Egg Capsule Hatching Success in Rapana venosa and Urosalpinx cinerea in Relation to Temperature and Salinity

Stephanie M. Gera
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Egg Capsule Hatching Success in *Rapana venosa* and *Urosalpinx cinerea* in Relation to Temperature and Salinity

A Thesis

Presented to

The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of

Master of Science

by

Stefanie M. Gera
APPROVAL SHEET

This thesis is submitted in partial fulfillment of
the requirements for the degree of

Master of Science

Stefanie M. Gera

Approved, by the Committee, December 2009

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# TABLE OF CONTENTS

ACKNOWLEDGEMENTS................................................................................................................v

LIST OF TABLES...............................................................................................................................vi

LIST OF FIGURES............................................................................................................................vii

ABSTRACT........................................................................................................................................ix

INTRODUCTION ................................................................................................................................2

  Overview of Gastropod Egg Capsule Development.................................................................2
  Niche Structure ............................................................................................................................4
  Atlantic Oyster Drill, *Urosalpinx cinerea*: Habitat, Life History, and Prey Field..............10
  Temperature, Salinity and Circulation in the Chesapeake Bay...........................................13
  Extant Ranges of *Rapana venosa* and *Urosalpinx cinerea* Within the Chesapeake Bay ....15
  Context........................................................................................................................................17
  Objectives.................................................................................................................................17
  Hypotheses...............................................................................................................................18

MATERIALS AND METHODS .......................................................................................................19

  Environmental Conditions and Collections.................................................................19
  Experimental Design........................................................................................................24
  Experimental Procedures.................................................................................................26
  Data Analyses.....................................................................................................................33

RESULTS........................................................................................................................................43

  Environmental Variation .................................................................................................43
  Exclusion of Data..............................................................................................................49
  Egg Capsule Height........................................................................................................50
# TABLE OF CONTENTS (CONTINUED)

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chance of Egg Capsule Hatching</td>
<td>52</td>
</tr>
<tr>
<td>Percentage of Egg Capsules to Hatch</td>
<td>52</td>
</tr>
<tr>
<td>Incubation Time</td>
<td>67</td>
</tr>
<tr>
<td>Percentage of Embryos Alive at Hatch From the Egg Capsule</td>
<td>67</td>
</tr>
<tr>
<td>Egg Capsule Dry Weight</td>
<td>72</td>
</tr>
<tr>
<td>Regional Context</td>
<td>75</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>87</td>
</tr>
<tr>
<td>Salinity</td>
<td>88</td>
</tr>
<tr>
<td>Temperature</td>
<td>90</td>
</tr>
<tr>
<td>Potential Overlap of <em>R. venosa</em> and <em>U. cinerea</em> in the Chesapeake Bay</td>
<td>94</td>
</tr>
<tr>
<td>APPENDIX</td>
<td>101</td>
</tr>
<tr>
<td>Selected Definitions</td>
<td>102</td>
</tr>
<tr>
<td>Mean Oyster Pier Temperature and Salinity from 2003 to 2008</td>
<td>103</td>
</tr>
<tr>
<td>Chesapeake Bay Map</td>
<td>104</td>
</tr>
<tr>
<td><em>U. cinerea</em> Broodstock Shell Length</td>
<td>105</td>
</tr>
<tr>
<td>Internal Temperature of Controlled Experimental Chamber</td>
<td>106</td>
</tr>
<tr>
<td>Distribution of <em>R. venosa</em> and <em>U. cinerea</em> Egg Capsule Height at Collection</td>
<td>107</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>108</td>
</tr>
</tbody>
</table>
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# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: Egg capsule collection dates for <em>R. venosa</em> and <em>U. cinerea</em></td>
<td>23</td>
</tr>
<tr>
<td>2: Description of normal and abnormal egg capsule color</td>
<td>27</td>
</tr>
<tr>
<td>3: Description of variables used in statistical analyses</td>
<td>36</td>
</tr>
<tr>
<td>4: Percentage of <em>R. venosa</em> and <em>U. cinerea</em> egg capsules to hatch</td>
<td>53</td>
</tr>
<tr>
<td>5: Summary table of statistical analyses for percentage of egg capsules to hatch</td>
<td>56</td>
</tr>
<tr>
<td>6: Range in the number of days required for incubation</td>
<td>61</td>
</tr>
<tr>
<td>7: Summary table of statistical analyses for number of days required for incubation</td>
<td>62</td>
</tr>
<tr>
<td>8: Summary table of statistical analyses for percentage of embryos alive at hatch</td>
<td>68</td>
</tr>
<tr>
<td>9: Summary table of statistical analyses for egg capsule dry weight</td>
<td>73</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: Line drawing of <em>Rapana venosa</em> egg capsules</td>
<td>7</td>
</tr>
<tr>
<td>2: Line drawing of <em>Urosalpinx cinerea</em> egg capsules</td>
<td>12</td>
</tr>
<tr>
<td>3: Rubbermaid® heating/cooling unit used as experimental chamber</td>
<td>23</td>
</tr>
<tr>
<td>4: Schematic of water change procedures used in experiments</td>
<td>29</td>
</tr>
<tr>
<td>5: Daily 2007 and 2008 York River water temperature (°C) at Gloucester Point, Virginia</td>
<td>44</td>
</tr>
<tr>
<td>6: Daily 2007 and 2008 York River salinity (ppt) at Gloucester Point, Virginia</td>
<td>46</td>
</tr>
<tr>
<td>7: Daily 2007 and 2008 day length (hours per day) at Gloucester Point, Virginia</td>
<td>48</td>
</tr>
<tr>
<td>8: Distribution of <em>R. venosa</em> and <em>U. cinerea</em> egg capsule height (mm) by CDD</td>
<td>51</td>
</tr>
<tr>
<td>9: <em>R. venosa</em> percentage of egg capsules to hatch in 2007 and 2008</td>
<td>46</td>
</tr>
<tr>
<td>10: Mean values and 95% confidence intervals for percentage of egg capsules to hatch</td>
<td>57</td>
</tr>
<tr>
<td>11: <em>U. cinerea</em> percentage of egg capsules to hatch</td>
<td>59</td>
</tr>
<tr>
<td>12: Mean values and 95% confidence intervals for incubation time</td>
<td>63</td>
</tr>
<tr>
<td>13: Mean values and 95% confidence intervals for percentage of embryos alive at hatch</td>
<td>69</td>
</tr>
<tr>
<td>14: Mean values and 95% confidence intervals for egg capsule dry weight (mg)</td>
<td>74</td>
</tr>
<tr>
<td>15: Chesapeake Bay bathymetry (m)</td>
<td>76</td>
</tr>
<tr>
<td>Figure</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
</tr>
<tr>
<td>16: Distribution of <em>C. virginica</em> and <em>M. mercenaria</em> in Chesapeake Bay prior to 1991</td>
<td>77</td>
</tr>
<tr>
<td>17: Chesapeake Bay salinity (ppt) 1984 to 2007</td>
<td>80</td>
</tr>
<tr>
<td>18: Historic distribution of <em>R. venosa</em> in the Chesapeake Bay</td>
<td>81</td>
</tr>
<tr>
<td>19: Historic distribution of <em>U. cinerea</em> in the Chesapeake Bay</td>
<td>83</td>
</tr>
<tr>
<td>20: Optimal temperature and salinity for the percentage of <em>R. venosa</em> egg capsules to hatch</td>
<td>85</td>
</tr>
<tr>
<td>21: Optimal temperature and salinity for the percentage of <em>U. cinerea</em> egg capsules to hatch</td>
<td>86</td>
</tr>
<tr>
<td>22: Potential habitat supporting spatial overlap of <em>R. venosa</em> and <em>U. cinerea</em> in the Chesapeake Bay</td>
<td>96</td>
</tr>
<tr>
<td>23: Optimal habitat supporting spatial overlap of <em>R. venosa</em> and <em>U. cinerea</em> in the Chesapeake Bay</td>
<td>97</td>
</tr>
</tbody>
</table>
ABSTRACT

The maintenance of a population within the geographic range is influenced by the physical and environmental conditions under which breeding occurs. It is hypothesized that environmental conditions under which egg capsules are successfully hatched will influence the range of potential habitat of the invasive veined rapa whelk, Rapana venosa, and the native Atlantic oyster drill, Urosalpinx cinerea, in the Chesapeake Bay. This study examines the environmental conditions of temperature, salinity, and the time of deposition of egg capsules within the reproductive period (here quantified as cumulative number of day degrees at egg capsule deposition). The range of R. venosa and U. cinerea habitat in the Chesapeake Bay, in relation to environmental conditions, is important given the potential impact of both species on native shellfish stocks. Egg capsule hatching success and egg capsule incubation time for R. venosa and U. cinerea were examined at temperatures (18°C, 22°C, 26°C, 30°C, and ambient York River water temperature) and salinities (7 ppt, 14 ppt, 21 ppt, 28 ppt, and ambient York River salinity) reflective of the Chesapeake Bay during egg capsule deposition.

Salinity is the greatest factor influencing R. venosa and U. cinerea egg capsule hatching success. Increasing salinities increase the percentage of R. venosa and U. cinerea egg capsules to hatch as well as the percentage of U. cinerea embryos alive at hatch. For R. venosa, the percentage of egg capsules to hatch is greatest at 21 ppt. For U. cinerea the percentage of egg capsules to hatch and the percentage of embryos alive at hatch are greatest at salinities of 21 ppt or 28 ppt. R. venosa and U. cinerea egg capsules do not hatch at 7 ppt in the temperature range examined. Temperature within the range examined was not found to be an important factor influencing egg capsule hatching success for either species. However, temperature was an important factor influencing the rate at which the egg capsule hatching process occurs.

The percentage of R. venosa egg capsules to hatch increases and the percentage of U. cinerea alive at hatch decreases along the egg capsule deposition time series. For R. venosa, an increase in the percentage of egg capsules to hatch occurs if egg capsules are deposited later in the time series. For U. cinerea the percentage of egg capsules to hatch is not affected by position in the egg capsule deposition time series, but the percentage of embryos alive at hatch decreases along the time series examined.

The temperatures and salinities for optimal R. venosa egg capsule hatching range from 18°C to 30°C and from 11 ppt to 28 ppt and vary based on the timing of egg capsule deposition. The temperatures and salinities for optimal U. cinerea egg capsule hatching range from 18°C to 30°C and from 20 ppt to 28 ppt and do not vary based on the timing of egg capsule deposition. Optimal egg capsule hatching for R. venosa and U. cinerea occur at different temperature-salinity combinations throughout the majority of the egg capsule deposition time series.
EGG CAPSULE HATCHING SUCCESS IN *RAPANA VENOSA* AND *UROSALPINX CINEREA* IN RELATION TO TEMPERATURE AND SALINITY
INTRODUCTION

Overview of Gastropod Egg Capsule Development

The Class Gastropoda, Phylum Mollusca, is comprised of sea and land snails, slugs, limpets, abalones, cowries, whelks and cone shells (Carpenter, 2002). With over 30,000 extant species, the members of the Class Gastropoda display a wide range of developmental patterns (Ruppert and Barnes, 1994; Carpenter, 2002). The Family Muricidae, within the Class Gastropoda, is comprised of over 2,500 temperate and tropical species of predatory marine snails (Radwin and D’Attilio, 1976). Muricid development begins with the encapsulation of embryos within the egg capsule (Appendix I) (Fretter and Graham, 1962).

The process of egg capsule production follows a general pattern. Fertilization of embryos occurs within the female. Embryos are transferred to the albumen and capsule glands. Next the embryos are embedded in secretions of mucus and protein and sealed in capsules. Sealed egg capsules pass through the mantle cavity before being transferred to the foot for deposition on appropriate substrate (Federighi, 1931; Carriker, 1955; Fretter and Graham, 1962; Sullivan and Maguel 1984; Butler, 1985; Roller and Stickle, 1988; Chung et al., 1993; Chung and Kim, 1997; Saglam and Duzgunes, 2007).

Gastropod development can be described as planktonic development, mixed development, direct development, or development with nurse eggs. Common developmental patterns in muricids include mixed development or direct development.
In mixed development, embryos incubate within the egg capsule and planktonic veliger larvae hatch via the egg capsule apical pore (Pechenik, 1986). Larvae remain in the plankton before settlement, loss of the velum, and metamorphosis (Pechenik, 1986). In direct development, embryos incubate within the egg capsule and metamorphosed juveniles hatch (Appendix I) via the egg capsule apical pore (Pechenik, 1986). Hatched juveniles are released directly from the egg capsule to the substrate on which the egg capsule was deposited (Pechenik, 1986). Encapsulation of embryos within the egg capsule occurs over a time period ranging from days to months depending on taxon and the environmental conditions during incubation for mixed and direct development (Fretter and Graham, 1962; Spight, 1975; Radwin and D’Attilio, 1976). Direct development, rather than mixed development, is considered the more derived developmental pattern, given the lack of feeding planktonic veliger larvae (Strathmann, 1978; Christiansen and Fenchel, 1979; Strathmann 1985, 1993). However, both mixed development and direct development are considered derived relative to most primitive developmental pattern, planktonic development.

The egg capsule is important for embryonic survival until hatch for three reasons. First, the egg capsule provides protection from predation (Pechenik, 1979), bacteria (Lord, 1986), ultraviolet radiation (Rawlings, 1996), and rapid changes in environmental conditions (Pechenik, 1982, 1983). Second, the egg capsule is permeable to inorganic solutes, salts, and oxygen (Rawlings, 1999) allowing exchange with the external environment. Third, the egg capsule contents may provide the developing embryos nutritional support during development (Thorson, 1950; Spight, 1976; Gallardo, 1979). Despite the importance of the egg capsule, variation in embryonic survival within the
same species may occur based on the environmental conditions during egg capsule production or incubation. Variable environmental conditions likely to be experienced by deposited egg capsules within an estuary include temperature, salinity, and dissolved oxygen (Carriker, 1955; Fretter and Graham, 1962; Scheltema, 1967; Lucas and Costlow, 1979; Pechenik, 1982, 1983; Moran and Emlet, 2001; Cancino et al., 2003; Pechenik et al., 2003).

Niche Structure

In marine and estuarine organisms, temperature and salinity are among the most important factors (Kinne, 1970, 1971) determining the dimension of the fundamental niche (Appendix I). The fundamental niche is therefore important in establishing the potential range of habitat (Appendix I) that may be occupied by a species. If temperature fluctuates beyond the species’ physiological tolerances, then changes in membrane structure, denaturation of proteins, inadequate oxygen supply, and/or thermal inactivation of enzymes or enzyme systems may result in death over time scales as short as hours (Schmidt-Nielsen, 1997). If salinity fluctuates beyond the species’ physiological tolerances then changes in ion regulation, osmoregulation, and/or rate of oxygen consumption may result in death in a matter of hours (Kinne, 1971; Schmidt-Nielsen, 1997). Adaptations for adult gastropod survival during periods of fluctuation include enclosure within the shell, osmoregulation, and the ability to physically move to other locations within the range of survival (Ruppert and Barnes, 1994).

The dimension of the realized niche (Appendix I) is partially shaped by ecological interactions among species. The realized niche is therefore important in partially
defining the actual range of habitat that may be occupied by a species. The niche exclusion principle states that two species cannot occupy the same ecological niche at the same time (Hardin, 1960). Thus, it is unlikely that two species will exist within the same realized niche even if both display similar physiological tolerances and have similar fundamental niches. As a result, the full range of environmental conditions that support a species within the fundamental niche will be partitioned to minimize overlap between competing species occupying similar realized niches (Kronfeld-Schor and Dayan, 1999; Lawler and Morin, 1993; Pyke, 1982). Reduction in realized niche overlap between competing species will help determine the occupied habitat for species with similar physiological tolerances.

Veined Rapa Whelk, *Rapana venosa*: Habitat, Life History, and Prey Field

Habitat

The veined rapa whelk, *Rapana venosa*, is a marine gastropod native to the Sea of Japan and surrounding waters, including the Gulf of Bohai, Yellow Sea, and East China Sea (Chung et al., 1993; Zolotarev, 1996). In addition to the native range, *R. venosa* has been introduced into the Black Sea (Drapkin, 1963), Mediterranean, Adriatic, and Aegean Seas (Bombace et al., 1994; Zolotarev, 1996), Chesapeake Bay (Harding and Mann, 1999), the Brittany coast of France (Dr. Philippe Goulletquer, IFREMER, personal communication to Mann and Harding, 2000), South America (Pastorino et al., 2000), and the Netherlands (Vink et al., 2005). Colonization of new habitats by *R. venosa* has been largely attributed to the use of ballast water during commercial and military shipping traffic (Zolotarev, 1996; Harding and Mann, 1999; Mann and Harding, 2000), as well as
oyster transportation to the Black Sea (Mann et al., 2004). Within the geographical range, *R. venosa* is known to exist in subtidal hard and soft bottom habitats (Bombace et al., 1994; Harding and Mann, 1999; Giberto et al., 2006). Newly settled *R. venosa* (< 10 mm shell length (SL), as measured from the tip of the spire to the siphonal canal) may occupy hard substrate including oyster reefs (Mann and Harding, 2000); whereas, larger adults (> 68 mm SL) prefer hard sand bottom habitats (Harding and Mann, 1999). Given available food and habitat, adult *R. venosa* may survive a range of temperatures from 4°C to above 28°C (Chung et al., 1993, Mann and Harding, 2000) and salinities from 10 ppt (for several hours Harding, unpublished) to 33 ppt (Chukhchin, 1984; KODC).

