queen and Burt (1955).

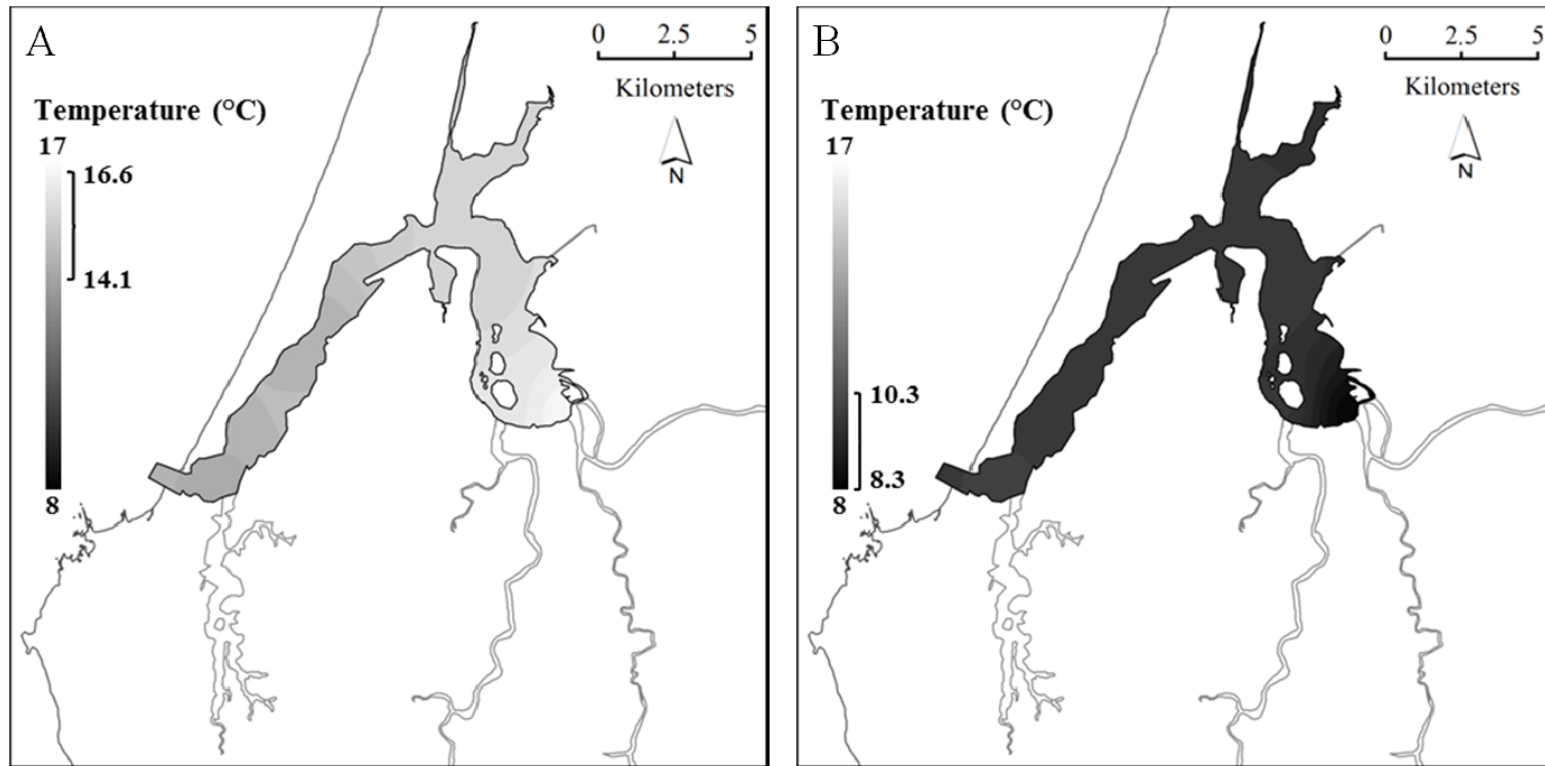


Figure 3.3. Average bottom temperatures in the Coos Bay Estuary during (A) the dry season (May-October) and (B) the wet season (November-April). Bracketed values on scale bars represent the temperature range present in each map. Data for these figures was taken from monthly surveys performed by Queen and Burt (1955).

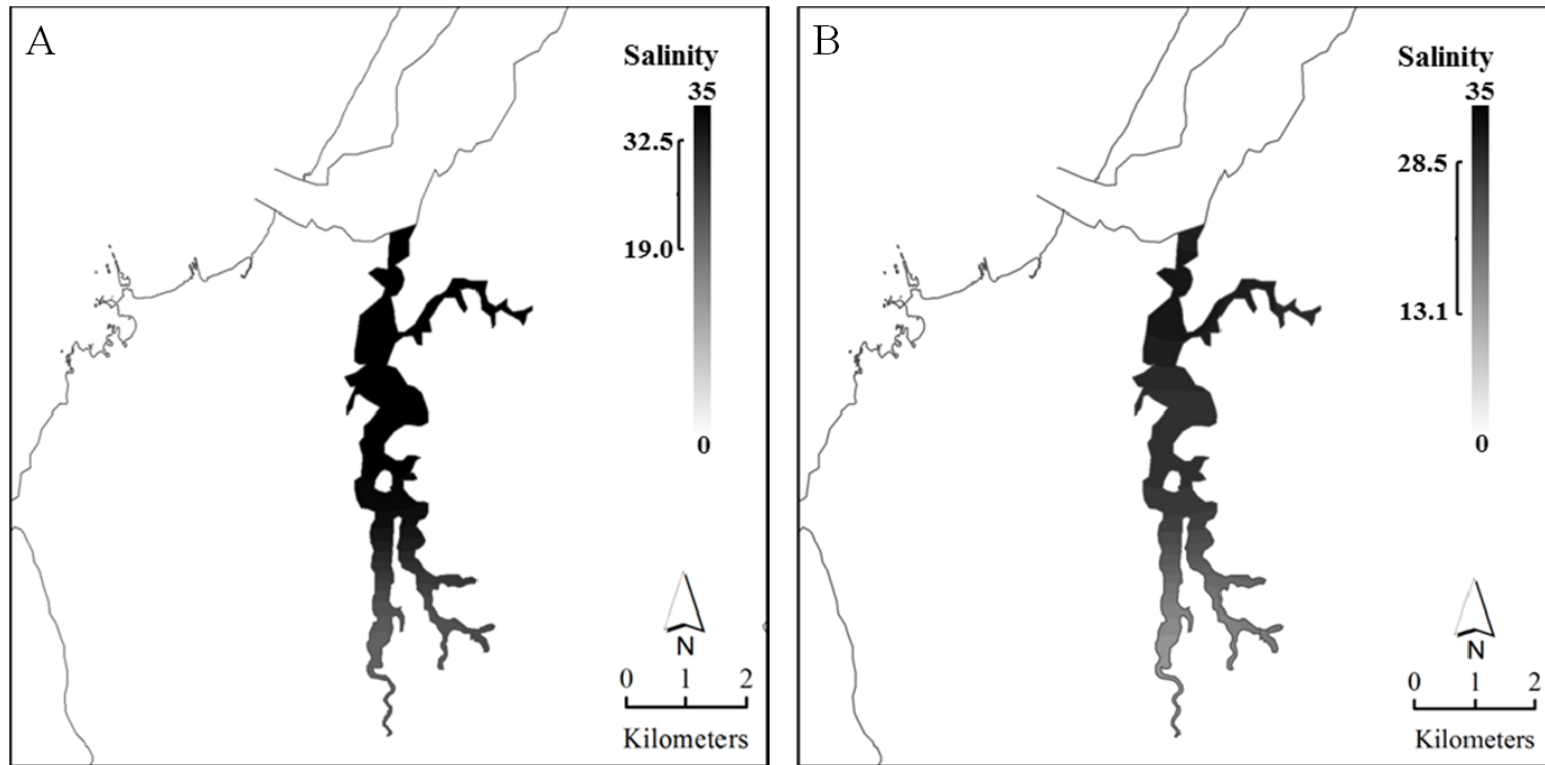


Figure 3.4. Average salinities in the South Slough during (A) the dry season (May-October) and (B) the wet season (November-April). Bracketed values on scale bars represent the salinity range present in each map. Data for these figures was taken from SWMP data monitoring stations (National Oceanic and Atmospheric Administration, 2008).

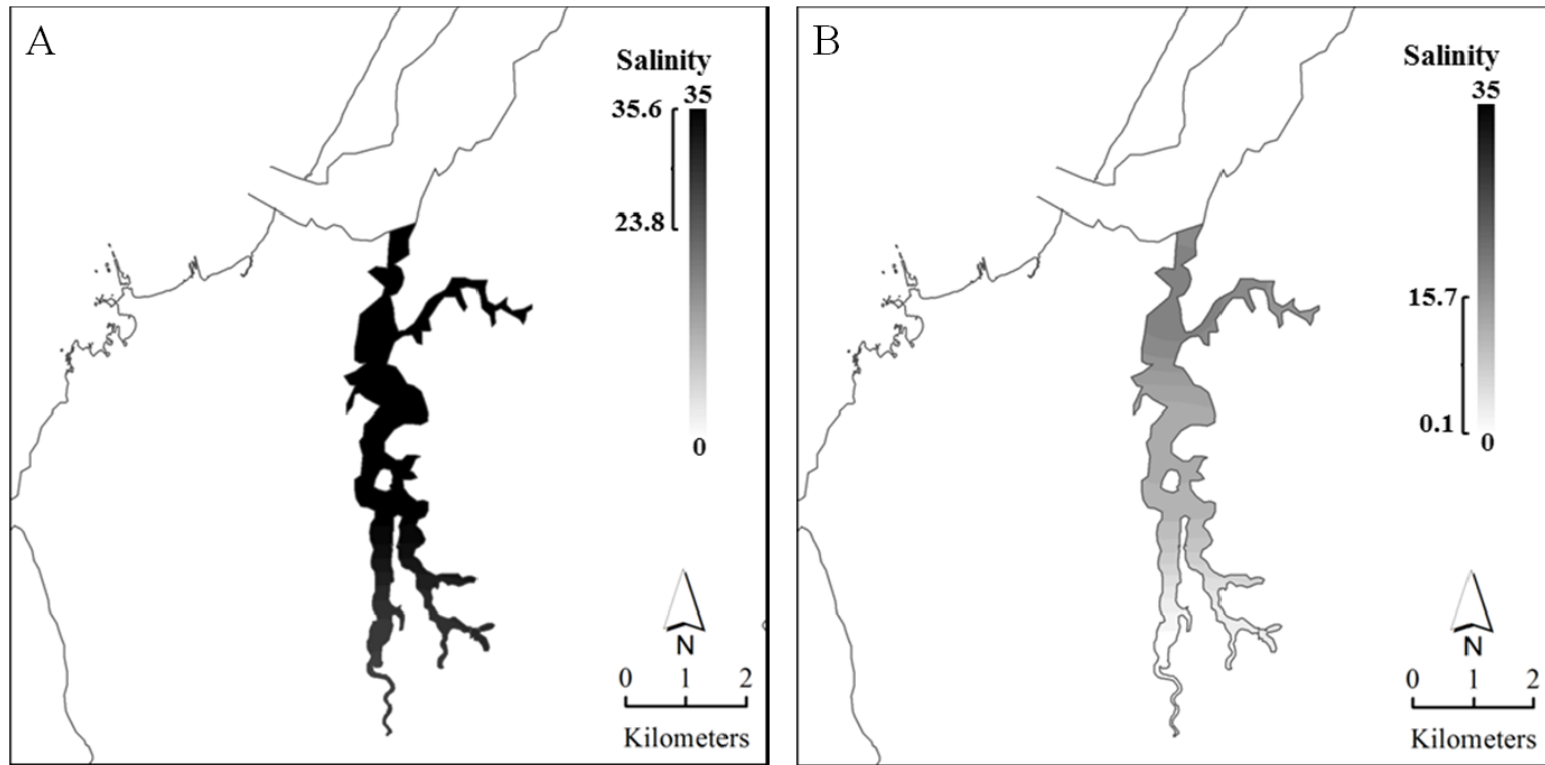


Figure 3.5. Salinity extremes experienced in the South Slough include (A) average salinity at high slack tide in the dry season (May-October) and (B) average salinity at low slack tide in the wet season (November-April). Bracketed values on scale bars represent the salinity range present in each map. Data for these figures was taken from SWMP data monitoring stations (National Oceanic and Atmospheric Administration, 2008).

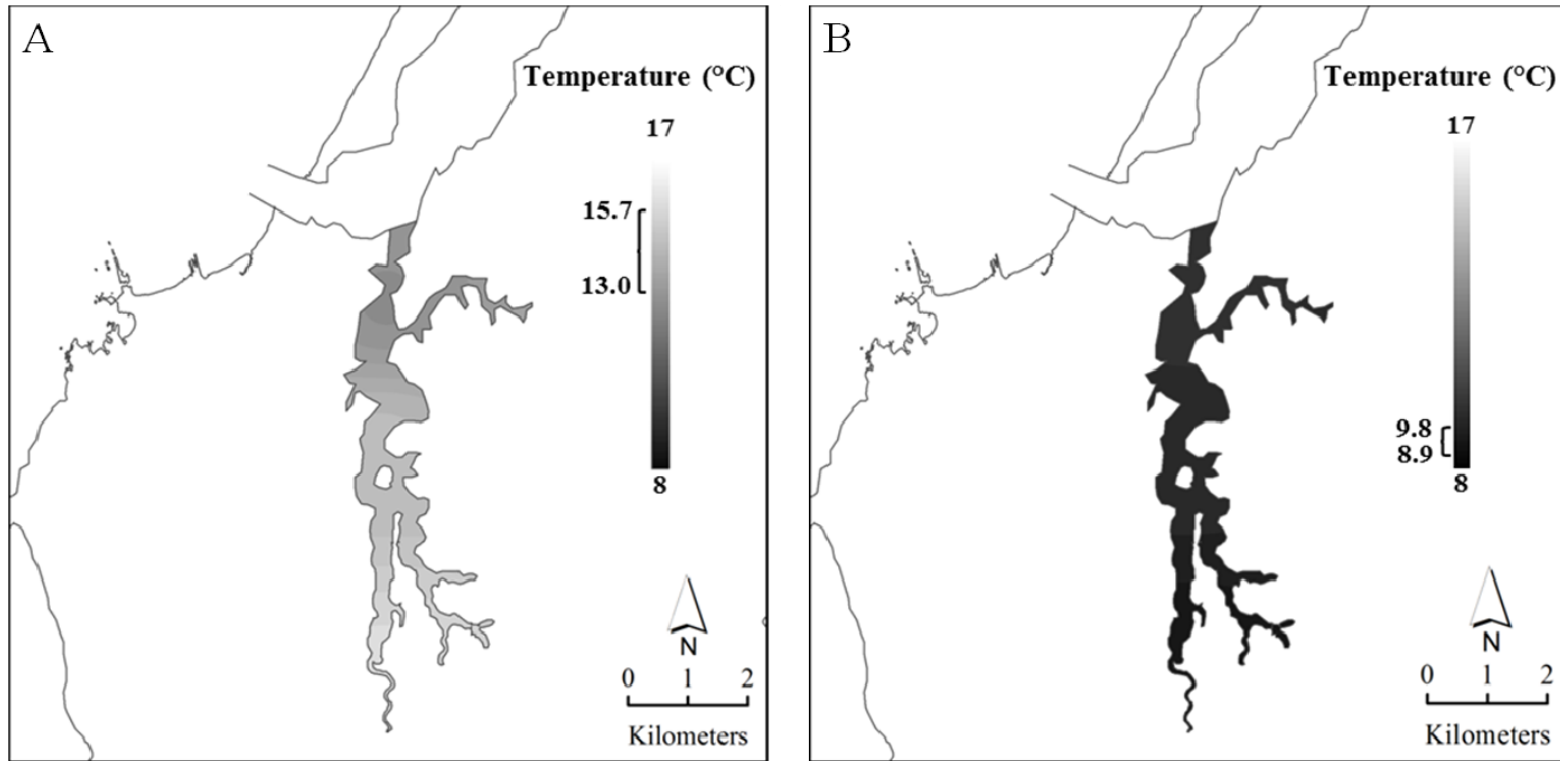


Figure 3.6. Average temperatures in the South Slough during (A) the dry season (May-October) and (B) the wet season (November-April). Bracketed values on scale bars represent the temperature range present in each map. Data for these figures was taken from SWMP data monitoring stations (National Oceanic and Atmospheric Administration, 2008).

The position of each of the 12 water baths in the sea table was determined using a random number table and can be seen in Fig. 3.7. Five 20-ml scintillation vials with lids, each containing one of the five salinity treatments and ten juvenile worms, were placed into each water bath. I checked each of the vials under a dissecting microscope every other day, noted any mortality that had occurred, and changed the water within each vial.

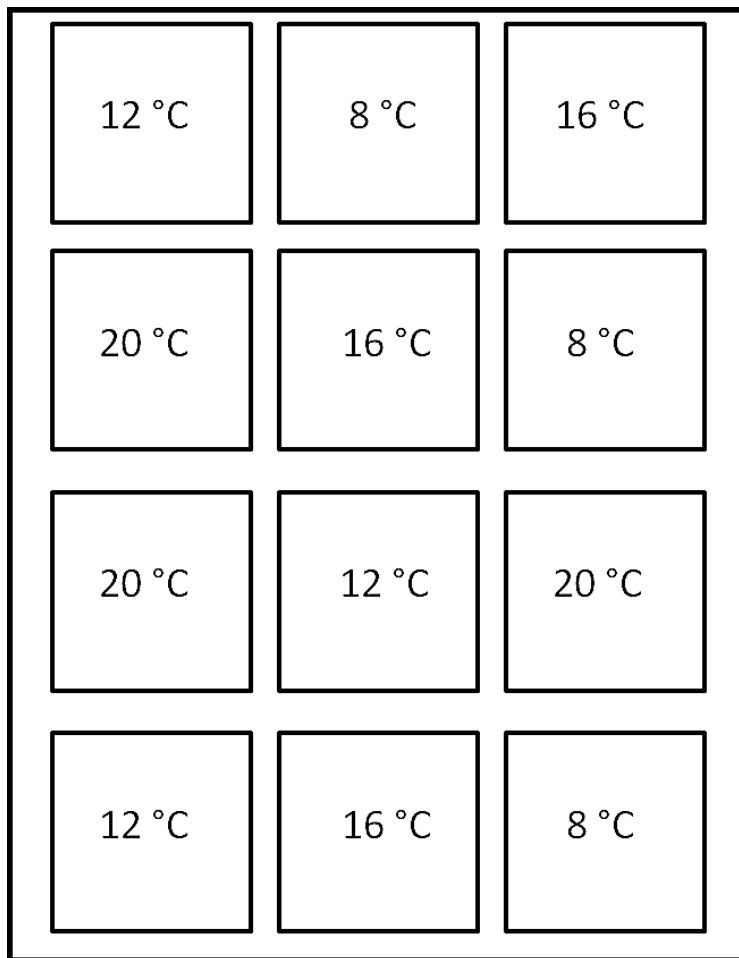


Figure 3.7. Design for temperature and salinity tolerance experiments. Each water bath was randomly assigned a temperature treatment and was kept at that temperature using aquarium heaters. Five vials, each containing a different salinity treatment as well as juvenile worms or larvae, were placed in each bath. Ambient water temperature of the seatable was 8 °C.

A worm was considered dead if it: 1) did not respond to being nudged with forceps, 2) was beginning to decay, or 3) was missing entirely from the vial. The experiment ran for 10 days, but because there was no change in worm mortality between Days 8 and 10, only data for the first 8 days are presented. Because each vial was checked multiple times during the experiment, I analyzed the results using an ANOVA of repeated measures (ANOVAR) with salinity and temperature as fixed factors (Zar, 2010). Post-hoc Bonferroni tests were used to detect significant differences between treatments. All percentages were arcsine square-root transformed for the analysis and were back-transformed for figures (Zar, 2010).

Larval salinity and temperature tolerance

I conducted two separate experiments to test the physiological tolerances of *Carcinonemertes errans* larvae to salinity and temperature. The larvae used for these experiments hatched from egg strings taken from the egg masses of female Dungeness crabs captured in the Coos Bay Estuary during the winter of 2009 and kept in a running seawater aquarium. Prior to the beginning of the experiment, all larvae were cultured in containers of 0.45 μm filtered seawater and stirred in sea tables at the Oregon Institute of Marine Biology.

The first experiment used the same methodology as the juvenile tolerance experiment described above with the following exceptions: 1) 20 one-week-old larvae (rather than 10 juvenile worms) were placed in each vial containing one of the five salinity treatments, and 2) the experiment lasted 24 hours, after which time I examined the contents of each vial under a dissecting microscope and determined how many larvae

were still alive. Due to the results of the 24-hour larval tolerance experiment, the two lowest salinity treatments (5 and 10) were not used in the second experiment and were replaced by a salinity 15 treatment in an attempt to determine more closely the lower salinity threshold that larvae could tolerate. The second experiment was allowed to run for 72 hours before I checked the vials for mortality. The 24-hour and the 72-hour experiments were analyzed using two two-way ANOVAs, with salinity and temperature as fixed factors. These were followed by post-hoc Tamhane tests, which do not assume equal variance. All percentages were arcsine square-root transformed for the analysis and were back-transformed for figures (Zar, 2010).

Results

Juvenile salinity and temperature tolerance

After two days, all of the juvenile worms in the salinity 5 treatment were dead, regardless of temperature (Fig. 3.8A). Survival at salinity 10 varied from 0.55 ± 0.03 SE at 20 °C to 0.7 ± 0.18 SE at 12 °C (Fig. 3.8B). Survival at salinity 20 was 0.91 ± 0.09 SE at 8 °C, but remained at 100% for the other temperature treatments (Fig. 3.8C). No mortality occurred at salinities 25 or 30 (Fig. 3.8D). By Day 4 of the experiment, survival at salinity 10 had dropped considerably, reaching zero at 16°C. At salinity 20, survival at 8 °C was 0.86 ± 0.13 SE, but remained at 100% for the other temperatures. No mortality occurred at the two higher salinity treatments. At Day 6, survival at salinity 10 was zero across all temperature treatments. Survival at salinity 20 also dropped below 100% in all of the temperature treatments for the first time, ranging from 0.82 ± 0.12 SE at 16 °C to 0.68 ± 0.10 SE at 12 °C. No mortality occurred at salinities 25 or 30.

