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Temporal and Spatial Variation in Reproductive Output of the Veined Rapa Whelk (Rapana venosa) in the Chesapeake Bay

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TEMPORAL AND SPATIAL VARIATION IN REPRODUCTIVE OUTPUT OF THE VEINED RAPA WHELK *(RAPANA VENOSA)* IN THE CHESAPEAKE BAY

A Thesis

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Catherine C. Ware

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This thesis is submitted in partial fulfillment of

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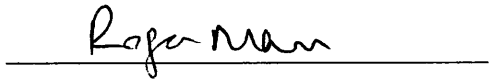

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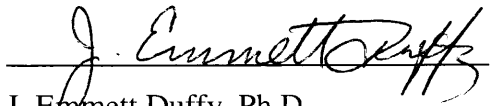
Committee Chairman / Advisor



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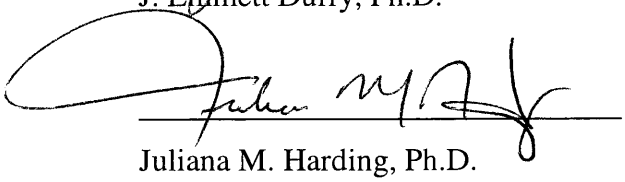

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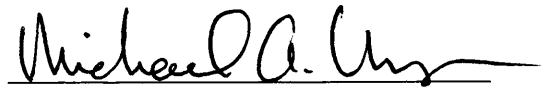

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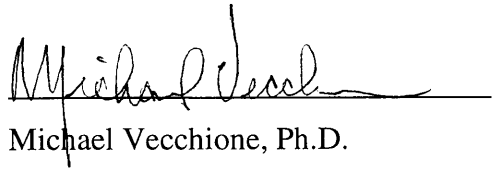

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ABSTRACT

Invading species face a number of challenges in receptor environments to become established members of the new ecosystem. The immediate distribution of adult life history stages reflects presence of available prey (in the case of invading predators) within the physical tolerances of the invading species; however, the functional reproductive range of the invader may be only a subset of the occupied range. It is critical to identify the functional reproductive range early in an invasion if control measures are to limit the range expansion of or eliminate the invader.

In order to assess the variation in propagule pressure (the rate at which individual progeny are released by adults) within the functional reproductive range of the invading predatory marine gastropod *Rapana venosa*, 19 individual adults from 3 areas (the James River, the Hampton Bar and the Ocean View areas of the Chesapeake Bay) were isolated during the spawning period, from May 15, 2001 to August 6, 2001. The number of egg masses laid and the number of egg cases per egg mass were recorded at an individual level in order to examine population differences among the three collection areas as well as temporal variation over the spawning period. Field collections of adult *R. venosa* were made in the months of April and May using opportunistic sampling based on bycatch from commercial fisheries. Once collected, the adults were maintained in the York River and fed to excess. Along with measurements of the number of egg masses and total egg cases produced in the season, three estimates of fecundity describing the egg mass were compared: the number of eggs per case, the hatch size of released veligers, and the ability of the egg mass to release veligers.

James River adults produced significantly more egg cases than either of the two sites and Ocean View produced the fewest egg masses. The adult *R. venosa* laid an average of 10 ± 4.09 egg masses over a spawning period characterized by water temperatures ranging from 19.2 °C to 27.7 °C. Individual egg masses had an average of 149 ± 104.9 egg cases containing about 1440 ± 689.9 eggs per case. The number of eggs per case was highly variable due to a significant dependence on the mean water temperature and the size of the egg case. Viability of the egg masses produced did not vary over space or time in terms of the size of released veligers and successful release of veligers. Twelve of the 19 adults observed exhibited signs of imposex, however, no variation in reproduction due to the presence of external male appendages on females was seen. These data indicate that a single adult *R. venosa* is capable of releasing over 2 million veligers per year and that fecundity increases with weight of the adult. Variation in environmental conditions as seen along the gradient from the mouth of the Chesapeake Bay into the James River has the potential to affect propagule pressure.

VARIATION IN REPRODUCTIVE OUTPUT OF *RAPANA VENOSA*

INTRODUCTION

The veined rapa whelk *Rapana venosa,* (Figure 1), a predatory marine gastropod, was discovered in the Chesapeake Bay in 1998 by the Virginia Institute of Marine Science (VIMS) trawl survey (Harding and Mann 1999). Native to the Sea of Japan, the Yellow Sea, the China Sea, and the Gulf of Bohai, *R. venosa,* of the family Muricidae, invaded the Black Sea in 1947 and within a decade dispersed along the Caucasian and Crimean coast (Zolotarev 1996). In spite of a large salinity difference between the Black Sea and the Mediterranean Sea (surface salinities of about 18 ppt and 36 ppt respectively; Leppakoski 1993), *R. venosa* has spread throughout the Mediterranean Sea, along the Adriatic coast of Italy (D. Savini and A. Occhipinti, University of Pavia, Pavia, Italy, pers. comm.), and to the southern coast of France (J. Joly, Laboratoire Conchylicole de Bretagne, France, pers. comm.). In addition, *R. venosa* has recently invaded another continent, as it was reported in Uruguay as recently as 1999 (Pastorino et al. 2000).

Voracious predators of bivalves, *R. venosa* have a rapid growth rate and adults may reach shell lengths (SL) in excess of 150 mm (Chung et al. 1993). The shell of *Rapana venosa* can be distinguished from gastropods native to the Chesapeake Bay by its heavy shell, large body whorl, and small teeth present along the edge of the outer lip of the aperture (Harding and Mann 1999). The known rapa whelk distribution in the Chesapeake Bay overlaps with commercially valuable shellfish populations (e.g., hard clams *Mercenaria mercenaria* and oysters *Crassostrea virginica*) (Harding and Mann

Figure 1. *Rapana venosa*, the veined rapa whelk, found in the Chesapeake Bay. Photograph copyright 1999 J. Harding, Virginia Institute of Marine Science, Molluscan Ecology, Gloucester Point, VA; used with permission.