Life History

The developmental pattern of *R. venosa* is mixed development. In the Chesapeake Bay copulation occurs year round (Mann et al., 2006) and egg capsule deposition occurs as water temperatures rise above 18°C (Harding et al., 2007; Harding et al., 2008) until late August or early September (Harding et al., 2008). At deposition, *R. venosa* egg capsules (Figure 1) can range in height from 5 mm to in excess of 40 mm as measured by the maximum distance from the basal plate to the apical pore (D’Asaro, 1991; Chung et al., 1993; Harding and Mann, 1999; Ware, 2002; Harding et al., 2007). Each group of *R. venosa* egg capsules, known hereafter as an egg mass (Appendix I), can be composed of 4 to 599 egg capsules (Ware, 2002). Incubation time for *R. venosa* embryos within the egg capsule ranges from 6 to 68 days (Chukhchin, 1984; Ware, 2002; Harding, 2006) at temperatures above 18°C. A power relationship exists between *R. venosa* egg capsule
Figure 1: Modified line drawing (D’Asaro, 1991) of *Rapana venosa* egg capsules with embryos. 1A. *R. venosa* egg capsules deposited on top of other egg capsules. 1B. Egg capsules attached directly to hard substrate.
height and the number of embryos contained within the egg capsule, as described by Harding et al. (2007). Between 113 and 3,673 embryos (Ware, 2002; Harding et al., 2007), ranging in size from 400 to 900 μm in diameter (Chukhchin, 1984; Harding, 2006), may be hatched from each egg capsule, depending on egg capsule height. Newly hatched *R. venosa* may remain in the water column for 24 (Harding, 2006) to 80 days (Giberto et al., 2006) as planktonic larvae, before settlement and metamorphosis. At the time of settlement, *R. venosa* range in size from 1.18 mm to 1.24 mm SL (Harding, 2006). Sexual maturity is reached between 1 to 2 years of age (> 35 mm SL) (Chukhchin, 1984; Westcott, 2001; Mann and Harding, 2003; Harding et al., 2007). Maximum adult sizes in excess of 165 mm SL have been observed in the Chesapeake Bay (Mann and Harding, 2000). The lifespan of *R. venosa* may range in excess of 15 years (Harding et al., 2007). Of the discussed life history characteristics, *R. venosa* may be successful as an invader of new habitat given high fecundity, possible high recruitment given the number of embryos contained within each egg capsule, early maturation, and long lifespan.

Prey Field

*Rapana venosa* is an opportunistic predator on bivalves (Chikina and Kucheruk, 2005). Prey, including species in the nonnative range, may include oysters (*Ostrea edulis, Crassostrea virginica*), scallops (*Chlamys glabra, Pecten ponticus*), mussels (*Mytilus galloprovincialis, Mytilus edulis*) (Drapkin, 1963; Harding and Mann, 1999), hard clams (*Mercenaria mercenaria*) (Harding and Mann, 1999; Savini et al., 2002), soft shelled clams (*Mya arenaria*) (Harding and Mann, 1999b), and barnacles (*Balanus*
eburneus, Balanus improvisus, Semibalanus balanoides (formerly B. balanoides). *Chthamalus fragilis*. *R. venosa* has been observed to be cannibalistic under food limed conditions (Micu and Todorova, 2007). As *R. venosa* increase in SL, the prey field progresses from smaller to larger prey (Harding et al., 2007; Harding et al., 2008). The predation rate from *R. venosa* (101 mm to 160 mm SL) on *M. mercenaria* (50 mm to 100 mm shell height) has been observed at a mean of 1.5 g prey wet weight per day, per *R. venosa* (Savini et al., 2002), or about 1 *M. mercenaria* per day (size range not noted; mean water temperature 26°C ± 1°C). Similar predation rates of 1.2 g prey wet weight per day, per *R. venosa* (99 mm to 110 mm SL) have been observed in the Northern Adriatic Sea (Savini and Occhipinti-Ambrogi, 2006) on the clam *Anadara inaequalvis* (17 mm to 57 mm SL), carpet clam, *Tapes philippinarum* (22 mm to 37 mm SL), and Mediterranean mussel, *M. galloprovincialis* (20 mm to 65 mm SL) (temperature not reported). In ecosystems lacking substantial predation pressure from bivalve consumers, the introduction and establishment of *R. venosa* may act as a novel source of mortality for shellfish resources. Such has been the case in the Black Sea as noted by Chukhchin (1984) who suggests that *R. venosa* had almost caused the local extinction of molluscs including *O. edulis*, *P. ponticus*, and *M. galloprovincialis*. In more recent years, *R. venosa* has acted as a consumer of bivalves such as *C. virginica* and *M. mercenaria* in the Chesapeake Bay (Harding and Mann, 1999; Harding and Mann, 1999b; Harding et al., 2007).
Atlantic Oyster Drill, *Urosalpinx cinerea*: Habitat, Life History, and Prey Field

Habitat

The Atlantic oyster drill, *U. cinerea*, is a marine and estuarine gastropod (Franz, 1971) native to the U.S. Atlantic Coastal Plain from Massachusetts to Florida (Carriker, 1955). In addition to the native range, *U. cinerea* has been introduced entirely along the Eastern Coast of the United States, southern portion of Canada, parts of the West Coast of North America, as well as portions of Great Britain (Carriker, 1955). Colonization of new habitats by *U. cinerea* has largely been attributed to oyster transplantation, cultivation, and harvesting operations in the 1940’s (Carriker, 1955) which inadvertently included *U. cinerea* in oyster shipments. Within the geographic range, *U. cinerea* is known to exist in subtidal locations with hard surfaces such as wood, rock, firm sand, and mud overlaid by shell, living oysters, mussels, or other epifauna (Federighi, 1931; Stauber, 1943; Mistakidis, 1951). Given available food and habitat resources, adults may survive temperatures from below 2°C (Galtsoff et al., 1937) to above 30°C (Sizer, 1936; Zachary and Haven, 1973) and salinities from 5 ppt to 40 ppt at summer temperatures (Sizer, 1936; Zachary and Haven, 1973).

Life History

The developmental pattern of *U. cinerea* is direct development. In Hampton Roads, Virginia (defined by the area surrounding 36°0’00"N, 76°0’00"W) copulation occurs year round (Federighi, 1931) and egg capsule deposition occurs as water temperatures rise above 20°C in May (Federighi, 1931; Carriker, 1955) through early November, with a brief pause in egg capsule deposition observed in August (Carriker, 1955). At
deposition, *U. cinerea* egg capsules (Figure 2) can range in height from 3.4 mm (Stauber, 1943) to greater than 10 mm (personal observation). Each *U. cinerea* egg mass contains 1 to 22 egg capsules (Carriker, 1955). Incubation *U. cinerea* of embryos ranges from 18 days (at 23°C to 29°C; Carriker, 1955) to 78 days (at 15°C to 30°C; Ganaros, 1958). A relationship exists between *U. cinerea* female SL, egg capsule height, and the number of embryos contained within the egg capsule such that female *U. cinerea* with decreased SL deposit shorter egg capsules with fewer embryos contained within each egg capsule (Carriker, 1955) when compared to *U. cinerea* with greater SL. Up to 35 embryos (Fretter and Graham, 1962), ranging in size from 0.8 mm to 1.0 mm SL (Federighi, 1931), may be hatched from each *U. cinerea* egg capsule, depending on egg capsule height. Sexual maturity is reached within the first 1 to 2 years of life (> 15 mm SL) (Stauber, 1943). Maximum adult sizes do not exceed 50 mm SL (Federighi, 1931; Carriker, 1955; Franz, 1971). The lifespan may range in excess of 10 years (Cole, 1942).

Prey Field

*Urosalpinx cinerea* is a generalist predator of bivalves. Prey, including species in the nonnative range, may include the oyster (*C. virginica*), soft clam (*M. arenaria*), mussel (*M. edulis*) and barnacles (*B. eburneus, B. improvisus, S. balanoides* (formerly *B. balanoides*, *C. fragilis*) (Carriker, 1955). *Urosalpinx cinerea* are capable of drilling and penetrating shelled prey items immediately upon hatching from the egg capsule (Carriker, 1955). Preference for larger prey items will occur with increasing SL (Carriker, 1955). *Urosalpinx cinerea* can act as an important consumer of commercially valuable shellfish
Figure 2: Modified line drawing (D’Asaro, 1991) of *Urosalpinx cinerea* egg capsules.
stocks. Historically, *U. cinerea* was a pest to the oyster industry in the Chesapeake Bay (Federighi, 1931; Stauber, 1943; Carriker, 1955); a single *U. cinerea* may consume in excess of 20 to 200 oysters in a season, depending on size (water temperature 19°C to 28°C; size range of oysters not noted; Galtsoff et al., 1937).

Temperature, Salinity and Circulation in the Chesapeake Bay

Temperature in the Chesapeake Bay varies over the course of a year from approximately 5°C to 28°C (Appendix I) based on factors such as the position of the Earth relative to the sun, water depth, and mixing. Globally, temperature is driven by exposure of the Earth to the sun's radiation. During the summer months, the Northern Hemisphere is exposed to more direct radiation relative to the spring, winter, and fall. An increase in the amount of radiation received in the Northern Hemisphere during the summer translates to an increase in temperature observed in the region. Locally, temperature within the Bay is based on a combination of factors including the water depth and mixing. Surface and shallow waters, which receive the majority of the sun's radiation, generally are warmer than water at depth. Water mixing attributed to wind and surface currents may circulate warmer upper waters with water at depth, homogenizing the water column. If water is not circulated between the upper and lower water column then a thermocline may become established and separate the two water masses.

Salinity in the Chesapeake Bay varies from near fresh conditions (0.5 ppt) to full ocean (35 ppt) (Appendix II) based on factors such as freshwater drainage, saltwater influx at depth, stratification, mixing, and storm events. Saltwater enters the Bay from the Atlantic Ocean via the Bay mouth. Salinity decreases towards the head of each
tributary relative to locations further towards the mainstem or mouth of the Bay. This is due primarily to the input of freshwater from streams, rivers, land runoff, and/or point sources. A gradient of salinities are found through the remainder of the tributaries and mainstem of the estuary where freshwater and saltwater mix. At any given location, the salinity is based on a combination of factors including the water depth and mixing. Freshwater is less dense than saltwater; thus, the less dense surface and shallow waters will generally contain less salty water when compared to the denser water at depth. Wind and surface currents may mix the upper layer of freshwater with saltwater at depth, homogenizing the water column. If the less dense freshwater is mixed with the more dense saltwater then a halocline may become established and separate the two water masses. Storm events are an important factor that may change the salinity of an estuary over short time scales. Small storms may decrease the salinity of an estuary a negligible amount; however, larger storms such as hurricanes or tropical storms can drastically reduce salinities over the course of hours or days. In 1972 Tropical Storm Agnes deposited in excess of 15 inches of rain on the region, prompting extensive flooding and very large amounts of freshwater runoff in the Chesapeake Bay watershed (Andrews, 1973). During this period Chesapeake Bay bottom salinities were reduced from 20 ppt to approximately 14 ppt in some locations. Chesapeake Bay surface salinities were reduced from 9 ppt to 3 or 4 ppt in some locations (Andrews, 1973).

Circulation in the Chesapeake Bay is attributed to large and small scale processes. On the large scale of the entire estuary the Coriolis Force drives the movement of freshwater out of the Bay and saltwater into the Bay. In the Northern Hemisphere, the Coriolis Force deflects water to the right. Saltwater entering the Bay from the south is
deflected towards northwards “to the right” along the Eastern Shore. Freshwater entering the Bay from the northern tributaries is pushed southwards along the Western Shore. Wind is a smaller scale process that drives circulation in the Chesapeake Bay. Moderate or strong winds may breakup stratification and mix the water column allowing turnover to evenly distribute heat, salt, nutrients, or free swimming organisms within the water column.

Extant Ranges of *Rapana venosa* and *Urosalpinx cinerea* Within the Chesapeake Bay

The extant distribution of *R. venosa* in the Chesapeake Bay extends to Tangier Light in Tangier Sound in the northern part of Chesapeake Bay, the James River below Wreck Shoal in the west, and to Cape Henry in the southeast (Harding and Mann, 2005) (For a map of the Chesapeake Bay with selected locations see Appendix III). Prior to the 1972, *U. cinerea* populations were commonly reported in the lower Rappahannock River below Towles Point (Andrews, 1973; Haven et al., 1981), the York River below Page’s Rock (Federighi, 1931; MacKenzie, 1961; Haven et al., 1981), and the James River below the James River Bridge (Andrews, 1973; Haven et al., 1981). *Urosalpinx cinerea* distributions were reduced after Tropical Storm Agnes in 1972 to higher salinity waters (Andrews, 1973; Haven et al., 1981) and no comprehensive studies have reported the distribution or abundance of *U. cinerea* since. Yearly oyster monitoring programs at VIMS since 1998 have collected individual *U. cinerea* as by-catch at the mouth of the Rappahannock River, Mobjack Bay, Lynnhaven River, Elizabeth River, and Tangier Sound (Southworth, Harding, and Mann, unpublished data); however, these data are not
comprehensive, nor is the target species of the VIMS oyster monitoring program *U. cinerea*.

Developmental pattern may be a factor in understanding how *R. venosa*, a mixed developer, and *U. cinerea*, a direct developer, have dispersed throughout the Chesapeake Bay. *R. venosa*, an invasive species in the Chesapeake Bay, is thought to have been introduced to the Chesapeake Bay via ballast water released in the vicinity of Ocean View located on the southern shore near the Bay mouth, and near the coal piers of Newport News Point located on the northern shore of the James River. Circulation patterns in the Chesapeake Bay, discussed previously in reference to temperature and salinity, may be an important factor transporting *R. venosa* veliger larvae away from the original source of introduction to more northerly locations in the Chesapeake Bay. Given long planktonic periods, planktonic feeding, and ability to depth regulate, large scale estuarine circulation is a plausible mechanism explaining transport of *R. venosa* from locations surrounding the original point of introduction to locations such as Butler’s Hole in the Rappahannock River or Tangier Light in Tangier Sound (Harding and Mann, 2005). Conversely *U. cinerea*, a native species in the Chesapeake Bay, does not have a planktonic period and is released directly to the hard substrate upon hatch. Thus, the distributional pattern of *U. cinerea* is likely based on centauries of dispersal by “foot”, literally crawling up the Bay, and transportation by humans rather than by dispersal in the plankton. Further, direct release of fully metamorphosed juvenile *U. cinerea* from the egg capsule rather than a planktonic stage may decrease *U. cinerea*’s ability to increase in density or abundance after localized decreases in population levels over short timescales.
Context

Juvenile *R. venosa* (<30 mm SL) and *U. cinerea* (<50 mm SL) may share similar tolerances to environmental conditions in the southern Chesapeake Bay (Carriker, 1955). The extant ranges of *R. venosa* and *U. cinerea* populations in the Chesapeake Bay are sharply influenced by environmental conditions, such as temperature and salinity, which allow for egg capsule hatching. The environmental conditions supporting successful *R. venosa* and *U. cinerea* egg capsule hatching may be important in understanding the potential and optimal ranges of *R. venosa* and *U. cinerea* populations in the Chesapeake Bay. Geographical overlap or separation of *R. venosa* and *U. cinerea* populations, based on potential and optimal egg capsule hatching conditions, may highlight differences in early embryonic tolerances. Further, insight into the potential and optimal environmental conditions supporting *R. venosa* and *U. cinerea* based on egg capsule hatching success may aid our understanding of the dynamics behind *U. cinerea* population recovery given the establishment of *R. venosa* in the Chesapeake Bay.

Objectives

This study quantitatively describes the effects of temperature, salinity, and position of egg capsules in the time series of egg capsule deposition on *R. venosa* and *U. cinerea* egg capsule hatching success (Appendix I). Egg capsule hatching success in this context is optimized under conditions where the greatest percentage of egg capsules hatch, and the greatest percentage of embryos are alive at hatch. For quantification purposes the position of egg capsules in the time series of egg capsule deposition is described by the
cumulative number of day degrees (CDD) at egg capsule collection. Comprehensive
description of the calculation of CDD is given in the Methods section of this text.

Hypotheses:

H$_1$: Environmental conditions (temperature; salinity or cumulative number of day
degrees at egg capsule collection) have an effect on the binary description (Y/N)
of egg capsule hatching.

H$_2$: Environmental conditions (temperature; salinity or cumulative number of day
degrees at egg capsule collection) have an effect on the percentage of egg
capsules to hatch.

H$_3$: Species (R. venosa or U. cinerea) has an effect on the percentage of egg
capsules to hatch.

H$_4$: Environmental conditions (temperature; salinity or cumulative number of day
degrees at egg capsule collection) have an effect on egg capsule incubation time.

H$_5$: Species (R. venosa or U. cinerea) has an effect on egg capsule incubation
time.

H$_6$: Environmental conditions (temperature; salinity or cumulative number of day
degrees at egg capsule collection) have an effect on the percentage of embryos
alive at hatch.

H$_7$: Species (R. venosa or U. cinerea) has an effect on the percentage of embryos
alive at hatch.

H$_8$: The cumulative number of degree days, calculated based on the ambient
temperature on the date of egg capsule collection and temperature at initiation of
gonad development, has an effect on egg capsule biomass as measured by the egg
capsule dry weight.

H$_9$: Species has an effect on the egg capsule biomass as measured by the egg
capsule dry weight.
METHODS

This study was completed at the Virginia Institute of Marine Science (VIMS) Molluscan Ecology laboratory at Gloucester Point (37°14'53" N, 76°29'59" W) on the York River, Virginia in 2007 and 2008.

Environmental Conditions and Collections

Daily, May through November, environmental conditions for Gloucester Point, Virginia (37°14'N, 76°29'W) were recorded. York River water temperature and salinity were recorded hourly at the VIMS oyster pier, 20 cm above the substrate, near the inflow to the laboratory seawater supply (VIMS Molluscan Ecology Program at http://web.vims.edu/mollusc/envmon/01VOY/01VOY.htm). York River water temperature and salinity flowing through the laboratory were recorded once per day at water changes (described later in experimental procedures). The day length, as given by the number of hours of daylight per day, was recorded for Gloucester Point once per day (U.S. Naval Observatory at http://aa.usno.navy.mil/data/docs/RS_OneYear.php).