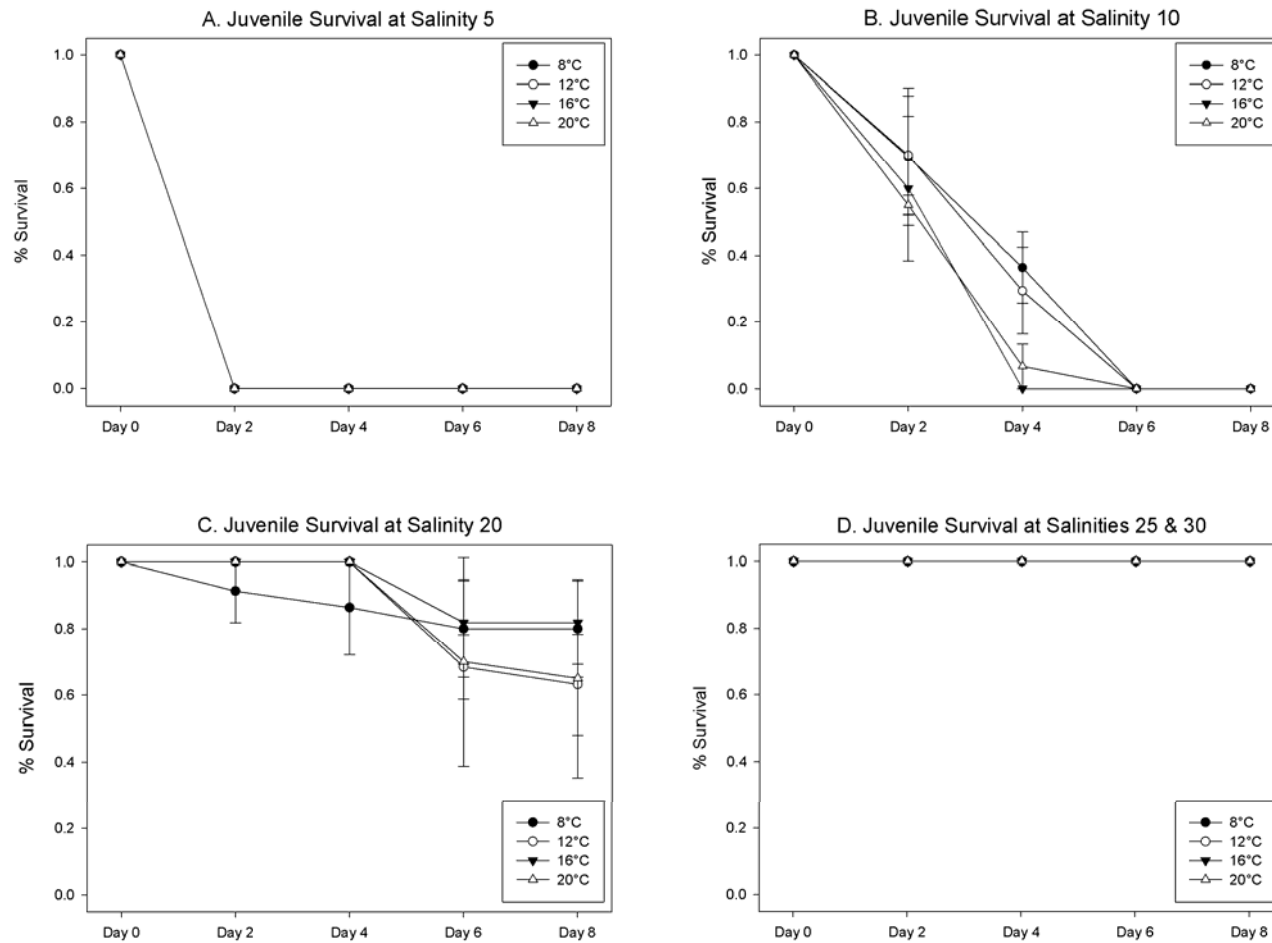


Figure 3.8 Average percent survival of juvenile *Carcinonemertes errans* over 8 days when exposed to four different temperature treatments at salinities of 5 (A), 10 (B), 20 (C), and 25 & 30 (D). Error bars represent one standard error.

No large changes in survival occurred between Days 6 and 8. Survival at salinity 20 decreased slightly, reaching as low as 0.63 ± 0.15 SE at 12 °C, but there was no change at salinities 25 or 30.

The results of the ANOVAR are shown in Table 3.1. The assumption of sphericity was violated (Mauchley's $W=0.23$, $p<0.001$), but the value of the Huynh-Feldt Epsilon was greater than 0.7 ($\epsilon = 0.702$). I therefore used the Huynh-Feld adjusted degrees of freedom for within-subject factors (Zar, 2010). Both the effect of day ($F = 45.506$, $p<0.001$) and the interaction between day and salinity ($F = 21.891$, $p < 0.001$) were highly significant, while all other within-subjects effects were not significant. The test of between-subject effects showed that salinity was highly significant ($F = 300.355$, $p < 0.001$), while temperature ($F = 0.204$, $p = 0.893$) and the salinity-temperature interaction ($F = 0.346$, $p = 0.974$) were not significant. Post-hoc Bonferroni tests ($\alpha = 0.05$) showed that all salinity treatments were significantly different from each other with the exception of salinities 25 and 30.

Larval salinity and temperature tolerance

At the end of the 24-hour tolerance experiment, all of the larvae in salinity treatments 5 and 10 were dead, regardless of temperature (Fig. 3.9A). Survival at salinities 20 (0.97 ± 0.01 SE) and 25 (0.99 ± 0.004 SE) was very high, and there was no mortality at salinity 30, again regardless of temperature. The ANOVA results are shown in Table 3.2. The transformed data violated the ANOVA assumptions of normality and equal variance (Levene's Test, $p < 0.01$), which could lead to rejecting the null

Table 3.1. *Salinity and temperature tolerance ANOVAR results for juvenile Carcinonemertes errans.*

A. Within-Subjects Effects					
Source of Variation	DF	SS	MS	F	P^H
day	2.105	3.504	1.665	45.506	<0.001
day x salinity	8.419	6.742	0.801	21.891	<0.001
day x temp	6.314	0.866	0.020	0.545	0.781
day x sal x temp	25.258	3.080	0.034	0.937	0.557
residual	84.193	0.397	0.037		
B. Between-Subjects Effects					
Source of Variation	DF	SS	MS	F	P
salinity	3	104.725	26.181	300.355	<0.001
temperature	3	0.053	0.018	0.204	0.893
temp x salinity	9	0.362	0.030	0.346	0.974
residual	32	3.487	0.087		

The two-way ANOVAR compared survival of juvenile worms exposed to four temperature treatments and five salinity treatments across eight days. Within-subject effects (A) show the differences between treatment days. Between-subjects effects (B) show the differences in survival with salinity and temperature as factors. P^H is the Huynh-Feldt adjusted P value, used because the data violated the assumption of sphericity.

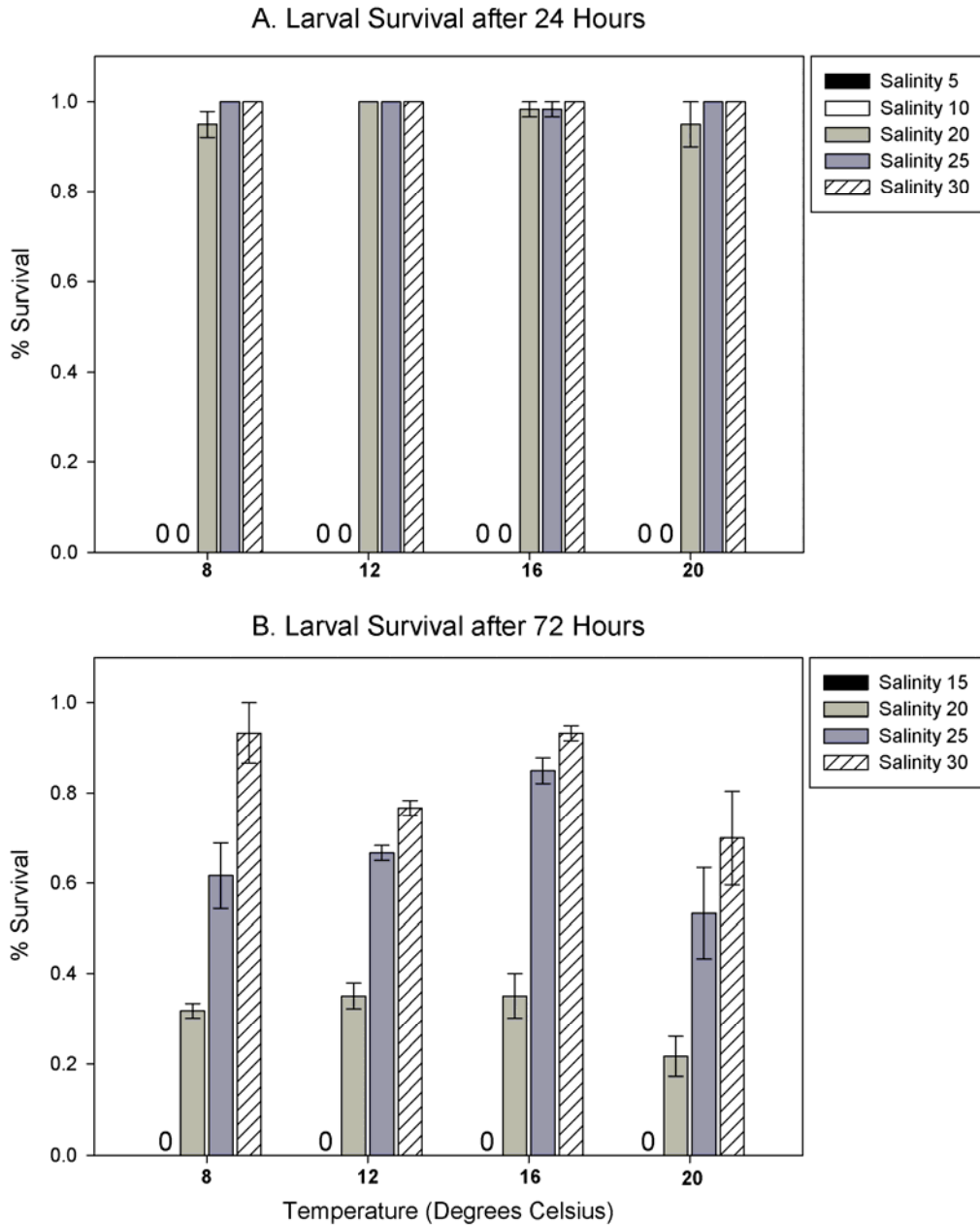


Figure 3.9. Average percent survival of larval *Carcinonemertes errans* when exposed to temperature and salinity treatment combinations for (A) 24 hours and (B) 72 hours. Error bars represent one standard error.

hypothesis of no difference between treatments when it should not be rejected (false positive; Sokal and Rohlf, 1981). I therefore adopted a more stringent α value of 0.01.

The effect of salinity was highly significant ($p < 0.001$), while the effects of temperature ($p = 0.583$) and the salinity-temperature interaction ($p = 0.644$) were not significant. The Tamhane tests showed that none of the temperature treatments were significantly different from one another, but that the salinity treatments were neatly divided into two significantly-different ($p < 0.001$) groups: salinities 5 and 10 and salinities 20, 25, and 30.

Table 3.2. Results of a two-way ANOVA testing survival of larval *Carcinonemertes errans* after 24 hours with salinity and temperature as fixed factors.

Source of Variation	DF	SS	MS	F	P
temp	3	0.0115	0.00384	0.658	0.583
salinity	4	33.283	8.321	1424.557	<0.001
temp x salinity	12	0.0564	0.00470	0.805	0.644
Residual	39	0.228	0.00584		
Total	58	33.726	0.581		

After the 72-hour exposure, the larvae at the lowest salinity treatment (15) were once again all dead, regardless of temperature (Fig. 3.9B). At salinity 20, average survival ranged from 0.22 to 0.35 (mean=0.31 \pm 0.02 SE), with no clear trend in the effect of temperature. Survival at salinities 25 and 30 was higher than at 20 (mean = 0.67 \pm 0.04 SE and mean = 0.83 \pm 0.04 SE, respectively) and varied with temperature, but with no clear trend as well. The ANOVA results are shown in Table 3.3.

Table 3.3. Results of a two-way ANOVA testing survival of larval *Carcinonemertes errans* after 72 hours with salinity and temperature as fixed factors.

Source of Variation	DF	SS	MS	F	P
temp	3	0.278	0.0928	7.490	<0.001
salinity	3	9.882	3.294	265.796	<0.001
temp x salinity	9	0.319	0.0354	2.859	0.014
Residual	32	0.397	0.0124		
Total	47	10.876	0.231		

Like the 24-hour experiment, the transformed data violated the ANOVA assumptions of normality and equal variance (Levene's Test $F = 5.199$, $p < 0.01$), so I adopted a more stringent α value of 0.01. This lower α value resulted in a non-significant interaction between temperature and salinity ($F = 2.859$, $p = 0.014$). The effect of temperature remained significant ($F = 7.490$, $p < 0.001$), but the effect of salinity was by far the leading explanation for the exhibited pattern ($F = 265.796$, $p < 0.001$). The Tamhane tests showed that none of the temperature treatments was significantly different from any other, but that all of the salinity treatments were significantly different ($p < 0.001$) except treatments 25 and 30 ($p = 0.057$).

Discussion

The ability to tolerate changes in temperature, salinity, or the combination of the two is often linked to ontogeny (Anger, 2003). Although the vast majority of tolerance

experiments in the literature have been performed on adult or sub-adult individuals, there is some evidence for drastic changes in physiological tolerance based on life stage (Kinne, 1971). Gametes and developing embryos typically show the lowest ability to tolerate large changes in either salinity or temperature, and salinity has been identified as one of the major causes of death in estuarine and coastal plankton (Calabrese and Davis, 1970; Metaxas, 1998; Anger, 2003; Kashenko and Korn, 2003; Bravo *et al.*, 2007; Nurdiani and Zeng, 2007). This is thought to result from insufficient development of systems for coping with physiological stress (Kinne, 1970). The trend is not universal, however, with the larvae of some species requiring lower salinities for proper development (Khlebovich, 1969).

In the case of *Carcinonemertes errans*, both the juvenile and the larval stages appear to exhibit a relatively high tolerance to salinities that would occur in the euhaline (salinity >30) and most of the polyhaline (salinity 18-30) regions while failing to survive exposure to mesohaline (salinity 5-18) conditions for extended periods of time. Juvenile worms all died within 48 hours when exposed to the salinity 5 treatment. When salinity increased to 10, however, some juveniles were able to survive for at least 4 to 6 days, suggesting that a brief foray by the host into salinity 10 waters would probably not be enough to eliminate its entire parasite load. Over sixty percent of the juvenile worms exposed to salinity 20 were still alive after 8 days, and not a single juvenile died at salinities 25 and 30, suggesting that juveniles of *C. errans* could survive at these salinities for longer periods or possibly indefinitely.

Although similar patterns in salinity tolerance were seen at the one-week-old larval stage of *Carcinonemertes errans*, the larval mortality rate at lower salinities was

higher than that of juvenile worms. This was clear at the salinity 10 level, where all larvae were dead within 24 hours, while some juveniles were able to survive at salinity 10 for up to 6 days. Survival among larvae exposed to salinity 20 for 72 hours was similar to that of juveniles exposed to the same salinity for 8 days. These results, which suggest a potential difference in the ability of different life stages to survive the salinity stresses tested, should be interpreted carefully, however, due to the increased mortality seen among larvae at all salinity treatments, including 25 and 30.

The effect of temperature, at least within the range that was tested, was not a significant source of mortality among juvenile worms. On Days 2 and 4 of the experiment, there appeared to be an inverse relationship between temperature and survival of worms given the salinity 10 treatment, but this was not found to be significant. The same pattern was not observed when mortality began to increase at salinity 20 beginning on Day 6.

After the 24-hour larval experiment, temperature treatments explained very little of the observed variance. During the 72-hour experiment, however, temperature did play a significant role in larval survival. More larvae died at 20 °C than at any other temperature across salinity treatments, but after that the trend is less clear. At salinity 25, survival increased from 8 °C to 16 °C, while at salinity 30, survival at 12 °C was lower than at 8 °C or 16 °C. The most likely explanation for this pattern is that the low sample size used for the experiment did not give me the power to differentiate a real pattern from the noise of natural variation. If I assume that the pattern is real, however, it appears that the ideal temperature for larval survival of *Carcinonemertes errans* is 16 °C. Although

temperature was a significant factor in the 72-hour experiment, it was clearly secondary to the effect of salinity.

This represents the first study to examine the physiological tolerances of *Carcinonemertes errans* to salinity and temperature. The ability of nemerteans to cope with salinity stress has been examined in just a handful of other species. The littoral nemertean *Lineus ruber* is known to survive salinities as low as 5 for up to seven days (Gibson, 1972). Charmantier *et al.* (1991) found that the nemertean *Pseudocarcinonemertes homari*, an egg predator on the American lobster, has a lethal salinity threshold of 11 at 7 °C, but a threshold of 19 at 14.5 °C. Scrocco and Fabianek (1970) found adult specimens of *Carcinonemertes carcinophila*, an Atlantic congener of *C. errans*, to be tolerant to salinities above 10. Below that threshold, however, all worms were dead within two days.

Osmoregulation in nemerteans is thought to involve both excretory nephridial networks (Gibson, 1972; Bartolomaeus and von Döhren, 2010) and the cerebral organs, which produce mucopolysaccharides hypothesized to offset the effects of body fluid dilution (Ling, 1970; Ferraris, 1979; Moore and Gibson, 1985; but see Amerongen and Chia, 1983). Unlike many freshwater, terrestrial, and brackish water nemerteans that are known to possess elaborate networks of nephridia that extend almost the entire length of their bodies (Moore and Gibson, 1985; Maslakova and Norenburg, 2008), members of the genus *Carcinonemertes* have only a single pair of protonephridia located in the foregut region, a common design among marine nemertean osmoconformers (Moore and Gibson, 1985; Bartolomaeus and von Döhren, 2010). The cerebral organs are also absent

in the Carcinonemertidae (Coe, 1902), further suggesting that members of *Carcinonemertes* are not capable of significant osmoregulation.

In order for a hypothesized salinity refuge to have any effect, the host must have higher tolerance to lower salinities than its parasite. The physiological tolerances and osmoregulatory abilities of the Dungeness crab have been well studied (Jones, 1941; Engelhardt and Dehnel, 1973; Hunter and Rudy, 1975; Brown and Terwilliger, 1992). *Cancer magister* is a weak osmoregulator as an adult, suggesting it may be a relative new-comer to estuarine life (Engelhardt and Dehnel, 1973). Cleaver (1949) reported that adult *Cancer magister* could not tolerate salinities below 11 for longer than a few hours, but did not provide any data to support this statement. Englehardt and Dehnel (1973) called the ionic regulatory system of *Cancer magister* “well-developed” and noted that while crabs left in salinity 8 water for four days exhibited 50% mortality, no mortality was seen in crabs kept at salinity 16 for up to nine days. Curtis and McGaw (2010) found that prolonged sub-lethal exposure of *Cancer magister* to low salinity water also leads to decreased oxygen uptake, increased digestion time, and decreased ability to forage. These authors proposed that crabs possibly adopt an “eat-and-run” strategy, moving into less saline conditions to forage when necessary, but retreating to less stressful conditions to digest.

Cancer magister also exhibits a clear ontogenetic component to its salinity tolerance (Brown and Terwilliger, 1992). As megalopae and young instars, crabs migrate to the upper reaches of the estuary. This occurs during the late spring and summer months when salinities are still relatively low compared to lower bay regions, but rarely reach zero (Lough, 1976). These life stages are better osmoregulators than adults and

often remain in shallow, intertidal waters where they forage and possibly avoid contact with larger members of their cannibalistic species (Holsman *et al.*, 2006). Larger crabs remain almost exclusively in subtidal habitats where salinity and temperature changes are less extreme and frequent. By the time the crabs reach maturity, they are almost exclusively found within the euhaline and polyhaline regions of the estuary (Chapter II).

Unlike the river-dominated Columbia River Estuary, the Coos Bay Estuary is tidally dominated much of the year, experiencing strong flushing each tidal cycle and exhibiting coastal conditions during high tides (Roegner and Shanks, 2001). This means that at least during the dry season, adult Dungeness crabs can move throughout much of the Coos Bay and South Slough without encountering bottom salinities below 20 (Queen and Burt, 1955; McAlister and Blanton, 1963). During the wet season, however, some polyhaline regions turn into mesohaline regions, with salinity values dipping below 20, particularly during low tides. Valino Island in the South Slough is one of these places (Figs. 3.4B, 3.5B), as are the upper bay sites of Jordan Cove and Highway (Chapter II; Figs. 3.1B, 3.2B). Although crabs probably would not remain for long periods of time in low saline conditions (Curtis and McGaw, 2010), any movement into or through low salinity areas to forage could possibly result in at least a partial removal of juvenile *Carcinonemertes*. Juvenile worms living on the crab carapace could die as a result of exposure to salinities below 25 (Fig. 3.8). The juvenile tolerance results suggest that each relatively short stint of two to four days in salinity 20 water (well within the ability of *Cancer magister* to tolerate) could potentially kill off around 15% of the crab's parasite load (Fig. 3.8). A longer stay or any time spent in less saline conditions, even if the crab were burrowed into the sand while waiting for conditions to improve, would be

even more effective at removing worms. A series of short-term low-salinity exposures could therefore represent a spacio-temporal refuge for the Dungeness crab from *Carcinonemertes errans* based on a salinity gradient within the Coos Bay Estuary. Given the clear difference in infections of *C. errans* seen within the regions of the estuary and the relatively similar salinity tolerance range of *C. magister* and *C. errans*, however, it seems unlikely that salinity stress on the parasite alone is accounting for the entire observed pattern in host infection. Other factors, particularly the ontogenetic movements of the host and the supply of competent larvae of *C. errans* in the estuary probably play a large role in the creation of the observed refuge (see Chapters II and IV).

Bridge

In Chapter III, I tested the physiological tolerances of *Carcinonemertes errans* to temperature and salinity stress and found that this species has a similar tolerance range to that of its host *Cancer magister*. It is therefore probable that other mechanisms contributed to the observed gradient in infections of *C. errans* on its host within the estuary. One possibility is that the gradient reflects the extent to which competent larvae of *C. errans* invade the estuary in search of hosts. In the following chapter, I present results of settlement experiments conducted within the estuary to test where and how *C. errans* infects *C. magister*. I also performed plankton tows within the estuary over one year to discover when competent larvae were available to recruit to crabs. Finally, I studied the process of larval settlement for *C. errans* in the laboratory and determined the stage at which larvae are competent to settle.