Figure 2. An egg mass of *Rapana venosa* made up of individual egg cases found in the Chesapeake Bay (from Harding and Mann 1999).

Figure 1.

Figure 2.

1999). In addition to having ecological and economic importance within the Chesapeake Bay, research on the reproduction of *R. venosa* presents an opportunity to understand aspects of invasion biology and a fundamental component of the biology of gastropods.

Rapana venosa Reproductive Biology

Rapa whelks are dieoecious and lay masses of individual egg cases (Figure 2) during months of warm water temperatures (>15 °C), from approximately May 15 to August 15 in the Chesapeake Bay (Mann and Harding 2000a). Individual egg cases vary from 6 to 24 mm in height and are attached basally to hard substrate (Chukchin 1984). Egg masses from *R. venosa* in their native range are reported to consist of 50 to >500 egg cases with each egg case containing 200 to 1000 eggs of about 200-500 microns diameter (Chukchin 1984, Chung et al. 1993). The tip of the egg case curves slightly and ends in a pore from which veliger larvae are released after a development period within the egg case of 14 to 28 days (Mann and Harding 2000b). The larvae leave the case as free-swimming veligers and after approximately 2 to 8 weeks metamorphose and settle on hard substrate as juveniles.

As mentioned before, adult rapa whelks prey on shellfish species, many of which are infaunal. Due to their limited motility (confined to the benthos as adults) and exposure to sediments, adults from different locations may be exposed to substantially different biotic and abiotic environments. Chung et al (1993) measured a gonadosomatic index (GSI), a ratio of the thickness of *R. venosa* gonad to the diameter of the posterior appendage including the gonad and liver, over a year and found that as water temperature decreases, the GSI gradually increases until spawning, when it drastically decreases. As

the animals apparently store lipids all year long to spawn in the summer months, the location of origin of an adult may affect its reproduction in the laboratory indefinitely.

Gastropod Egg Cases

Although the process has not been examined in *R. venosa*, the production of egg capsules, composed predominantly of protein and carbohydrate, has been described in the muricid species *Ilynassa obsoleta* and *Nucella lapillus* (Sullivan and Maguel 1984; Fretter and Graham 1962). The general process is as follows: eggs are released from the ovary and fertilized in the upper oviduct. Once fertilized, the eggs move down the oviduct in groups of several hundred to the albumen gland where they are embedded in secretions. A sphincter between the albumen gland and the next region of the oviduct, the capsule gland, relaxes and embryos are forced into the protein and mucus secretions of the capsule gland. A plug subsequently seals the opening in these secretions. The soft case passes out of the oviduct and through the mantle cavity along a temporary groove on the right side of the foot to the ventral pedal gland. Within the ventral pedal gland, the egg cases of *Ilynassa obsoleta* and *Nucella lapillus* are sculpted into a species-specific shape, hardened, and attached to the substrate. The end of the case deepest in the pedal gland becomes the apical end containing the plug while the basal disk protrudes from the foot and is cemented to the substratum (Sullivan and Maguel 1984). The strength of the egg case of *N. lapillus* is attributed to the association of polysaccharide with protein in the wall of the case, possibly forming a glycoprotein through covalent bonds (Bayne1968).

The larvae develop within the egg cases into shelled veligers with no signs of adelphophagy (personal observation). Once the larvae have darkened and have begun to swim within the egg case, they are generally close to release from the egg case. Three possible mechanisms for release of larvae from the egg cases of muricids have been identified (Sullivan and Maguel 1984). The egg cases of *Littorina littorea* have been observed to swell prior to hatching, perhaps indicating that the egg case takes up water and ruptures as a result of increased osmotic pressure (Davis, 1968). A second possibility for release of larvae is the use of radulas or other parts of the body of the embryo to mechanically tear the case (*Lymnaea stagnalis*; Vaughn 1953). The final possibility is that the larvae chemically dissolve a pre-designated region of the case, the operculum, or mucoid plug. This may be a result of proteases digesting the protein of the case. This method has been documented for several gastropod species including the muricid *Urosalpinx cinerea* (Hancock 1956) and *Nassarius obsoletus* (Pechenik 1975).

Although many muricids create species-specific egg cases, egg cases from *R. bezoar* in the Gunnar Thorson collection of prosobranch egg capsules are smaller but otherwise identical to those of *R. venosa* (D'Asaro 1991). These cases (*Rapana* spp.), collected from southern Japan, are described as "ampulliform" (resembling a flask or bottle having a wide body tapering to a narrow neck with a terminal escape aperture closed by a mucoid plug; D'Asaro 1991), with a short stalk and pointed apex curving sharply to one side. The sharply curved part of the case is bordered by ridges that meet and fuse opposite the pointed apex, forming the apical plate of the case (see Figure 3; D'Asaro 1991). The possibility that *R. venosa* and *R. bezoar* are the same species has been refuted based on genetic and morphological comparisons (Gensler 2001; Green

Figure 3. *Rapana venosa* (Valenciennes). a. Different forms of egg masses and cases from southern Japan. b. (A) Lateral view of egg case. (B) Side view. (C) Side opposite the one shown in (B). (D) Apical view. Illustrations and legends from D'Asaro (1991).