The number of cumulative day degrees (CDD) for each species were calculated from daily average York River bottom temperature data above 10°C, which denotes the start of gametogenesis for both species (Carriker, 1955; Harding et al., 2008). The number of CDD is used as a surrogate for position in the egg capsule deposition time series. It is necessary to use a surrogate for position in the egg capsule deposition series since egg
capsules are deposited along a seasonal progression. Along the seasonal progression, temperature and the length of exposure to temperatures supporting gametogenesis, rather than date, are important in determining the start and end of the reproductive period (Mann, 1979). The number of CDD is a good surrogate for the position in the egg capsule deposition time series since egg capsule deposition will begin each year at approximately the same number of CDD. The number of CDD at egg capsule collection has been used previously to act as a surrogate for the position in the reproductive period in other molluscs such as the Pacific oyster *Crassostrea gigas* (Mann, 1979), the European flat oyster *Ostrea edulis* (Wilson and Simons, 1985), the blue mussel *Mytilus edulis* (Bayne and Worrall, 1980), the green mussel *Perna viridis* (Lee, 1986), *R. venosa*, *U. cinerea*, and the thick lipped oyster drill *Eupleura caudate* (Harding et al., 2008). The number of CDD’s at each egg capsule collection was calculated using a modification of the Mann (1979) formula proposed by Wilson and Simons (1985):

\[ D = \sum_{n=i}^{n} (t_i - t_0) \]

where \( D \) is the thermal constant in day degrees, \( n \) is the number of days required to reach ripeness, \( t_i \) is the average daily temperature to which adult *R. venosa* or *U. cinerea* were exposed (°C), and \( t_0 \) is the estimated temperature for initiation of gonad development. For *R. venosa* and *U. cinerea*, \( t_0 \) was set at 10°C, which denotes the start of gametogenesis for both species (Carriker, 1955; Harding et al., 2008). Note that as part of the calculation, the number of CDD includes a temperature component.
Broodstock Sources and Collection

*Rapana venosa* broodstock was obtained from the southern Chesapeake Bay, USA as donations to the VIMS rapa whelk bounty program. *Rapana venosa* broodstock used in 2007 were collected prior to June 2007. *Rapana venosa* broodstock used in 2008 were collected between October 2007 and June 2008. *R. venosa* broodstock (> 50 mm SL) was composed of approximately 150 and 100 *R. venosa* in 2007 and 2008, respectively. All *R. venosa* were maintained in laboratory flow-through seawater conditions at VIMS and fed *M. mercenaria* and *C. virginica* to excess.

*Urosalpinx cinerea* broodstock was obtained on May 15, 2008 from the seaside of Virginia’s Eastern Shore (36° 37' 36"N, 75° 37' 34"W), near Wachapreague. *Urosalpinx cinerea* broodstock was acclimated from salinities of 27 ppt with a 0.33% (2 out of 600 individuals) mortality rate. Acclimation occurred over the period May 15, 2008 to June 3, 2008 such that the salinity within each acclimation chamber was reduced by 2 ppt every other day until ambient York River salinity (17 ppt) was reached. *U. cinerea* broodstock (19.2 mm to 43.9 mm SL; Appendix IV) was composed of approximately 600 *U. cinerea*. After acclimation, *U. cinerea* were maintained in laboratory flow-through seawater conditions at VIMS and fed *C. virginica* to excess.

Egg Capsule Collection and Handling

During 2007 and 2008, a randomly selected size range of freshly deposited *R. venosa* and *U. cinerea* egg capsules were collected from broodstock at VIMS. Freshly deposited egg capsules are described as a pale to light yellow color for *R. venosa* (Harding and Mann, 1999) and a clear bluish white to yellow for *U. cinerea* (Carriker, 1955).
capsules were collected from below the flow-through water line at approximately one month intervals throughout the egg capsule deposition time series, for a total of 3 collection dates each year (Table 1). Collected egg capsules were used for egg capsule culture or egg capsule dry weight analysis.

Egg capsules designated for culture were disinfected using a set sequence of dips that included filtered seawater and filtered seawater plus 1 tablespoon bleach, then separated, measured, and placed into experimental vials. The disinfection procedure, described by Ware (2002), was composed of a series of 5 to 30 second filtered seawater and/or bleach dips. Filtered seawater was raw York River water processed through filters with pore sizes of 5 and 1 μm. Dipped egg capsules from the same mass were placed in a finger bowl and separated by cutting between the basal plate (Figures 1, 2). Given differences in the maximum egg capsule height between species, a single *R. venosa* or *U. cinerea* egg capsule was placed into a 50 mL or 20 mL experimental vial, respectively. For each vial, an identification code indicated the culture temperature-salinity combination, the egg mass from which the egg capsules came, and the date of egg capsule collection. At the time of egg capsule placement into the experimental vial, each vial contained the designated salinity water at the designated temperature (± 1°C). Egg capsules were not acclimated to the culture conditions prior to placement into the experimental vials.
Table 1: Egg capsule collection dates for *Rapana venosa* and *Urosalpinx cinerea* in relation to environmental conditions. *U. cinerea* egg capsules collected on 609 CDD in 2008 were terminated on July 24, 2008 (day 43) due to observation of ciliates across multiple temperature-salinity combinations.
<table>
<thead>
<tr>
<th>Species</th>
<th>Cumulative day degrees at harvest</th>
<th>Ambient temperature (°C)</th>
<th>Ambient salinity (ppt)</th>
<th>Day length (hours) at harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. venosa</em></td>
<td>2007</td>
<td>23</td>
<td>18</td>
<td>14.5</td>
</tr>
<tr>
<td><em>R. venosa</em></td>
<td>2007</td>
<td>25</td>
<td>20</td>
<td>14.6</td>
</tr>
<tr>
<td><em>R. venosa</em></td>
<td>2007</td>
<td>28</td>
<td>22</td>
<td>14.0</td>
</tr>
<tr>
<td><em>R. venosa</em></td>
<td>2008</td>
<td>27</td>
<td>15</td>
<td>14.7</td>
</tr>
<tr>
<td><em>R. venosa</em></td>
<td>2008</td>
<td>21</td>
<td>21</td>
<td>14.4</td>
</tr>
<tr>
<td><em>R. venosa</em></td>
<td>2008</td>
<td>28</td>
<td>28</td>
<td>14.0</td>
</tr>
<tr>
<td><em>U. cinerea</em></td>
<td>2008</td>
<td>27</td>
<td>15</td>
<td>14.7</td>
</tr>
<tr>
<td><em>U. cinerea</em></td>
<td>2008</td>
<td>21</td>
<td>21</td>
<td>14.4</td>
</tr>
<tr>
<td><em>U. cinerea</em></td>
<td>2008</td>
<td>28</td>
<td>28</td>
<td>14.0</td>
</tr>
</tbody>
</table>
Experimental Design

Egg Capsule Culture Temperature

Egg capsule culture temperatures of 18°C, 22°C, 26°C, 30°C and ambient York River water temperature were chosen to reflect potential conditions experienced by egg capsules deposited by wild *R. venosa* and *U. cinerea* within the Chesapeake Bay.

Temperature Chambers

Temperature chambers were created by modifying Rubbermaid® heating/cooling units (model # VEC212FRB) or using a flow-through seawater flume. One Rubbermaid® chamber was modified to maintain each of the four culture temperatures (± 1°C) (Figure 3). Ambient conditions were created using a flow-through seawater system that continually circulated raw York River water, at ambient temperature, past experimental vials. The daily recorded temperature in each chamber are reported in Appendix V. Each temperature chamber held a total of 60 vials for *R. venosa* or 100 vials for *U. cinerea*.

Egg Capsule Culture Salinities

Egg capsule culture salinities of 7 ppt, 14 ppt, 21 ppt, and 28 ppt and ambient York River salinity were chosen to reflect potential conditions limiting *R. venosa* and *U. cinerea* adult distributions. These conditions were also reflective of conditions experienced by egg capsules deposited by wild *R. venosa* and *U. cinerea* within the Chesapeake Bay.
Figure 3: Rubbermaid® heating/cooling unit (model # VEC212FRB) used as a temperature controlled experimental chamber.
Experimental Salinities

Salinities of 7 ppt, 14 ppt, 21 ppt, and 28 ppt were mixed using distilled water and Instant Ocean®. Ambient salinity water was produced by filtering raw York River seawater through filters with pore sizes of 5 and 1 μm. After filtering, ambient water may have included ciliates, or harmful algal blooms depending on ambient York River conditions at collection. Water was stored in partially closed 20 L Nalgene® carboys with air stones to aerate the stored water. Salinity levels were recorded daily before water changes.

Experimental Procedures

Progression of Embryos to Hatch

*Rapana venosa* and *U. cinerea* egg capsules from each experiment were examined daily for progression of embryos to hatch and egg capsule color until removal from the culture series. Normal observation of embryo progression to hatch included 4 stages observed along a progression of time that ranged from days to months. At stage 1, tight clusters of embryos, without noticeable shells were observed. At stage 2, embryos were shelled. Embryos displayed movement at stage 3. At stage 4, embryos hatched via the apical pore of each egg capsule. Normal observation of egg capsule color included a progression of egg capsule color from yellow to grey, or black for *R. venosa* and from white to yellow, or brown for *U. cinerea* (Table 2). Observations of abnormal embryo progression to hatch included the disintegration of embryos within the egg capsule. Abnormal egg capsule color included purple, green, pink, or brown (Table 2) throughout the egg capsule height. Egg capsules were removed from the culture series at hatch or if
Table 2: A description of egg capsule color as an indicator of *Rapana venosa* and *Urosalpinx cinerea* egg capsule progression to hatch. Note that the progression of egg capsule color and the number of days for which the egg capsule typically displays each color will vary depending on environmental conditions during egg capsule incubation from days to months.
<table>
<thead>
<tr>
<th>Egg Capsule Color</th>
<th>Normal progression of color (deposition to onset of hatch) (&gt; 18°C, &gt; 7 ppt)</th>
<th>Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. venosa</em></td>
<td>Light to lemon yellow, pale grey, black (Harding and Mann, 1999)</td>
<td>Purple, green, brown, pink (Harding and Mann, 1999)</td>
</tr>
<tr>
<td><em>U. cinerea</em></td>
<td>Bluish-white, pale yellow, yellow, yellowish brown (Carriker, 1955)</td>
<td>Purple, green, brown, pink (Carriker, 1955)</td>
</tr>
</tbody>
</table>
abnormal states persisted for multiple days. Experiments were conducted 3 times per year. Each year the period of time, beginning with the first egg capsule collection to the last egg capsule hatch or death from the 3rd egg capsule collection, spanned from approximately May to November.

Water Changes

At daily intervals each egg capsule was removed from the experimental vial, and placed into another experimental vial containing water of the designated temperature and salinity, requiring approximately 6 hours per day. Prior to water changes, vials were given 24 hours to equilibrate at the chosen temperature. Transfer of an egg capsule between vials was accomplished by pouring the vial contents through a 500 μm sieve, and lifting the egg capsule by the basal plate using forceps (Figure 4). To prevent contamination, the sieve and forceps were rinsed in a dilute bleach solution (1 tablespoon bleach per 4 L tap water, with tap water rinse after bleach dip) between vial water changes. Empty vials were washed with warm soapy water, rinsed with tap water, and left to air dry for 24 hours before being refilled and placed back into the designated temperature chamber the following day.

Quality Control

In 2007 and 2008, as each experiment was in progress, water samples from a selection of experimental vials at each temperature-salinity combination were examined for ciliates under a dissecting microscope (magnification 30x). Ciliates are naturally occurring in the York River and were not entirely removed from the ambient water by
Figure 4: A schematic of the daily egg capsule water change procedure used for *Rapana venosa* and *Urosalpinx cinerea* in 2007 and 2008.
Fill vial with designated salinity water

Add egg capsule to vial at designated temperature

Remove egg capsule from vial

Clean empty vial

Let vial dry for 24 hr

Transfer egg capsule to fresh vial at set environmental condition

Refill clean/dry vial with 30 mL or 15 mL of designated salinity water for *R. venosa* or *U. cinerea* respectively.
filtering. Ciliates within Instant Ocean® mixed water samples were present if the
disinfection process failed to kill 100% of the ciliates present at the time of egg capsule
collection. Cross-contamination among vials was minimized by bleaching equipment
between vial water changes, using separate air supplies in each carboy containing water
samples, and washing used vials before refilling. An entire experiment was terminated if
ciliates were observed in across multiple temperature-salinity combinations.

Processing of Egg Capsules After Hatch

After hatch *R. venosa* larvae were determined to be alive or dead using a neutral red
dye, counted, and then preserved. Neutral red, a 2,8-substituted aminophenazine
(Fernando et al., 1966), when added to living tissue permanently stains cell nuclei red.
Neutral red powder (10 mg) dissolved in seawater (20 mL) of the appropriate salinity was
given 4 hours to stain at the temperature-salinity combination in which egg capsule
hatching occurred. Four drops of neutral red per hatched vial was sufficient to stain all
hatched embryos. Four drops of 10% neutral buffered formalin were added to fix the
sample after staining. Twenty-four hours after preservation, *R. venosa* larvae were
transferred to a 1.5 mL centrifuge vial containing 10% ethanol. Forty-eight hours after
preservation, the 10% ethanol was replaced with 50% ethanol and the vial was archived.
Egg capsules from which *R. venosa* embryos hatched were preserved and archived in
individual 3 mL centrifuge vials beginning with the addition of ethanol, and following the
following the protocol described above.

After hatch *U. cinerea* juveniles were assessed as alive or dead, counted, and
preserved. *Urosalpinx cinerea* were assessed as alive or dead based on attachment (alive)
or non-attachment (dead) to a hard surface. All juvenile *U. cinerea* hatched from a single egg capsule were transferred to a 1.5 mL centrifuge vial containing a dilute seawater-neutral buffered formalin mixture equivalent to the strength used to preserve *R. venosa* veligers (4 drops 10% neutral buffered formalin to 30 mL seawater of the correct salinity). Twenty-four hours after preservation, the dilute neutral buffered formalin mixture was swapped for 10% ethanol. Forty-eight hours after preservation, the 10% ethanol was replaced with 50% ethanol and the vial was archived. Egg capsules from which *U. cinerea* embryos were hatched were preserved and archived in individual 1.5 mL centrifuge vials beginning with the addition of ethanol, and following the following the protocol described above.

Enumeration of Embryos Per Egg Capsule Alive at Hatch

The total number of *R. venosa* embryos hatched alive from each egg capsule was counted and the percentage of embryos alive determined relative to the total number of embryos within the egg capsule. Stained and unstained *R. venosa* were counted a minimum of three times (the coefficient of variation between counts was less than 2%) under a dissecting microscope (magnification 64x). The number of *R. venosa* embryos that retained the neutral red stain (alive) was divided by the total number of embryos hatched from each egg capsule and multiplied by 100 to determine the percentage of embryos alive at hatch. After hatch, egg capsules were examined under a dissecting microscope to determine if any embryos remained within the egg capsule.

The total number of *U. cinerea* embryos hatched alive from each egg capsule was counted and the percentage of embryos alive determined relative to the total number of
embryos per egg capsule counted at the time of collection. Accurate counting of *U. cinerea* was achieved without multiple counts due to the low number of *U. cinerea* contained within each egg capsule (Fretter and Graham, 1962). After hatch, egg capsules were examined under a dissecting microscope (magnification 64x) to determine if any embryos remained within the egg capsule.

**Processing of Egg Capsules After Embryo Death**

Unhatched *R. venosa* and *U. cinerea* egg capsules that did not release any embryos were preserved and archived in 3 mL or 1.5 mL centrifuge vials, respectively, beginning with the addition of ethanol, and following the following the protocol described above.

**Egg Capsule Dry Weight**

Observations of the egg capsule dry weight were made for *R. venosa* in 2007 and 2008 and for *U. cinerea* in 2008 to determine if egg capsule biomass changed over the course of the egg capsule deposition time series. Egg capsules used in egg capsule dry weight analyses were from the same egg masses (refer to Figure 2, Appendix I) as those used in the culture experiments. Thus, egg capsule collection dates, egg capsule collection procedure, and environmental conditions at egg capsule collection were identical between experimental egg capsules and egg capsules used in egg capsule dry weight analysis. Wet weight of an egg capsule was determined by placing each egg capsule on a pre-weighed, pre-labeled pan. Pans with egg capsules were placed in a drying oven at 80°C for 72 hours. Egg capsules and pans were reweighed after drying to determine the dry weight of each egg capsule after correcting for the pan weight.
Data Analyses

Data were analyzed using significance testing including separate one-way or 3-way ANOVAs (or Kruskal-Wallis non-parametric test) as well as mean and 95% confidence intervals. ANOVAs and associated test statistics (p-values, F or H statistics, degrees of freedom) are presented to examine if statistically significant results exist between treatments. Mean and 95% confidence intervals are presented to indicate the reliability of estimating the mean within the given interval range (Zar, 1999), as well as to display the range of values in which the mean likely falls. Just as p values above 0.05 indicate data are likely consistent between treatments, overlapping confidence intervals indicate consistencies data between treatments as well. The reverse is true with p values below 0.05 and non-overlapping confidence intervals, as each indicate the likelihood that differences exist between the treatments. Newman (2008) suggests that confidence intervals be used instead of significance testing where possible based on “incorrect inferences” and “indefensible regulatory decisions” resulting from confused application of significance testing. In these analyses both methods are presented so that that significant results attained from significance testing can be reinforced or refuted by an alternative method, mean and 95% confidence intervals.

Significance Level

Significance levels for all statistical tests were established at $\alpha = 0.05 \text{ a priori.}$ Statistically significant values ($p < 0.05$) indicate that differences between observations taken from populations are unlikely to have occurred by chance, assuming that the null hypothesis is true. Normality (describing how closely the data follow a normal
distribution), and homogeneity of variance (describing the variance between populations) are two of the assumptions tested for an ANOVA. For normality, statistically significant values were determined using a Kolmogorov-Smirnov test (Minitab v.15). For homogeneity of variance, statistically significant values were evaluated with Levene’s Test (Minitab v.15). Violations in the assumptions of an ANOVA may lead to a type I error (reject the null hypothesis when the null hypothesis is true) or type II error (failure to reject the null hypothesis when the null hypothesis is false) (Zar, 1999). If a data set failed to achieve normality and homogeneity of variance, a Kruskal-Wallis nonparametric test was performed at $\alpha = 0.05$ a priori. A Kruskal-Wallis test does not assume normality or homogeneity of variance, although power is reduced compared to using an ANOVA. When necessary, a Fisher’s posthoc multiple comparison test was used for parametric data to compare significantly different populations. A Dunn’s posthoc comparison test was used for nonparametric data to compare significantly different populations.

Power

Power was estimated following the method described by Zar (1999) and determined to be sufficient for the number of *R. venosa* and *U. cinerea* egg capsules cultured at each temperature-salinity combination. A total of 6 *R. venosa* egg capsules were cultured at each temperature-salinity combination of the 5x5 temperature-salinity matrix in 2007. For each *R. venosa* egg capsule collection in 2007 a total of 150 egg capsules were required. Given 3 egg capsule collections in 2007, a total of 450 *R. venosa* egg capsules were required. A total of 10 *R. venosa* egg capsules were cultured at a single
temperature-salinity combination in 2008. Given 3 egg capsule collections in 2008, a total of 30 *R. venosa* egg capsules were required. A total of 10 *U. cinerea* egg capsules were cultured at each temperature-salinity combination of the 5x5 temperature-salinity matrix in 2008. For each *U. cinerea* egg capsule collection in 2008 a total of 250 egg capsules were required. Given 3 egg capsule collections in 2008, a total of 750 *U. cinerea* egg capsules were required.