CHAPTER IV

LARVAL SETTLEMENT OF THE NEMERTEAN EGG PREDATOR

CARCINONEMERTES ERRANS ON THE DUNGENESS CRAB, *CANCER MAGISTER*

Introduction

Over 90,000 species of marine invertebrates have biphasic life histories, with benthic adults producing planktonic larvae (Thorson 1964). For some of these species, the planktonic larval phase lasts only a few minutes to a few hours (e.g. many ascidian and bryozoan larvae), while the larvae of other species (e.g. many crab zoeas and snail veligers) can remain in the plankton from weeks to months (Crisp 1976). At some point, however, all larvae must either leave their pelagic existence and begin life on the substratum or perish. Larval settlement is one of the most critically important events in the life of a biphasic organism and has been the subject of many studies and excellent reviews (e.g. Crisp 1974; Scheltema 1974; Chia & Rice 1978; Pawlik 1992; Hadfield 1998). Not only does settlement bring about major changes in the physical and biotic interactions that the animal will experience, it often coincides with a dramatic metamorphosis wherein larval structures are lost and a new body plan is created (Herrmann 1995). In many invertebrate groups (e.g. bryozoans, sponges, ascidians, and barnacles) settlement also means the end of the motile stage and the beginning of a sedentary life (Thorson 1950).

How larvae find their way to an appropriate habitat for their next life stage has been the focus of studies dating back to the beginning of the last century (reviewed in Wilson 1952). Originally, it was widely supposed that larvae acted as passive particles,

the distribution of settlers being entirely random and controlled by the currents and waves (Nelson 1928). The work of D. P. Wilson (1952), Knight-Jones (1953), Crisp (1955), Scheltema (1961) and others, however, showed that larvae were equipped with sensory structures and, at least under laboratory conditions, were capable of active substrate choice. The present understanding of settlement suggests that larvae are moved by currents in the large scale and actively select substrata on the small scale (e.g. Pawlik et al. 1991; Koehl 2007).

The search for the stimuli, or cues, that induce larval settlement responses has also been a subject of focused research (for reviews, see Morse 1990; Pawlik 1992; Hadfield & Paul 2001). Stimuli may include physical factors, such as light, gravity, hydrostatic pressure, temperature, salinity, and properties of the substratum itself (Pawlik 1992). Cues also include biogenic chemicals such as those produced by microbial films, conspecifics, and food sources (Crisp 1974). Crisp (1965) argued that larvae must necessarily rely solely on tactile stimuli for the induction of settlement because 1) any water soluble cue released from a substratum would be immediately diluted to negligible concentrations directly above the viscous boundary layer, and 2) the small size of larvae makes it difficult to detect a chemical gradient across their bodies, and also makes them move with parcels of water rather than through them. Recent studies have shown, however, that larvae are not only able to detect dissolved cues in flowing water conditions, but that they can respond rapidly to these cues (Turner et al. 1994; Hadfield & Koehl 2004; Elbourne & Clare 2010; Koehl & Hadfield 2010).

Although often overlooked, parasites represent a huge fraction of marine biodiversity (Rohde 1982). Like their terrestrial counterparts, marine parasites can affect

the size and distributions of host populations, thus potentially altering entire ecosystems (Price 1980). This becomes particularly apparent when the animals affected happen to be an important fishery species (Haskin et al. 1966). In order to disperse from one host to another, marine parasitic species also tend to have planktonic larvae (Pawlik 1992). While finding an appropriate place to settle is important for any larva, this is especially true for the larvae of parasites. Here, settlement represents the end of the free-living phase and the beginning of the parasitic one, and failure to locate the appropriate host will almost certainly result in death (Boone et al. 2004). In the case of a parasite, the host itself represents both the food source and a site where conspecifics may potentially be found. This idea led Chia (1978) to predict that settlement cues for parasitic larvae should be imperative and are likely associated with the host species. Clearly, parasitic species offer excellent opportunities to study specificity in the patterns of larval settlement, but thus far studies involving settlement for parasitic species have mostly been confined to digenean flukes (James 1971) and some crustacean groups, especially rhizocephalan barnacles (Boone et al. 2003; 2004).

The nemertean worm *Carcinonemertes errans* WICKHAM 1978 presents an excellent opportunity to better understand parasitic settlement patterns in marine systems. This species is an egg-predator on the Dungeness crab, *Cancer magister* DANA 1852, an important fishery species along the Pacific Northwest of the United States and Canada (Wickham 1979a). Although some have argued that the egg-predator nemerteans of the genus *Carcinonemertes* are not true parasites (Kuris 1997), their dependence on crab hosts for food and subsequent completion of their life cycle is certainly parasitic in nature (Roe 1988). Since *C. errans* has the potential to cause an average of 50-60% brood loss

on infected female Dungeness crabs (Wickham 1979a; b), the larval biology of *Carcinonemertes*, particularly at the time of settlement, has been singled out as an area of much-needed research (Kuris 1997).

Unlike their free-living hoplonemertean relatives that produce relatively large, yolky, short-lived planuliform larvae similar in form to the adults (Norenburg & Stricker 2002), reproduction in *Carcinonemertes errans* and its congeners appears to reflect an r-selected parasitic lifestyle; larvae are produced in much higher numbers, and are small (~110µm at hatching), contain relatively little yolk, and possibly remain planktonic for long periods of time (Stricker & Reed 1981; Roe 1988). Although several studies have attempted to induce larval settlement in *C. errans* and its congeners (Roe 1979; Stricker & Reed 1981; Bauman 1983), none has done so successfully. This has left the questions of timing and specificity of larval settlement in the genus *Carcinonemertes* unresolved. Most known nemertean species are free-living benthic predators that are unlikely to show considerable specificity in substratum selection during settlement (Stricker 1987). Given their dependence on their crab hosts, it seems likely that members of *Carcinonemertes* would exhibit much higher specificity in substratum choice. It is, however, possible that worms could settle less discriminately and then seek out their host by crawling, as has been suggested for the larvae of *Gononemertes australiensis*, a parasite of an ascidian (Egan & Anderson 1979).

The goals of the present study were fourfold: 1) to discover the stage at which larvae of *Carcinonemertes errans* are capable, or competent, to settle, 2) to induce larval settlement of *C. errans* in the laboratory, 3) to observe patterns of settlement of *C. errans* in the field and test for evidence of associative and gregarious settlement, and 4) to

determine when the competent larvae are available to infect crab hosts in the coastal ocean and estuaries.

Methods

Study organisms

Larvae of *Carcinonemertes errans* were collected from egg masses of Dungeness crabs captured in Oregon coastal waters during the winter and spring of 2009-2011 and reared in aquaria at the Oregon Institute of Marine Biology, Charleston, Oregon. Larvae were kept in 1.5 liter containers of 0.45 μm filtered seawater that were placed in a flowing seawater table with a stirring rack. Water in the cultures was changed every 3-4 days. Competent larvae of *C. errans* were collected in plankton tows in the Coos Bay Estuary, as described below.

Dungeness crabs, *Cancer magister*, used in both lab and field studies were captured in the Coos Bay Estuary, Oregon, using trapping methods described in Chapter II of this dissertation. Crabs were brought back to the Oregon Institute of Marine Biology and kept in tanks with flowing seawater until use in the experiments. Other species of crabs used in settlement trials were also taken from the Coos Bay, by either hand (*Hemigrapsus nudus* and *Hemigrapsus oregonensis*) or trap (*Cancer productus* and *Carcinus maenas*).

Laboratory settlement experiments

Larvae of *Carcinonemertes errans* were exposed to possible settlement cues beginning one day after hatching. Potential cues included 1) small living *Cancer*

magister juveniles that had been cleaned of all worms, 2) juveniles of *C. magister* that carried a known number of juvenile nemerteans, 3) pieces of exoskeleton from *C. magister*, 4) tissue from *C. magister*, 5) water from containers where individuals of *C. magister* were kept, and 6) juvenile conspecific worms. Living crabs were carefully examined for worms prior to use in experiments. Only male crabs were used in settlement trials because they are known to carry the parasite with at least equal frequency as females (Chapter II) and are much easier to examine thoroughly under their abdominal flap. Each crab was placed in a clean closed-system container along with a known number of larvae. At the end of the experiment, the crab was carefully examined under a dissecting microscope for signs of larval settlement. To test for the presumed host specificity of *C. errans* (Wickham 1980), living species of other crabs inhabiting the Coos Bay Estuary (shore crabs, *Hemigrapsus nudus* and *Hemigrapsus oregonensis*, the red rock crab, *Cancer productus*, and the invasive green crab, *Carcinus maenas*) were also tested using the same procedures. Cues other than living crabs (i.e. conspecific worms, *C. magister* exoskeleton, *C. magister* tissue, and water with crab “essence”) were tested using a Latin square design (Sokal & Rohlf 1981). Small dishes containing one cue each and filled with 0.45 μm filtered seawater were placed on a tray in rows. Each row contained only one replicate of each cue. A known number of larvae were introduced into each dish. The tray was kept in a table with running seawater to ensure constant temperature. After 24 hours, each dish was removed from the tray and examined under a dissecting microscope for settled larvae.

To test for larval age at competency, settlement trials were repeated on larvae each week after hatching until larvae were six weeks old. After six weeks, too few larvae

remained in cultures to conduct experiments. Prior to each of these trials, a subsample of larvae were relaxed and examined with a compound microscope (Olympus BX50) for evidence of morphological changes that could signal the arrival of competency.

Micrographs of larvae were taken using a camera mounted on the microscope (Optronics MicroFire True Color firewire digital camera).

Advanced larvae of *Carcinonemertes errans* were collected in plankton tows as described below, examined with the compound microscope, measured, photographed, and kept in finger bowls to use in settlement experiments. These trials were performed in closed containers with live male Dungeness crabs known to be uninfected with juvenile *C. errans*. Larvae were added to the containers and left for 24 hours. On the occasion of successful larval settlement, a subsample of newly-settled worms was removed from the crab, relaxed, and again measured and photographed under the compound microscope. These worms were then placed in a finger bowl of filtered seawater and observed for swimming or crawling behavior for 24 hours. This procedure was repeated 48 and 72 hours after successful larval settlement.

Field settlement experiments

Study site

Coos Bay is a drowned river estuary 54 km² in area located along the southern coast of Oregon. Input from rivers and streams varies seasonally, from 150 m³ s⁻¹ during the rainy winter to <3 m³ s⁻¹ in the dry summer months (Roegner et al. 2007). Regions of the lower estuary are well flushed during each tidal cycle (Roegner & Shanks 2001), but 2-3 tidal cycles are needed to flush areas of the upper bay (S. Rumrill, pers. comm.) and

South Slough branch of the estuary (Rumrill 2006). The bay can be divided into four distinct salinity regimes: the euhaline regime (>30) which is located near the mouth of the bay, the polyhaline regime (18-30) which stretches from about river mile 5 to river mile 12, the mesohaline regime (5-18) which consists of most of the upper-bay sloughs, and the oligohaline regime (<5) which is riverine (Davidson 2006).

Field trials

To determine the settlement patterns of *Carcinonemertes errans* in the Coos Bay Estuary, three caging trials were conducted. The first and second trials occurred in August and September of 2009. In the first trial, I examined: 1) whether larvae of *C. errans* could infect their hosts directly from the water column or only when the host was in contact with the substratum, and 2) whether crabs that were previously infected with juvenile worms were more likely to attract new parasites. I deployed three cages at each of six sites around the bay, each corresponding with one of my trapping sites described in Chapter II of this dissertation (Fig. 4.1). Three of the sites (Boathouse, Clam Island, and Empire) were in the most marine-influenced part of the estuary where prevalence of juvenile *C. errans* on *Cancer magister* was always at or near 100% and intensities were typically high (Chapter II). The other three sites were in the upper bay (Jordan Cove and Highway) and the South Slough (Valino Island) where both prevalence and mean intensity of *C. errans* was much lower (Chapter II). For cages, I used Fukui fish traps (60cm x 45cm x 20cm, ½ inch mesh) with their entrances wired shut (see Chapter II for image). Two of the cages at each site were weighted down on the substratum, and a line with a float was attached to each. Floatation was attached to the third cage to make it

positively buoyant. The floating cage was held in place by a line attached to an anchor that had been drilled into the substratum (Fig. 4.2). To ensure that cages remained submerged throughout the experiment, all were deployed during the lowest tide cycle of the month in which the trial took place. One male Dungeness crab was placed in each of

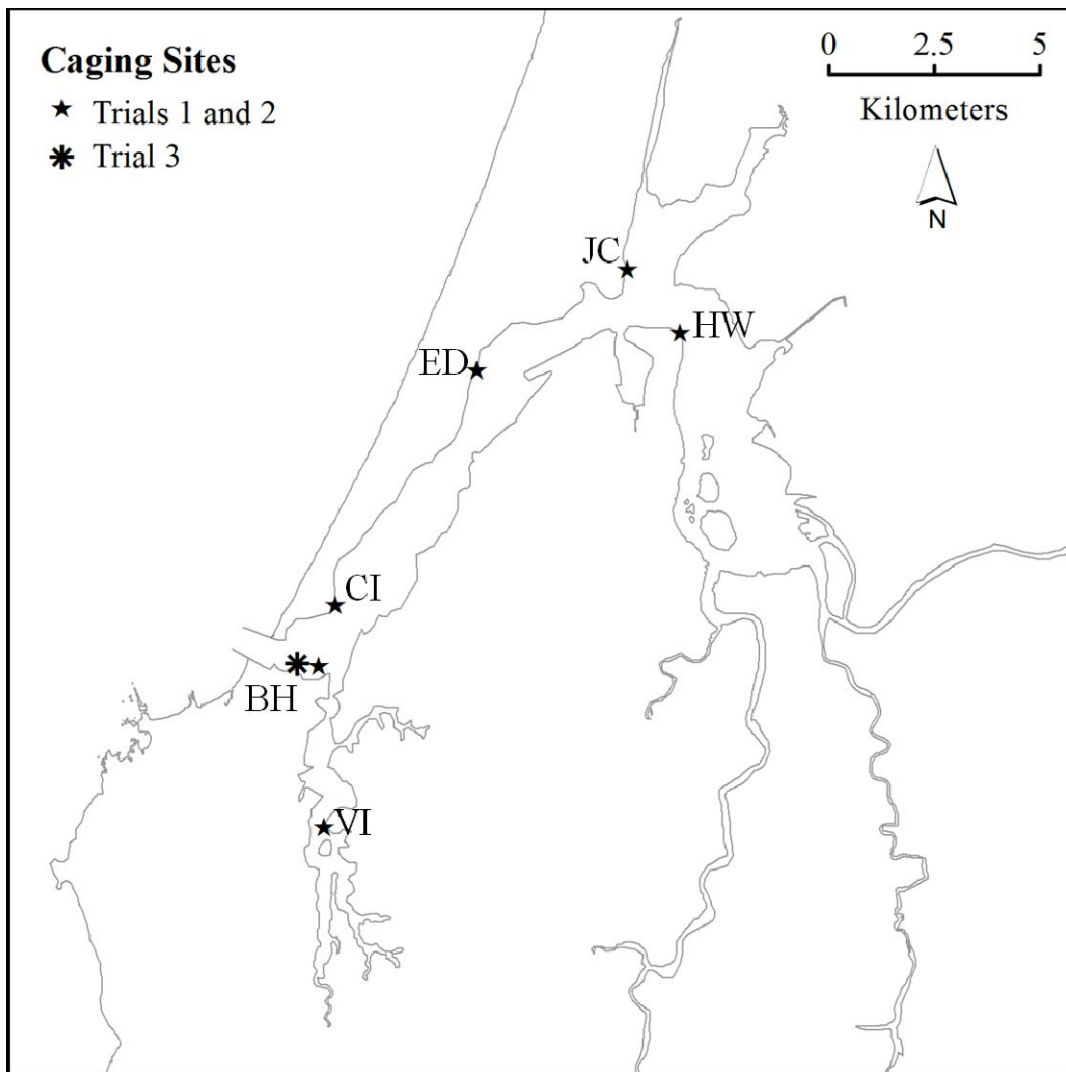


Fig.4.1. Location of caging sites during field settlement trials for *Carcinonemertes errans* in Coos Bay and South Slough, Oregon. Abbreviations: BH = OIMB Boathouse, CI = Clam Island, VI = Valino Island, ED = Empire Docks, JC = Jordan Cove, HW = Highway.

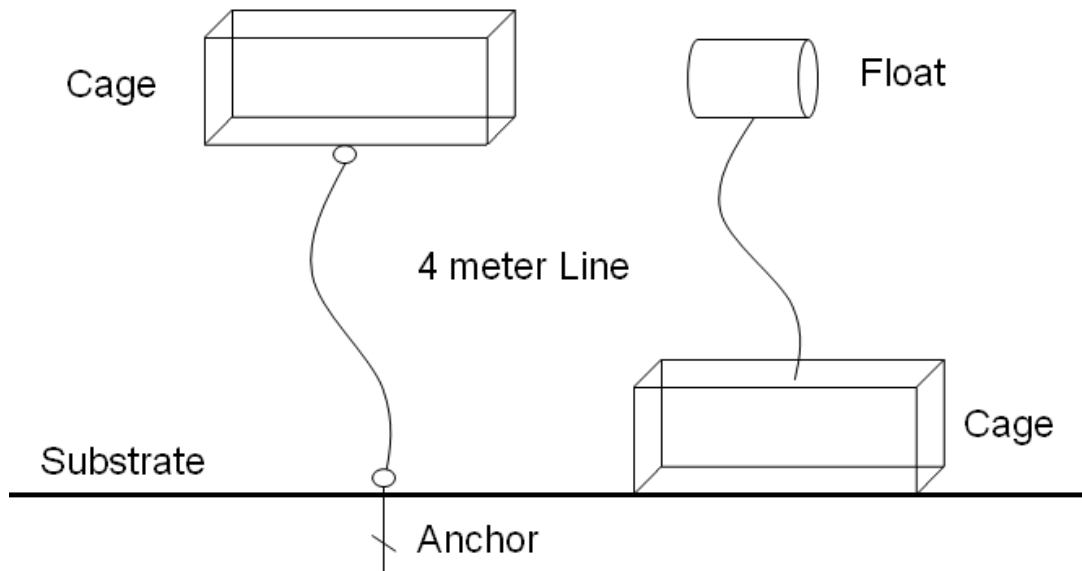


Fig.4.2. Design for field caging experiment. During the first trial, one floating cage and two bottom cages were deployed at each of six sites. The floating cage and one of the bottom cages held a previously-uninfected *Cancer magister* individual. The other bottom cage held a previously-infected *C. magister* carrying a known number of nemertean. For the second trial, only the two bottom cages were deployed at each site. Both trials lasted one month.

the traps at all of the sites. Crabs placed in the floating cage and one of the two bottom cages were checked on three separate occasions and known to be free of any juvenile *C. errans*. The crab placed in the second bottom cage was infected with a known number of worms. The carapace width of all crabs was measured prior to the beginning of the trial. I checked the cages once a week, recorded the infestation level of each crab, and fed the crabs. Crabs were monitored for one month.

The second round of caging experiments was conducted because the losses sustained during the first trial were too heavy for a clear pattern to emerge. The second

trial was nearly identical to the first, but the floating cages were omitted. Bottom cages still contained one crab with a known parasite load and one uninfected crab.

The third caging experiment was conducted in September 2010. Ten cages (25cm x 15cm x 10cm) were constructed out of ½ inch Vexar mesh and weighted down using scrap iron and rocks attached to the bottom of each cage with zip ties. The cages were attached every 1.3 meters along a 20 meter-long line that was anchored to the end of the dock at the OIMB Boathouse, near the mouth of the Coos Bay (Fig. 4.1). Each crab being used in the experiment was measured and carefully examined on three separate occasions to be sure of its infection state (5 infected, 5 uninfected). Once again, only male crabs were used in the trial. The size of infected crabs was not significantly different from that of uninfected crabs (t-test, $p=0.349$). Each crab was randomly assigned its own cage and wired inside. I then lowered the cages into the water off the south side of the dock and checked them daily for one week to make sure all of the crabs were still present and alive. After one week, I removed each crab and carefully counted the number of worms present.

The results of the first two trials were not analyzed separately due to losses described below. For the third trial, a t-test was performed to test for a difference in the mean change of intensity between previously infected and previously uninfected crabs (Sokal & Rohlf 1981). Combining the data from the three trials along with the pilot conducted before the third trial (identical methods to those described for Trial 3), I ran two regression analyses (Zar 2010). The first examined the relationship of crab carapace width on the change in parasite load during the trials. The second included only crabs that were previously-infected before the beginning of the trial and examined the

relationship between the initial parasite load and the number of new larval recruits that a crab acquired during the trials.

Plankton tows

Beginning in July 2010 and ending in June 2011, I conducted plankton tows over my trapping sites in the Coos Bay Estuary. Not every site was sampled every month, but all sites were sampled at least five times throughout the year. From March through June 2011, I also took plankton tows in coastal waters just outside of the Coos Bay Estuary. The purpose of these tows was to: 1) discover if and when competent larvae of *Carcinonemertes errans* are present in the water column at each site, and 2) to collect larvae to use in settlement experiments. All tows were taken by boat using a 150 μ m net and lasted five minutes. In preliminary surveys, larvae were found most often in subsurface water and were more common during flood tides than ebb cycles. For this reason, the depth of each tow was usually ~2-3 meters off the bottom, and plankton were collected either during high slack water or during an incoming tide.

Plankton from each tow were brought back to the laboratory at the Oregon Institute of Marine Biology and sorted live with a dissecting microscope. Larvae of *Carcinonemertes errans* from Coos Bay plankton were initially identified by S. A. Maslakova using sequence data from COI and 16S genes (Maslakova pers. comm.), and sequences of *C. errans* were kindly provided by J. Norenburg (Smithsonian Institution). Larvae thus identified possessed characteristic coloration, size, and two pairs of eyes. Larvae I collected were morphologically identical to those identified by the sequence data as belonging to *C. errans*, and thus are assumed to belong to the same species. Any

larvae of *C. errans* found in a sample were photographed as described above and kept for settlement experiments.

Results

Laboratory settlement experiments

No larvae of *Carcinonemertes errans* raised from hatching in the laboratory were induced to settle during experimental trials. This was true for every trial up to the death of the cultures around six weeks after hatching. Larvae in dishes were always observed to be swimming, never crawling along the bottom or exhibiting anything that might be identified as “searching” behavior. Directly after hatching, larvae were nearly round, an average of $104.67 \pm 1.02\text{SE } \mu\text{m}$ long ($n=30$), opaque due to yolk, and had one pair of ocelli directly over the brain as well as posterior and anterior cirri (Fig. 4.3A). By the third week after hatching, larvae had elongated to a mean length of $272.73 \pm 9.25\text{SE } \mu\text{m}$ long ($n=30$) and begun to look more worm-like (Fig. 4.3B). The first pair of ocelli and cirri were still present, but most of the yolk reserves were gone, presumably having been used for elongation and metabolism. This resulted in a somewhat transparent appearance. Little to no change occurred in larval morphology from this point until larvae in all cultures died around week 6.

Larvae collected in plankton tows differed substantially from those in raised in laboratory cultures. Larvae from tows were much larger (mean length= $643.85 \pm 14.61\text{SE } \mu\text{m}$, $n = 20$), lacked posterior and anterior cirri, and contained a pink-orange pigment concentrated especially around the gut. The first pair of ocelli observed in laboratory specimens was present, but a second pair of ocelli, positioned anterior to the

first pair and spaced farther apart, was also observed in all larvae (Fig. 4.3C). Internal organs, especially the brain, lateral nerve cords, and proboscis structure, were more clearly developed than in any of the specimens raised in the laboratory (Fig. 4.3C).