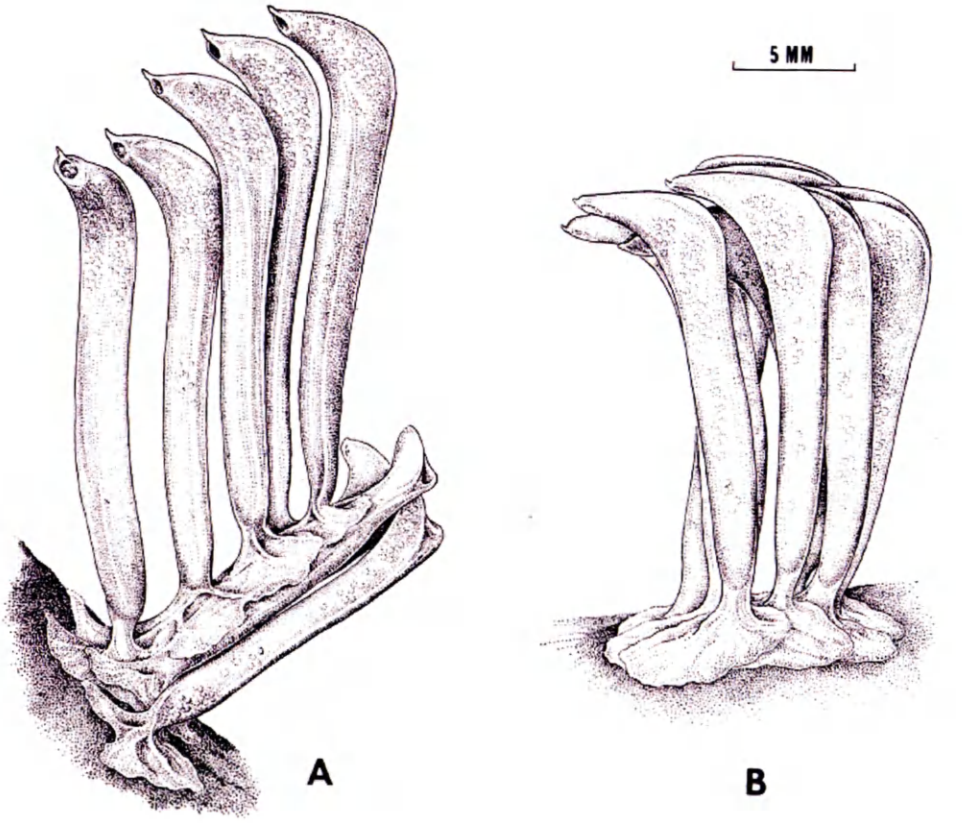


Figure 3a.

THORSON COLLECTION - MURICID EGG CAPSULES

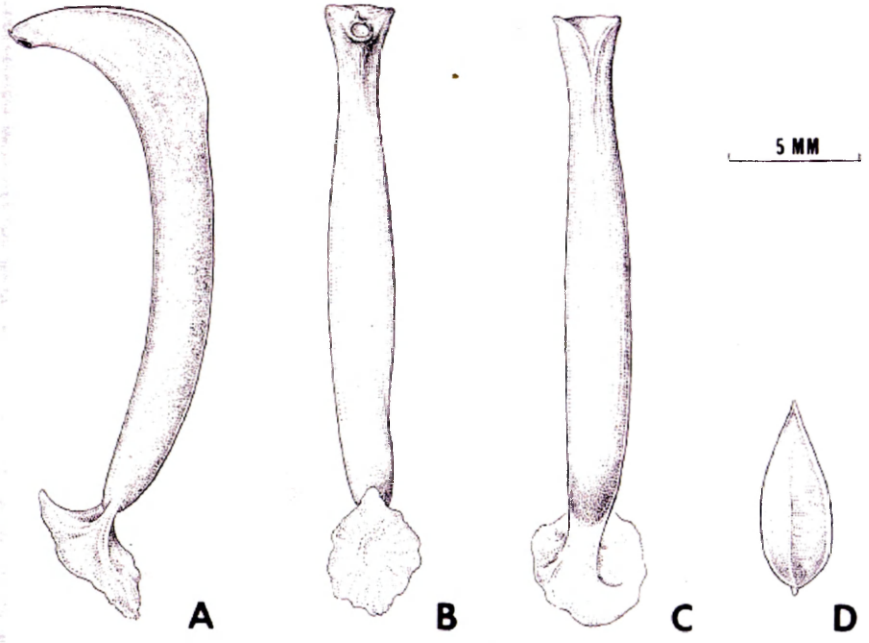


Figure 3b.

2001), but the possibilities that an error exists in the identification in Thorson's collection or that egg-case morphology of *Rapana* spp. is not species-specific have not been eliminated.

Variation in Egg Case Production

Within- and between-population variation in fecundity (number of embryos produced) of certain marine gastropod species has been previously documented. Within British populations of the nudibranch *Adalaria proxima,* variation in reproductive performance (a weight-adjusted index of each individual's spawning products summed over the reproductive period) has been shown to differ among sites (Lambert et al. 2000). In this species, the adults produce their largest spawn mass first, deploying up to 50% of an individual's total reproductive potential, possibly as a "bet-hedging" strategy (Lambert et al. 2000). Large nudibranchs produced larger first spawn masses and more total spawn than did smaller nudibranchs, concordant with other studies showing size of egg cases dependent on the size of the female producing them (Gallardo 1979, Ito 1997), but Lambert et al. (2000) saw no relationship between maximum body size and the amount of spawn after the first spawning. In the case of encapsulated embryos of *Nucella crassilabrum*, the number of eggs per case and the size of the egg case are significantly correlated (Gallardo 1979). The variation in allocation of energy in terms of the size and number of eggs produced in many spawns in a season differs both among populations of the same species and among individuals within a population (Lambert et al. 2000, Ito 1997, Gallardo 1979). Studies of annual species (Lambert et al. 2000, Ito 1997) tend to

find a temporal trend associated with fecundity for one reproductive season while this has not necessarily been noted in gastropods that reproduce for several years (Gallardo 1979).