Identification of Variables

The variables used in the statistical analyses consist of the following: cumulative day degrees (CDD), egg capsule dry weight, egg capsule height, egg capsule incubation time, percentage of embryos alive at hatch, salinity, species, and temperature (Table 3).

Exclusion of Data

*R. venosa* and *U. cinerea* egg capsules from a temperature-salinity combination in which no egg capsule hatched for either species across all collection dates in addition to egg capsules from experiments terminated prior to the 75th day of incubation were excluded from data analyses (specific examples are given in the Results section). Successful egg capsule hatching was observed to occur on at least the 75th day of egg capsule incubation. Inclusion of data from experiments terminated prior to the 75th day of incubation may bias the results given the potential period of incubation was not reached.
Table 3: A description of the variables used in the statistical analyses.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
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<tbody>
<tr>
<td>Cumulative day degrees</td>
<td>The cumulative number of degree days (beginning as York River bottom temperatures ≥ 10°C). Used as a proxy for position in egg capsule time series.</td>
</tr>
<tr>
<td>Egg capsule dry weight</td>
<td>Weight of an egg capsule (mg) after drying at 80°C for 72 hours.</td>
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<tr>
<td>Egg capsule height</td>
<td>The egg capsule height (mm) measured by the distance between the basal plate and the apical pore.</td>
</tr>
<tr>
<td>Incubation time</td>
<td>The number of days between egg capsule deposition and hatch. In the case that hatching occurred over more than one day the incubation time was given as the mean number of days incubation time.</td>
</tr>
<tr>
<td>Percentage to hatch</td>
<td>The percentage of egg capsules at a given temperature salinity combination that released at least embryo alive at the time of hatch.</td>
</tr>
<tr>
<td>Percentage of embryos alive</td>
<td>The percentage of embryos alive from a single egg capsule at the time of hatch.</td>
</tr>
<tr>
<td>Salinity</td>
<td>The culture salinity (7 ppt, 14 ppt, 21 ppt, 28 ppt, or ambient York River salinity ranging from 17 ppt to 24ppt in 2007 and from 15 ppt to 30 ppt in 2008).</td>
</tr>
<tr>
<td>Species</td>
<td>R. venosa or U. cinerea.</td>
</tr>
<tr>
<td>Temperature</td>
<td>The culture temperature (18°C, 22°C, 26°C, 30°C, or ambient York River temperature ranging from 23 °C to32°C in 2007 and 15°C to 3°C in 2008).</td>
</tr>
</tbody>
</table>
Culture Temperatures and Salinities

In 2007 for *R. venosa*, and 2008 for *U. cinerea* egg capsules were cultured at the 25 temperature-salinity combinations described above. In 2008, *R. venosa* egg capsules were cultured at 21°C and 21 ppt to compare consistency of handling and all procedures between years with *R. venosa* egg capsule hatched in 2007.

Binary Description of Egg Capsule Hatching

A binomial test (Zar, 1999) was performed for each temperature-salinity combination in each experiment to determine if the percentage of egg capsules to hatch deviated from an expected 50% chance of egg capsule hatching. The assumptions of a binomial test include a dichotomous distribution for the variable of interest, and random sampling (Zar, 1999). Egg capsule hatching success was recorded in a binary fashion where 1 = successful hatch and 0 = unsuccessful hatch.

Percentage of Egg Capsules to Hatch

The percentage of *R. venosa* and *U. cinerea* egg capsules to hatch was evaluated in relation to incubation temperature, salinity, and the number of CDD at egg capsule collection using two species specific 3-way ANOVAs in addition to mean and 95% confidence intervals. In each 3-way ANOVA, temperature, salinity, a temperature-salinity interaction, and the number of CDD at egg capsule collection were used as factors and the percentage of egg capsules to successfully hatch was used as the response. Please note that the calculation used to determine the number of CDD includes a
temperature component such that an increase in temperature results in an increase in the calculated number of CDD.

For each species, egg capsules cultured at 7 ppt were removed from the analyses based on no egg capsules hatching at any temperature for either species. For *R. venosa*, the percentage of egg capsules to hatch data satisfied assumptions of normality and homogeneity without transformation after the removal of all egg capsules cultured at 7 ppt. For *U. cinerea*, transformed percentage of egg capsules to hatch data (ln, log, (log + 1), square root, arcsin, sin, Box-Cox (Box and Cox, 1964) and reciprocal) and untransformed failed to meet the assumption of normality; however, untransformed data satisfied the assumption of homogeneity. Examination of the residual plot of the percentage of *U. cinerea* egg capsules to hatch expressed normality, and analyses were performed on untransformed *U. cinerea* data. A 4-way ANOVA with species as an additional factor was not used due to the inability to achieve normality and homogeneity of variance for the combined data set.

The percentage of egg capsules to hatch was compared between species using a Kruskal-Wallis test in addition to mean and 95% confidence intervals. A Kruskal-Wallis test was used since data did not satisfy the assumptions of normality or homogeneity of variance required for an ANOVA. These data met the assumption of independence and were from treatments having the same continuous distribution which are required for the Kruskal-Wallis test.
Incubation Time

The incubation time (days) to successful hatch of *R. venosa* and *U. cinerea* egg capsules was evaluated relative to incubation temperature, salinity, and the number of CDD at egg capsule collection using two species specific 3-way ANOVAs in addition to mean and 95% confidence intervals. In each 3-way ANOVA, temperature, salinity, a temperature-salinity interaction, and the number of CDD at egg capsule collection were used as factors and the incubation time of successfully hatching egg capsules was used as the response. Please note that the calculation used to determine the number of CDD includes a temperature component such that an increase in temperature results in an increase in the calculated number of CDD. It was not possible to compute a temperature-salinity interaction for *U. cinerea* incubation time data due to an unbalanced design resulting from no recorded hatches at some temperature-salinity combinations (see Table 4). For *R. venosa*, incubation time data satisfied the assumption of normality and homogeneity with Box-Cox transformation (Box and Cox, 1964). For *U. cinerea*, untransformed and transformed incubation time data (ln, log, \((\log + 1)\), square root, arcsin, sin, Box-Cox (Box and Cox, 1964) and reciprocal) failed to meet the assumption of normality; however, untransformed data satisfied the assumption of homogeneity. Examination of the residual plot for *U. cinerea* incubation time expressed normality, and analyses were performed on untransformed *U. cinerea* data. A 4-way ANOVA with species as an additional factor was not used due to the inability to achieve normality and homogeneity of variance for the combined data set.

The incubation time to successful hatch of egg capsules was compared between species using a Kruskal-Wallis test in addition to mean and 95% confidence intervals. A
Kruskal-Wallis test was used since data did not satisfy the assumptions of normality or homogeneity of variance required for an ANOVA. These data met the assumption of independence and were from treatments having the same continuous distribution which are required for the Kruskal-Wallis test.

Percentage of Embryos Alive at Hatch

The percentages of embryos alive counted from successfully hatching *R. venosa* and *U. cinerea* egg capsules were evaluated in relation to incubation temperature, salinity, and the number of CDD at egg capsule collection using two species specific 3-way ANOVAs in addition to mean and 95% confidence intervals. In each 3-way ANOVA, temperature, salinity, a temperature-salinity interaction, and the percentage of embryos alive at hatch was used as the response. Please note that the calculation used to determine the number of CDD includes a temperature component such that an increase in temperature results in an increase in the calculated number of CDD. It was not possible to compute a temperature-salinity interaction for *U. cinerea* percentage of embryos alive at hatch data due to an unbalanced design resulting from no recorded hatches at some temperature-salinity combinations (Table 4). For *R. venosa* untransformed and transformed percentage of embryos alive at hatch data (ln, log, (log + 1), square root, arcsin, sin, Box-Cox (Box and Cox, 1964) and reciprocal) failed to meet the assumption of normality; however, untransformed data satisfied the assumption of homogeneity. Examination of the residual plot of the percentage of *R. venosa* embryos alive at hatch expressed normality, and analyses were performed on untransformed *R. venosa* data. For *U. cinerea* untransformed and transformed percentage of embryos alive at hatch data (ln,
failed to meet the assumption of normality; however, untransformed data satisfied the assumption of homogeneity. Examination of the residual plot for the percentage of *U. cinerea* alive at hatch expressed normality, and analyses were performed on untransformed *U. cinerea* data. A 4-way ANOVA with species as an additional factor was not used due to the inability to achieve normality and homogeneity of variance for the combined data set.

The percentage of embryos alive at hatch was compared between species using a Kruskal-Wallis test in addition to mean and 95% confidence intervals. A Kruskal-Wallis test was used since data did not satisfy the assumptions of normality or homogeneity of variance required for an ANOVA. These data met the assumption of independence and were from treatments having the same continuous distribution which are required for the Kruskal-Wallis test.

**Egg Capsule Dry Weight**

*R. venosa* and *U. cinerea* egg capsule dry weight (mg) was evaluated in relation to the number of CDD at egg capsule collection using two species specific one-way ANOVAs in addition to mean and 95% confidence intervals. In each one-way ANOVA, for each species, the number of CDD at egg capsule collection was the factor, and the response was the standardized egg capsule dry weight (mg/mm of egg capsule height). Egg capsule dry weight (mg) was standardized by egg capsule height (mm) to account for differences in egg capsule height based on species. For *R. venosa* untransformed and transformed dry weight data (ln, log, (log + 1), square root, arcsin, sin, Box-Cox (Box
and Cox, 1964) and reciprocal) failed to meet the assumption of normality; however, untransformed data satisfied the assumption of homogeneity. Examination of the residual plot for \textit{R. venosa} egg capsule dry weight expressed normality, and analyses were performed on untransformed \textit{R. venosa} data. For \textit{U. cinerea} dry weight data satisfied the assumption of normality and homogeneity of variance without transformation. A 2-way ANOVA with species as an additional factor was not used due to the inability to achieve normality and homogeneity of variance with the combined data set.

Egg capsule dry weight was compared between species using a Kruskal-Wallis test in addition to mean and 95\% confidence intervals. A Kruskal-Wallis test was used since data did not satisfy the assumptions of normality or homogeneity of variance required for an ANOVA. These data met the assumption of independence and were from treatments having the same continuous distribution which are required for the Kruskal-Wallis test.
RESULTS

Environmental Variation

From 2007 to 2008, the York River daily mean water temperature and the York River water temperature as recorded in seawater flowing through the laboratory were similar (Figure 5A-D) and consistent with data across the 5 year period from 2003 to 2008 (Appendix II). The York River daily water temperature was recorded every 15 minutes at the VIMS oyster pier 20 cm above the substrate and averaged. The York River water temperature as recorded in seawater flowing through the laboratory supply was recorded once daily, between noon and 2 pm, in the ambient temperature chamber during water changes. The York River daily mean water temperature fluctuated between 8°C to 30°C across the period May 31, 2007 to November 4, 2007 and from 11°C to 29°C across the period June 12, 2008 to November 19, 2008 (Figure 5). Maximum temperatures of approximately 29°C were reached in August 2007 and 2008 (Figure 5). Differences between recorded York River daily mean water temperature and the York River water temperature as recorded in seawater flowing through the laboratory supply (Figure 5A) may be due to the frequency of recorded measurements or daily fluctuations such as the stage of the tidal cycle, recent storm events, water flow in the flume, or ventilation that affect the recorded temperature at the time of observation.

From 2007 to 2008, the York River daily mean salinity and the salinity of seawater flowing through the laboratory supply were similar from July through November
Figure 5: Average daily York River bottom water temperature and York River temperature recorded once daily in laboratory flow-through seawater (°C) during the experimental period in 2007 and 2008. The experimental period in 2007 was from May 31, 2007 to November 4, 2007. The experimental period in 2008 was from June 12, 2008 to November 19, 2008. A. 2007 mean bottom water temperature. B. 2007 water temperature as recorded in laboratory flow-through seawater conditions. C. 2008 mean bottom water temperature. D. 2008 water temperature as recorded in laboratory flow-through seawater conditions. The x-axis scale denote the 1st of each month. Data were accessed on January 20, 2009 (VIMS Molluscan Ecology Program at http://web.vims.edu/mollusc/envmon/01VOY/01VOY.htm).
A.

Water Temperature (°C)

York River Oyster Pier

Month

2007

B.

Water Temperature (°C)

York River Laboratory Supply

Month

2007

C.

Water Temperature (°C)

York River Oyster Pier

Month

2008

D.

Water Temperature (°C)

York River Laboratory Supply

Month

2008
(Figure 6A-D). In 2007, recorded salinities from June through July were as much as 10 ppt greater than salinities from the same period in 2008. Increases in the observed York River salinities from June to July 2007 when compared to the same period in 2008 were not due to low precipitation in 2007 (4.87 inches precipitation in 2007; 0.25 inches precipitation in 2008; mean of 7.20 inches precipitation from available data 1988 to 2008) as recorded at New Quarter Farm Airport, Gloucester, Virginia (Weather Underground at http://www.wunderground.com/history/airport/New%20quarter%20farm). Data from 2007 more closely follow the trend given by the mean monthly salinities across the 5 year period from 2003 to 2008 (Appendix II). The York River daily mean salinity fluctuated between 18 ppt and 24 ppt across the period May 31, 2007 to November 4, 2007 and from 15 ppt and 23 ppt across the period June 12, 2008 to November 19, 2008 (Figure 6). Maximum salinities of 23 ppt to 24 ppt were reached in November of 2007 and 2008. Some differences between recorded York River salinity and the salinity as recorded in seawater flowing through the laboratory supply may be due to differences in the equipment used to record salinity (electronic readings for York River bottom salinity versus a refractometer, calibrated weekly, for seawater flowing through the laboratory supply). Other sources of variation may include factors such as the stage of the tidal cycle or recent storm events at the time of observation.

Over the course of the experimental period in 2007 and 2008 (May 31, 2007 to November 4, 2007; June 12, 2008 to November 19, 2008) the day length, given by number of hours from sunrise to sunset, for Gloucester Point, Virginia (37°14′N, 76°29′W) ranged from 10.55 hours to 14.72 hours and 10.07 hours to 14.72 hours in
Figure 6: Mean York River bottom water salinity and York River salinity recorded once daily in laboratory flow-through seawater (ppt) during the experimental period in 2007 and 2008. The experimental period in 2007 was from May 31, 2007 to November 4, 2007. The experimental period in 2008 was from June 12, 2008 to November 19, 2008. A. 2007 mean bottom water salinity. B. 2007 salinity as recorded in laboratory flow-through seawater conditions. C. 2008 mean bottom water salinity. D. 2008 salinity as recorded in laboratory flow-through seawater conditions. The x-axis scale denote the 1st of each month. Data were accessed on January 20, 2009 (VIMS Molluscan Ecology Program at http://web.vims.edu/mollusc/envmon/01VOY/01VOY.htm).
2007 and 2008, respectively (Figure 7). Maximum day length of 14.72 hours was recorded for the days directly prior to, and directly after the summer solstice each year (June 18, 2007 to June 23, 2007 and June 17, 2008 to June 22, 2008) (U.S. Naval Observatory at http://aa.usno.navy.mil/data/docs/RS_OneYear.php). A one day offset in the period describing the maximum day length between 2007 and 2008 was due to a leap year occurring in 2008.

In 2007 and 2008 the environmental conditions (water temperature, salinity, day length, and the number of CDD at egg capsule collection) observed at the start and end of _R. venosa_ egg capsule deposition were recorded. In 2007, _R. venosa_ egg capsule deposition began with one individual depositing at VIMS on May 7, 2007 (16°C, 15 ppt, 13.92 hours day length, 185 CDD). The majority of the population began depositing egg capsules on May 14, 2007 (19°C, 15 ppt, 14.13 hours day length, 242 CDD). _Rapana venosa_ egg capsule deposition ended on August 20, 2007 at VIMS (28°C, 23 ppt, 13.42 hours day length, 1720 CDD). In 2008, _R. venosa_ egg capsule deposition began with one individual depositing at VIMS on April 28, 2008 (18°C, 15 ppt, 13.63 hours day length, 153 CDD). The majority of the population began depositing egg capsules on May 7, 2008 (19°C, 18 ppt, 13.93 hours day length, 225 CDD). In 2008, the period of egg capsule deposition for _R. venosa_ at VIMS ended on August 27, 2008 (25°C, 21 ppt, 13.13 hours day length, 1878 CDD). In 2007, the period between the beginning and end of the egg capsule deposition, or the egg capsule deposition duration, lasted 106 days and 1535 CDD from May 7, 2007 to August 20, 2007. In 2008, the egg capsule deposition duration lasted 122 days and 1725 CDD from April 28, 2008 to August 27, 2008.
Figure 7: 2007 and 2008 day length (hours per day from sunrise to sunset) for Gloucester Point, Virginia 37°14'N, 76°29'W) during the experimental period in 2007 and 2008. The experimental period in 2007 was from May 31, 2007 to November 4, 2007. A. 2007 day length. B. 2008 day length. The x-axis scale denotes the 1st of each month. Arrows indicate the dates of egg capsule collection. Data were accessed on April 23, 2009 (U.S. Naval Observatory at http://aa.usno.navy.mil/data/docs/RS_OneYear.php).
In 2008 the environmental conditions (water temperature, salinity, day length, and the
number of CDD at egg capsule collection) observed at the start and end of *U. cinerea* egg
capsule deposition at VIMS were recorded. Evidence of *U. cinerea* egg capsule
deposition was observed on the Eastern Shore of Virginia on the day of broodstock
collection (May 17, 2008) (17°C, 27 ppt, 14.17 hours day length). However, deposition
of *U. cinerea* egg capsules at VIMS under laboratory flow-through seawater conditions
did not begin until one day after the acclimation period ended on June 4, 2008 (22°C, 17
ppt, 14.6 hours day length, 487 CDD). The majority of the *U. cinerea* population at
VIMS began depositing egg capsules on June 9, 2008, 6 days after the acclimation period
ended (26°C, 16 ppt, 14.7 hours day length, 559 CDD). The summer period of egg
capsule deposition for *U. cinerea* at VIMS ended on August 25, 2008 (26°C, 21 ppt, 13.2
hours day length, 1847 CDD). The summer egg capsule deposition duration lasted 83
days and 1360 CDD from June 4, 2008 to August 25, 2008.