Larvae taken from the plankton were competent to settle. During one trial in which 30 larvae of *Carcinonemertes errans* collected in a plankton tow were incubated for 24 hours with a juvenile male *Cancer magister*, 12 new recruits were found under the crab's abdominal flap. These newly-settled worms closely resembled the competent larvae taken in plankton tows (Figs. 4.4A, 4.4B): no cirri, pink-orange coloration, and two pairs of ocelli. New settlers were found to be slightly longer than pre-settled larvae from the plankton ($833 \pm 13.86\text{SE } \mu\text{m}$, $n = 3$). When these three newly-settled worms were placed in a finger bowl of filtered seawater and observed over 24 hours, all swam like pre-settled larvae rather than crawling on the bottom.

When newly-settled worms were allowed to remain on the crab for 48 hours before removal, a clear morphological change occurred (Figs. 4.4C, 4.4D). The first pair of ocelli disappeared in all individuals. These worms were also longer on average ($945 \pm 31.80\text{SE } \mu\text{m}$, $n = 3$) than worms examined 24 hours after introduction to the crab host. When these nemerteans were placed in a finger bowl and checked over 24 hours, all of them consistently crawled on the bottom of the dish rather than swimming.

No noticeable change occurred between worms left on the host for 48 hours and those left on the host after 72 hours. Worms in the latter group also lost the first pair of eyes, were longer on average than worms left for 24 hours, and crawled rather than swam when placed in finger bowls.

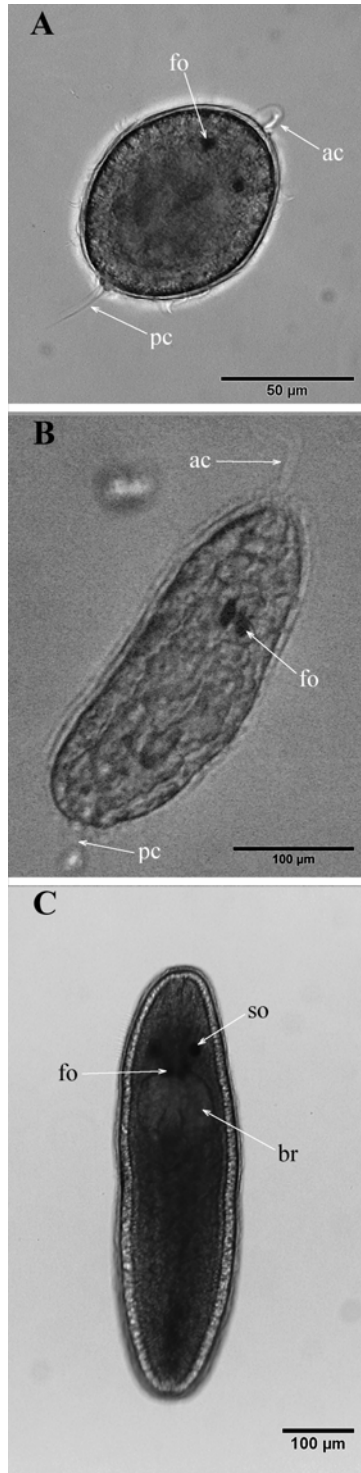


Fig. 4.3. Larval stages of *Carcinonemertes errans*. **A.** Larva from laboratory culture at hatching. **B.** Larva from laboratory culture three weeks after hatching. **C.** Competent larva of unknown age from a plankton tow in the Coos Bay Estuary. Note the differences in scale. Abbreviations: ac = anterior cirrus, pc = posterior cirrus, fo = first pair of ocelli, so = second pair of ocelli, br = brain.

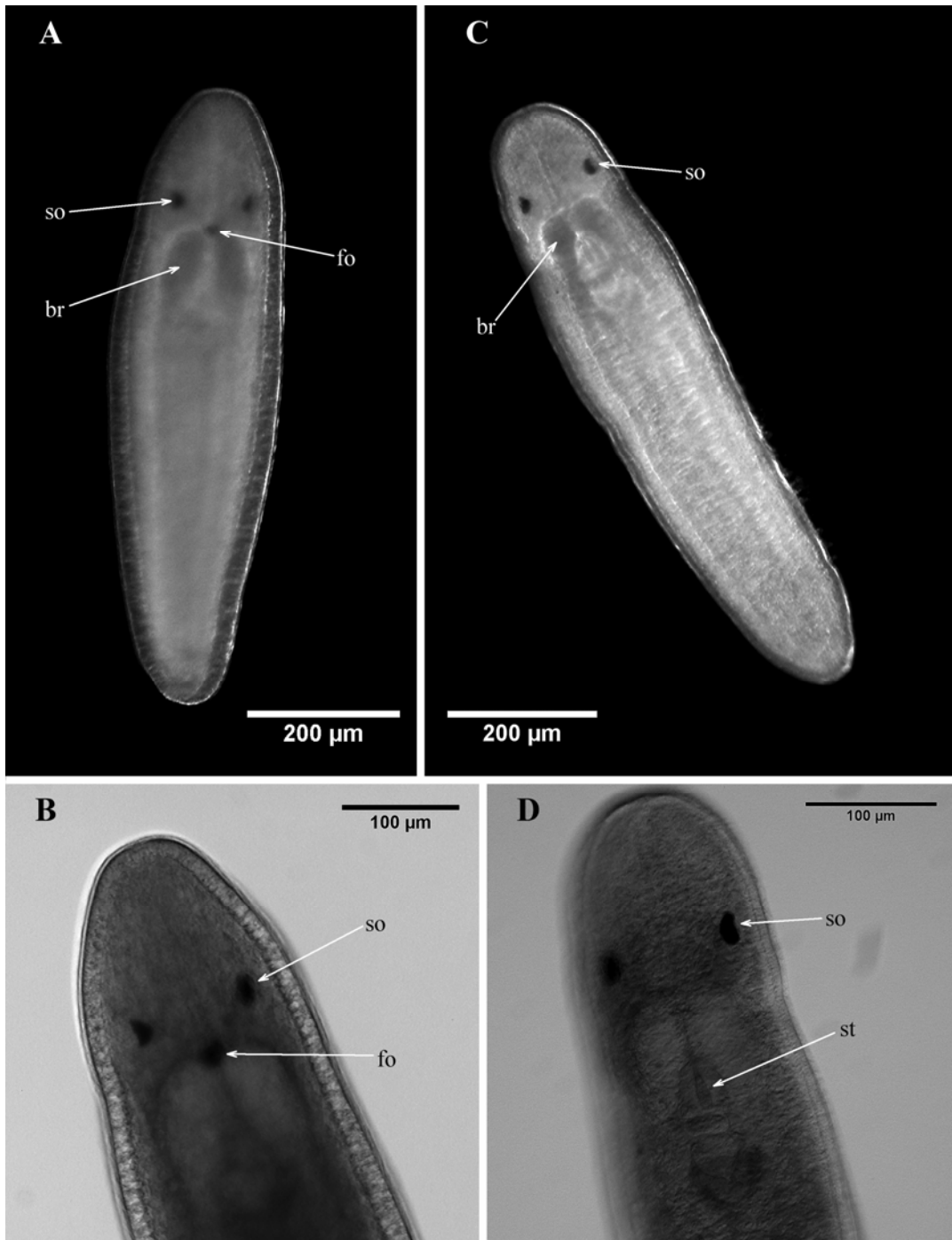


Fig. 4.4. Metamorphosis of *Carcinonemertes errans*. **A.** Settled larva removed from *Cancer magister* 24 hours after the beginning of the trial. **B.** Magnification of cephalic region of 24- hour larva. **C.** Metamorphosed juvenile removed from *C. magister* 48 hours after the beginning of the trial. **D.** Magnification of cephalic region of 48-hour juvenile. Abbreviations: fo = first pair of ocelli, so = second pair of ocelli, br = brain, st = stylet.

Table 4.1. Results for field caging experiment Trial 1. Number of individual *Carcinonemertes errans* observed on crabs placed in floating and bottom cages at six sites. All crabs in floating cages were uninfected at the beginning of the trial. Half of the bottom cages contained uninfected crabs, while the other half contained crabs infected with a known number of worms. Abbreviations: Int_0 = initial parasite intensity (number of worms) each crab carried, Int_F = final parasite intensity, ΔInt = change in parasite intensity during the trial, NA = lost cages.

Site	Floating Cage			Bottom Cage Uninfected			Bottom Cage Infected		
	Int_0	Int_F	ΔInt	Int_0	Int_F	ΔInt	Int_0	Int_F	ΔInt
OIMB Boathouse	0	NA	NA	0	14	14	7	86	79
Clam Island	0	11	11	0	18	18	21	115	94
Empire Docks	0	10	10	0	NA	NA	13	NA	NA
Jordan Cove	0	NA	NA	0	11	11	5	12	7
Highway	0	5	5	0	NA	NA	7	NA	NA
Valino Island	0	NA	NA	0	2	2	440	450	10

Table 4.2. Results for field caging experiment Trial 2. Number of individual *Carcinonemertes errans* observed on crabs placed in bottom cages at six sites. Half of the cages contained uninfected crabs, while the other half contained crabs infected with a known number of worms. Abbreviations: Int₀ = initial parasite intensity (number of worms) each crab carried, Int_F = final parasite intensity, ΔInt = change in parasite intensity during the trial, NA = lost cages.

Site	Bottom Cage Uninfected			Bottom Cage Infected		
	Int ₀	Int _F	ΔInt	Int ₀	Int _F	ΔInt
OIMB Boathouse	0	15	15	7	73	66
Clam Island	0	NA	NA	20	48	28
Empire Docks	0	NA	NA	9	NA	NA
Jordan Cove	0	NA	NA	12	17	5
Highway	0	3	3	11	NA	NA
Valino Island	0	7	7	16	26	10

Field settlement experiments

In the first and second field settlement trials, a combination of strong tidal currents, high waves, and equipment theft left me with about half of my original cages. Despite these losses, a few patterns emerged (Tables 4.1, 4.2). First, all of the crabs in the bottom cages picked up new settlers by the end of the month (range = 2 to 94). Second, crabs in floating cages also became infected with *Carcinonemertes errans*. Third, the change in infection did vary from site to site, but all sites saw at least some new settlement. And finally, for those sites in which both a previously-infected and a previously-uninfected crab were available at the end of the experiment, previously-infected crabs tended to show a larger increase in new settlers, especially in lower bay sites.

The results of the third field trial are shown in Table 4.3. All of the crabs became infected after one week of exposure. The five crabs that were uninfected at the beginning of the experiment had an average increase in parasite intensity of 10.2 ± 2.06 SE (range 6 to 18). The average increase of previously-infected crabs was twice as large (mean=20, ± 3.24 SE, range = 13 to 32). A t-test found the difference between infection state to be statistically significant when $\alpha = 0.05$ ($p = 0.034$).

Table 4.3. Results for field caging experiment Trial 3. Number of individual *Carcinonemertes errans* observed on crabs placed in Vexar cages deployed along a line from the dock of the OIMB Boathouse. Half of the cages contained uninfected crabs (shaded rows), while the other half contained crabs infected with a known number of worms. Abbreviations: CW= crab carapace width measured just anterior to the 10th lateral spine, Int₀ = initial parasite intensity (number of worms) each crab carried, Int_F = final parasite intensity, Δ Int = change in parasite intensity during the trial.

Crab	CW (mm)	Int ₀	Int _F	Δ Int	% Increase
1	112	72	88	16	22.22
2	123	0	8	8	
3	126	2	15	13	650
4	116	0	10	10	
5	127	0	18	18	
6	107	19	39	20	105.26
7	96	17	49	32	188.24
8	120	2	21	19	950
9	126	0	6	6	
10	103	0	9	9	

A slightly negative relationship was observed between the change in parasite intensity and crab carapace width (Fig. 4.5), but the trend was not significant ($r^2 = 0.052$, $p = 0.102$; $n = 52$). As shown in Fig. 4.6, among previously-infected crabs only, the negative relationship between initial parasite intensity and the change in intensity over one week was found to be significant ($r^2 = 0.303$, $p < 0.001$; $n = 37$).

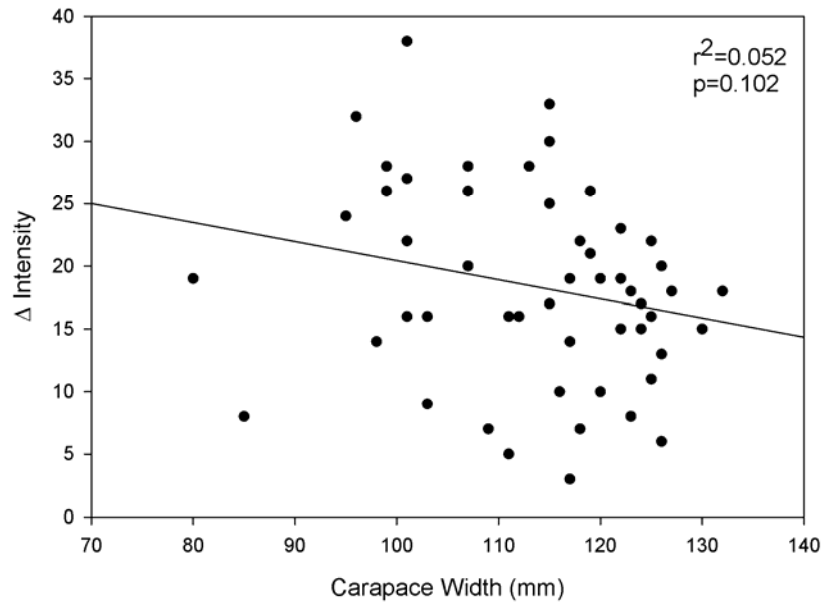


Fig. 4.5. Regression analysis of the change in parasite intensity of *Carcinonemertes errans* in relation to host size. Crabs used in the regression included both previously-infected and previously-uninfected individuals from a combination of Trials 1, 2, and 3 as well as pilot experiments ($n=52$).

Plankton tows

The results of the plankton tows conducted in the Coos Bay Estuary and offshore waters are shown in Table 4.4. There was a clear gradient in where larvae were found

within the bay. No larvae were ever found at Jordan Cove, Highway, or Valino Island. However, larvae were present in tows performed at Boathouse, Clam Island, Empire, and Collver Point sites. The number of larvae at Boathouse, Clam Island, and Empire were similar, but relatively few larvae were found at Collver Point. A clear seasonal pattern was observed as well, with larvae only present in plankton samples from August through early November, with peak occurrence in October 2010 (Figure 4.7). No larvae of *Carcinonemertes errans* were taken in offshore plankton tows.

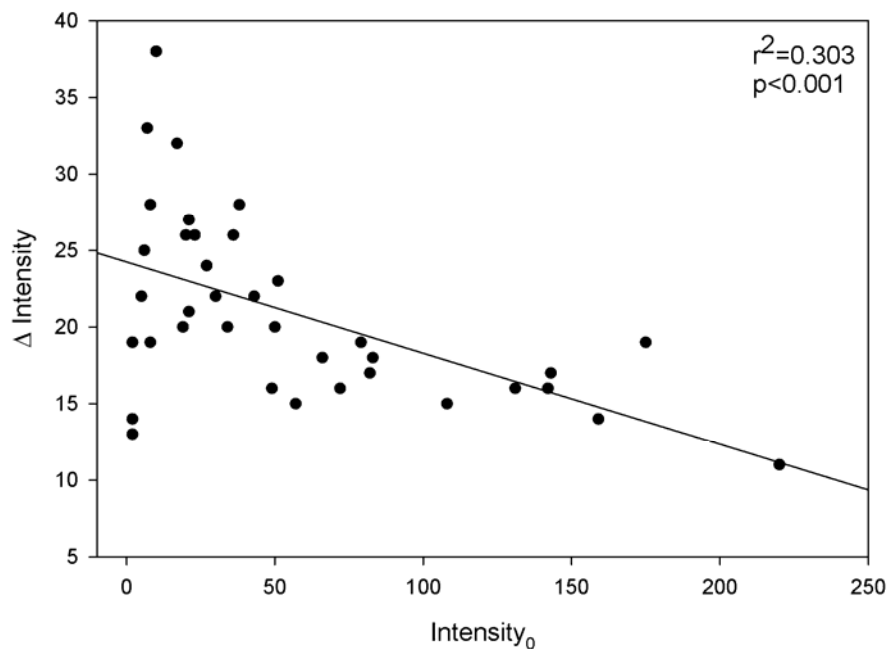


Fig.4.6. Regression analysis of the change in parasite intensity of *Carcinonemertes errans* in relation to initial intensity. Only crabs that were infected at the beginning of the trials were included. Data from a combination of Trials 1, 2, and 3 as well as pilot experiments (n=37).

Table 4.4. Results for plankton tows performed in Coos Bay Estuary and offshore. Number of individual *Carcinonemertes errans* larvae taken in plankton samples at each of 8 sampling sites. Sampling began in July of 2010 and continued until June 2011. Blank spaces represent months in which no tow was performed at a given site.

Site	Jan '11	Feb '11	Mar '11	Apr '11	May '11	Jun '11	Jul '10	Aug '10	Sep '10	Oct '10	Nov '10	Dec '10
Boathouse	0	0		0		0	0	10	15	16	0	
Clam Is.	0	0	0		0	0		3	12	16	0	
Empire	0		0	0		0	0	7	8	30	1	
Jordan			0			0		0	0		0	
Highway			0			0		0		0	0	
Collver Pt.	0		0			0	0	1	1	1	0	
Valino Is.	0		0			0		0	0	0		
Offshore			0	0		0						

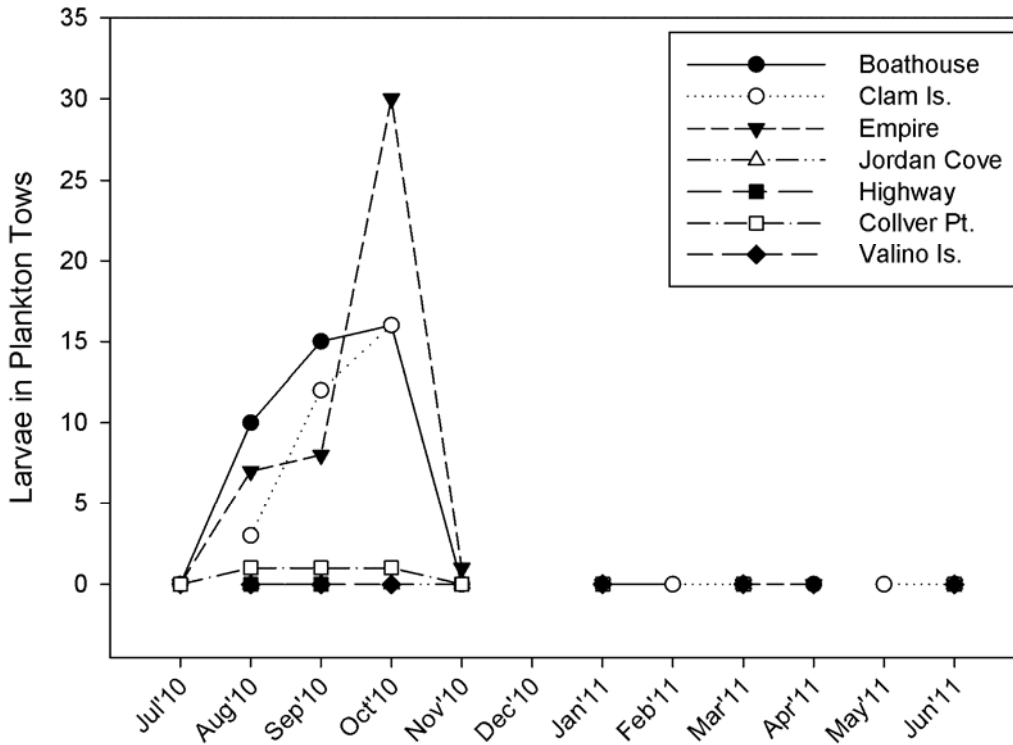


Fig. 4.7. Results from plankton tows in the Coos Bay Estuary. The total number of larvae of *Carcinonemertes errans* that were taken at each sampling site between July 2010 and June 2011. No tows were performed in December 2010.

Discussion

Laboratory settlement experiments

Larval settlement of *Carcinonemertes errans* is described here for the first time under laboratory conditions. There remains, however, a gap between those larvae taken in plankton tows that were induced to settle and those that were raised from hatching in the laboratory, which never settled during experiments. This disparity could have been the result of several factors (or combinations of factors) present in the laboratory cultures including the lack of the appropriate larval food source, failure to be provided with the

appropriate substratum for settlement, failure to provide the correct conditions for normal development, or contamination and subsequent death of laboratory cultures. The last possibility seems quite unlikely based on the consistent timing of the death of nearly all of my larval cultures in two separate years. In every case, cultures appeared healthy for several weeks and then deteriorated quickly. A culture that was started two weeks after another culture typically crashed around two weeks after the first rather than both crashing simultaneously.

The substratum choice also seems unlikely because field-caught larvae settled in response to the live crab cue to which laboratory-reared larvae failed to respond. Given the clear morphological differences present between field-caught larvae and lab-raised larvae, the difference in environmental conditions, particularly possible food sources, seems the likely explanation. Larvae taken in plankton tows were nearly twice as large, on average, than the largest larvae ever observed in laboratory cultures. The relatively clear appearance of older lab-reared larvae is also in stark contrast to the pink-orange coloration of the larvae from the plankton. This coloration, which appears to be concentrated around the gut in some specimens, could very well be a by-product of planktivorous feeding. It is therefore likely that lab-reared larvae did not settle when exposed to the correct settlement cues because they had failed to reach the stage at which they were competent to settle.

Competency represents an example of convergent evolution; many different phyla have developed a larval stage that is, in effect, a loaded gun (Hadfield et al. 2001). In a typical competent larva, the development of juvenile structures is all or mostly complete, making a rapid transition possible. In this way, larvae can begin the change from pelagic

life to benthic almost immediately upon contacting the appropriate substrate. Clearly, *Carcinonemertes errans* has also developed a morphologically-distinct competent stage. The most telling features of this stage are the two pairs of eyes, the first pair being present from the time of hatching and lost following settlement and the second pair present beginning in the competent larval stage and remaining during the juvenile and adult stages.

The process of larval settlement in *Carcinonemertes* is distinctive. Competent larvae settle directly onto their crab host within 24 hours of exposure in the laboratory. The exact location of initial settlement is unknown, but all new settlers were found underneath the abdomen after 24 hours. Larval settlement in *Carcinonemertes errans* does appear to be reversible, at least for a limited period of time. Individuals taken off the crab after only 24 hours of exposure still looked and behaved like competent larvae, possessing two pairs of eyes and swimming continually in a finger bowl. Continued contact with the host, however, appears to induce the process of metamorphosis, here defined as the phenomenon involved with the morphological changes an animal experiences as it permanently transitions from a swimming larva to a benthic juvenile or adult (Pawlik 1992). The worm loses its first set of larval eyes, which were presumably used for navigating in a pelagic world, but retains the second pair. As a side note, juvenile and adult *C. errans* are negatively phototactic. It therefore stands to reason that the development of this second pair of eyes could signal the beginning of photonegative behavior in the competent larva. This could potentially bring the larva into contact with the host at the exact time when it is capable of infecting a crab (Chapter V).