In addition to maternal body size and temporal patterns affecting egg production, food availability and other factors associated with varying physical and biotic environments have been shown to affect the number and size of egg cases, and the number of eggs per case (Cheung and Lam 1999, Rawlings 1990). When food availability in the laboratory decreased for the prosobranch *Nassarius festivus,* both the number and size of egg cases and the number of eggs per case decreased as well (Cheung and Lam 1999). Egg case morphology, in terms of thickness of the egg case wall, has been shown to vary as much as 25% among populations of *Nucella emarginata*, an intertidal muricid (Rawlings 1990). The predatory isopod *Idotea wosnesenskii*, which preferentially opens thin-walled cases in the laboratory, were present at the populations (2 of 3) that had thicker walled egg cases (Rawlings 1990). Although the presence of isopods was not tested for causation of thick-walled egg cases, which would show that the change in the egg case morphology was adaptive to the biotic environment, D'Asaro (1970) suggests that oothecae with adaptive modifications do occur. Within the gastropod family Olividae, the egg cases of *Oliva sayana* are similar in shape to those of the genus *Olivella,* but the former's egg cases are unattached. D'Asaro (1970) presents the hypothesis that unattached egg cases are less affected by shifting sands, as they move with turbulent water and can become part of the plankton, whereas the attached cases of *Olivella* may be covered. Based on the evidence presented by these studies, intra-specific and intra-generic egg case morphology varies with, and possibly as adaptations to, the conditions of the local environment.

Reproductive Strategy

With at least 30,000 extant species (Ruppert and Barnes 1994), the Gastropoda exhibit a diversity of life histories. The reproductive strategy of prosobranch gastropods can be divided into two categories: direct development and "mixed," or indirect, development (Pechenik 1986). In direct development, the parent provides nutrients to the embryo until the embryos reach juvenile stage. Two forms of direct development are viviparity and ovoviviparity. For viviparous species, the embryos develop within the body of the parent with a direct tissue connection to the parent for nutrients to support development. Ovoviviparity requires that the parent indirectly provide nutrients through albumen material, yolk or nurse eggs (adelphophagy), often within an egg case, to sustain the embryo through its final metamorphosis. Direct development is advantageous in that it insures that the embryos will have energy and nutrients until they are juveniles. One disadvantage, however, is that it does not employ a planktonic stage, which is a potentially critical stage for dispersal of the species.

Mixed development, as used by Pechenik (1986), refers to larvae that develop within egg cases or egg masses and subsequently develop as planktonic larvae before metamorphosis to benthic form. This allows for greater dispersal of the species, although it can also apparently (but not necessarily) lead to a life history in which the planktonic stage is omitted. The veliger may feed solely through planktotrophy or may be sustained by yolk (lecithotrophy) in addition to feeding in the plankton, but is not entirely reliant on parental sustenance. The veligers may be released directly from gelatinous eggs or the embryos may be encapsulated for a period before being released as veligers. *Rapana venosa* is unique among gastropods in the Chesapeake Bay in that it has mixed

development. The native gastropods, including *Busycon carica, Busycotypus canaliculatum, Urosalpinx cinerea,* and *Nucella lapillus,* all encapsulate and supply nutrients to their embryos until the young can crawl away. The various life histories of the species affect energetic allocations and dispersal capabilities of the species. Both of these aspects of species' ecological profiles are crucial in studying an invasion. The study of the fecundity of *R. venosa* is therefore especially intriguing due to the aspect of mixed development unique to a foreign environment.

An interesting competition within the Chesapeake Bay between "invaders," one of mixed development (*R. venosa*), and one with direct development (the native muricid *U. cinerea*) is currently taking place (R. Mann, VIMS, Gloucester Point, VA, pers. comm.). The freshwater flows associated with Hurricane Agnes in 1972 restricted the distribution of the native population of *U. cinerea* to the proximity of the mouth of the Chesapeake Bay. Since that time, the species has effectively been "reinvading" the Bay. The introduction of *R. venosa* may have occurred more than 10 years after the start of the reinvasion by *U. cinerea*, but is aided by dispersal in the planktonic stage and hence may establish populations throughout the Chesapeake Bay before the *U. cinerea* population entirely recovers its historical range.

Bioinvasions

On any one day, it has been estimated that more than 3000 species may be in transit in ballast water world-wide (Carlton and Geller 1993). This number is growing yearly, as factors increasing the risks of both terrestrial and marine species introductions include an expanding global economy, increasing trade volume, and international trade

agreements facilitating commodity movements worldwide (Robinson 1999). Numerous efforts have been made to raise awareness about and prevent the global spread of non-indigenous species. The problem, however, is far from solved, as the rapid and recent inter-continental spread of *R. venosa* can demonstrate.