Exclusion of Data

*R. venosa* and *U. cinerea* egg capsules cultured at 7 ppt and egg capsules from
temperature-salinity combinations found to be contaminated with ciliates were excluded
from data analyses. For *R. venosa* and *U. cinerea* no egg capsules cultured at 7 ppt and
any temperature hatched. Including data from an entire salinity combination with 0% of
egg capsules to hatch for both *R. venosa* and *U. cinerea* did not allow for normality,
despite transformation. *U. cinerea* egg capsules collected on 609 CDD and cultured at all
temperature-salinity combinations and *U. cinerea* egg capsules collected on 1153 CDD
and cultured at ambient salinity were excluded based on observation of ciliates.
Termination occurred on July 24, 2008 (day 43 for egg capsules collected on 609 CDD; day 9 for egg capsules collected on 1153 CDD). As previously stated, egg capsule hatching was observed to occur on at least the 75th day of incubation. Data from collection dates noted were excluded from analyses since termination occurred prior to the 75th day of incubation, and the full period for potential egg capsule hatching was not reached.

Egg Capsule Height

*Rapana venosa* and *U. cinerea* egg capsule height varied based on the size range of females depositing egg capsules at the time of egg capsule collection (Carriker, 1955; Harding et al., 2007). Differences in egg capsule height between collection periods are important when comparing factors (such as egg capsule dry weight) that may vary by egg capsule height (Harding et al., 2007). In 2007, collected *R. venosa* egg capsules ranged in height from 9.0 to 34.7 mm (Figure 8A-C). In 2008, collected *R. venosa* egg capsules ranged in height from 14.7 to 30.2 mm (Figure 8D-F). Collected *U. cinerea* egg capsules ranged in height from 5.1 to 10.7 mm (Figure 8G-I). *Rapana venosa* egg capsules collected on 898 CDD (July 4, 2007) had a shorter egg capsule height frequency distribution than all other collection dates based on the size range of egg capsules available on the date of egg capsule collection. *Urosalpinx cinerea* egg capsules from all collection periods had a shorter egg capsule height frequency than *R. venosa* egg capsules.
Figure 8: Distribution of *R. venosa* and *U. cinerea* egg capsule height by the number of CDD at egg capsule collection. A. 2007 *R. venosa* 413 CDD; N = 150. B. 2007 *R. venosa* 898 CDD; N = 150. C. 2007 *R. venosa* 1381 CDD; N = 150. D. 2008 *R. venosa* 609 CDD; N = 16. E. 2008 *R. venosa* 1153 CDD; N = 10. F. 2008 *R. venosa* 1442 CDD; N = 10. G. 2008 *U. cinerea* 609 CDD; N = 250. H. 2008 *U. cinerea* 1153 CDD; N = 250. I. 2008 *U. cinerea* 1442 CDD; N = 250. Bin sizes were based on the maximum range of egg capsule height for each species. Mid-point values for each bin (5 mm for *R. venosa* or 1 mm for *U. cinerea*) are given along the x-axis. Note differences in x-axis scale between *R. venosa* and *U. cinerea* based on bin sizes.
Urosalpinx cinerea 2008

609 CDD.

G. 2008

1153 CDD.

H. 2008

1442 CDD.

I. 2008
Chance of Egg Capsule Hatch

Deviations in the total number of observed successfully hatching egg capsules (from a 50% chance of successful hatch expected) occurred based on the temperature-salinity combination during egg capsule culture. In 2007 and 2008, differences from 50% hatch occurred in 63 temperature-salinity-CDD combinations. The temperature-salinity combinations in which the total number of successfully hatching egg capsules deviated significantly above or below the expected 50% hatch are presented in bold in Table 4. The null hypotheses stating that environmental conditions have no effect on the chance of an egg capsule to hatch were not accepted.

Percentage of Egg Capsules to Hatch

*Rapana venosa* egg capsules did not hatch at 7 ppt. The percentage of successfully hatching *R. venosa* egg capsules at each temperature-salinity combination varied from 17% to 100% and increased significantly with increased salinity and number of CDD at egg capsule collection. The null hypotheses stating that salinity or the number of CDD at egg capsule collection have no effect on the percentage of *R. venosa* egg capsules to hatch were not accepted. The null hypotheses stating that temperature had no effect on the percentage of *R. venosa* egg capsules to hatch was accepted. The lowest percentages of egg capsules to hatch (17%) were recorded from egg capsules collected on May 31, 2007 at 413 CDD and the temperature-salinity combinations of: 18°C and 14 ppt; 30°C and 21ppt; 30°C and ambient salinity; ambient temperature and 14 ppt (Figure 9A). The highest percentages of *R. venosa* egg capsules to hatch (100%) were recorded for the temperature-salinity combinations of: ambient temperature and ambient salinity (413

52
Table 4: Percentage (%) of *Rapana venosa* and *Urosalpinx cinerea* egg capsules to hatch within a given temperature-salinity combination. Temperatures and salinities in which the percentage of egg capsules to hatch significantly deviated from 50% are given in bold. The maximum numbers of *R. venosa* and *U. cinerea* egg capsules in each temperature-salinity combination were 6 egg capsules at 413, 898, and 1381 CDD, 16 egg capsules at 609 CDD, or 10 egg capsules at 1153 and 1142 CDD. *U. cinerea* egg capsules collected on 609 CDD (all temperature-salinity combinations), and 1153 CDD (ambient salinity only) were removed from analyses based on observation of ciliates. ND = no data are available. T = termination of experiment.
Figure 9: Percentage of *Rapana venosa* egg capsules to hatch in 2007 and 2008 based on
the number of CDD at egg capsule collection. A. 413 CDD; N = 60 egg capsules;
Experiment duration = 97 days. B. 898 CDD; N = 72 egg capsules; Experiment duration
= 96 days. C. 1381 CDD; N = 99 egg capsules; Experiment duration = 94 days. D. 609
CDD; N = 16 egg capsules; Experiment duration = 31 days; 898 CDD; N = 10;
Experiment duration = 27 days; 1381 CDD; N = 10 egg capsules; Experiment duration =
31 days. N = number of egg capsules to hatch at least one embryo. The maximum
number of egg capsules in each temperature-salinity combination is 6 at 413 CDD, 898
CDD, and 1381 CDD. The maximum number of egg capsules in each temperature-
salinity combination is 16, 10, and 10 at 609 CDD, 1153 CDD, and 1442 CDD
respectively. Data are not displayed at 7 ppt given no egg capsules hatching.
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<td>ND</td>
<td>T</td>
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</tr>
<tr>
<td>Ambient</td>
<td>Ambient</td>
<td>100</td>
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<td>ND</td>
<td>83</td>
<td>ND</td>
<td>T</td>
<td>80</td>
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</table>
Successful hatching of *R. venosa* egg capsule occurs at salinities of 14 ppt, 21 ppt, and 28 ppt (Table 5, Figure 10B-C). The percentage of egg capsules to hatch is significantly higher (with overlapping confidence intervals) at ambient salinity, relative to 14 ppt or 28 ppt and at 21 ppt relative to 28 ppt. Given survival of *R. venosa* populations across the geographic range in environments with salinities ranging from > 12 ppt (Rio de la Plata estuary; Giberto et al., 2006) to 33 ppt (Korea; Chukhchin, 1984; KODC), successful hatching of *R. venosa* egg capsules at salinities of 14 ppt, 21 ppt, and 28 ppt are within the salinity range supporting reproductive populations along the geographic range.

Along the egg capsule deposition time series in 2007 (413 CDD to 1381 CDD), the percentage of *R. venosa* egg capsules to hatch increased significantly (with non-overlapping confidence intervals) with the number of CDD at egg capsule collection and within the same temperature-salinity combination (Tables 4-5, Figures 13, 14C). The percentage of egg capsules to hatch is significantly higher (with non-overlapping confidence intervals) at 1381 CDD relative to 413 CDD. Instances in which the percentage of egg capsules to hatch did not increase with increasing number of CDD at egg capsule collection include egg capsules cultured at 7 ppt and any temperature, 21 ppt and 18°C, 21 ppt and 26°C (in 2007), ambient salinity and 22°C, and ambient temperature and ambient salinity (Figure 9). An increase in the percentage of egg capsules to hatch along the egg capsule deposition time series was also observed in 2008 (Table 4, Figures 9, 14C). Possible explanations for the increased percentage of egg capsules to hatch with increasing number of CDD could include differences in ambient salinity at the time of
Table 5: Summary table of statistical analyses regarding the percentage of *Rapana venosa* and *Urosalpinx cinerea* egg capsules to hatch. Analyses include all experiments in which the egg capsules were given in excess of 75 days to hatch. NA = not applicable. DF = degrees of freedom. The F statistic and Fisher’s multiple comparison test are associated with the ANOVA test. The H statistic and the Dunn’s multiple comparison test are associated with the Kruskal-Wallis test.
<table>
<thead>
<tr>
<th>Test</th>
<th>Factor(s)</th>
<th>Response</th>
<th>DF</th>
<th>F or H statistic</th>
<th>p-value</th>
<th>Fisher’s or Dunn’s Multiple Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-way ANOVA</td>
<td>Temperature</td>
<td>Percentage of <em>R. venosa</em> egg capsules to hatch</td>
<td>4</td>
<td>1.42</td>
<td>0.25</td>
<td>NA</td>
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<td></td>
<td>Salinity</td>
<td>Percentage of <em>R. venosa</em> egg capsules to hatch</td>
<td>3</td>
<td>5.90</td>
<td>&lt;0.01</td>
<td>14 ppt &lt; 21ppt, Ambient; 21 ppt &gt; 28 ppt; 28 ppt &lt; Ambient</td>
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<td></td>
<td>Cumulative Day Degrees (CDD)</td>
<td>Percentage of <em>R. venosa</em> egg capsules to hatch</td>
<td>5</td>
<td>5.01</td>
<td>&lt;0.01</td>
<td>413 CDD &lt; 1381 CDD</td>
</tr>
<tr>
<td></td>
<td>Temperature-salinity interaction</td>
<td>Percentage of <em>R. venosa</em> egg capsules to hatch</td>
<td>12</td>
<td>0.82</td>
<td>0.63</td>
<td>NA</td>
</tr>
<tr>
<td>3-way ANOVA</td>
<td>Temperature</td>
<td>Percentage of <em>U. cinerea</em> egg capsules to hatch</td>
<td>4</td>
<td>1.21</td>
<td>0.35</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Salinity</td>
<td>Percentage of <em>U. cinerea</em> egg capsules to hatch</td>
<td>3</td>
<td>74.81</td>
<td>&lt;0.01</td>
<td>14 ppt &lt; 21ppt, 28 ppt, Ambient</td>
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<td>Cumulative Day Degrees (CDD)</td>
<td>Percentage of <em>U. cinerea</em> egg capsules to hatch</td>
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<td>3.74</td>
<td>0.07</td>
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<td>Temperature-salinity interaction</td>
<td>Percentage of <em>U. cinerea</em> egg capsules to hatch</td>
<td>12</td>
<td>0.24</td>
<td>0.99</td>
<td>NA</td>
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<tr>
<td>Kruskal-Wallis</td>
<td>Species</td>
<td>Percentage of egg capsules to hatch</td>
<td>1</td>
<td>0.06</td>
<td>0.81</td>
<td>NA</td>
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</table>
Figure 10: Mean values and 95% confidence intervals for factors regarding the percentage of *Rapana venosa* and *Urosalpinx cinerea* egg capsules to hatch. A. *R. venosa* percentage of egg capsules to hatch versus temperature. B. *R. venosa* percentage of egg capsules to hatch versus salinity. C. *R. venosa* percentage of egg capsules to hatch versus the number of CDD. D. *U. cinerea* percentage of egg capsules to hatch versus temperature. E. *U. cinerea* percentage of egg capsules to hatch versus salinity. F. *U. cinerea* percentage of egg capsules to hatch versus the number of CDD. G. Percentage of egg capsules to hatch versus species. Data are not displayed at 7 ppt given no egg capsules hatching for either species. *U. cinerea* egg capsules collected on 609 CDD (all temperature-salinity combinations), and 1153 CDD (ambient salinity only) were removed from analyses based on observation of ciliates. CDD = cumulative day degrees at egg capsule collection. ND = no data are available. T = termination of experiment. Ambient temperature range was 13°C - 30°C. Ambient salinity range was 15 ppt – 24 ppt.
egg capsule deposition, or a change in egg capsule quality or maternal investment over the egg capsule deposition time series. N values were the same at each CDD of egg capsule collection and are likely not a contributing factor for the increased percentage of egg capsules to hatch with increasing number of CDD.

_**Urosalpinx cinerea** egg capsules did not hatch at 7 ppt. The percentage of successfully hatching _U. cinerea_ egg capsules varied from 10% to 100% and decreased significantly at salinities of 14 ppt (with non-overlapping confidence intervals). The null hypothesis stating that salinity has no effect on the percentage of _U. cinerea_ egg capsules to hatch was not accepted. The null hypotheses stating that temperature or the number of CDD at egg capsule collection have no effect on the percentage of _U. cinerea_ egg capsules to hatch was accepted. The lowest percentage of _U. cinerea_ egg capsules to hatch (10%) was recorded at ambient temperature and 14 ppt (1153 CDD) (Figure 11A). The highest percentage of _U. cinerea_ egg capsules to hatch (100%) was recorded at 30°C and 28 ppt (1442 CDD) (Figure 11B).

Successful _U. cinerea_ egg capsule hatching was observed at salinities of 14 ppt, 21 ppt, and 28 ppt, depending on temperature. The percentages of egg capsules to hatch were significantly higher at ambient salinity, 21 ppt and 28 ppt relative to 14 ppt (with non-overlapping confidence intervals) (Table 5, Figure 10E). Given survival of _U. cinerea_ populations in environments with salinities ranging ≥ 12 ppt (Hampton Roads, Virginia; Federighi, 1931) to ≤ 35 ppt (under laboratory controlled conditions; Engle, 1935-1936) successful hatching of _U. cinerea_ egg capsules at 14 ppt, 21 ppt, and 28 ppt are well within the salinity ranges that support adult populations.
Figure 11: Percentage of *Urosalpinx cinerea* egg capsules to hatch in 2008. A. 1153 CDD; N = 89 egg capsules; Experiment duration = 127 days. B. 1442 CDD; N = 115 egg capsules; Experiment duration = 111 days. ND = no data are available. N = the number of egg capsules to hatch at least one embryo. Data are not displayed at 7 ppt given no egg capsules hatching. *U. cinerea* egg capsules collected on 609 CDD (all temperature-salinity combinations), and 1153 CDD (ambient salinity only) were removed from analyses based on observation of ciliates. The maximum number of egg capsules in each temperature-salinity combination is 10.
A. Percentage of Egg Capsules to Hatch

- Salinity 14 ppt
- Salinity 21 ppt
- Salinity 28 ppt
- Salinity Ambient

100 80 60 40 20

Temperature (°C)

B. Percentage of Egg Capsules to Hatch

- Salinity 14 ppt
- Salinity 21 ppt
- Salinity 28 ppt
- Salinity Ambient

100 80 60 40 20

Temperature (°C)
The null hypothesis stating that species has no effect on the percentage of egg capsules to hatch was accepted.

**Incubation Time**

The incubation time of *R. venosa* egg capsules varied from 9 days to 68 days and increased significantly at lower temperatures and increased significantly at a lower number of CDD at egg capsule collection. A significant interaction occurred between temperature and salinity. The null hypotheses stating that temperature or the number of CDD at egg capsule collection have no effect on *R. venosa* egg capsule incubation time was not accepted. The null hypothesis stating that salinity has no effect on *R. venosa* egg capsules incubation time was accepted. The shortest *R. venosa* incubation time (9 days) was recorded at 30°C and 28 ppt (898 CDD) (Table 6A). The longest incubation time (68 days) was recorded at 18°C and 28 ppt (1381 CDD) (Table 6A).