Along with the loss of the first pair of eyes, the metamorphosing settler also ceases to swim when taken off its crab host and adopts a longer, thinner body plan (just under 1 mm long). Such elongation is common among the larvae of hoplonemerteans at the time of settlement (Stricker 1987). In short, it looks exactly like a slightly smaller version of any juvenile worm taken from a crab host. Although far from the dramatic metamorphosis observed in nemerteans with a pilidium larva, the physical changes following settlement in *Carcinonemertes errans* are the most dramatic of any hoplonemertean for which settlement has been described (Stricker 1987). The loss of the first pair of eyes is particularly interesting, as the larval eyes are typically retained in hoplonemertean juvenile and adult stages (Norenburg & Stricker 2002). The clear morphological change between larva and juvenile can act as a helpful indicator in any future studies involving settlement of *C. errans*, as any examined settlers retaining larval features will be known to have settled in the last 48 hours.

Field settlement experiments

In his two-tiered caging experiment to determine whether larvae of *Carcinonemertes epialti* infect crabs from the water column or the substrate, Bauman (1984) observed that shore crabs in the bottom tier became more infected with *C. epialti* than those in the upper tier. Hosts in the upper tier (30 cm off the bottom) did carry some worms, however, suggesting that either some worms do infect from the water column or they are capable of crawling 30 cm to the second tier. This mixed result left the issue unresolved. The results of my first field trial clearly showed that the larvae of *C. errans* are capable of infecting their crab host directly from the water column. The height of my

floating cages above the substratum would have varied through tidal cycles, but at low tide no cage would have been closer than one meter off the bottom. It remains a possibility that at least some competent larvae may settle on the substrate and seek out their hosts by crawling, but in all of my laboratory observations of competent larvae, I never saw any of them stop swimming and begin crawling until after metamorphosis.

In his seminal review on larval settlement, Crisp (1974) described the patterns of associative settlement and gregarious settlement, both of which are commonly observed among the settling larvae of marine invertebrates. Associative settlement, the enhanced or specific settlement of one species on another, has been observed for many marine species (Crisp 1974; Hadfield & Paul 2001), and is probably the rule for parasitic larvae (Chia 1978; Pawlik 1992). The results of both field and laboratory work in this study clearly show that *Carcinonemertes errans* exhibits associative settlement with its host, *Cancer magister*.

The second pattern, gregarious settlement, is defined as the process wherein larvae of the colonizing species are induced to settle by established individuals of the same species (Knight-Jones 1953). Such a behavior would clearly result in several advantages such as choosing a habitat that is likely to support postlarval growth (Jensen 1989), increased likelihood of reproductive success (Crisp 1979; Pennington 1985), and even protection from predation (Highsmith 1982). Gregarious settlement has been reported in at least 35 invertebrate species in 8 phyla (Burke 1986) and is particularly prevalent among hard-bottom, sessile animals such as barnacles (Knight-Jones 1953), bivalves (Bayne 1969), polychaetes (Wilson 1968), and ascidians (Young & Braithwaite 1980). This study represents the first clear evidence for gregarious settlement in a

nemertean. The infection of previously worm-free crabs during all field trials proves that larvae of *Carcinonemertes errans* do not require the presence of conspecifics to settle, but significantly more larvae did settle on hosts that already carried at least a few juvenile worms. *Carcinonemertes errans* has separate sexes, and although Roe (1986) showed that some females are capable of reproducing parthenogenetically, the low success rate of this strategy made sexual reproduction the most viable reproductive option. Natural selection should therefore favor larvae that seek out hosts where they would be more likely to encounter a worm of the opposite sex. A similar pattern is seen for rhizocephalan barnacles, where the host is first infected by a female larva and then by a male (Boone et al. 2003).

Gregariousness has its costs as well. Aggregated individuals must compete for space and resources, which may decrease individual fitness (Pawlik 1992). To minimize this effect, some species that exhibit gregarious settlement also exhibit a “spacing-out” behavior (Hui & Moyse 1987) sometimes called “territoriality” in the literature (Knight-Jones & Moyse 1961). The results of the regression analysis (Figure 4.6) suggest that something similar may be happening with *Carcinonemertes errans*. Wickham (1979b) reported that there is a negative relationship between the number of worms present in a crab egg mass and the number of eggs that each worm eats, even when food does not appear to be limiting. Fewer crab eggs eaten translates into fewer offspring produced per worm (Wickham 1979b). He suggested that worms may produce some sort of intraspecific feeding inhibitor, similar to those seen in some amphibians (Rose 1960). Another possibility may be an increase in time spent in agonistic behaviors as worms increase in density. Whether chemical or behavioral in nature, this potential decrease in

fitness could provide the selective force necessary for *C. errans* to develop territorial behavior at the time of larval settlement, rejecting hosts that carry too many conspecifics.

The field trials also showed that the size of the host crab did not determine how many new settlers it gained in a given time (Figure 4.5). This is a particularly interesting result given the clear positive relationship between size and parasite prevalence and intensity observed during the three-year trapping survey in the Coos Bay Estuary (Chapter II). If the size of the crab itself is not a good predictor for larval settlement, then the observed pattern must be attributable to one or both of the controlled factors during the field experiments: location within the estuary and length of exposure. Although sample sizes were small for field trials, it appeared that the increase in mean intensity of *Carcinonemertes errans* on crabs left in cages for one month during trials 1 and 2 was much higher than that of crabs left for only one week in trial 3 (Tables 4.1, 4.2, 4.3). Also, crabs in cages at sites in the lower bay had a larger increase in mean intensity on average than crabs at upper bay or South Slough sites (Tables 4.1, 4.2). It therefore seems likely that both location and exposure time contribute to the number of worm larvae settling on a crab host. Since larger crabs in the Coos Bay Estuary have potentially been exposed to larvae longer than smaller crabs and also tend to spend more time in waters closer to the mouth of the bay (Chapter II, this dissertation), both factors could be working to create the observed pattern.

Plankton tows

The larvae of *Carcinonemertes errans* were the most common nemertean larvae found in plankton tows conducted in the Coos Bay Estuary. Given that individual worms

can produce hundreds to thousands of larvae (Wickham, 1980) and infections on an ovigerous female Dungeness crab can number in the thousands to tens of thousands (Wickham 1979a), this is not particularly surprising. The distribution of larvae collected in tows appeared to mirror that of both field settlement trials and field trapping surveys, with the most larvae present in lower bay sites where both prevalence and mean intensity of juvenile *C. errans* were highest (Chapter II). Occasional larvae were also found at the Collver Point site, which showed intermediate infection rates during the trapping survey. Although no competent larvae of *C. errans* were found in tows at Jordan Cove, Highway, or Valino Island sites, some settlement did occur there during caging experiments (Tables 4.1, 4.2), proving that the competent larvae can reach these areas and that the infected crabs trapped there were not necessarily migrants from another part of the bay.

Pulling on the observation that young-of-the-year *Cancer magister* first became infected with *Carcinonemertes* in August and September, Wickham (1980) suggested that the larval duration for *C. errans* may be around 8 to 9 months, an exceptionally long larval period. Given the small number of young-of-the-year crabs Wickham was able to examine, however, this proposition seemed tenuous at best. However, my plankton tows for 2010-2011 showed a clear peak in larval abundance during August-October, exactly 8-10 months after the peak of larval hatching for *C. errans* on Dungeness crabs (Wickham 1980). The fact that larvae of *C. errans* still have not been raised from hatching to competency in the lab means that the actual length of larval duration for this species remains unknown. However, larvae were completely absent from bay tows after the start of November 2010 and were never present in coastal ocean tows between March-June 2011. If the larvae do reach competency within a few months of hatching,

one would definitely expect their presence in March tows. Interestingly, both Kuris (1978) and Roe (1979) saw peaks in abundance of *C. epialti* on *Hemigrapsus oregonensis* in September-November as well. This peak in larval abundance can also help explain the counterintuitive seasonal patterns observed at some sites within the estuary during the trapping survey (Chapter II).

In summary, *Carcinonemertes errans* is now known to have a competent larval stage that is significantly larger than the early-stage larvae that have been raised in the laboratory. The increased size and pink-orange coloration of the competent stage suggest that these larvae are planktotrophic and grow considerably while in the plankton. Competent larvae retain the first pair of larval eyes but have also developed their juvenile pair of eyes which could possibly alter their phototactic behavior in favor of seeking out a benthic host. Upon contact with *Cancer magister*, competent larvae of *C. errans* settle on the crab's exoskeleton and migrate under the abdominal flap within 24 hours. When removed from the host, recently-settled worms retain their larval characteristics. If larvae remain on a host for 48 hours, however, a metamorphosis takes place and worms look and behave like juveniles. In the field, competent larvae are present in the waters of the Coos Bay Estuary during the months of August through early November, can infect crab hosts from the water column, and exhibited density-dependent gregarious settlement on caged Dungeness crabs.

Many questions still remain regarding the larval life and settlement of *Carcinonemertes errans*. Filling in the current life-cycle gap between the 6-week-old post-hatching larvae raised in the laboratory and the competent larvae from the plankton will most likely involve finding the required food source for larval development and

growth (Chapter V). One possible avenue to discovering this source would be to perform a genetic analysis on the contents of the pink-orange larval gut. If the food source is non-animal in nature (e.g. bacteria, phytoplankton) it may be distinguishable from the genes of the larva itself. Raising larvae from hatching to settlement would also answer the question of how long the larvae of *C. errans* actually remain in the plankton, proving if the estimated 8-9 month larval duration is accurate.

Genetic work on the genus *Carcinonemertes* now in progress (J. Norenburg, pers. comm.), suggests the possibility that *Carcinonemertes errans* and *C. epialti* are the same species. If this is true, larvae hatching from *Cancer magister* hosts could possibly infect other crab species and vice versa. This leaves the possibility that the larvae found in plankton tows in August-November were hatched from the egg masses of *Hemigrapsus* spp. or *Cancer productus*, and were therefore only a few months old. However, with Dungeness crabs in coastal waters numbering in the tens of thousands, the complete absence of larvae from March through June makes this hypothesis less likely. A study in which some larvae from a plankton tow are sequenced while others from the same tow are used in settlement experiments with different species of potential hosts could help to test this possibility.

Using competent larvae of *Carcinonemertes errans* to further examine the details of larval settlement could yield interesting results. It is quite possible that settlement in *C. errans* involves at least three separate cues: 1) an associative settlement cue that begins the process settlement process on the crab, 2) a gregarious settlement cue to help the worm decide if it's going to stay on the crab, and 3) another crab-produced cue that induces worm metamorphosis. The specificity of these cues is also an interesting question

that warrants further research. While it may seem obvious that parasite settlement cues should be host-specific, this is not always the case. James (1971) found surprisingly little specificity in some fluke larvae, and the same is true of at least some rhizocephalan barnacle species (Boone et al. 2004). While lower specificity may result in large numbers of larvae dying in the short-term, over evolutionary time this strategy may result in a successful transition to infecting a new host species. Such a strategy may explain the ability of *Carcinonemertes epialti* to infect so many host species while many of its congeners remain host-specific.

Bridge

In Chapter IV, I examined the settlement patterns of *Carcinonemertes errans* in the field, determined when competent larvae are present in the estuary, and documented the process of larval settlement and metamorphosis. I found that larvae raised in the laboratory are morphologically distinct from competent larvae found in plankton tows, suggesting that the larval stage of *C. errans* is likely long-lived and planktophic. In the following chapter, I tested the ability of lab-reared larvae to ingest a variety of particulate food sources as well as dissolved organic matter (DOM). The ability of larvae of *C. errans* to detect a variety of light stimuli under simulated natural light conditions was also examined, helping me to predict the possibility of vertical migration in larvae of this species.

CHAPTER V

PHOTOTAXIS AND FEEDING IN LARVAE OF THE OOPHAGOUS NEMERTEAN

CARCINONEMERTES ERRANS

Introduction

The life cycles of many benthic marine invertebrates include a planktonic larval stage (Thorson, 1950). The selective advantages of such a stage may include 1) broadening the range of a species through dispersal, 2) avoiding direct competition with parents and siblings, and 3) increasing access to energy and materials beyond what parents provide (Garstang, 1928; Thorson, 1950; Scheltema, 1971; Jägersten, 1972; Strathmann, 1978; Wray, 1995). However, these potential benefits are countered by considerable costs; larval wastage, or death during the larval stage, is estimated to be enormous for most species (Thorson, 1950; Rumrill, 1990). Environmental stressors (e.g. UV radiation, unfavorable temperatures or salinities, low dissolved oxygen, and pollution), starvation, sinking, advection away from suitable habitats, and predation can all reduce larval survival (Thorson, 1950; Morgan, 1995). Through adaptive behaviors, however, larvae are capable of controlling their vertical position in the water column, thus enhancing their access to prey, promoting avoidance of predators, and controlling horizontal transport (Thorson, 1964; Forward, 1988; Young 1995).

Larval orientation is accomplished by reacting to environmental stimuli such as pressure, salinity, temperature, currents, gravity, and light (Young, 1995). Responses to these stimuli function in depth regulation by creating positive and negative feedback systems, with the former resulting in net migration and the latter leading to maintenance

of a particular vertical position (Sulkin, 1984). Gravity is probably the most important of the vector cues used for larval orientation (Young, 1995). Larvae respond to gravity by moving toward the center of the earth (positive geotaxis) or away from it (negative geotaxis). Light intensity, wavelength, and angular distribution change considerably with time of day, atmospheric conditions, water turbidity, and depth (Thorson, 1964). Still, the downward ambient flux of light at a given depth is ~100 times that of the upward flux (Clarke and Denton, 1962), and many larvae are able to detect and use this stimulus for orientation and depth regulation. Larvae may be attracted to light (positive phototaxis) or repelled by it (negative phototaxis), or they may change their activity patterns at different light intensities (photokinesis).

Stimuli can act in concert with one another or be in direct competition. There are several species, for example, that exhibit geonegative and photonegative behavior simultaneously. If the light stimulus dominates during the day and only gravity is available at night, one would observe the nocturnal migration pattern that is common among many zooplankters (Young, 1995). A negative interaction between two stimuli could also be used to maintain a given depth. For example, the larvae of the mud crab *Rhithropanopeus harrisi* stay in an isolume by being negatively phototactic at low light intensities then exhibiting geonegative behavior when they can no longer detect light (Forward, 1985).

One major issue with most phototaxis experiments in the literature is the use of a narrow beam of highly directional light as a stimulus (Forward, 1988). This method often induces phototaxis, but it does not accurately simulate the angular distribution of light in the ocean where photons converge on a given point from different directions in

space (Buchanan and Goldberg, 1981). In studies where light fields that do simulate angular light distribution are used, few species have exhibited positive phototaxis (Buchanan and Goldberg, 1981; Buchanan *et al.*, 1982; Stearns and Forward, 1984). Forward (1986) compared the larval phototactic response of *Rhithropanopeus harrisi* in a natural light distribution field to the response in a narrow beam. Whereas larvae showed a negative response to low intensity light and a positive response to high intensity light in narrow beams, larvae in the simulated natural light field failed to show the positive phototactic response. Given the growing evidence, Forward (1988) concluded that under natural conditions, positive phototaxis is probably not common among zooplankton.

In addition to depth regulation and orientation behaviors, the ability of a larva to obtain food is vital to its success in the plankton. As a larva develops, its nutritional requirements may change (Boidron-Metairon, 1995). Some stages of larval development may be lecithotrophic while others are planktotrophic (e.g. barnacle cyprids vs. nauplii). Two feeding stages of a life cycle may also have different mechanisms for capturing food and may target different food sources (e.g. crab zoea vs. megalops; Lough, 1976). For many meroplankters, the dominant food source is phytoplankton (Boidron-Metairon, 1995), but other food options are often available to and exploited by larvae (Olson *et al.*, 1987). Bacteria are selectively ingested in Antarctic echinoderm, polychaete, and nemertean larvae (Rivkin, 1991). Many larvae are also predators on other zooplankters, from ciliates and flagellates to copepods and other meroplankters (Baldwin and Newell, 1991).

Along with feeding on particulate matter, marine invertebrate larvae across several taxa also tap into the supply of dissolved organic material (DOM) present in seawater (Wendt and Johnson, 2006). DOM is readily available in seawater; reduced carbon concentration is ten times that of particulate matter (Stephens, 1981). Many soft-bodied invertebrates (both larvae and adults) are known to absorb DOM via transepidermal transport (for a review, see Wendt and Johnson, 2006). This supply can supplement, or in some cases fully supply, a larva's nutritional needs for both metabolism and growth (Cowell and Jaekle, 1990; Shilling and Manahan, 1991).

Worms of the genus *Carcinonemertes* are egg predators on decapod crustaceans (Coe, 1902). During the juvenile stage, these worms live encysted on the exoskeleton or between the gill lamellae of their crab hosts, presumably surviving from dissolved organics leaking out of the host (Crowe *et al.*, 1982). When the female crab lays her eggs, the worms become active, move into the egg mass, and begin to feed. At this time they mature, mate, and lay egg strings of their own among the eggs of their host (Wickham, 1980). Several days to a few weeks later, worm larvae hatch out of these strings and begin the planktonic stage of the life cycle. In the case of *Carcinonemertes errans* Wickham 1978, which infects the Dungeness crab, *Cancer magister* Dana 1852, along the west coast of North America, this planktonic stage can last several months before the larvae become competent to settle and infect a new host (Wickham, 1980). Larvae of *C. errans* are quite small at hatching (~100 µm long) and are filled with a small amount of yolk (Stricker and Reed, 1981). Larvae also hatch with one pair of simple ocelli positioned directly above the brain (Hyman, 1951).

This combination of characteristics, along with the potential importance of the species as an egg predator on a commercially important shellfish species, makes *Carcinonemertes errans* a good candidate for studies examining possible larval phototaxis and feeding behaviors. Bauman (1983) described the phototactic behavior of the larvae of *C. errans* as being positively phototactic under lighted conditions and positively geotactic in the dark for the first two weeks following hatching. This study, however, had the following limitations: 1) only narrow beam light was used to test phototactic responses, 2) only white light was tested, 3) only one intensity of light was tested, 4) most of the results were for larvae five days old and younger, and 5) larval mortality was very high during the experiments, making the results difficult to interpret.

The purpose of this study was to examine several aspects of the larval ecology of *Carcinonemertes errans* including: 1) phototactic responses in a more natural light field generated in the laboratory, 2) spectral responses of larvae to blue, green, and red wavelengths of light, 3) the effect of light intensity on larval phototaxis, 4) the effect of pressure on larval phototaxis, and 5) the feeding behavior of *C. errans* larvae when offered a variety of potential food sources, including DOM.

Materials and Methods

Study organisms

Larvae of *Carcinonemertes errans* were collected from egg masses of Dungeness crabs captured in Oregon coastal waters during winter and spring 2009-2011 and reared in aquaria at the Oregon Institute of Marine Biology, Charleston, Oregon. Larvae were held in 1.5 liter containers of 0.45 μ m filtered seawater that were placed in a flowing

seawater table and kept stirred with a stirring rack. Water in the cultures was changed every 3-4 days.

Particulate feeding

The ability of larvae of *Carcinonemertes errans* to consume particulate prey items was tested. Larvae were offered several possible food items, including the phytoplankters *Dunaliella tertiolecta*, *Isochrysis galbana*, *Monochrysis lutheri*, *Rhodomonas lens*, *Skeletonema costatum*, and *Thalassiosira pseudonana*, various ciliates, and natural plankton mixes (seawater filtered through a 200 μm nylon mesh). In each case, ~ 100 *Carcinonemertes* larvae were placed in a custard dish and incubated with the potential food item for 24 hours. A subsample of larvae was then examined with a compound microscope to determine whether any food items could be detected in the larval gut. To test whether larvae might be ingesting small particles indiscriminately, 100 larvae were incubated in a dish with fluorescent spheres $\sim 2 \mu\text{m}$ in diameter for 24 hours. Larvae were then examined with an epifluorescence microscope to see if any of these spheres could be found in the larval gut.

Dissolved organics

Two methodologies were applied to assess whether larvae of *Carcinonemertes errans* may feed on dissolved organic material (DOM). First, 200 two-week-old larvae were placed in custard dishes of 0.45 μm filtered seawater containing one of four treatments: no DOM or antibiotics added (control), only antibiotics added (antibiotic control), a 1 to 9 mixture of cell culture media and filtered seawater and antibiotics (low

DOM), and a 1 to 4 mixture of cell culture media and filtered seawater and antibiotics (high DOM). All antibiotics added to treatments consisted of 100 µg each of penicillin G (Sigma, P-3414) and streptomycin sulfate (Sigma, S-0890) and were included to control bacterial growth. Antibiotics are known to be somewhat toxic to some larvae (M. Strathmann, 1987), so the antibiotics only treatment acted as a control for possible effects caused by their addition. Dissolved organics added to treatments consisted of minimum essential cell medium (Invitrogen, 41061-029), chosen because it contained several sugars, ribonucleosides, deoxyribonucleosides, fatty acids, and 21 different amino acids. Both the high and low DOM treatments represented higher concentrations of dissolved organics than are normally found in seawater, but were within the range of those tested for DOM uptake in marine invertebrate larvae (Wendt and Johnson, 2006). Three replicate dishes of each treatment were randomly assigned positions in a 3x4 grid (Fig. 5.1). The grid was kept in a flowing seawater table at the Oregon Institute of Marine Biology.