Vermeij (1996) defines an invasion as "the geographical expansion of a species into an area not previously occupied by that species" and identifies three successive stages of an invasion: arrival, establishment and integration. "Arrival" describes the dispersal of individuals to the receptor region either naturally or with the aid of humans (Vermeij 1996), rather than "introductions" which are human-mediated invasions, not natural range expansions (Carlton 1996). Establishment requires the persistence of an immigrant, or invasive, population in the receptor region by means of local reproduction and recruitment as opposed to repeated arrivals. The final step in an invasion, integration, occurs when the species incorporates itself into the ecology of the receptor region so much that evolution reflects the changed selective regime in the receptor community (Vermeij 1996). The factors distinguishing species that fail to invade from those that successfully invade may be examined at each step in the process. Success and failure can be influenced by circumstances in the donor biota, receptor biota, and by characteristics of the invading species (Vermeij 1996). Unfortunately, the studies of successful marine invasions rarely combine extensive descriptive data and quantitative results (Grosholz and Ruiz 1996). Records of failed marine invasions (arrival without establishment and integration) are scarce. Thus, comparisons between potentially invasive species that fail and those that succeed are difficult to make. In order to succeed, however, the species must successfully reproduce (to produce new generations)

in the receptor region. To evaluate the current invasion of the Chesapeake Bay by *R. venosa* and potential future invasions into similar habitats, an understanding fundamental aspects of reproductive biology in the receptor region is needed.

If the invasive species reproduces successfully within the receptor region, the rate of reproduction can influence whether the species expands its range in the receptor region and to what extent it may influence the biota. Spatial and temporal variability in reproduction may indicate the circumstances in the receptor region in which the species is successful in producing young and may predict under what circumstances the species may invade other areas. A broad understanding of the reproduction of the invasive species can be helpful in this respect. For example, by sheer weight of numbers the freshwater Asian clam, *Corbicula fluminea* was reported to be the most important aquatic pest species in the United States in 1982 (McMahon 1982). The first living population was found near the mouth of the Columbia River separating Washington and Oregon in 1938, and by the early 1980s *C. fluminea* was found in 35 of the United States and in northern Mexico (McMahon 1982). The fast spread of this invader was in part possible due to its reproductive strategy: *C. fluminea* are monoecious, releasing benthic pediveliger larvae or planktonic veligers that become benthic within 48 hours (Dressler and Cory 1980). There are typically two spawning periods per year, with one study reporting peak production of over 800 larvae/clam/day and an average of 1,140,820 larvae/m^2/year for established adult populations (Aldridge and McMahon 1978). The success of this invader may also be attributable to its highly variable phenotype and its ability to withstand harsh environments, but in order to attain Vermeij's second stage of

invasion (establishment) reproduction and recruitment must be possible in the receptor regions.

As mentioned previously, *R. venosa* has invaded the coasts of several continents and inland seas. The suspected vector of introduction into the Chesapeake Bay is ballast water transport of larval stages from the eastern Mediterranean or Black Sea (Harding and Mann 1999). The Chesapeake Bay, including the ports of Baltimore and Norfolk, receives more ballast water of foreign origin from commercial vessels (approximately 15 million metric tons (3 billion gallons) in 1991) than any other port system on the Atlantic or Pacific coasts (G. Ruiz, Smithsonian Environmental Research Center, pers. comm.). This places the Chesapeake Bay in an increasingly vulnerable position as a receptor region for marine invaders' arrivals. *Rapana venosa* appears established in the Chesapeake Bay, as egg masses have been found in the field, larval forms have been successfully cultured in the laboratory under prevailing local conditions, and an increasing number of size classes are represented in field collections of adult animals (Harding and Mann 1999 and unpublished data). To estimate the potential ecological and economic impact of this species in the receptor region, the Chesapeake Bay, reproduction of *R. venosa* in the Chesapeake Bay needs to be understood.

Native Gastropod Species

The two large native predatory gastropods common in the Chesapeake Bay are *Busycon carica*, the knobbed whelk, and *Busycotypus canaliculatum*, the channeled whelk. Both of these species lay eggs enclosed in cases that are laid in long strings from August to October, from which crawl-away young are released either late in the fall if the

egg cases were laid early, or the next spring from March to May (Castagna and Kraeuter 1994). The end of the string of cases is buried under the surface of the sand or mud about 10-20 cm (Magalhaes 1948). A string of *B. carica* cases can vary from 10 to 200 egg cases, each containing albuminous fluid, food eggs and 30-70 developing embryos (Magalhaes 1948). The embryos hatch at about 4 mm in shell length (Magalhaes 1948, Kraeuter et al. 1989). *Busycon carica* first sexually mature at 9 years of age (Castagna and Kraeuter 1994).

Urosalpinx cinerea, the Atlantic oyster drill is a small muricid gastropod native to the Chesapeake Bay that also deposits its eggs in cases. As in the knobbed and channeled whelks, these cases release crawl-away young. *Urosalpinx cinerea* produces approximately 20-38 egg cases per year, with about 12 eggs per case (Spight et al. 1974). Maturing at 2 years of age, this species is not expected to live much beyond 5 years of age (Spight et al. 1974).

Very little is known about the reproduction of *R. venosa* in the Chesapeake Bay. Description of reproduction in its native range gives a baseline of what may be expected, however, variable physical and biotic environments have been shown to alter reproductive output of gastropods. In order to learn more about the reproduction of *R. venosa* in the Chesapeake Bay, the Virginia Institute of Marine Science' Molluscan Ecology Program has maintained adults in the laboratory since 1999.

Collection and Distribution of Rapana venosa within the Chesapeake Bay

To date, collection of adult rapa whelks (shell length >100 mm) has depended on the commercial clam and crab fisheries (which collect the whelks as bycatch), using a

bounty of $2-$5 offered by VIMS since 1998, and 2001 for each rapa whelk. Collection has been limited spatially and seasonally to areas that are open to commercial shellfish harvest (e.g., hard clams, blue crabs, oysters). There were no reports of animals <68 mm SL as bycatch in 1998, 1999 and 2000, most likely due to the size selectivity of the watermen's gear (crab pots, dredges, patent tongs) or a difference in habitat preference by small whelks.