*Rapana venosa* incubation time decreased significantly (with non-overlapping confidence intervals) by as much as 70% (18°C versus 30°C) over the temperature range examined (Table 7, Figure 12A). The egg capsule incubation time was significantly higher at 18°C relative to 22°C, 22°C relative to 26°C, and 26°C relative to 30°C. Temperature has long been known to be an important factor affecting rate processes in molluscs (Kinne, 1970, 1971; Spight, 1975; Schmidt-Nielson, 1997) such as the Atlantic slipper snail, *Crepidula fornicata*, (Pechenik, 1984), Eastern white slipper snail, *Crepidula plana* (Zimmerman and Pechenik, 1991), Eastern mudsnail, *Nassarius obsoletus* (Scheltema, 1967), and dog whelk, *Thais lapillus* (Largen, 1967). In general at high temperatures, rate processes occur faster than at low temperatures. *R. venosa*
Table 6: Range in the incubation time (days) for successful hatching of *Rapana venosa* and *Urosalpinx cinerea* egg capsules, with N egg capsules in parentheses. A. *R. venosa* in 2007. B. *R. venosa* in 2008. C. *U. cinerea* in 2008. NHO = no hatch observed. ND = no data are available. Analyses include all experiments in which the egg capsules within the matrix were given in excess of 75 days to hatch.
<table>
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<tr>
<th>Culture Salinity</th>
<th>18°C</th>
<th>22°C</th>
<th>26°C</th>
<th>30°C</th>
<th>Ambient</th>
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<tbody>
<tr>
<td>7 ppt</td>
<td>NOH</td>
<td>NOH</td>
<td>NOH</td>
<td>NOH</td>
<td>NOH</td>
</tr>
<tr>
<td>14 ppt</td>
<td>56 (1)</td>
<td>27 - 57 (13)</td>
<td>18 - 41 (13)</td>
<td>14 - 20 (9)</td>
<td>12 - 22 (11)</td>
</tr>
<tr>
<td>21 ppt</td>
<td>39 - 60 (11)</td>
<td>22 - 44 (14)</td>
<td>14 - 31 (12)</td>
<td>13 - 22 (12)</td>
<td>15 - 34 (14)</td>
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<tr>
<td>28 ppt</td>
<td>41 - 68 (7)</td>
<td>22 - 42 (9)</td>
<td>17 - 30 (9)</td>
<td>9 - 18 (8)</td>
<td>11 - 16 (6)</td>
</tr>
<tr>
<td>Ambient</td>
<td>41 - 67 (15)</td>
<td>23 - 48 (12)</td>
<td>11 - 29 (17)</td>
<td>12 - 22 (11)</td>
<td>14 - 41 (15)</td>
</tr>
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</table>

| 21 ppt | ND | ND | 21 - 24 (26) | ND | ND |

<table>
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<tr>
<th>7 ppt</th>
<th>18°C</th>
<th>22°C</th>
<th>26°C</th>
<th>30°C</th>
<th>Ambient</th>
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<tr>
<td>14 ppt</td>
<td>NOH</td>
<td>50 - 64 (3)</td>
<td>NOH</td>
<td>28 - 37 (2)</td>
<td>21 - 54 (2)</td>
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<tr>
<td>21 ppt</td>
<td>43 - 67 (14)</td>
<td>25 - 54 (17)</td>
<td>14 - 36 (14)</td>
<td>19 - 37 (13)</td>
<td>19 - 49 (17)</td>
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<tr>
<td>Ambient</td>
<td>39 - 63 (7)</td>
<td>32 - 39 (9)</td>
<td>16 - 26 (8)</td>
<td>16 - 24 (8)</td>
<td>16 - 25 (8)</td>
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Table 7: Summary table of statistical analyses for incubation time (days) of *Rapana venosa* and *Urosalpinx cinerea* egg capsules. NA = not applicable. Analyses include all experiments in which the egg capsules within the matrix were given in excess of 75 days to hatch. The F statistic and Fisher’s multiple comparison test are associated with the ANOVA test. The H statistic and the Dunn’s multiple comparison test are associated with the Kruskal-Wallis test.
<table>
<thead>
<tr>
<th>Test</th>
<th>Factor(s)</th>
<th>Response</th>
<th>DF</th>
<th>F or H statistic</th>
<th>p-value</th>
<th>Fisher’s or Dunn’s Multiple Comparison</th>
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<td>95.61</td>
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<td>18°C &gt; 22°C &gt; 26°C &gt; 30°C; 18°C &gt; 22°C &gt; Ambient</td>
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<td>Salinity</td>
<td><em>R. venosa</em> incubation time</td>
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<td>2.12</td>
<td>0.10</td>
<td>NA</td>
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<td>Cumulative day degrees (CDD)</td>
<td><em>R. venosa</em> incubation time</td>
<td>2</td>
<td>15.25</td>
<td>&lt;0.01</td>
<td>413 CDD &gt; 609 CDD, 898 CDD, 1153 CDD, 1381 CDD, 1442 CDD</td>
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<td></td>
<td>Temperature-salinity interaction</td>
<td><em>R. venosa</em> incubation time</td>
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<td>3.91</td>
<td>&lt;0.01</td>
<td>NA</td>
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<tr>
<td>3-way ANOVA</td>
<td>Temperature</td>
<td><em>U. cinerea</em> incubation time</td>
<td>4</td>
<td>141.25</td>
<td>&lt;0.01</td>
<td>18°C &gt; 22°C &gt; 26°C &gt; 30°C; 23 = 30; 18°C &gt; 22°C &gt; Ambient</td>
</tr>
<tr>
<td></td>
<td>Salinity</td>
<td><em>U. cinerea</em> incubation time</td>
<td>3</td>
<td>18.15</td>
<td>&lt;0.01</td>
<td>14ppt &gt; 28 ppt, Ambient; 21ppt = 28 ppt = Ambient</td>
</tr>
<tr>
<td></td>
<td>Cumulative day degrees (CDD)</td>
<td><em>U. cinerea</em> incubation time</td>
<td>1</td>
<td>7.14</td>
<td>&lt;0.01</td>
<td>1153 CDD &lt; 1442 CDD</td>
</tr>
<tr>
<td>Kruskal-Wallis</td>
<td>Species</td>
<td>Incubation time</td>
<td>1</td>
<td>18.69</td>
<td>&lt;0.01</td>
<td><em>R. venosa</em> &lt; <em>U. cinerea</em></td>
</tr>
</tbody>
</table>
Figure 12: Mean values and 95% confidence intervals for incubation time (days) of *Rapana venosa* and *Urosalpinx cinerea* egg capsules. A. *R. venosa* incubation time versus temperature. B. *R. venosa* incubation time versus salinity. C. *R. venosa* incubation time versus the number of CDD at egg capsule collection. D. *U. cinerea* incubation time versus temperature. E. *U. cinerea* incubation time versus salinity. F. *U. cinerea* incubation time versus the number of CDD at egg capsule collection. G. Incubation time versus species. CDD = cumulative day degrees at egg capsule collection. Data are not displayed at 7 ppt given no egg capsules hatching. ND = no data are available. T = termination of experiment. ◇ = high variability due to low sample size (N = 6). Ambient temperature range was 13°C - 30°C. Ambient salinity range was 15 ppt - 24 ppt.
incubation times from this study fall within the previously observed range of 5 days to 60
days incubation at similar temperatures of 22°C to 27°C (Ware, 2002; Harding, 2006).
Given previous descriptions of the relationship between an increase in culture
temperature and a decrease in egg capsule incubation time for other muricids with direct
development (Kinne, 1970, 1971; Spight, 1975; Schmidt-Nielson, 1997) the observed
relationship for \textit{R. venosa} is consistent with previous work (e.g. \textit{Thais bufo} 24 days at
24°C, salinity not noted: Chari, 1950; \textit{Thais haemastoma} (= \textit{S. haemastoma}) 24 days at
24°C, salinity not noted: D’Asaro, 1966; \textit{Ocenebra poulsoni} 21 to 28 days at 17 to 20°C
and \textit{Shaskyus festivus} 21 to 28 days at 17 to 20°C, salinity not noted: Fotheringham,
1971).

Along the egg capsule deposition time series, \textit{R. venosa} egg capsule incubation time
decreased significantly by as much as 34% at 413 CDD relative to 1381 CDD (with non­
overlapping confidence intervals) (Table 7; Figure 12). A decrease in the incubation time
across the egg capsule deposition time series is not isolated to the egg capsule deposition
time series in 2007. Similar observations were recorded for \textit{R. venosa} egg capsules
cultured at 26°C and 21 ppt in 2008 (Figure 12C). Egg capsule incubation time may
decrease with an increasing number of CDD at egg capsule collection given higher water
temperatures during egg capsule production and possible development of embryos prior
to egg capsule deposition. Scheltema (1967) previously noted that increased temperature
increased the stage of embryonic development for \textit{Nassarius obsoletus} at the time of egg
capsule deposition. A potential increase in embryonic development of \textit{R. venosa} prior to
egg capsule deposition may occur in a similar fashion to \textit{N. obsoletus} given that egg
capsule production follows the same general pattern and both species express mixed development (Scheltema, 1967; Sullivan and Maugel, 1984).

The number of days required for *U. cinerea* embryos to hatch from the egg capsule varied from 11 days to 76 days and increased with decreased temperature, decreased salinity, and increased number of CDD at egg capsule collection (Table 6, Figure 12D - F). The null hypotheses stating that temperature, salinity, or the number of CDD at egg capsule collection have no effect on *U. cinerea* egg capsule incubation time were not accepted. The shortest incubation time (11 days) was recorded at ambient York River temperature and 28 ppt (1153 CDD) (Table 6C). The longest incubation time (76 days) was recorded at 26°C and 28 ppt (1442 CDD) (Table 6C).

*Urosalpinx cinerea* incubation time decreased significantly (with non-overlapping confidence intervals) by as much as 58% (18°C versus 30°C, or ambient temperature) over the temperature range examined (Table 7, Figure 12D). The egg capsule incubation time was significantly higher at 18°C relative to 22°C, and 22°C relative to 26°C.

Observations of *U. cinerea* incubation time from this study fall within the previously observed range of 18 days (Carriker, 1955 at 23°C to 29°C) to 78 days incubation (Ganaros, 1958 at 15°C to 30°C) and are consistent with incubation times for other muricids with direct development (e.g. *Ocenebra erinacea* 84 to 91 days at 10 to 15°C, salinity not noted: Hancock, 1960; *Torvamurex territus* 90 days at 20°C, salinity not noted: Murray and Goldsmith, 1963; *Ceratostoma foliatum* 120 days at 10 to 12°C, salinity not noted: Spight et al., 1974).

*Urosalpinx cinerea* egg capsule incubation time decreased significantly (with overlapping confidence intervals) by as much as 33% (14 ppt versus 28 ppt or ambient)
over the salinity range examined (Table 7, Figure 12E). The egg capsule incubation time was significantly higher at 14 ppt relative to 28ppt and ambient salinity. Field observations of *U. cinerea* egg capsule development previously noted limited embryonic development to hatch at salinities < 14 ppt in Hampton Roads, Virginia (ambient temperatures >18°C; Federighi, 1931) and Delaware Bay (ambient temperatures > 13°C; Stauber, 1943). An increased number of days required for egg capsule incubation (Figure 12E) may be associated with decreased percentage of embryos hatched alive at 14 ppt (Federighi, 1931; Stauber, 1943).

*Urosalpinx cinerea* egg capsule incubation time decreased significantly (with overlapping confidence intervals) by 2% along the egg capsule deposition time series (Table 7; Figure 12F). Egg capsule incubation time was significantly higher at 1442 CDD relative to 1153 CDD (Table 7, Figure 12F). Although statistically significant, a 2% variation in egg capsule incubation time may not be biologically meaningful given the large differences in incubation time based on culture temperature or salinity.

Species significantly affect the egg capsule incubation time (with non-overlapping confidence intervals) such that *U. cinerea* egg capsules require an average of 6 additional days to hatch relative to *R. venosa* egg capsules (Table 6, Figure 12G). The null hypothesis that species has no effect on the number of days required for incubation was not accepted. Given the planktonic period for *R. venosa* after hatch (> 24 days; Harding, 2006), the statistically significantly increased incubation time required by *U. cinerea* egg capsules may not be largely relevant biologically, since newly hatched *U. cinerea* will likely reach the benthos before *R. venosa* while avoiding potential mortality associated with a planktonic existence. A difference in 6 days incubation when comparing a mixed
developing species to a direct developing species supports Spight’s (1975) observation that the incubation periods are “little, if at all, longer for metamorphosed hatchings [direct developing species] than for swimming hatchlings [mixed developing species]”. Spight’s (1975) observations were based on a literature review of the incubation time required for the egg capsules of 12 direct developing muricids (Torvamurex territus, Ocenebra erinacea, O. japonica, O. aciculate, U. cinerea, E. caudate, Ceratostoma foliatum, Bedova hanleyi, Thais emarginata, T. lamelllose, T. dubia, Acanthina spirata) and 13 mixed developing species (Murex incarnates, M. ramosus, M. virgineus, M. trapa, Ocenebra poulsoni, Shaskyus festivus, Bedevina biriliffi, R. thomasiana (= R. venosa), Jopas francolinum, Thais haemastoma (= S. haemastoma), T. bufo, Morula marginalba, Purpura patula) across a range of temperatures from 6°C to 32°C, depending on species. The range of salinities required for embryonic development was not reported.

**Percentage of Embryos Hatched Alive From the Egg Capsule**

The percentage of embryos hatched alive from successfully hatching *R. venosa* egg capsules ranged from 67% to 100% and was not significantly affected by temperature, salinity, or the number of CDD at egg capsule collection (Table 8; Figure 13). The null hypotheses stating that the temperature, salinity, or the number of CDD at egg capsule collection have no effect on the percentage of embryos alive at hatch were accepted.

Of the 246 out of 450 *R. venosa* egg capsules to successfully hatch, only 3 egg capsules hatched less than 100% embryos alive at hatch (67%, 90% and 99% embryos alive, respectively). The lowest percentage of embryos alive at hatch (67%) was recorded from one egg capsule collected on 413 CDD and cultured at 22°C and 21 ppt.
Table 8: Summary table of statistical analyses for the percentage of embryos alive at hatch from the egg capsules of *Rapana venosa* and *Urosalpinx cinerea*. Analyses include all experiments in which the egg capsules within the matrix were given in excess of 75 days to hatch. NA = not applicable. DF = degrees of freedom. The F statistic and Fisher’s multiple comparison test are associated with the ANOVA test. The H statistic and the Dunn’s multiple comparison test are associated with the Kruskal-Wallis test.
<table>
<thead>
<tr>
<th>Test</th>
<th>Factor(s)</th>
<th>Response</th>
<th>DF</th>
<th>F or H statistic</th>
<th>p-value</th>
<th>Fisher’s or Dunn’s Multiple Comparison</th>
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<tbody>
<tr>
<td>3-way ANOVA</td>
<td>Temperature</td>
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<td>0.50</td>
<td>0.74</td>
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<td></td>
<td>Salinity</td>
<td>Percentage of <em>R. venosa</em> embryos alive at hatch</td>
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<td>0.78</td>
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<td>Cumulative day degrees (CDD)</td>
<td>Percentage of <em>R. venosa</em> embryos alive at hatch</td>
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<td>1.17</td>
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<td>Temperature-salinity interaction</td>
<td>Percentage of <em>R. venosa</em> embryos alive at hatch</td>
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<td>0.87</td>
<td>0.58</td>
<td>NA</td>
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<td>3-way ANOVA</td>
<td>Temperature</td>
<td>Percentage of <em>U. cinerea</em> embryos alive at hatch</td>
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<td>0.88</td>
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<td>NA</td>
</tr>
<tr>
<td></td>
<td>Salinity</td>
<td>Percentage of <em>U. cinerea</em> embryos alive at hatch</td>
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<td>&lt;0.01</td>
<td>14 ppt &lt; 21 ppt, 28 ppt, Ambient</td>
</tr>
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<td></td>
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<td>Percentage of <em>U. cinerea</em> embryos alive at hatch</td>
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<td>&lt;0.01</td>
<td>1153 CDD &lt; 1442 CDD</td>
</tr>
<tr>
<td>Kruskal-Wallis</td>
<td>Species</td>
<td>Percentage of embryos alive at hatch</td>
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<td>179.58</td>
<td>&lt;0.01</td>
<td><em>R. venosa &gt; U. cinerea</em></td>
</tr>
</tbody>
</table>
Figure 13: Mean values and 95% confidence intervals for the percentage of *R. venosa* and *U. cinerea* embryos alive at hatch. A. *R. venosa* number of embryos at hatch versus temperature. B. *R. venosa* number of embryos at hatch versus salinity. C. *R. venosa* number of embryos at hatch versus the number of CDD at egg capsule collection. D. *U. cinerea* number of embryos at hatch versus temperature. E. *U. cinerea* number of embryos at hatch versus salinity. F. *U. cinerea* number of embryos at hatch versus the number of CDD at egg capsule collection. G. Number of embryos at hatch versus species. Data are not displayed at 7 ppt given no egg capsules hatching. ND = no data are available. T = termination of experiment. ◊ = high variability due to low sample size (N = 6). Ambient temperature range was 13°C - 30°C. Ambient salinity range was 15 ppt – 24 ppt.
One egg capsule collected on 413 CDD and cultured at 26°C and ambient salinity hatched 90% of the embryos alive at the time of hatch. Finally, one egg capsule collected on 413 CDD and cultured at ambient temperature and 14 ppt hatched 99% of the embryos alive at hatch. At least one egg capsule in all temperature-salinity combinations (excluding those at 7 ppt and any temperature) hatched 100% embryos alive (Figure 13).

The percentage of embryos hatched alive from successfully hatching *U. cinerea* egg capsules ranged from 10% to 100% and decreased significantly at a salinity of 14 ppt and decreased significantly with increased number of CDD (Table 8; Figure 13). The null hypotheses stating that salinity or the number of CDD have no effect on the percentage of embryos alive at hatch were not accepted. The null hypothesis stating that temperature has no effect on the percentage of embryos alive at hatch was accepted. The lowest percentages of *U. cinerea* embryos hatched alive from a single egg capsule (10%) were recorded on 1153 CDD and cultured at 30°C and 28 ppt and one egg capsule collected on 1153 CDD and cultured at 30°C and 14 ppt. One hundred percent embryos alive at the time of hatch were recorded from at least one egg capsule cultured at 21 ppt and all temperatures (1153 CDD); 28 ppt and all temperatures (1153 CDD); 21 ppt and temperatures of 22°C, 30°C, and ambient temperature (1442 CDD); 28 ppt and all temperatures (1442 CDD); ambient salinity and temperatures of 22°C, 26°C, and 30°C (1442 CDD).

The percentage of *U. cinerea* embryos alive at hatch decreased significantly (with non-overlapping confidence intervals) at 14 ppt relative to 21 ppt, 28 ppt or ambient salinity (Table 8, Figure 13B). Previous studies have estimated *U. cinerea* embryonic mortality rates during encapsulation in the wild between 13% (Cole, 1942) in British
oyster beds (ambient temperature, salinity not noted) to 50% (Haskin, 1935) in Barnegat Bay, New Jersey (ambient temperature, salinity not noted). Embryonic mortality was higher in this study than the observations by Cole (1942) or Haskin (1935). A significantly decreased number of embryos hatched from the egg capsules at salinities of 14 ppt indicate the lower tolerance of \textit{U. cinerea} embryos to salinities of 14 ppt.

The percentage of embryos alive at hatch decreased significantly (with overlapping confidence intervals) by as much as 12% (Table 8, Figure 13F) at 1442 CDD relative to 1153 CDD. \textit{Urosalpinx cinerea} embryonic mortality has not previously been described in relation to the egg capsule deposition time series. A decrease in the percentage of embryos alive at hatch according to the CDD at egg capsule collection could be influenced by maternal investment in the egg capsules or other external conditions prior to egg capsule collection.