Every two days, the living larvae in each dish were counted and the percent surviving from the original 200 was calculated. Larvae were then placed in a new dish with new treatment materials. A subset of 10 larvae from each of the four treatments was relaxed in 7.5% MgCl₂ and larval length along the longest body axis was estimated using an ocular micrometer on a compound microscope. This continued until Day 12 of the experiment when most of the larvae had died. Larval survival data were analyzed using a repeated measures analysis of variance (ANOVAR) with treatment as a fixed factor (Zar, 2010). Because survival values were measured in percentages, all values were arcsine square root transformed prior to analysis and back-transformed for

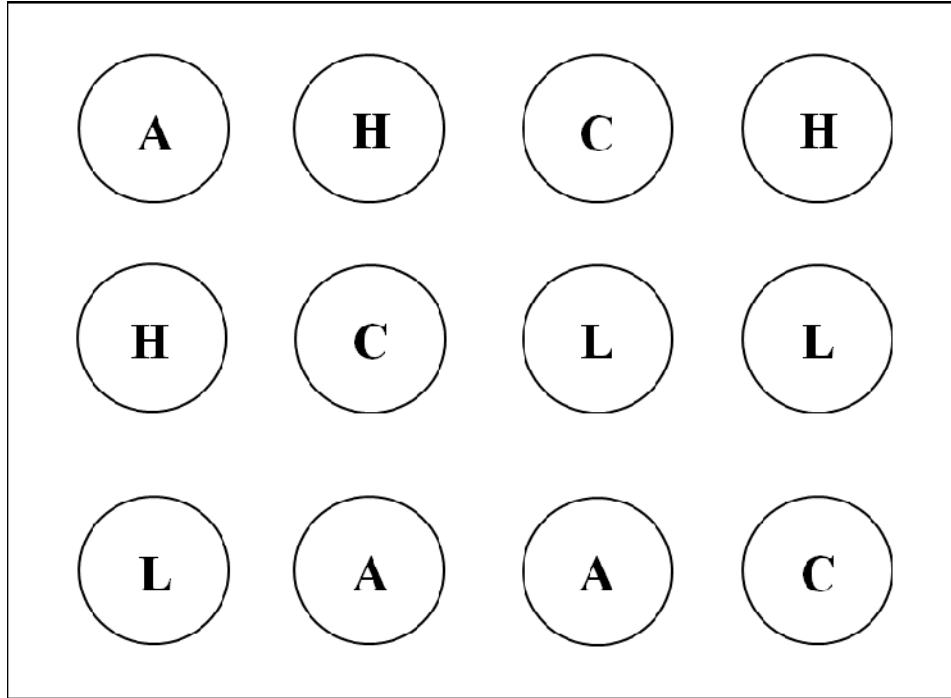


Figure 5.1. Design for larval dissolved organics (DOM) experiment. Dishes containing one of four treatments were randomly assigned positions on the tray. Treatments: H= high DOM, L= low DOM, A= antibiotics only, C= control.

figures (Sokal and Rohlf, 1981). Larval length data were analyzed with a two-way ANOVA with treatment dish and day as fixed factors. Bonferroni post-hoc tests were used to test between treatment groups (Zar, 2010).

Fluorescent labeling was also used to test for larval uptake of dissolved proteins. Larvae were exposed to bovine serum albumin (BSA) with a fluorescein conjugate (Invitrogen, A23015). Concentrations of BSA tested ranged from 20 nM to 1 μ M (W. Jaeckle, Illinois Wesleyan University, pers. comm.). In each test, ten larvae were placed in each of three replicate wells containing one of the BSA treatments or a control of filtered seawater and were allowed to soak for 24 hours. After this time, larvae were examined under an epifluorescence microscope for glowing label in the epidermis, the gut, or both.

Phototaxis experiments: wavelength (λ)

The phototactic response of larvae of *Carcinonemertes errans* to light of varying wavelengths was quantified. A 12.7 cm x 11.43 cm x 10.795 cm Plexiglas container (experimental column) was filled with 0.45 μm filtered seawater and ~2000 dark-acclimated larvae of *C. errans* and sealed. The column was then placed in a 40 cm x 28 cm x 17 cm water bath with glass sides that was filled with water until it was level with the top of the Plexiglas container. Larvae were allowed to acclimate to their surroundings for 15 minutes in the dark and were then exposed to one of five light treatments: white light, red light ($\lambda = 650\text{nm}$), green light ($\lambda = 550\text{nm}$), blue light ($\lambda = 465\text{nm}$), or a darkened control. Each cohort of larvae was exposed to all five treatments in random order, with a 15-minute dark acclimation period between subsequent treatments. Light for this experiment was provided by a Kodak Carousel 4600 slide projector with a 300 W 82 V bulb, chosen because it closely mimicked natural light intensity (Johnson and Forward, 2003). Light of specific wavelengths was projected through a red, green, or blue gel filter mounted in a 35 mm slide. The transmission peak for each of these gels was tested using a spectrophotometer.

As described in Buchanan *et al.* (1982), a mirror was positioned above the water bath so that light from the projector reflected off of the mirror and onto the water bath and experimental column from above, mimicking the angular light distribution in the natural environment. Prior to sampling, the swimming behavior of larvae during lighted trials was examined with a dissecting microscope mounted on a side arm next to the experimental column. Fifteen minutes after each treatment trial began, I simultaneously sampled the water at three vertical levels with syringes attached to the column with

valves. The valves were located 1.27 cm (bottom), 5.08 cm (middle), and 8.89 cm (top) from the bottom of the column, respectively. A 4-ml sample of water was taken from each position in the column and examined under a dissecting microscope. The number of larvae in each sample was recorded. Because the number taken across samples varied, these values were converted to the percent of the total larvae in a given water column sample (top, middle, and bottom combined) for all figures. Larvae were tested the day of hatching (Day 1) and then subsequently on Days 2, 4, 7, 14, and 28 after hatching. Each trial was repeated twice with new cohorts of larvae for a total of 3 replicates for each treatment x age combination.

Using the positions of the three sampling valves, I calculated the average height from the bottom of the column for the total sample of larvae in each treatment replicate. I then compared these average height responses of larvae to the different wavelength treatments using a two-way ANOVA with day and wavelength as fixed factors (Sokal and Rohlf, 1981). Post-hoc Bonferroni tests were used to detect differences between days. Post-hoc Dunnett's t-tests were performed to determine if a given wavelength treatment varied significantly from the darkened control (Zar, 2010).

Phototaxis experiments: light intensity

I tested for the potential effect of varying light intensity on the phototactic behavior of larval *Carcinonemertes errans*. Light intensity levels for each light treatment were measured with a LI-193SA spherical quantum sensor attached to a LI-COR LI-250A light meter (Table 5.1). High-intensity light trials were conducted as described above in the wavelength trials, with white light as well as red, green, and blue wavelength

light as treatments. During the medium- and low-light intensity trials, neutral density filters were placed in front of the projector to reduce the amount of light available to the larvae while retaining the same spectral distribution. All sampling methods were identical to those described above. Because light was transmitted selectively through the colored filters, the light intensity was higher in some wavelength treatments than others. However, the relative amount of light between intensity treatments remained the same across wavelength treatments. Medium-intensity light was always ~60% as bright as high intensity-light, and low-intensity light was always ~25% as bright as medium-intensity light. Intensity trials were performed for Day 7, Day 14, and Day 28 after hatching.

The average height off the bottom of the column for the total sample of larvae in each treatment replicate was calculated. Because the light intensity values were not equal across wavelength treatments, a two-way ANOVA was performed for each wavelength treatment with day and light intensity as fixed factors (Sokal and Rohlf, 1981). Bonferroni tests were used for post-hoc analyses.

Field light measurements

To determine the practical relevance of the light intensity experiments to natural conditions, I measured light intensity at different depths within the Coos Bay Estuary. A LI-193SA spherical quantum sensor was attached to a LI-COR LI-250A light meter and lowered into the water column of the channel near the mouth of the bay. A ~4 kg weight was tied to the end of the rope to prevent drifting. Data were collected every 1.5 meters

to 18 m. Data collection occurred during midday on a sunny day, an overcast day when the water was relatively clear, and an overcast day when the water was turbid.

Table 5.1. *Irradiance values for the treatment levels used in light intensity trials.*

Light Treatment	Intensity Treatment	$\mu\text{mol s}^{-1} \text{m}^{-2}$	Photons $\text{s}^{-1} \text{m}^{-2}$
White Light	High	68.06	4.1E+19
	Medium	41.93	2.52E+19
	Low	9.22	5.55E+18
Red Light ($\lambda = 650 \text{ nm}$)	High	21.72	1.31E+19
	Medium	13.51	8.13E+18
	Low	3.2	1.93E+18
Green Light ($\lambda = 550 \text{ nm}$)	High	2.83	1.7E+18
	Medium	1.77	1.07E+18
	Low	0.48	2.89E+17
Blue Light ($\lambda = 465 \text{ nm}$)	High	3.05	1.84E+18
	Medium	1.9	1.14E+18
	Low	0.5	3.01E+17
Darkened Control	NA	0	0

Values can be expressed in $\mu\text{mol s}^{-1} \text{m}^{-2}$ or photons $\text{s}^{-1} \text{m}^{-2}$. Although intensity values varied between light treatments, the relative values of intensities within treatments (high to medium to low) remained relatively constant.

Phototaxis experiments: effects of pressure

The effect of pressure on phototaxis in *Carcinonemertes errans* was tested. The methods were the same as described above for the wavelength experiments with the following exceptions: 1) half of the trials were conducted while the container was pressurized, and 2) only white light and darkened control treatments were tested. Hydrostatic pressure was added to the sealed experimental column by attaching tubing to one of the valves, attaching a water-filled syringe to the other end of the tubing, and applying constant force to the syringe. The pressure within the container was measured by placing a SCUBA depth gauge inside with the larvae. All pressure experiments were conducted at a pressure mimicking 3 meters depth (1.3 atmospheres). Larvae were tested on Days 1, 2, 4, 7, 14, and 28 after hatching.

The average height off the bottom of larvae during each trial was calculated as described above. The effect of pressure in both the lighted and the darkened experimental column was examined using 2 two-way ANOVAs with day and pressure as fixed factors (Sokal and Rohlf, 1981). Bonferroni tests were used for post-hoc analyses.

Results

Particulate feeding

No larvae were observed with any particulate food material in their guts. Regardless of whether they were offered potential food items or not, larval cultures tended to survive for 3-4 weeks if left in large, stirred containers. Following 3-4 weeks, larvae typically deteriorated within 15 days. No larvae lived longer than 53 days. The results of the fluorescent ball experiments were also negative.

Dissolved organics

The results of the ANOVAR are shown in Table 5.2. There was a significant difference between sampling days in the within-subjects effects ($F = 271.308$, $p < 0.001$) because of steady mortality during the experimental trial. However, no significant difference between any of the treatment levels was detected ($F = 0.327$, $p = 0.806$).

Table 5.2. *Effect of dissolved organics on larval survival of Carcinonemertes errans.* Results of ANOVAR showing within-subjects effects (A) across six days of sampling and between-subjects effects (B). Treatment refers to amount of dissolved organics or antibiotics larvae received.

A. Within-Subjects Effects					
Source of Variation	DF	SS	MS	F	<i>P</i>
day	6	22.965	3.827	271.308	<0.001*
day x treatment	18	0.097	0.005	0.382	0.986
residual	48	0.677	0.014		

B. Between-Subjects Effects					
Source of Variation	DF	SS	MS	F	<i>P</i>
treatment	3	0.044	43.523	0.327	0.806
residual	8	0.360	0.015		

*represents statistically significant values at $\alpha = 0.05$

Larvae in all cultures, irrespective of antibiotic or DOM treatments died at similar rates and most larvae (99.5%) were dead after 12 days (Fig. 5.2A). Larvae in all treatments exhibited similar growth patterns, elongating with age regardless of the presence or absence of DOM (Fig. 5.2B). A two-way ANOVA (Table 5.3) showed that the effect of day on larval length was significant ($F = 44.847$, $p < 0.001$), but that of the DOM treatments was not ($F = 1.001$, $p = 0.401$). Larvae did not exhibit any observable uptake of fluorescently-labeled BSA. Individuals placed under the epifluorescent microscope did not fluoresce after 24 hours of exposure, suggesting no uptake in the epidermis or the gut.

Phototaxis experiments: wavelength (λ)

The effect of wavelength on average larval height in the experimental column was significant ($F = 10.349$, $p < 0.001$). Results for the two-way ANOVA are shown in Table 5.4. Dunnett post-hoc tests ($\alpha = 0.05$) showed that larvae responded to the blue wavelength light treatment by moving significantly closer to the bottom of the column when compared to the darkened control ($p = 0.008$). Average larval height also varied significantly with the age of the larvae ($F = 21.725$, $p < 0.001$; Fig. 5.3). Bonferroni tests ($\alpha = 0.05$) confirmed that the average height of larvae on Day 1 was significantly lower than the average height on all other days tested ($p < 0.001$). There was also a significant difference between the average height of larvae on Day 28 and the average height of all other ages except Day 14.

A general trend of larval movement was apparent, with larvae moving from the bottom position at hatching to the top position by the end of the trial period. As shown in

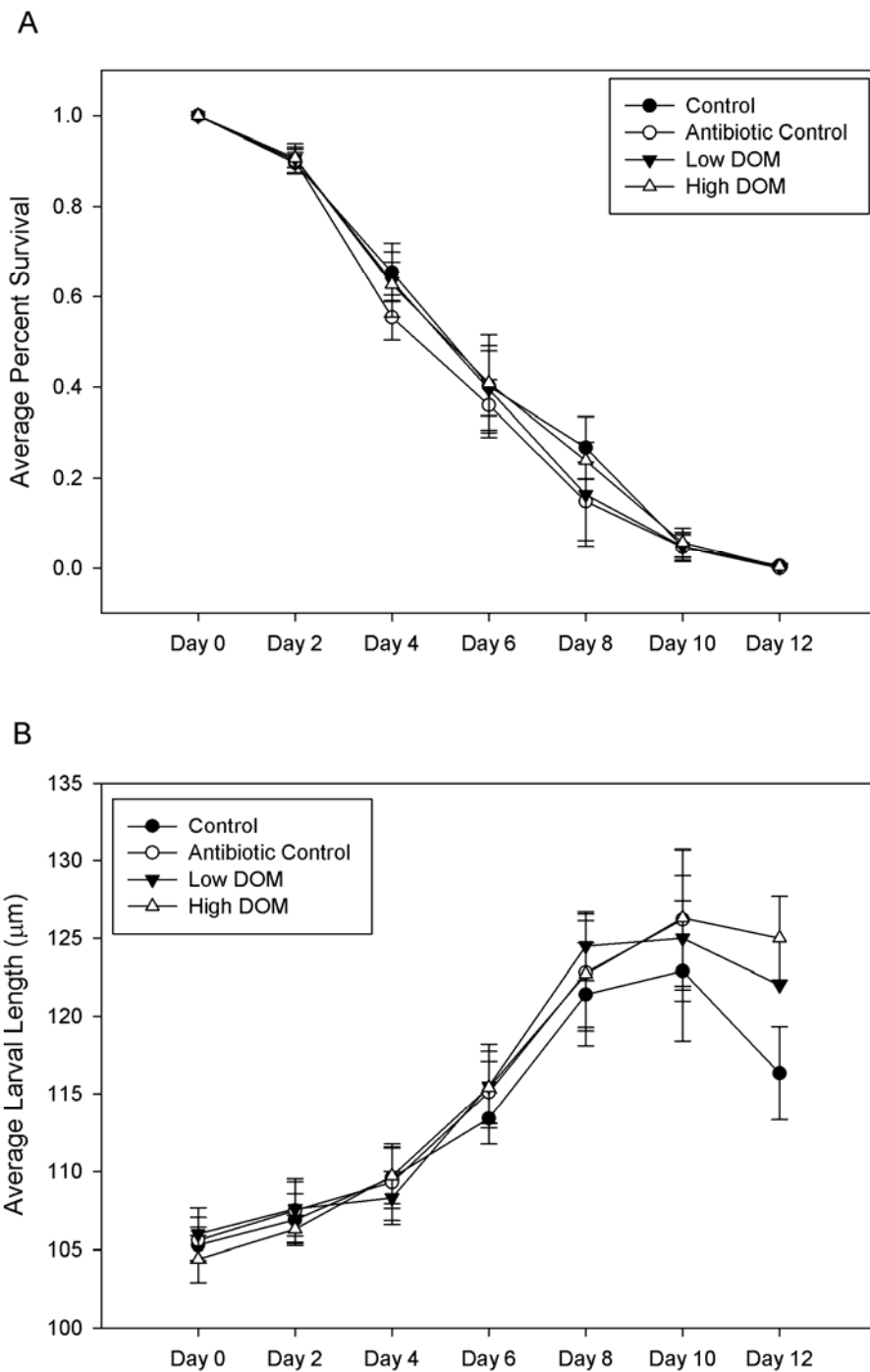


Figure 5.2. Results for dissolved organics (DOM) experiment. (A) The average percent survival of larvae during the trial. (B) The average relaxed length of larvae given DOM and antibiotic treatments. Error bars represent one standard error.

Table 5.3. Results of a two-way ANOVA examining larval length during the dissolved organics feeding experiment with treatment and day as fixed factors.

Source of Variation	DF	SS	MS	F	P
day	6	4,266.261	711.044	44.847	<0.001*
treatment	3	47.593	15.864	1.001	0.401
day x treatment	17	70.240	4.132	0.261	0.998
residual	48	761.037	15.855		

*represents statistically significant values at $\alpha = 0.05$

Table 5.4. Results of a two-way ANOVA examining average larval height in the water column with day and wavelength treatments as fixed factors.

Source of Variation	DF	SS	MS	F	P
wavelength	4	15.909	3.977	10.349	<0.001*
day	5	41.746	8.349	21.725	<0.001*
wavelength x day	20	13.227	0.661	1.721	0.055
residual	60	23.058	0.384		

*represents statistically significant values at $\alpha = 0.05$

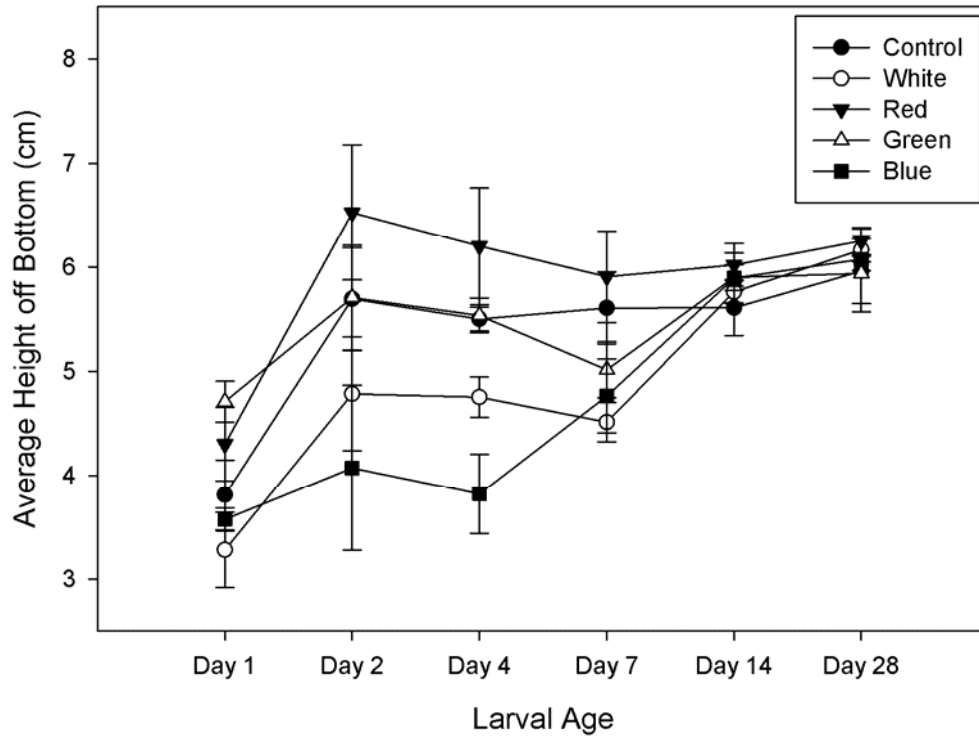


Figure 5.3. Larval Phototaxis in *Carcinonemertes errans* exposed to different wavelengths of light. The average height of larvae sampled in the water column is shown from hatching (Day 1) to 28 days after hatching. Error bars represent one standard error.

Fig. 5.4A, a plurality of larvae was found in the bottom sample on Day 1 for all treatments except green light, which was evenly split between the middle and bottom positions. One day later, the highest percent of larvae were taken from the top position for the control, red light, and green light treatments. For the white and blue light treatments, however, the highest percent of larvae were still found in the bottom samples (Fig. 5.4B). Larvae continued to exhibit similar phototactic behaviors on Day 4 (Fig. 5.5A). More larvae were taken from the top samples than the middle or the bottom samples for the control, red light and green light treatments. The percentage of larvae was again highest in bottom samples in the blue light treatment, but in the white light

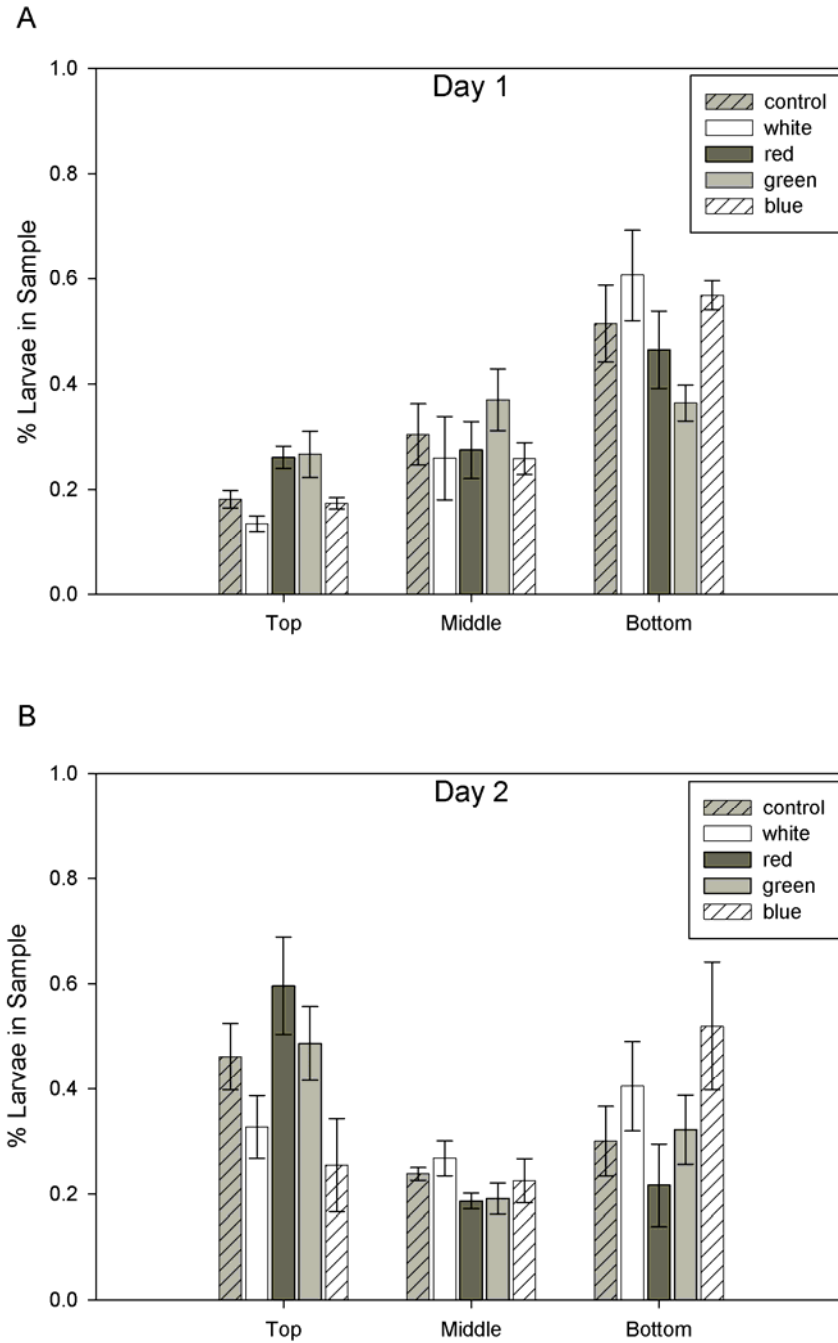


Figure 5.4. Response of larval *Carcinonemertes errans* to different wavelengths of light. (A) 1-day-old larvae. (B) 2-day-old larvae. All larvae were sampled from the three positions in the water column simultaneously. Error bars represent one standard error.

treatment, the percentage of larvae in the middle and the bottom treatments were approximately equal. There were no major changes in phototactic behavior between Day 4 and Day 7 (Fig. 5.5B). A major shift in phototactic behavior occurred between Day 7 and Day 14 (Fig. 5.6A). At least 40% of the larvae sampled were taken from the top for all treatments, including blue and white light. Very little change occurred in the percentages of larvae found in the different positions between Day 14 and Day 28 (Fig. 5.6B).