The current known distribution of *R. venosa* in the Chesapeake Bay extends to the mouth of the Rappahannock River in the north, the Lafayette River in the south, the Chesapeake Bay Bridge tunnel in the southeast and the James River Bridge in the southwest (Harding and Mann 1999). On isolated occasions rapa whelks have been reported on the Eastern Shore. The majority of collections are from the lower James River, Hampton Roads and the Ocean View region (Figure 4). This area represents a spatial cline of environmental conditions including substrate type and salinity. Table 1 describes salinity (typical annual range), depth and sediment type in the regions that provide the basis for sampling in the current study (J. Harding, VIMS, Gloucester Point, VA, pers. comm.).

Table 1. Three collection areas of *R. venosa* in the lower Chesapeake Bay.

Site	**James River**	**Hampton Bar**	**Ocean View**
Salinity	15-20 ppt	22-28 ppt	25-35 ppt
Depth	20-60 feet	15-30 feet	18-30 feet
Sediment	Soft Sand	Firm Sand	Hard Sand

Figure 4. Collection sites of *R. venosa* in the Chesapeake Bay: 1. James River. 2. Hampton Bar. 3. Ocean View. 4. Lafayette River. 5. Lynnhaven River (after Harding and Mann 1999).

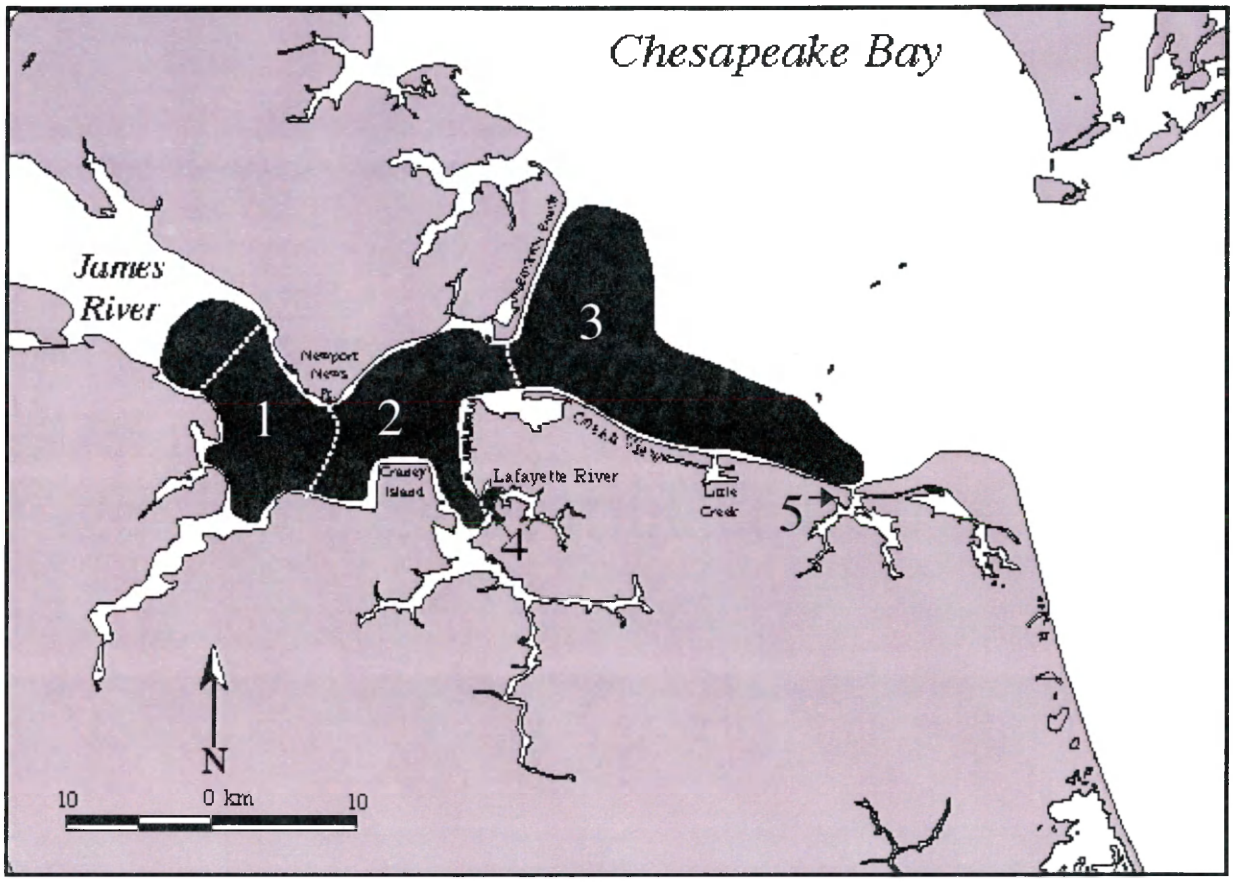

Chesapeake Bay
James River
Newport News
1
2
3
4
5
Crouch Island
Lafayette River
Little Creek
N
10
0 km
10

Figure 4.

Quantifying the variability in reproductive output of *R. venosa* over time and space at the individual level

Experimental Design and Objectives

The objective of this study was to measure egg mass production, number of egg cases per egg mass, number of eggs per egg case, and the viability of veligers for individual, gravid females from known locations (representing the spatial variability within the documented range) over one summer reproductive period. An optimal design would use equal numbers of female adults from each site; however, both the opportunistic collection of adults, and the criteria for inclusion of individuals in the experiment (see below) required changes in this design.