The percentage of embryos alive at hatch was significantly increased (with non-overlapping confidence intervals) in \textit{R. venosa} egg capsules relative to \textit{U. cinerea} egg capsules (Table 8, Figure 13G). The null hypothesis stating that species has no effect on the percentage of embryos alive at hatch was not accepted. Developmental strategy and/or \textit{r} versus \textit{k} selection may be important factors influencing the greater percentage of \textit{R. venosa} embryos to be hatched alive from the egg capsule. Since metamorphosis must be completed within the egg capsule rather than in the plankton, incubation may require a longer period (Spight, 1975); thus, increasing the probability for embryonic mortality.
Egg Capsule Dry Weight

*Rapana venosa* egg capsule dry weight ranged from 0.08 mg/mm (1381 CDD) to 1.23 mg/mm (413 CDD) and decreased significantly (with non-overlapping confidence intervals) at 413 CDD relative to 898 CDD and 1381 CDD (Table 9; Figure 14A). The null hypothesis stating that the number of CDD has no effect on egg capsule dry weight was not accepted for *R. venosa*. *Rapana venosa* egg capsules designated for dry weight analysis ranged from 8.8 mm to 33.8 mm in height. A height frequency distribution of egg capsule height used for *R. venosa* dry weight analyses can be found in Appendix VI (A-C, and F-H). The greatest dry weight of *R. venosa* egg capsules was observed at the beginning of the egg capsule deposition time series (Figure 14).

*Urosalpinx cinerea* egg dry weight ranged from 0.26 mg/mm (1442 CDD) to 0.61 mg/mm (1442 CDD) (with overlapping confidence intervals) and no significant variation was observed based on the number of CDD at egg capsule collection (Table 9; Figure 14B). The null hypothesis stating that the number of CDD has no effect on egg capsule dry weight was accepted for *U. cinerea*. *Urosalpinx cinerea* egg capsule designated for dry weight analysis ranged from 5.8 mm to 9.5 mm in height. A distribution of egg capsule height used for egg capsule dry weight analyses can be found in Appendix VI (D-E). The hypotheses stating that the number of CDD has an effect on egg capsule dry weight was rejected for *U. cinerea*.

*Urosalpinx cinerea* egg capsules contained significantly more dry weight (mg/mm) than *R. venosa* (with non-overlapping confidence intervals) (Table 9, Figure 14C). The null hypothesis stating that species has no effect on egg capsule dry weight was not
Table 9: Summary table of statistical analyses for the dry weight of *Rapana venosa* and *Urosalpinx cinerea* egg capsules (mg dry weight / mm egg capsule height). Analyses include all experiments in which the egg capsules within the matrix were given in excess of 75 days to hatch. NA = not applicable. DF = degrees of freedom. The F statistic and Fisher’s multiple comparison test are associated with the ANOVA test. The H statistic and the Dunn’s multiple comparison test are associated with the Kruskal-Wallis test.
<table>
<thead>
<tr>
<th>Test</th>
<th>Factor(s)</th>
<th>Response</th>
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<th>F or H statistic</th>
<th>p-value</th>
<th>Fisher’s or Dunn’s Multiple Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>One-way ANOVA</td>
<td>Cumulative day degrees (CDD)</td>
<td><em>R. venosa</em> dry weight (mg/mm)</td>
<td>5</td>
<td>10.46</td>
<td>&lt;0.01</td>
<td>413 CDC &gt; 898 CDD, 1381 CDD; 609 CDD &gt; 898 CDD, 1381 CDD; 898 CDD &lt; 1153 CDD, 1442 CDD</td>
</tr>
<tr>
<td>One-way ANOVA</td>
<td>Cumulative day degrees (CDD)</td>
<td><em>U. cinerea</em> dry weight (mg/mm)</td>
<td>2</td>
<td>0.33</td>
<td>0.57</td>
<td>NA</td>
</tr>
<tr>
<td>Kruskal-Wallis</td>
<td>Species</td>
<td>Dry weight (mg/mm)</td>
<td>1</td>
<td>5.36</td>
<td>0.02</td>
<td><em>R. venosa</em> &lt; <em>U. cinerea</em></td>
</tr>
</tbody>
</table>
Figure 14: Mean values and 95% confidence intervals for the egg capsule dry weight of *Rapana venosa* and *Urosalpinx cinerea* egg capsules across the egg capsule deposition time series. A. *R. venosa* egg capsule egg capsule dry weight. B. *U. cinerea* egg capsule egg capsule dry weight. C. Comparison of egg capsule egg capsule dry weight by species. ND = no data are available. T = termination of experiment.
**Rapana venosa**

![Graph A](image)

**Urosalpinx cinerea**

![Graph B](image)

![Graph C](image)
accepted. Differences in egg capsule dry weight between species could be influenced by
differences in absolute egg capsule height, organic and/or inorganic material invested in
the egg capsules by the female, and developmental pattern expressed by either species.

**Regional Context**

This study describes the conditions necessary for *R. venosa* and *U. cinerea* egg
capsule hatching. The distributions of adult *R. venosa* and *U. cinerea* in the southern
Chesapeake Bay; and thus, the potential sites for egg capsule deposition, are influenced
by bathymetry, food resources, and salinity. With respect to bathymetry, large sections
of the Chesapeake Bay are available as potential habitat, given that the mean depth of the
Bay is 6 m and *R. venosa* and *U. cinerea* populations along the geographic range may
occur at depths to 20 m (Mann and Harding, 2000) or 36 m (Carriker, 1955), respectively
(Figure 15).

Food resources are necessary to support populations of *R. venosa* and *U. cinerea* in
the southern Chesapeake. A primary prey item for *R. venosa* and *U. cinerea* is *C.
virginica*. Within the depth range of 0-6 m *C. virginica* habitat has been observed in
portions of the lower James River below Hog Island, the lower York River below Page’s
Rock, portions of Mobjack Bay, the Rappahannock River below Towles Point, and along
the bayside of the Eastern Shore below Tangier Sound (Andrews, 1973; Haven et al.,
1981; Berman et al., 2002; Mann et al., 2009; Southworth, Harding, and Mann,
unpublished data) (Figure 16). In addition to *C. virginica*, adult *R. venosa* also consume
*M. mercenaria*. In the lower Chesapeake Bay, *M. mercenaria* habitat has been observed
in portions of the lower James River below Goodwin Point, the lower York River below
Figure 15: Chesapeake Bay bathymetry from available depth soundings prior to 2008.

Figure 16: Chesapeake Bay oyster and hard clam distributions prior to 1991. Data are based on the work of Haven et al., 1981 and Roegner and Mann, 1991.
Page’s Rock, portions of Mobjack Bay, and the lower half of the Eastern Shore peninsula on the bayside (Rogner and Mann, 1991; Mann et al., 2005) (Figure 16). Figure 16 shows liberal estimates of habitat supporting *M. mercenaria* based the location of *M. mercenaria* populations during the survey work of Roegner and Mann (1991). More recent work by Mann et al. (2005) report *M. mercenaria* populations throughout the same river systems as reported by Roegner and Mann (1991) and shown on Figure 16. In contrast to Roegner and Mann (1991), Mann et al. (2005) do not report *M. mercenaria* populations extending past the mouth of each river system nor along the Eastern Shore. However, Mann et al. (2005) surveyed a limited region compared to the data reported by Roegner and Mann (1991).

Salinities, particularly during the spring when low values are associated with freshets and runoff, limit the upstream distribution for survival of adult *R. venosa* and *U. cinerea*. As stated previously, adult *R. venosa* survival is limited to salinities greater than 10 ppt (Harding, unpublished data), for several hours, and greater than or equal to 12 ppt (Giberto et al., 2006) for longer term population survival. Adult *U. cinerea* survival is limited to salinities greater than 7 ppt for short term survival (Stauber, 1943) and greater than or equal to 15 ppt (Federighi, 1931; Engle, 1935-1936) for longer term population survival. Egg capsules will likely be deposited where adult survival occurs, or at salinities above 12 ppt for *R. venosa* and salinities above 15 ppt for *U. cinerea*. Based on the results of this study, deposited egg capsules will hatch at salinities of at least 14 ppt, 21 ppt, and 28 ppt. Given adult survival and egg capsule hatching at salinities ranging from 14 ppt to 28 ppt, there is high probability that self-sustaining populations of either species will exist in habitat at least within this salinity range which also include habitat of
the appropriate depth with food resources. In the lower Chesapeake Bay spring salinities \( \geq 14 \) ppt exist in the lower James River below the Nansemond River, the lower York River below Page’s Rock, Mobjack Bay, the Rappahannock River near Broad Creek, and the lower half of the Eastern Shore peninsula on the bayside (Figure 17). Please note that salinity was classified in groups with a range of 7 ppt in each group for ease of presentation and application to the salinity ranges examined in this study. The regional scales supporting self-sustaining populations, as noted above, are likely to fluctuate on seasonal and yearly scales based on precipitation or amount of runoff. Additionally, populations may extend above or below the areas noted since salinity in the Chesapeake Bay is a continual gradient, and not classified in groups.

The known distribution of \( R. \) \( venosa \) (since 1998) in the Chesapeake Bay corresponds with the location of resources (bathymetry, food, salinity) described above. Since the discovery of \( R. \) \( venosa \) in the Chesapeake Bay, the majority (99\%) of donations received at VIMS have been collected in the James River below Wreck Shoal, York River below Gloucester Point, Mobjack Bay, and mainstem Chesapeake Bay near Ocean View (Harding and Mann, 2005) (Figure 18). Slight variation in the recorded distribution of \( R. \) \( venosa \) may occur on a seasonal or yearly basis based on bathymetry, food, and salinity.

The historic and current distribution of \( U. \) \( cinerea \) in the Chesapeake Bay corresponds with the location of resources (bathymetry, food, salinity) described above. Historically, the majority of \( U. \) \( cinerea \) populations in the Chesapeake Bay have been observed in the James River below Brown Shoal, the York River below Page’s Rock, Mobjack Bay, the Rappahannock River below Towles Point, and along the bayside of the Eastern Shore (Andrews, 1973; Haven et al., 1981; Southworth, Harding, and Mann, unpublished data)
Figure 17: Chesapeake Bay salinity from March through May (1984-2007) at 2 m depth.

Data were accessed on May 20, 2009 (Division of Shellfish Sanitation at

80
Figure 18: Current distribution 99% of the more than 18,000 *R. venosa* collected in the Chesapeake Bay since 1998 as indicated by the light grey shading (based on Harding and Mann, 2005). Map accessed on September 20, 2007 (VIMS Molluscan Ecology Program at http://web.vims.edu/mollusc/research/rapaw/merapmap.htm).
After Tropical Storm Agnes, “dramatic reduction in the distribution and abundance of drills” (Andrews, 1973) occurred. In the 3 decades since Tropical Storm Agnes in 1972, the limited survey data on the distribution and abundance of *U. cinerea* suggest that *U. cinerea* populations have not recovered to historic levels (Andrews, 1973; Southworth, Harding, and Mann, unpublished). However, *U. cinerea* have been collected from the James River below Brown Shoal and the Elizabeth River, the York River below Clay Bank, Mobjack Bay, the Rappahannock River below Towles Point (Andrews, 1973; Southworth, Harding, and Mann, unpublished). Figure 19 does not suggest that a continuous distribution of *U. cinerea* existed prior to or after Tropical Storm Agnes in 1972, as noted collection sites were not part of a continuous survey. Figure 19 only notes sites within the Chesapeake Bay where a limited number of *U. cinerea* have been collected since Tropical Storm Agnes. Further, since noted collection sites were not part of a larger, continuous survey, it is possible that the current distribution of *U. cinerea* within the Chesapeake Bay may expanded beyond that reported by Andrews (1973) or Southworth, Harding, and Mann (unpublished). Within the region directly surrounding the Chesapeake Bay, *U. cinerea* have been collected along the seaside of the Eastern Shore near Wachapreague (Figure 19).

Optimal conditions for egg capsule hatching

The percentage of egg capsules to hatch above 75%, herein defined as optimal conditions, occur in over different temperature-salinity combinations for *R. venosa* and *U. cinerea* across the majority of the egg capsule deposition time series. For *R. venosa*, the number of CDD at egg capsule collection was a significant factor affecting the
Figure 19: Historic distribution of *U. cinerea* in the Chesapeake Bay based on Andrews, 1973; Haven et al., 1981; Southworth, Harding, and Mann, unpublished data.
percentage of egg capsules to hatch. For this reason, the optimal conditions for the percentages of *R. venosa* egg capsule to hatch are discussed in relation to the number of CDD at egg capsule collection. At 413 CDD, optimal conditions for the percentage of *R. venosa* egg capsules to hatch were restricted to a limited temperature-salinity combination: between 22°C to 27°C and 14 ppt to 21 ppt or between 18°C to 19°C and 20 ppt to 24 ppt (Figure 20A). At 898 CDD, the optimal conditions for the percentage of *R. venosa* egg capsules to hatch ranged from 19°C to 30°C and 12 ppt to 24 ppt, depending on temperature (Figure 20B). At 1381 CDD, optimal conditions for the percentage of *R. venosa* egg capsules to hatch were located at temperatures of 21, 26 and 30°C and at salinities ranging from 11 ppt to 28 ppt, depending on temperature (Figure 20C).

Differences in the optimal location for the percentage of *R. venosa* egg capsules to hatch according to the number of CDD at egg capsule collection may be due to differences in salinity during egg capsule production, maternal nutrition, genetics of the parent, genetic bottlenecking of *R. venosa* populations in the Chesapeake Bay (Chandler, 2007), or environmental history during the period of egg capsule production.

Significant differences in the proportion of egg capsules to hatch did not occur for *U. cinerea* in relation to the number of CDD at egg capsule collection. The optimal conditions (> 75% hatch) for the percentage of *U. cinerea* egg capsules to hatch were located at salinities of 21 and 28 ppt across all culture temperatures examined (Figure 21A, B). Please note that due to termination of egg capsules collected at 609 CDD data from only 2 egg capsule collection dates (1153 and 1442 CDD) are presented.
Figure 20: Optimal conditions for the percentage of *R. venosa* egg capsules to hatch in 2007. A. Egg capsules collected on 413 CDD. B. Egg capsules collected on 898 CDD. C. Egg capsules collected on 1381 CDD.
Percentage of egg capsules to hatch:

- 0% - 25%
- 25% - 50%
- 50% - 75%
- > 75%

(A) 413 CDD
(B) 898 CDD
(C) 1381 CDD
Figure 21: Optimal conditions for the percentage of *U. cinerea* egg capsules to hatch from 1153 to 1442 CDD in 2008. A. Egg capsules collected on 1153 CDD. B. Egg capsules collected on 1442 CDD. Note that egg capsules collected on 609 CDD were terminated due to contamination with ciliates.
Percentage of egg capsules to hatch:

- 0% - 25%
- 25% - 50%
- 50% - 75%
- > 75%

Terminated, 609 CDD

1153 CDD

1442 CDD
DISCUSSION

Salinity and not temperature is the primary factor influencing \textit{R. venosa} and \textit{U. cinerea} egg capsule hatching success. The salinities examined ranged from 7 ppt to 28 ppt. For \textit{R. venosa}, salinities of 21 ppt or ambient salinity (ranging from 18 ppt to 24 ppt) increased the percentage of egg capsules to hatch relative to 14 ppt. For \textit{U. cinerea}, salinities of 21 ppt, 28 ppt, and ambient (ranging from 15 to 23 ppt) increased the percentage of egg capsules to hatch and the percentage of embryos alive at hatch relative to 14 ppt. The number of cumulative day degrees at egg capsule collection, a proxy for position of egg capsules in the deposition time series, was an additional factor influencing egg capsule hatching. For \textit{R. venosa} egg capsules collected later in the egg capsule deposition time series (having a greater number of CDD at egg capsule collection), the percentage of egg capsules to hatch increased relative to earlier points in the egg capsule deposition time series. For \textit{U. cinerea} egg capsules collected later in the egg capsule deposition time series (having a greater number of CDD at egg capsule collection) the percentage of embryos alive at hatch was decreased. For \textit{R. venosa} and \textit{U. cinerea}, temperature within the examined range (18°C to 30°C) was not a significant factor affecting egg capsule hatching success, although temperature was a significant factor determining the rate at which the hatching process occurs.

Physiological tolerances are important in determining distribution of many sessile fauna. Such is the case for many benthic invertebrates since populations are unlikely to
become established unless environmental conditions are within the range supporting embryonic development during the spawning season, or embryonic development can take place under favorable conditions and be larvae transported through dispersal (Calabrese, 1969; Kinne, 1971). Salinity is important since it may limit embryonic survival to hatch and help determine the distribution of self-perpetuating populations. Temperature is important since it may determines the duration of reproduction across latitudes, the rate of egg capsule hatching, and the larger latitudinal range of each species.

Salinity

Salinity determines the distribution of self-perpetuating populations of *R. venosa* and *U. cinerea* in estuaries by limiting adult survival and embryonic survival to hatch. *R. venosa* embryos (hatching at salinities of 14 ppt, 21 ppt, and 28 ppt), exhibit lower tolerance to salinity when compared to larval (≥ 7 ppt; Mann and Harding, 2003) or adult (>12 ppt in the Río de la Plata estuary, Uruguay; Giberto et al., 2006) life history stages. Similarly, *U. cinerea* embryos (hatching at salinities of 14 ppt, 21 ppt, and 28 ppt, depending on temperature) exhibit decreased tolerance to low salinity when compared to the adult (>12 ppt; Federighi, 1931) life history stage. In the Chesapeake Bay, spring salinities of 14 ppt, which support hatching of embryos from the egg capsule of *R. venosa* and *U. cinerea*, are located in the lower James River below the Nansemond River, lower York River below Page’s Rock, Mobjack Bay, mouth of the Rappahannock River, and Eastern Shore below Smith Island, Maryland (Figure 17). *Urosalpinx cinerea* egg capsule hatching occurs at salinities of 14 ppt (at temperatures ≥ 22°C) and at salinities of 21 ppt and 28 ppt (at all temperatures). Thus, although *U. cinerea* may exist in all areas
described above as supporting *R. venosa* populations, egg capsule hatching is likely to be less successful than if egg capsules were deposited at salinities of 21 ppt or 28 ppt. This supports Wass’s (1972) observations of *U. cinerea*, being a “upper meso- to eurohaline”, species that is likely to be more abundant around areas with higher salinities such as the lower tip of Virginia’s Eastern Shore below Cherrystone Creek, and sites surrounding the Bay mouth (Figure 17). In the Chesapeake Bay region, hatching of *R. venosa* and *U. cinerea* embryos may additionally be supported along the seaside of the Eastern shore given high salinities within the observed range of hatching.