Phototaxis experiments: light intensity

The results of the light intensity trials are shown in Fig. 5.7 for Day 7, Fig. 5.8 for Day 14, and Fig. 5.9 for Day 28. The effect of light intensity was significant for all wavelength trials except red light (Table 5.5), with lower light intensities resulting in lower average heights in the experimental column. For white light (Table 5.5A), the effect of larval age ($F = 12.668$, $p < 0.001$) and the interaction between age and intensity were also significant ($F = 12.516$, $p < 0.001$). Bonferroni tests showed that all three light intensities produced significantly different average larval heights from each other ($p < 0.001$), and larvae on Day 28 were significantly higher in the water column than those on Day 7 or Day 14.

There was no significant change in average height of larvae in the water column after exposure to the different intensities of red light (Table 5.5B). Both light intensity and larval age significantly affected the average height of larvae when exposed to green light (Table 5.5C). Bonferroni tests showed significant differences between high-intensity green light and the medium- and low-intensity treatments, but no significant

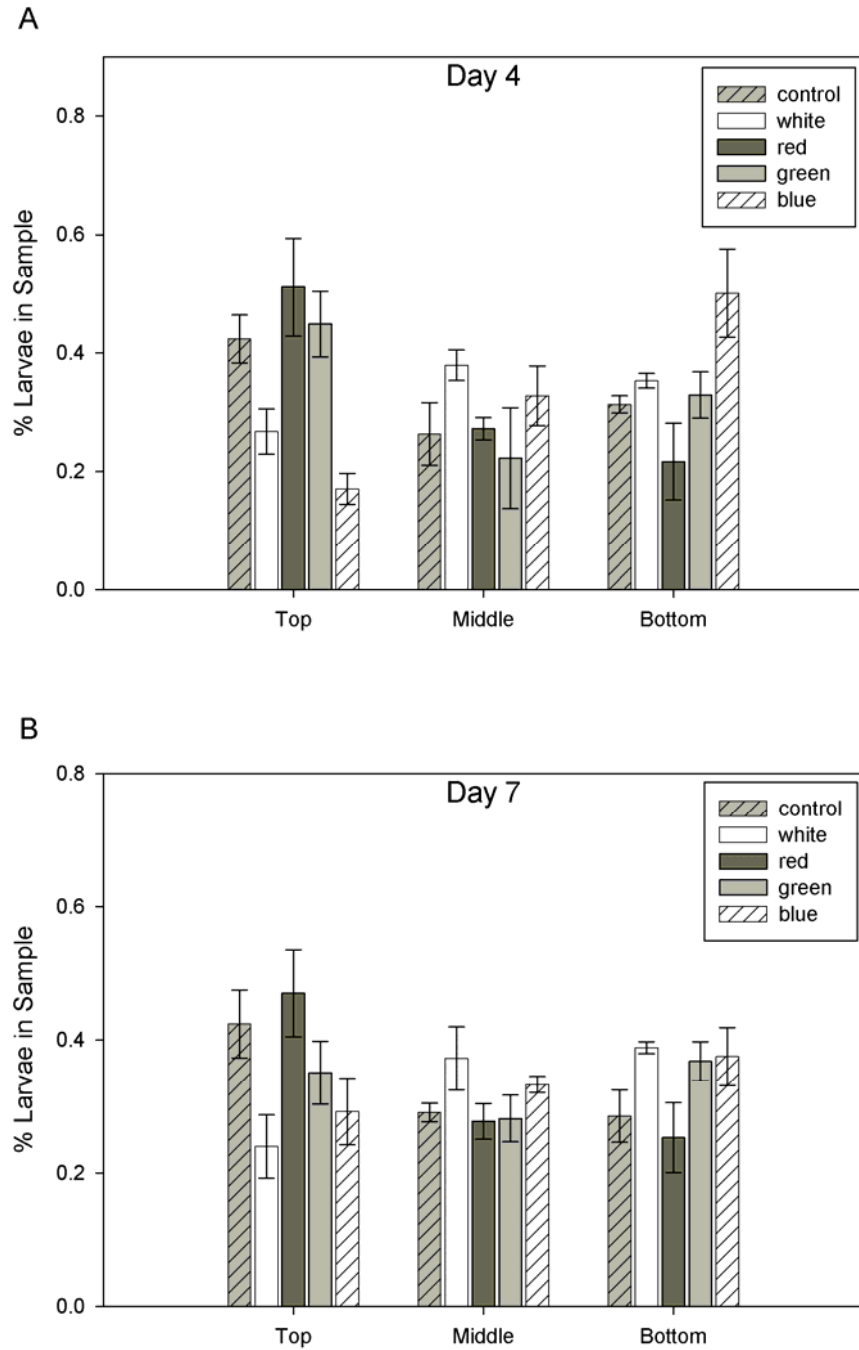


Figure 5.5. Response of larval *Carcinonemertes errans* to different wavelengths of light. (A) 4-day-old larvae. (B) 7-day-old larvae. All larvae were sampled from the three positions in the water column simultaneously. Error bars represent one standard error.

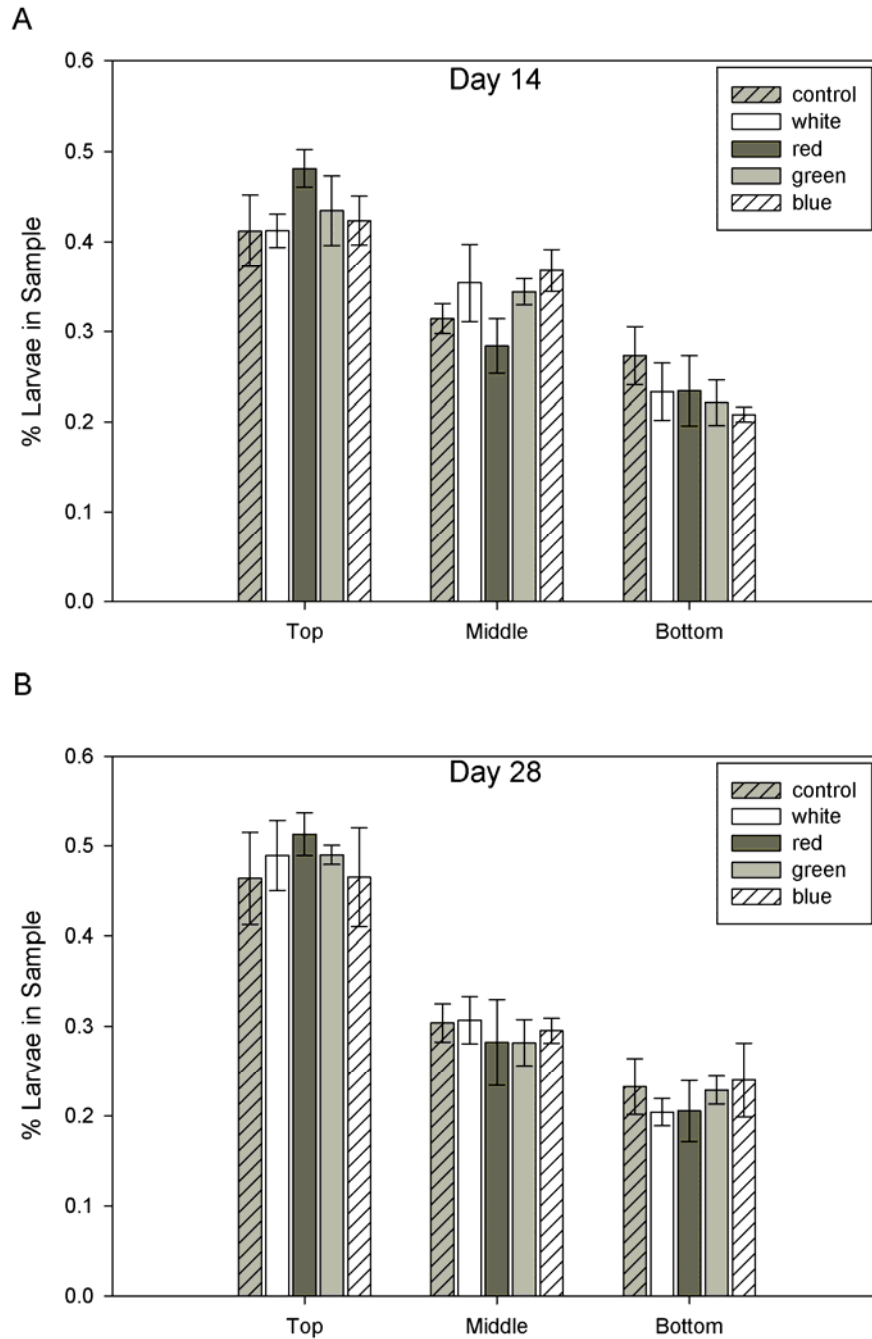


Figure 5.6. Response of larval *Carcinonemertes errans* to different wavelengths of light. (A) 14-day-old larvae. (B) 28-day-old larvae. All larvae were sampled from the three positions in the water column simultaneously. Error bars represent one standard error.

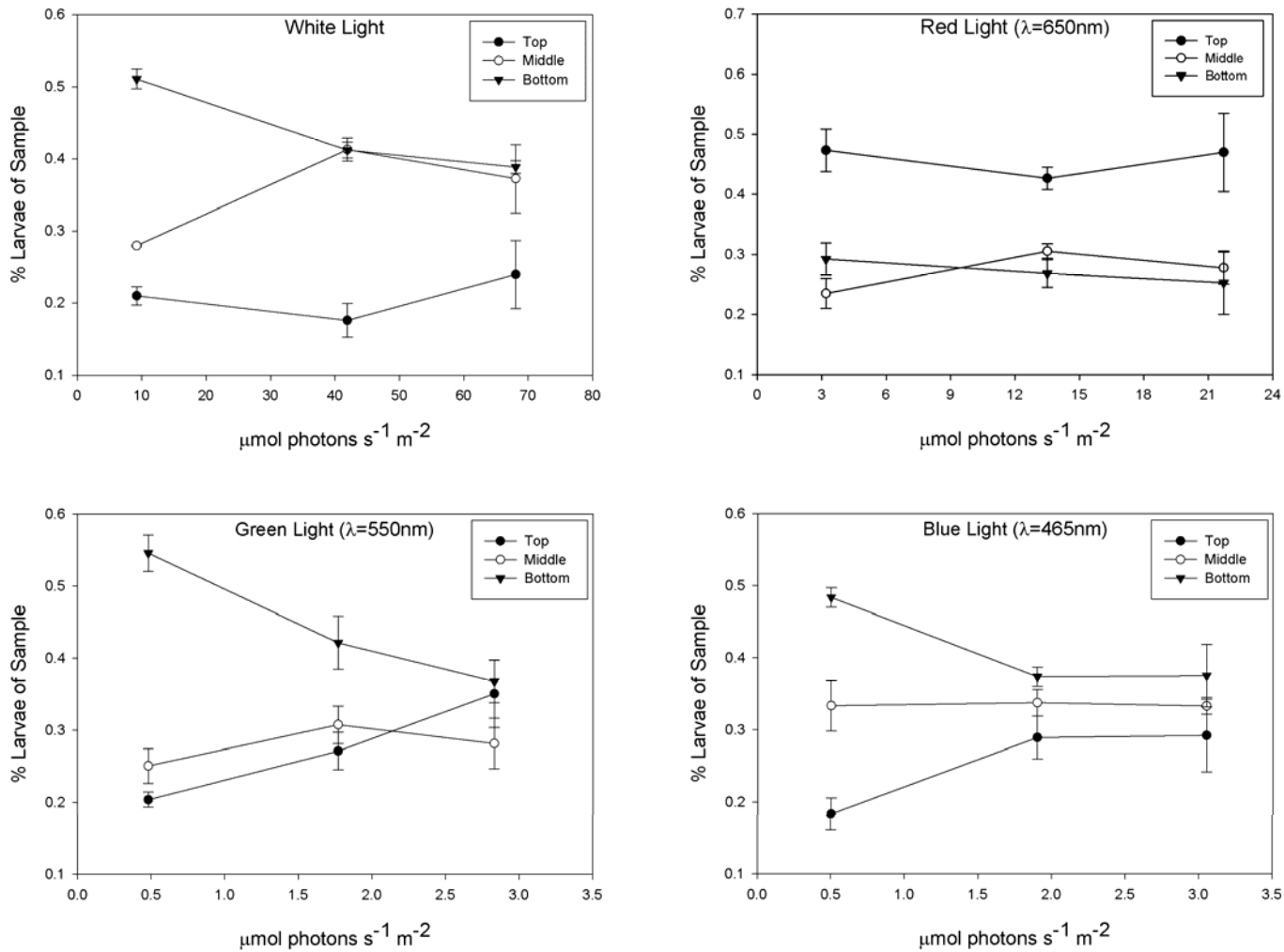


Figure 5.7. Phototaxis in larval *Carcinonemertes errans* during Day 7 trials. Three light intensities were tested with each of the four light wavelength treatments. Note the scale for each of the wavelengths. Error bars represent one standard error.

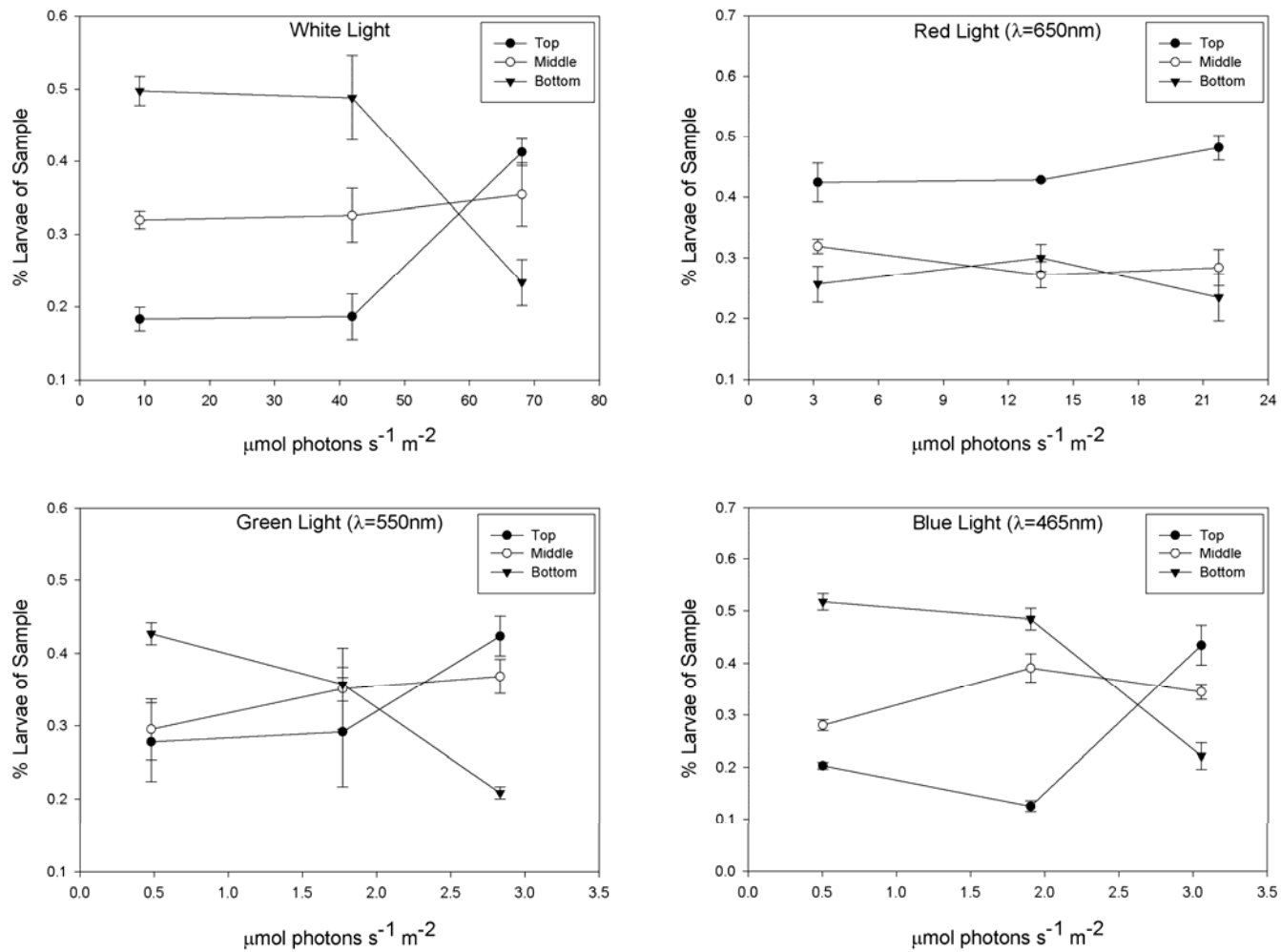


Figure 5.8. Phototaxis in larval *Carcinonemertes errans* during Day 14 trials. Three light intensities were tested with each of the four light wavelength treatments. Note the scale for each of the wavelengths. Error bars represent one standard error.

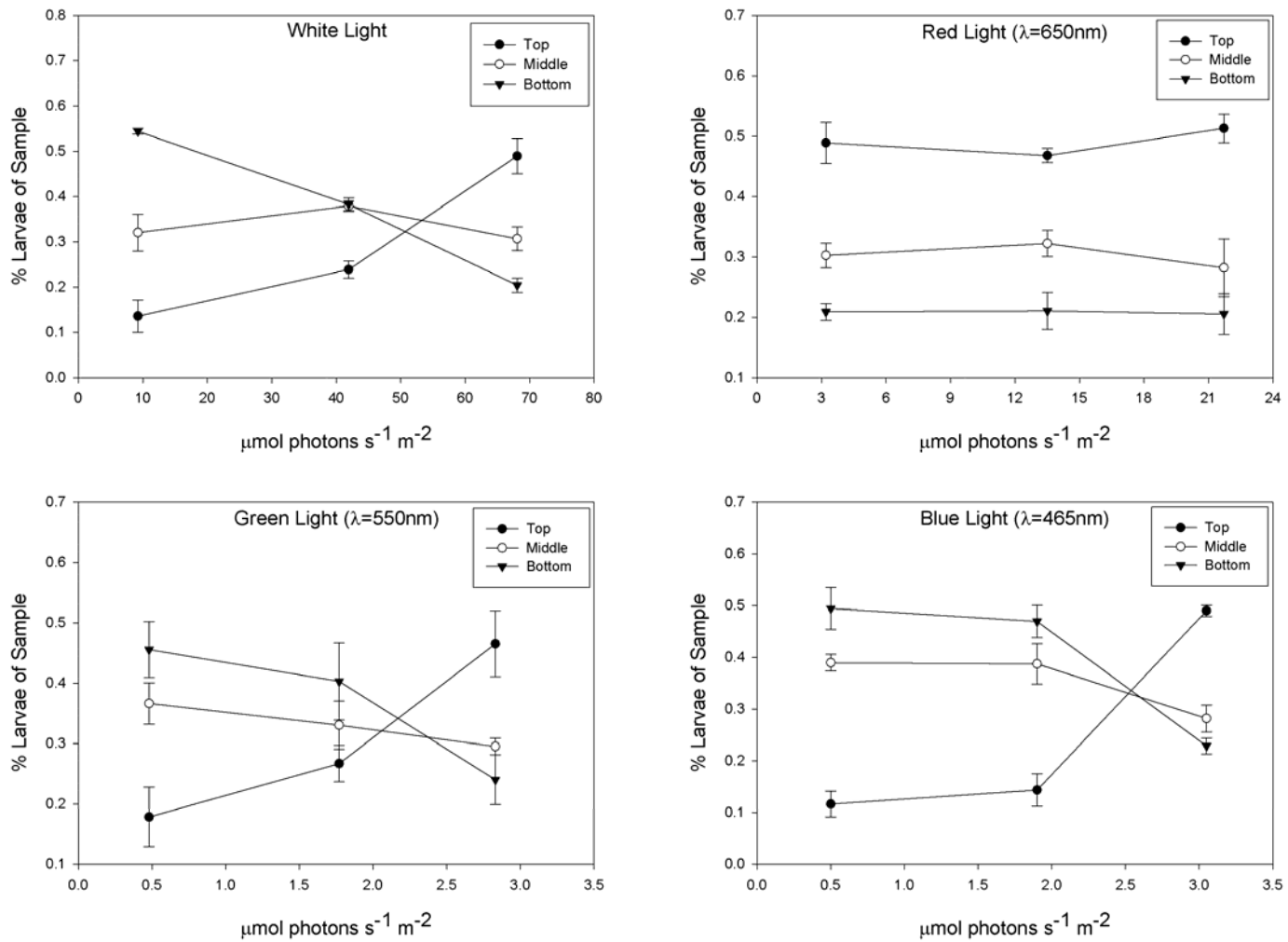


Figure 5.9. Phototaxis in larval *Carcinonemertes errans* during Day 28 trials. Three light intensities were tested with each of the four light wavelength treatments. Note the scale for each of the wavelengths. Error bars represent one standard error.