Examination of *R. venosa* reproductive output can be effected by comparing the viability among eggs, egg cases, or egg masses. In the first option (eggs), counting the number of veligers hatched from any given egg case or any given egg mass provides an estimate of reproductive output. At the egg case level, noting whether each individual egg case in an egg mass releases swimming larvae and comparing the morphology of these cases contributes valuable information. Although these two options would yield high fidelity data, they could logistically only be examined on a low number of animals. In order to answer questions about spatial and temporal variability in reproductive output, examination of egg masses allows qualitative and quantitative description of three geographic sites represented by five to ten animals from each site for an entire season of egg laying. Therefore, reproductive output was analyzed at the level of the egg mass: three estimates of fecundity described each egg mass. The three assays of fecundity were

the average number of eggs per case an egg mass contained, the average hatching size of the released veligers from an egg mass, and whether the egg mass released veligers. These three characteristics have great validity as predictors of fecundity. Although in some situations nurse eggs may be present in capsules releasing planktonic veligers (Rivest 1983), no evidence of nurse egg consumption has been observed for *R. venosa*. Therefore, unlike the species *Nucella crassilabrum* where about 7% of the embryos are released from the egg case (Gallardo 1979), the number of eggs per case of *R. venosa* should be an accurate estimate of the potential number of embryos released from the case.

A larger hatching size of the released veligers may indicate higher potential survival according to Spight (1976) because the large hatchling: 1) tolerates physical stresses more readily; 2) is susceptible to fewer predators; 3) can withstand starvation longer; 4) can travel further to find food or shelter (for organisms traveling equal numbers of body lengths per unit time, the larger will cover more ground than the smaller one) and 5) has a larger food supply (large prey taken in addition to smaller prey). Although several of these points have been challenged (Perron 1981 found that smaller veligers survived starvation conditions longer), in planktonic species a large size will usually mean a shorter time until metamorphosis to a benthic stage, and therefore less time for mortality in the plankton. Hatching size is also an especially appropriate measure of fecundity among populations within species that do not utilize nurse eggs. Species that produce viable eggs of uniform size with no nurse eggs produce offspring of relatively uniform size (Rivest 1983). To influence hatching size in these species,

selection would have to act on egg size, albumen availability, or growth form, all of which may reflect inter-population variability (Rivest 1983).

The third estimate of fecundity, whether an egg mass will release veligers, is clearly a critical step in the propagation of the population. Although variation in this predictor has not been explicitly quantified in the literature, the low rate of release of *R. venosa* veligers from egg masses observed in the laboratory in 2000 prompted further study. These three estimates of fecundity assess the reproductive output, in terms of number of and potential viability (as predicted by hatching size) of propagules, put forth by an individual *R. venosa* adult.

MATERIALS AND METHODS

General Observations of Temporal and Spatial Variation of *Rapana venosa* egg case morphology and release of larvae (1999-2000)

Prior to the present study, 1200 adult rapa whelk specimens were collected from the lower Chesapeake Bay from July 1998 until September 2000 (J. Harding, VIMS, Gloucester Point, VA, pers. comm.). Larvae were successfully cultured through metamorphosis in 1999 and 2000 at local water temperatures and salinities (Harding and Mann unpub. data). Egg cases laid in the laboratory by wild-collected adults during 1999 and 2000 were used to describe potential variations in egg case morphology with respect to the origin of the parents.

Typically 30 to 50 adult animals from each of five sites (James River, Hampton Bar, Ocean View, Lafayette River, and Lynnhaven River (Figure 4)) were maintained in site-specific tanks in the laboratory. The animals were kept in a flow-through laboratory system and fed to excess. Egg masses were collected twice per week from May to August, 1999 and 2000, and were subsequently maintained in individual containers, with static filtered seawater that was changed every other day.

Upon harvest, the egg cases' morphology was categorized as common (the most common morphology), or as one of several rare morphologies: tall-thin, short-flat, short-pointed or petite (Figure 5; note: cases with embryos killed by physical stress change

from the normal yellow color to pink or purple (Gallardo 1979)). Observations also included whether the egg mass released veligers.

Quantifying the variability in reproductive output of R. venosa over time and space at the individual level (2001)

Adult Collection

Selection of adult animals was opportunistic. Whelks were collected as bycatch by watermen during April and May 2001 and set aside for the experiment. In choosing individuals for study certain criteria had to be met. All animals had to be freshly collected so that there was minimal bias from being held in the laboratory and maximal site-related effect on gametogenesis and vitellogenesis. Once egg laying had begun in any of the animals, no more experimental animals could be selected as prior egg laying history for the 2001 season would be unknown. Sex is difficult to determine externally, but as best could be established, females were selected. Although imposex (the imposition of male sexual characters on females such as the presence of a penis and vas deferens; Smith 1971) has been observed in *R. venosa* collected from the lower Chesapeake Bay (Westcott 2001), imposex was not a factor that could be accounted for *a priori* in the experimental design.