Early life history stages (embryonic and/or larval stages) have commonly been observed to limit the range of other benthic invertebrate species in the Chesapeake Bay. Prior to Tropical Storm Agnes, egg capsule deposition and embryonic development in *E. caudate*, set the distributional limit for self-perpetuating populations of this species to salinities > 15 ppt in the Delaware Bay (Stauber, 1943); although adult survival can occur > 12.5 ppt under laboratory conditions (Manzi, 1970). The distribution of reproductively active *C. virginica* is limited to habitats with salinities ≥ 5 ppt (Loosanoff, 1953; Shumway, 1996). Below these salinities, gametogenesis for *C. virginica* is depressed which negatively affects spawning. However, *C. virginica* can survive fresh conditions (0 ppt) (Kennedy, 1991) for short periods of time. The distribution of *M. mercenaria* is limited to salinities ≥ 17 ppt where larval metamorphosis is not impeded (Davis, 1958; Roegner and Mann, 1991); although adult survival can occur ≥ 12 ppt (Roegner and Mann, 1991). For the barnacle species, *B. eburneus*, *B. improvises*, and *S. balanoides* (formerly *B. balanoides*) the known distribution is determined in part by the tolerance of developing nauplii within the plankton. Survival for *B. eburneus*, *B. improvises*, and *S.*
balianoides occurs between 25 ppt and 40 ppt, between 30 ppt and 45 ppt, and between 6 ppt and 30 ppt respectively (Holmes and Pryor, 1938; Crisp and Costlow, 1963).

Temperature

Temperature determines the duration of egg capsule deposition for many gastropods, since reproductive activity will occur at approximately the same temperature regardless of latitude (Scheltema, 1967; Conover, 1992) if populations are present. At higher latitudes there are fewer days in which water temperatures support gonad development, copulation, egg capsule deposition, and embryonic development, relative to lower latitudes. As a result, the number of egg capsules deposited per female may be decreased when compared to populations at lower latitudes. The period of time supporting gonad development and reproduction may be an important factor in determining species dispersal or population densities over time.

The relationship between the number of days supporting gonad development, copulation, egg capsule deposition, and embryonic development in regard to latitude has been observed for R. venosa and U. cinerea along the species’ geographic range. For R. venosa in the Chesapeake Bay (37°N; Harding and Mann, 1999) or Korea (38°N; Chung et al., 1993) the egg capsule deposition time series begins in April or May at temperatures ≥ 18°C (Chung et al., 1993; Harding et al., 2007; Harding et al., 2008). The egg capsule deposition time series ends in late July or August as temperatures approach 26°C to 30°C in the Chesapeake Bay and Korea (Chung et al., 1993; Harding et al., 2007; Harding et al., 2008). At higher latitudes, R. venosa egg capsule deposition occurs from July through September in the Black Sea (36 to 40°N; Chukhchin, 1984) at temperatures of
19°C to 25°C. In the southern hemisphere, in the Rio de la Plata estuary, at lower latitudes (34 to 36°S) the period for reproduction follows the same pattern and is driven by temperatures of 18 to 22°C (Giberto et al., 2006) which occur from November to February. For *U. cinerea* in the Chesapeake Bay (Carriker, 1955) the egg capsule deposition time series begins in April or May at temperatures $\geq 20^\circ$C. The egg capsule deposition time series for *U. cinerea* in the Chesapeake Bay ends in October or until ambient temperature decreases below 12°C (Carriker, 1955; MacKenzie, 1961). At higher latitudes *U. cinerea* egg capsule deposition occurs from May to June in Delaware Bay (39°N; Stauber, 1943) and Mystic River near Noank Connecticut (41°N; Franz, 1971) as temperatures rise above 15°C (Stauber, 1943).

Decreased temperature may decrease survival by prolonging embryonic development within the egg capsule. Prolonged development may be associated with increased embryonic mortality due to egg capsule predation (Spight, 1975). Predation on *R. venosa* or *U. cinerea* egg capsules has not been described. However, predation on gastropod egg capsules have been described for the emarginated dogwinkle, *Nucella emarginata* and the mud snail *Ilyanassa obsoleta*. Predators of the egg capsules of *Nucella emarginata* along the geographic range include the shore crabs, *Hemigrapsus nudus*, *H. oregonensis*, and isopod *Idotea wosnesenskii* (Rawlings, 1990). Predators of the egg capsules of *Ilyanassa obsoleta* along the geographic range include the hermit crab *Pagurus longicarpus*, green crab *Carcinus maenas*, and periwinkle *Littorina littorea* (Brenchley, 1982). Given the predators on the egg capsules of *N. emarginata* and *I. obsoleta* potential predators of the egg capsules of *R. venosa* and *U. cinerea* in the Chesapeake Bay may include local species such as the Asian shore crab, *Hemigrapsus sanguineus*, and hermit crabs *Pagurus*
longicarpus, P. annulipes, and P. pollicaris. The predation rate of embryos during encapsulation may be high, or as much as 85% in as little as 10 days for I. obsoleta at water temperatures of 13°C to 24°C (salinity not noted) (Brenchley, 1982). Despite the differences in egg capsule shape and height between I. obsoleta, R. venosa, and U. cinerea (3 mm, > 40 mm, and < 10 mm, respectively) and potential differences in the predator field, if mortality rates for R. venosa or U. cinerea are similar to predation experienced by I. obsoleta during encapsulation, then a faster rate of hatching may greatly increase the chance of embryos to hatch alive from R. venosa or U. cinerea egg capsules.

Prolonged development may further decrease embryonic survival by increasing the likelihood that developing embryos encounter physical stress during encapsulation (Spight, 1975). Physical stress may be related to fluctuations in environmental conditions such as change in salinity related to freshets, low dissolved oxygen, increased temperature, risk of desiccation that may occur over short time scales, or differences in egg capsule organic content. It has been argued that egg capsules may be important in protecting embryos from predation (Pechenik, 1979), bacteria (Lord, 1986), ultraviolet radiation (Rawlings, 1996) and rapid changes in environmental conditions (Pechenik, 1982, 1983). However, the longer an egg capsule requires for incubation, the more likely it is that embryos will experience deleterious changes in environmental conditions or internal stressors within the egg capsule resulting in embryonic mortality.

After hatch, the rate at which the egg capsule hatching process occurs may continue to play an important role in species survival by influencing access to available habitat, access to food resources, and likely predators. Hard substrate is limiting in the lower
Chesapeake Bay. *Rapana venosa* or *U. cinerea* hatching earlier in the reproductive time series may have a better chance of reaching available habitat when compared to embryos hatched later before available substrate is colonized. Access to available food resources may also vary based on the timing of hatch. The prey match-mismatch hypothesis of Cushing (1990) suggests that the timing of hatch and the availability of food resources will influence post settlement survival. Primary food sources of juvenile *R. venosa* and *U. cinerea* include bryozoans, barnacles (*B. eburneus, B. improvisus, S. balanoides* (formerly *B. balanoides*), *C. fragilis*), the mussel *M. edulis*, and the juvenile oyster *C. virginica*. Settlement of barnacles in the Chesapeake Bay has been observed from May through July (>18°C) (Harding, 2001). Settlement of *M. edulis* occurs in the early spring >10°C (Chipperfield, 1953). Settlement of *C. virginica* occurs as water temperatures increase above approximately 20°C (Kennedy and Krantz, 1982), beginning in late April or May in the Chesapeake Bay. *R. venosa* and *U. cinerea* embryos from egg capsules hatching either before prey items have settled, or after prey items have grown too large for either species to penetrate the prey valves will be more limited in access to available food items. For newly hatched *R. venosa* or *U. cinerea* the timing of hatch is also important in determining likely predators. For example, *R. venosa* (<35 mm SL) are vulnerable to predation by blue crabs (*Callinectes sapidus*) (Harding, 2003). A faster rate of egg capsule hatching may allow for a longer period of growth for *R. venosa* outside of the egg capsule such that refuge from predation may be reached sooner than egg capsules requiring a longer incubation time.

Temperature is known to limit species distribution over geographic scales based on tolerance of all life history stages to the annual temperature cycle of the region (Schmidt-
The Chesapeake Bay is a temperate estuary that experiences temperature fluctuations from 2°C to ≥ 25°C over the course of a year (VIMS Molluscan Ecology Program at www.vims.edu/mollusc; Appendix II). The distribution of self-perpetuating populations of *R. venosa* and *U. cinerea* should be limited to areas supporting successful egg capsule deposition and development of embryos to hatch, if dispersal is not considered. *Rapana venosa* and *U. cinerea* are observed to successfully hatch at temperatures of 18°C to 30°C. Geographically, this range of temperature tolerance for egg capsule hatching places the potential survival range of both species in the Northern hemisphere from Boston, Massachusetts (42°N) (annual temperature range 0°C to 20°C; NOAA at http://www.ndbc.noaa.gov/) to semi-tropical or tropical latitudes such as St. Augustine, Florida (29°N) (annual temperature range 17 to 30°C; NOAA at http://www.ndbc.noaa.gov/). Temperatures in more southern latitudes such as the Eastern Caribbean (15°N) (annual temperature range 26 to 30°C; NOAA at http://www.ndbc.noaa.gov/) may support populations if either species were to be introduced to these latitudes. However, there may be other constraints to the northern and southern distribution limits in addition to temperature. This may be one factor influencing why *R. venosa* may not currently be found in Hong Kong, despite suitable annual temperatures (Mann and Harding, 2000).

**Potential Overlap of *R. venosa* and *U. cinerea* in the Chesapeake Bay**

Based on bathymetry, food resources, spring salinity, and the percentage of egg capsule hatching from this study (Figures 15 – 17; Figures 20 - 21), potential overlap of self-sustaining *R. venosa* or *U. cinerea* populations will likely occur in the lower
Chesapeake Bay where salinities are equal to at least 14 ppt, 21 ppt, or 28 ppt and food resources are available. These areas in the Chesapeake Bay include portions of the lower James River below the Nansemond River, lower York River below Page’s Rock, Mobjack Bay, and mouth of the Rappahannock River near Broad Creek (Figure 22). The shaded areas in Figure 22 are based on egg capsule hatching at 14 ppt, 21 ppt, and 28 ppt within area that also includes available food resources. The shaded area may not represent all areas where adults of either species have been collected to date based on limitations with the data mapping food resources. Despite this, the shaded areas depicted in Figure 22 are consistent with previous observations regarding the location of *R. venosa* and *U. cinerea* populations (Wass, 1972; Harding and Mann, 2005), and should be considered estimates of the potential habitat to be occupied by either species. The potential range of either species (Figure 22) is likely to increase or decrease in area based on hatching of *R. venosa* and/or *U. cinerea* egg capsules at salinities between 7 ppt and 14 ppt or if reproductive populations of either species exist in areas not denoted as having appropriate food resources.

The optima for hatching of egg capsules differ for *R. venosa* and *U. cinerea* in the Chesapeake Bay, these being at spring salinities of 14 ppt or 21 ppt and 21 or 28 ppt, respectively. Differences are also noted in prey preferences, and the lifetime dispersal capabilities of each species (Figure 23). The optimal salinity and season conditions for *R. venosa* egg capsule hatching in the Chesapeake Bay correspond to portions of the lower James River below the Nansemond River, lower York River below Page’s Rock, Mobjack Bay, the mouth of the Rappahannock River near Broad Creek and along the seaside of the Eastern
Figure 22: Potential habitat supporting overlap of *R. venosa* and *U. cinerea* in the Chesapeake Bay. Data based on the following: NOAA at [http://www.ngdc.noaa.gov/](http://www.ngdc.noaa.gov/), Haven et al. (1981) and Roegner and Mann (1991), and Division of Shellfish Sanitation at [http://www.vdh.virginia.gov/EnvironmentalHealth/Shellfish](http://www.vdh.virginia.gov/EnvironmentalHealth/Shellfish).
Figure 23: Optimal habitat supporting overlap of *R. venosa* and *U. cinerea* in the Chesapeake Bay. Data based on the following: NOAA at [http://www.ngdc.noaa.gov/](http://www.ngdc.noaa.gov/), Haven et al. (1981), Roegner and Mann (1991), and Division of Shellfish Sanitation at [http://www.vdh.virginia.gov/EnvironmentalHealth/Shellfish](http://www.vdh.virginia.gov/EnvironmentalHealth/Shellfish). Please note, optimal habitat for *R. venosa* is primarily located within the mainstem of the Chesapeake Bay and western tributaries. Optimal habitat for *U. cinerea* is primarily located along the seaside of the Eastern Shore of Virginia.
Shore (Figure 23). Optimal conditions for *R. venosa* egg capsule hatching may also occur outside of the Chesapeake Bay along the seaside of the Eastern Shore based on *R. venosa* egg capsule hatching data collected on 1381 CDD (Figure 20). Optimal conditions for *U. cinerea* egg capsule hatching correspond to those found on the seaside of the Eastern Shore rather than within the Chesapeake Bay (Figure 23); however, this does not preclude successful hatching in the Bay as demonstrated by both the current study and historical distributions. Based on optimal conditions for egg capsule hatching, possible overlap of the two species could occur outside of the Chesapeake Bay along the lower seaside of the Eastern Shore, where food resources are available and spring salinities are higher (Figure 23). This option is supported by reported salinity tolerances of *R. venosa* and *U. cinerea* (Wass, 1963; Mann and Harding, 2003). However, to date, that the majority of *R. venosa* donations (99%) are collected within the Chesapeake Bay rather than along the seaside of the Eastern Shore (Harding and Mann, 2005). It is reasonable to expect continued success of *R. venosa* in the western tributaries of the Chesapeake Bay (Figure 24A), and *U. cinerea* within the coastal bays and lagoons along the Eastern Shore (Figure 24B). Species overlap will occur given that potential habitat for both species does exist within the Chesapeake Bay (Figure 22).

It is noteworthy that although *R. venosa* and *U. cinerea* display similar embryonic tolerance to the salinities examined in this study, *R. venosa*, the invasive species, and not *U. cinerea*, the native species, is likely to be the more successful of the two species to occupy habitat within the western tributaries of the Chesapeake Bay. Success of *R. venosa* in adapting to novel environments, as well as biological differences between the two species (including developmental pattern and presence or absence of a planktonic
Figure 24: Optimal and potential habitat supporting \textit{R. venosa} and \textit{U. cinerea} in the Chesapeake Bay. \textbf{A.} \textit{R. venosa}. \textbf{B.} \textit{U. cinerea}. 
larval stage, incubation time, and the percentage of embryos alive at hatch) may contribute to *R. venosa* becoming the more successful of the two species in the western tributaries in which *U. cinerea* was once common (Carriker, 1955; MacKenzie, 1961; Wass, 1972; Andrews, 1973). Further, the “decimation” of *U. cinerea* populations by Tropical Storm Agnes highlights how the boundaries of species distributions that were stable over historical or even geological time may be reset for extended periods by single, traumatic events. *R. venosa* appears to have been successful in adapting to conditions present in a defined portion of the Chesapeake Bay. In contrast, the reoccupation of portions of the Chesapeake Bay previously occupied by *U. cinerea* prior to Tropical Storm Agnes would appear to be impeded by the additional challenge of competition from the newly established invader occupying similar habitat in the Chesapeake Bay.
APPENDIX I: Definitions.
**Egg capsule:** Formed by the parental reproductive tract (Pechenik, 1986) and used to encapsulate developing embryos. The size and shape of the case, texture of the wall (leathery or gelatinous) and number of cases attached together are variable between species (Rupert and Barnes, 1994).

**Egg capsule hatching success:** Given by the percentage of egg capsules to hatch and the percentage of embryos alive at hatch.

**Egg mass:** an isolated group of egg capsules attached to the side of the holding container (Harding et al., 2007).

**Embryo:** The product of the reproductive process of an animal or plant (Merriam-Webster, 2009). In this context the term will be used to represent developing *R. venosa* or *U. cinerea* encapsulated within the egg capsule.

**Fundamental niche:** the full range of environmental and physiological conditions (biological and physical) under which an organism may exist (Elton, 1927; Hutchinson, 1957; Whittaker et al., 1973).

**Habitat:** the natural environment where an organism naturally or normally lives (Merriam-Webster).

**Hatch:** The release of at least one embryo from the egg capsule without assistance under controlled laboratory setting.

**Realized niche:** “the range of all environmental conditions, including microclimatic regimes and resources characteristics actually occupied or utilized by a species” (Barrows, 2001).
APPENDIX II: Mean York River temperature and salinity (37°14'51"N, 76°29'58"W) (SD) from 2003 to 2008. *Please note data gaps exist from the later part of September 2003 to January 2005 due to disruptions in the data stream. All other data were calculated using data collected without gaps. SD = standard deviation.
APPENDIX III: Map of the Chesapeake Bay.
APPENDIX IV: *U. cinerea* broodstock shell lengths as of May 15, 2008, the day they were first collected from the Eastern Shore, Virginia (36° 37' 36" N, 75° 37' 34" W), near Wachapreague. Values along the x-axis are given as median values of each bin. N = 600 *U. cinerea*. 
18 °C • 22 °C ◆ 26 °C □ 30 °C ○ Ambient

Controlled Chamber

Ambient Chamber
APPENDIX VI: Distribution of *R. venosa* and *U. cinerea* egg capsule height by the number of CDD at egg capsule collection and used in egg capsule dry weight analyses.

Egg capsule height for *R. venosa* was binned in 5 mm size bins. Egg capsule height for *U. cinerea* was binned in 1 mm size bins. A. *R. venosa* 413 CDD; *N* = 60. B. *R. venosa* 898 CDD; *N* = 53. C. *R. venosa* 1381 CDD; *N* = 60. D. *U. cinerea* 1153 CDD; *N* = 9. E. *U. cinerea* 1442 CDD; *N* = 15. F. *R. venosa* 609 CDD; *N* = 6. G. *R. venosa* 1153 CDD; *N* = 10. H. *R. venosa* 1442 CDD; *N* = 10. Mid-point values for each bin are given along the x-axis. Note differences in scale between *R. venosa* and *U. cinerea*. 
Rapana venosa

A.

Number of Egg Capsules in Each Bin

Urosalpinx cinerea

D.

Number of Egg Capsules in Each Bin

Egg Capsule Height (mm)

C.

Number of Egg Capsules in Each Bin

Egg Capsule Height (mm)
Rapana venosa

F.

Number of Egg Capsules in Each Bin

G.

H.
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110


Mann, R., J.M. Harding, and E. Westcott. 2006. Occurrence of imposex and seasonal patterns of gametogenesis in the invading veined rapa whelk Rapana venosa from Chesapeake Bay, USA. Marine Ecology Progress Series. 310: 129-138.