Table 5.5. Results of 4 two-way ANOVAs examining the difference between the average heights of larvae in the water column when exposed to varying intensities of (A) white light, (B) red light, (C) green light, and (D) blue light on Days 7, 14, and 28 after hatching

A. Light Intensity: White Light					
Source of Variation	DF	SS	MS	F	P
intensity	2	7.205	3.603	40.433	<0.001*
day	2	2.257	1.129	12.668	<0.001*
intensity x day	4	4.461	1.115	12.516	<0.001*
residual	18	1.604	0.0891		
B. Light Intensity: Red Light ($\lambda=650\text{nm}$)					
Source of Variation	DF	SS	MS	F	P
intensity	2	0.386	0.193	1.301	0.297
day	2	0.850	0.425	2.868	0.083
intensity x day	4	0.0672	0.0168	0.113	0.976
residual	18	2.666	0.148		
C. Light Intensity: Green Light ($\lambda=550\text{nm}$)					
Source of Variation	DF	SS	MS	F	P
intensity	2	10.617	5.308	22.068	<0.001*
day	2	1.928	0.964	4.008	0.036*
intensity x day	4	0.724	0.181	0.753	0.569
residual	18	4.330	0.241		
D. Light Intensity: Blue Light ($\lambda=465\text{nm}$)					
Source of Variation	DF	SS	MS	F	P
intensity	2	16.033	8.017	75.385	<0.001*
day	2	0.00474	0.00237	0.0223	0.978
intensity x day	4	5.134	1.284	12.070	<0.001*
residual	18	1.914	0.106		

*represents statistically significant values at $\alpha = 0.05$

difference between medium-intensity and low-intensity green light. Bonferroni tests also found a significant difference between the average height of larvae on Day 7 and Day 14. When exposed to lower intensities of blue light, larvae showed significantly lower average heights in the water column (Table 5.5D). There was also a significant interaction between larval age and light intensity. This was because the average height of larvae exposed to high-intensity blue light from Day 7 (4.764 cm) to Day 28 (6.075 cm) increased while the average height of larvae exposed to low-intensity blue light decreased across this same period (from 3.936 cm on Day 7 to 3.641 on Day 28). Bonferroni tests found significant differences between the high light intensity treatment and the two lower intensity treatments.

Field light measurements

Light intensity readings measured in the field are shown in Table 5.6. In general, intensity varied with day, atmospheric conditions, and the turbidity of the water. The amount of light attenuation with increasing depth remained relatively constant however, around a 30-40% decrease in light intensity per 1.5 meters increase in depth. Measured values were comparable to those tested in the laboratory experiments.

Phototaxis experiments: effects of pressure

The average height of larvae in the water column did not change significantly when the container was pressurized to the equivalent of 3 meters depth (Table 5.7). This was true for larvae exposed to white light ($F = 0.003$, $p = 0.954$) and those kept in dark conditions ($F = 1.110$, $p = 0.303$). A significant effect of larval age was observed,

reflecting the age-related patterns described above in the wavelength section. The results of the pressure experiment on Day 7 shown in Fig. 5.10 were typical.

Table 5.6. *Field light measurements from the mouth of the Coos Bay Estuary. All light measurements are given in $\mu\text{mol s}^{-1} \text{m}^{-2}$*

Depth (m)	Sunny	Overcast, Low Turbidity	Overcast, High Turbidity
0	944.2	223	154.7
1.5	734.1	158.8	109.8
3	462.4	110.4	76.6
4.5	310.7	78.9	52.5
6	203	53.4	36.6
7.5	139.8	38.8	27.8
9	86.1	24.1	14.9
10.5	57.3	15.2	10.7
12	34.9	10.7	6.8
13.5	25.2	6.1	4.2
15	16.5	4.2	3
16.5	9.3	2.2	1.9
18	5.7	1.3	1.1

Table 5.7. Results of 2 two-way ANOVAs examining the difference between the average heights of larvae in the water column when exposed to hydrostatic pressure under (A) white light and (B) no light on Days 1, 2, 4, 7, 14, and 28 after hatching.

A. White Light					
Source of Variation	DF	SS	MS	F	P
pressure	1	0.00105	0.00105	0.00339	0.954
day	5	33.723	6.745	21.749	<0.001*
pressure x day	5	0.537	0.107	0.346	0.880
residual	24	7.443	0.310		
B. Darkened					
Source of Variation	DF	SS	MS	F	P
pressure	1	0.365	0.365	1.110	0.303
day	5	21.045	4.209	12.795	<0.001*
pressure x day	5	0.204	0.0408	0.124	0.986
residual	24	7.895	0.329		

* represents statistically significant value at $\alpha = 0.05$

Discussion

Larval feeding biology

Hoploneurtean planuliform larvae typically originate from large, yolky eggs and live a short lecithotrophic planktonic existence before settling without a noticeable metamorphosis event (Norenburg and Stricker, 2002). As hoploneurteans, members of the genus *Carcinonemertes* would be expected to produce lecithotrophic, short-lived larvae as well (Kuris, 1993). However, like their small size and modified proboscis structure, the reproductive biology of these worms appears to be modified to better meet their parasitic existence (Wickham, 1980; Roe, 1988). The oocytes of *Carcinonemertes*

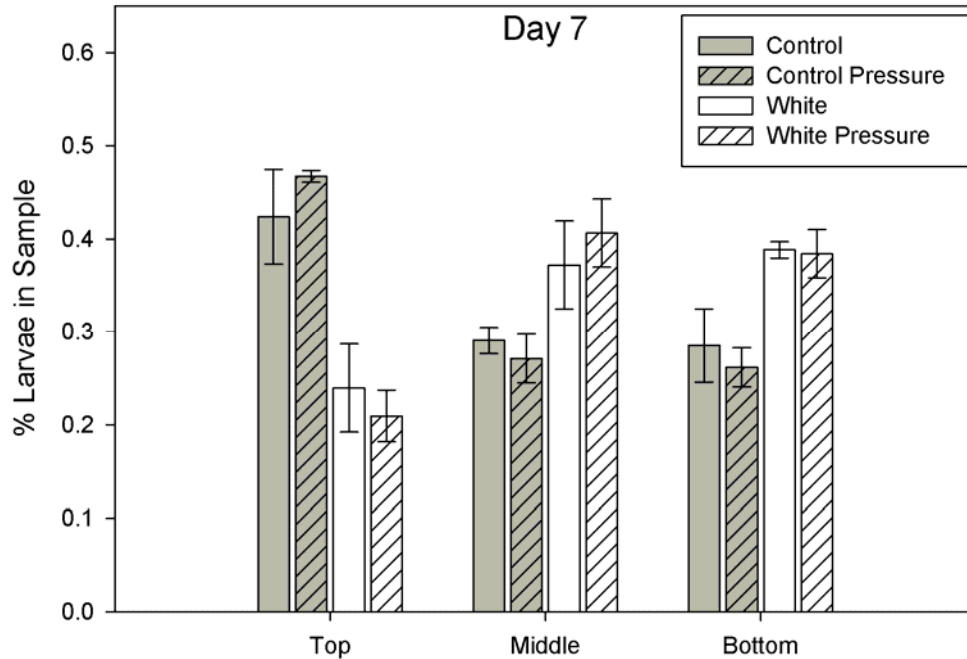


Figure 5.10. Effect of pressure on larval phototaxis of *Carcinonemertes errans* on Day 7. The pressurized treatment was the equivalent of 3 meters depth. Error bars represent one standard error.

are smaller than those of other hoplonemertean (70 μm in *Carcinonemertes errans* compared to 300 μm for *Paranemertes peregrina* and 350 μm in *Amphiporus formadabilis*), and females produce many more eggs than the typical hoplonemertean (Stricker, 1987). The larval morphology of *C. epialti* was described by Stricker and Reed (1981). Although these larvae are small and contain limited yolk, some authors have argued that they are lecithotrophic (Bauman, 1983).

The larvae of *Carcinonemertes errans* used in my experiments were nearly identical to those of *C. epialti*. Yolk reserves were present upon hatching, but within a few weeks, this resource was exhausted. At this stage, larvae were somewhat elongated and appeared mostly transparent (Chapter IV). Once the yolk was gone, growth and

development became arrested. Larvae continued to survive for 2-3 weeks past this point in laboratory cultures, but all of these larvae eventually died, presumably of starvation. Larvae were not observed to consume any of the offered food items and “fed” larvae died at the same rate as larvae in filtered seawater. The larvae of *C. errans* did not appear to have any obvious mechanism for capturing food. Most planktotrophic larvae have bands of cilia or appendages for concentrating food and directing it to the mouth (Hart and Strathmann, 1995), but no such bands or appendages were present in the uniformly ciliated larvae of *C. errans*. Some anthozoan planulae, which have a similar morphology to *C. errans*, capture food by trailing a thread of mucus behind them, allowing particles to stick to the thread, and then pulling it into their posteriorly-located mouth. Other planulae temporarily attach themselves mouth down to a substratum, evert their stomodeum, and take food directly off the surface (R. Strathmann, 1987). Although I observed larvae of *C. errans* both trailing mucus threads and attaching themselves to the bottom of culture dishes with these threads, it seems unlikely that this is a normal feeding behavior for the following reasons: 1) these behaviors do not occur often; seen in ~20 larvae out of thousands observed, 2) the mouth of *C. errans* was in the anterior position rather than the posterior and larvae always swim anterior end forward, and 3) larvae that did attach themselves to the bottom or sides of the culture dish with mucus threads and were not liberated died within one to two days. I therefore concluded that thread trailing in *C. errans* was indicative of a moribund condition rather than food collection.

The planuliform larvae of some palaeonemerteans have been observed feeding on a large range of particles. Norenburg and Stricker, (2002) described the larval feeding of *Carinoma tremaphoros*, which concentrates microplankton by flattening and extending

the body margin on each side of the mouth to form “pseudo lappets.” Palaeonemertean larvae are also capable of capturing much larger prey; Jägersten (1972) observed one such larva feeding on a pilidium of its own size. If the early larval stages of *Carcinonemertes errans* are capable of either of these types of feeding behaviors, they did not exhibit this capacity during feeding trials.

Larvae did not benefit from exposure to elevated dissolved organics in my experiments, nor did they show any evidence of DOM uptake in the labeling experiment. Given the current hypothesis that juvenile worms of many *Carcinonemertes* species subsist on organic matter leaking from their hosts (Crowe *et al.*, 1982), this result was especially surprising. Larvae used in the DOM survival experiment did not survive in any of the treatments for longer than 12 days, which falls within the time frame of measurable DOM uptake results in the literature (Wendt and Johnson, 2006).

Larval phototaxis

During the course of my experiments under a simulated natural light field, larvae never demonstrated positive phototaxis. If, however, I repeated Bauman’s (1983) experiments with larvae in a column and narrow beam light, I observed the photopositive behavior he reported. I therefore conclude that the photopositive response currently ascribed to the larvae of *Carcinonemertes errans* is an artifact of the narrow beam light stimulus and should not be considered when discussing the larval ecology of *C. errans* in its natural environment. Rather than being photopositive, the dominant pattern exhibited by larvae was one of positive geotaxis directly after hatching transitioning to a negative geotactic pattern as demonstrated by the darkened controls. Distinguishing between

active geotactic behavior and passive floating and sinking can be difficult (Young and Chia, 1987). Although I could not observe the behavior of larvae during darkened control trials, observations made during red light trials, which never produced significantly different results from darkened controls, showed that larvae continued to swim actively rather than to passively sink, drift, or float.

Unless the intensity of the light treatment was altered, the only detectable larval response to light was negative phototaxis, and even this was limited in duration (Days 2-7). Only the blue wavelength (465 nm) induced a significant photonegative response. The lack of response to red light, the limited response to green and white lights, and the short-term response to blue light corroborate with what is known about larval spectral sensitivity. A majority of larvae across several taxa exhibit sensitivity maxima in the blue to blue-green light range (wavelengths that attenuate least in the ocean), but are usually not sensitive to red light (Young and Chia, 1987; Forward, 1988).

The response of larvae of *Carcinonemertes errans* to light of varying intensities was consistent with the hypothesis that the larvae are capable of detecting changes in intensity and responding appropriately. Decreasing the intensity of white light by 40% in Day 14 and Day 28 caused negative phototaxis in the larvae of *C. errans* that overrode the negative geotactic response and brought more of them from the top of the container to the middle or the bottom. A further 35% decrease in intensity invoked a similar response on Days 7, 14, and 28, with larvae swimming down from the top and middle positions to the bottom (Figs. 5.7, 5.8, 5.9). Similar responses were seen for green and blue wavelengths, suggesting that these are likely the wavelengths driving the response in the observed pattern for white light. No significant responses were seen with changes to the

intensity of red light, further supporting the hypothesis that larvae of *C. errans* do not respond to this wavelength.

The effect of pressure was not significant at the simulated depth of 3 meters. Larvae of *Carcinonemertes errans* are known to occur at depths greater than 3 meters in the estuary (Chapter IV, this dissertation). It is possible that higher pressures play a role in determining the vertical distribution of larvae, whether by providing a direct cue at a certain depth (barokinesis) or interacting with another cue to produce a response of varying intensity (Young and Chia, 1987). For example, most decapods become more sensitive to light cues as pressure increases (Rice, 1964). This could help larvae maintain their vertical position within the water column (Crisp, 1974). However, at depths that larvae are likely to encounter in Oregon estuaries, the larvae of *C. errans* showed no barokinetic response.

The larvae of *Carcinonemertes errans* occur in the temperate coastal ocean and estuaries where they must develop for several months before seeking a new host. The positively geotactic response exhibited by larvae of *C. errans* immediately after hatching would likely retain larvae near the bottom. Since larvae are not competent to settle on new crab hosts during this time (Chapter IV) and they transition to negative geotaxis one day later, this response is puzzling. Perhaps the response is linked to some aspect of larval development. Larvae of *C. errans* do not have any statocyst-like structures, so the mechanism whereby they detect gravity is unknown (Gibson, 1972). It is possible that this unknown mechanism is still developing at the time of hatching and is not functional until 2 days after hatching. Another hypothesis is that newly hatched larvae are weak swimmers. Tests with individuals relaxed in MgCl₂ showed that the larvae of *C. errans*

are negatively buoyant and will sink if they stop swimming. The weak swimmer hypothesis does not seem likely, however, because observations made with an arm-mounted microscope during the experiments failed to reveal any difference between the vertical swimming speeds of larvae on Day 1 compared to those on other days. A third possibility is that positive geotaxis is an ancestral artifact preserved in *C. errans*; the larvae of some species of *Carcinonemertes* are thought to have a very brief larval stage and might even practice autoinfection on their hosts (Kuris, 1993).

After the switch to geonegative behavior on Day 2, larvae could begin to make their way toward the surface (at least at night) where they would experience greater dispersal potential and come in contact with potential food sources. During the day, larvae in the ocean would potentially move into deeper water as they detect and move away from the available blue light. Within estuaries, this pattern may be less distinct due to higher attenuation of blue light in these environments, particularly during times of high freshwater runoff (Forward, 1988). Such a pattern aligns with the well-studied phenomenon of diel vertical migration, common among many mero- and holoplanktonic organisms. The most common pattern, nocturnal migration, brings zooplankters to the surface at night to feed and to depth during the day to avoid predation (Temple and Fischer, 1965; Pennington and Emlet, 1986; Shanks, 1986; Hobbs and Botsford, 1992). Beginning on Day 7, however, the photonegative response to blue light begins to disappear, and on Days 14 and 28, larvae are more likely to be at the surface than at depth at all times of day.

The effect of varying light intensity on larval behavior predicts another pattern, however. Under the highest intensity tested on Day 7, there was a slight pattern of

negative phototaxis already occurring for white, green, and blue treatments. Decreasing the intensity strengthened this existing pattern, suggesting that a decrease in light intensity might promote a photokinetic response (Young and Chia, 1987) and cause larvae that were already heading away from the light to do so more strongly (e.g. beat cilia at a faster rate). On Days 14 and 28, the decrease in light intensity to medium and low levels actually reversed the behavior seen at the higher light intensity and larvae moved down to the middle and lower positions. A photokinetic response may still be active in this case, but there is almost certainly a phototactic response occurring as well.

Lower light intensities are experienced in surface waters during certain times of day (e.g. dawn or dusk) and at greater depths (Forward, 1988). In the first case, the observed response would serve to shift larvae away from the surface water during the first hours of sunlight (negative geotactic behavior would concentrate them there during the night). Once at depth, the low intensity light would continue to provide a positive feedback loop, keeping larvae deep. If larvae reached a depth where light was no longer detectable, however, their geonegative behavior would bring them back toward the surface until light was again available, creating a negative feedback loop.

The light intensities tested do appear to be within the range a larva could experience within an estuary and coastal waters. From measurements taken in the Coos Bay Estuary on a sunny day at midday, the highest intensity of white light tested in the laboratory corresponded to a depth of 9.5 meters, the medium intensity to 11 meters, and the lowest intensity to 16.5 meters (Table 5.6). On an overcast day, the values tested as high, medium, and low intensities in the laboratory occurred at depths around 5, 7, and

12.5 meters, respectively, in the estuary. When overcast conditions were coupled with turbid estuarine waters, corresponding depths were around 3.5, 5, and 11 meters.

In conclusion, there is still much to learn about the larval ecology of *Carcinonemertes errans*. Larvae began their lives with a small yolk reserve, but this was exhausted within only a few weeks, suggesting that another nutritional source was needed for larvae to complete development. The nature of this food source remains unknown, as does the mechanism by which it is collected. Despite the negative results of my experiments, dissolved organic matter remains an intriguing possibility, especially considering the current hypothesis that juvenile worms subsist on DOM leaking from crab hosts.

Larvae of *Carcinonemertes errans* were not positively phototactic when placed in a column simulating natural light conditions. They were, however, negatively geotactic beginning two days after hatching and had a spectral sensitivity maximum in the blue to blue-green wavelength range. This spectral sensitivity could result in larvae conducting diel vertical migrations during the early morning and at dusk, when light intensities are low enough to activate the intensity dependent photonegative response. It is important to note that all experiments were conducted with larvae that were not yet competent to settle on crab hosts. Competent larvae retain their original set of eyes, but have also developed a second pair of eyes, which they then retain as juveniles (see Chapter IV). Since juvenile worms are negatively phototactic, development of this second set of eyes suggests that competent larvae might be negatively phototactic as well. Examining the phototactic and geotactic responses of competent larvae would be a highly relevant addition to the work reported here.

CHAPTER VI

GENERAL CONCLUSION

Host-parasite interactions provide excellent opportunities to study evolutionary and ecological interactions between species and their environment (Price 1980). In this dissertation project, I examined several aspects of the interaction between the nemertean egg predator *Carcinonemertes errans* and its host, *Cancer magister*.

Although *Carcinonemertes errans* is known to occur on coastal Dungeness crabs from Alaska to central California (Wickham 1980), the distribution of the worm in estuarine populations of *Cancer magister* has not been well studied (McCabe et al. 1987). I examined the infection status of *C. magister* within the Coos Bay Estuary, Oregon, for three years, trapping adult and juvenile crabs from the mouth of the bay to the edge of the adult range in the upper estuary. Crabs nearest the ocean carried the heaviest parasite loads, both when measured in terms of parasite prevalence and mean intensity. Larger crabs were more likely to be infected and also carried greater numbers of nemerteans. One site within the bay showed a significant difference in parasite prevalence between the wet season and the dry season, and another site showed a significant seasonal effect in mean intensity. In both cases, infection rates were higher in the wet season than the dry, suggesting that salinity changes within the estuary were not the sole cause of the estuarine gradient in infection with *C. errans*. The likely source of seasonal variation is a combination of crab migration, both ontogenetic and otherwise, as well as an increased number of competent *Carcinonemertes* larvae present in the plankton during the beginning of the wet season. Throughout the study, crabs taken from coastal waters

carried significantly more worms than crabs from the bay, suggesting that the estuary may be acting as a parasite refuge for estuarine crabs.

Some hosts experience salinity refuges from their parasites within estuaries (e.g. Haskin and Ford 1982; Barber et al. 1997; Tolley et al. 2006). To examine whether the estuarine refuge of *Cancer magister* might be linked with the physiological tolerances of *Carcinonemertes errans*, juvenile and larval worms were subjected to temperature and salinity tolerance experiments in the laboratory. Salinities tested ranged from 5 to 30 and temperatures ranged from 8 °C to 20 °C. Both larvae and juveniles showed low tolerance to salinities 10 and lower, although juveniles were slightly more robust to these insults. Salinity 20 was near the limit of physiological tolerance for *C. errans*, especially in the larval stage. Temperature did not play a significant role in juvenile survival and only factored significantly into larval survival after prolonged exposure to the treatment. Since *C. magister* can survive in salinities from 11 to 35 but prefers salinities above 20 (Cleaver 1949; Engelhardt and Dehnel 1973; Hunter and Rudy 1975; Curtis and McGaw 2010), these results suggest that salinity may play a role in creating the estuarine gradient of *C. errans* in Coos Bay, but probably does not act alone.

Settlement is an important event for all planktonic larvae of marine invertebrates, but this may be especially true for parasitic larvae that must find an appropriate host or perish (Pawlik 1992). I examined the process and ecology of larval settlement in *Carcinonemertes errans* using both laboratory and field experiments. Larvae of *C. errans* raised from hatching were never induced to settle despite being offered the appropriate settlement substratum (live *Cancer magister*). This was a function of their not having reached competency. Larvae taken in plankton tows were morphologically

distinct from larvae raised in laboratory cultures and did successfully settle on *C. magister* under laboratory conditions. These competent larvae possessed two pairs of simple eyes and the pink-orange coloration observed in juvenile worms. Initial settlement was reversible within a 24-hour window. After 48 hours, however, a non-reversible metamorphosis occurred wherein newly-settled worms lost one pair of eyes as well as the propensity to swim when removed from their host crab. This represents the first description of larval settlement of *Carcinonemertes* in the literature.

In field settlement experiments where live crabs of known infection status were placed in cages in the Coos Bay Estuary, larvae of *Carcinonemertes errans* were shown to be capable of infecting hosts directly from the water column and exhibited a preference to settle on crabs already infected with juvenile conspecifics. However, this gregarious settlement behavior appeared to be density-dependent, with lightly infected hosts more likely to gain more worms than heavily infected hosts. Settlement also occurred near all of my trapping sites, suggesting that parasite settlement as well as host migration contributed to infection rates throughout the bay. In monthly plankton tows, larvae of *C. errans* were found only between August and November. Since most larvae of *C. errans* hatch out between January and March along the Oregon coast, this suggests that Wickham's prediction (1980) that larvae of *C. errans* may remain in the plankton for 8-9 months may be correct. Larvae were mostly collected near lower bay sites, confirming that the coastal ocean is the likely source of competent larvae.

Larvae of *Carcinonemertes errans* in laboratory cultures survived around six weeks and then died, suggesting possible starvation following the exhaustion of yolk

resources. Despite being offered a variety of food choices, larvae did not feed under laboratory conditions, and trials using dissolved organics were negative.

To better understand their ecology and distribution potential, larvae of *Carcinonemertes errans* were exposed to laboratory tests designed to mimic the natural angular light distribution of a water column. In these conditions, larvae of *C. errans* were rarely photopositive, directly contradicting the findings of Bauman (1983), who conducted his experiments with narrow beam light. When larvae did respond to light, they were most sensitive to blue-green wavelengths, which is common among many taxa (Forward 1988). Low intensity light invoked photonegative responses. Larvae were geopositive at hatching but geonegative from the second day until Day 28 after hatching. This combination of geonegative behavior in the dark and photonegative behavior under low light conditions could result in daily vertical migrations.

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