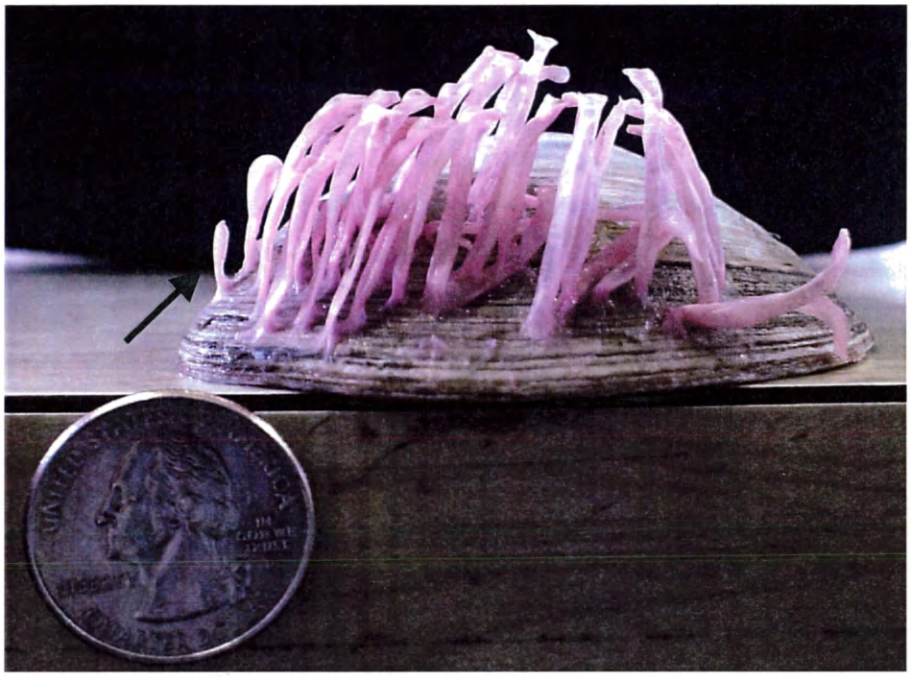
Five individuals meeting the above criteria were obtained from each of the Ocean View and James River sites, while nine such individuals were obtained from Hampton Bar.

Figure 5. Commonly observed morphologies of *Rapana venosa* egg cases from the Chesapeake Bay. Photographs courtesy of J. Harding, Virginia Institute of Marine Science, Molluscan Ecology, Gloucester Point, VA.

a. Common morphology

b. Tall-thin morphology

c. Short-flat morphology



d. Arrow to short-pointed morphology

e. Petite morphology on the right

Figure 5.

Collection Sites

The lower Chesapeake Bay is characterized by a cline of chemical and physical gradients including substrate, salinity, and depth. We chose three sections of the lower Bay that have high occurrence of *R. venosa* and are along an environmental cline (see Table 1 and Figure 4). These areas were the James River site, located between the Monitor-Merrimac Bridge Tunnel and State Route 258 James River Bridge; Hampton Bar, located between the Monitor-Merrimac Bridge Tunnel and the Hampton Roads Bridge tunnel; and Ocean View, located off Willoughby Spit, outside of the Hampton Roads Bridge Tunnel and up to but not beyond the Chesapeake Bay Bridge Tunnel. The deepest site, 20-60 feet, is the James River Site and has mostly soft sand with one rock pile and a salinity of 15-20 ppt. The Hampton Bar site has a depth of 15-30 feet with a firm sand bottom (not as much mud or silt as the James River location) and a salinity of 22-28 ppt. The final site, Ocean View, has a depth similar to Hampton Bar, 18-30 feet, and a hard sand bottom. The salinity is 25-35 ppt, the highest of any of the sites.

Adult Maintenance

To quantify the variability in reproductive output of *R. venosa* over time and space at the individual level, egg production over time was related to food and temperature in the York River. Each adult female was isolated and maintained in a 2-gallon bucket with 2-inch diameter holes cut in the sides and bottom to facilitate water exchange. The buckets were kept in Taylor floats (large cages typically used for oyster culture) immersed in the York River (Figure 6). Floats were moored in ~1 m depth of water adjacent to the shoreline at VIMS. At no time were the floats stranded at low tide.

Figure 6. Taylor float with buckets containing individual adult *R. venosa* in the York River, Chesapeake Bay, U.S.A.

Figure 7. Whole *R. venosa* egg mass positioned on grid for random selection of individual cases.

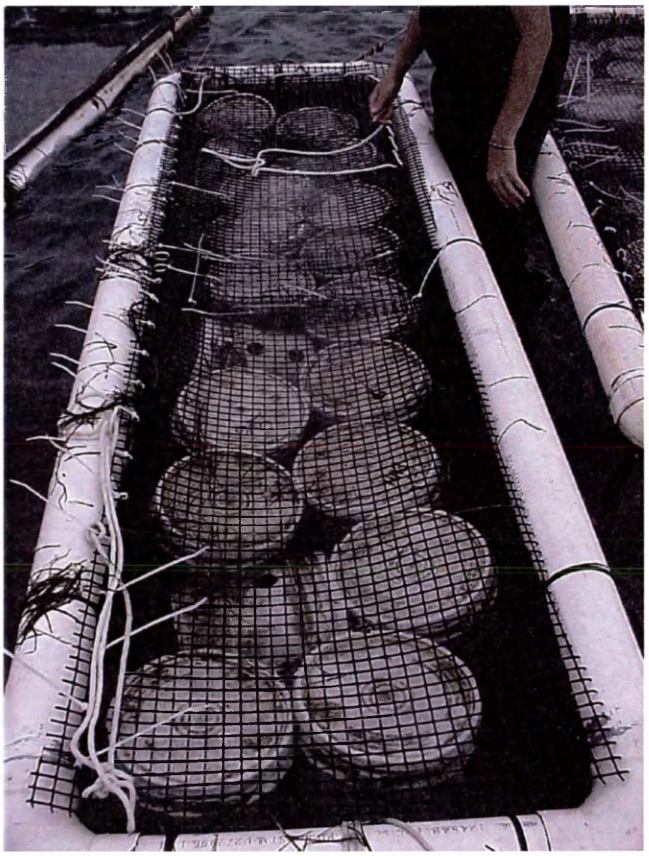


Figure 6.

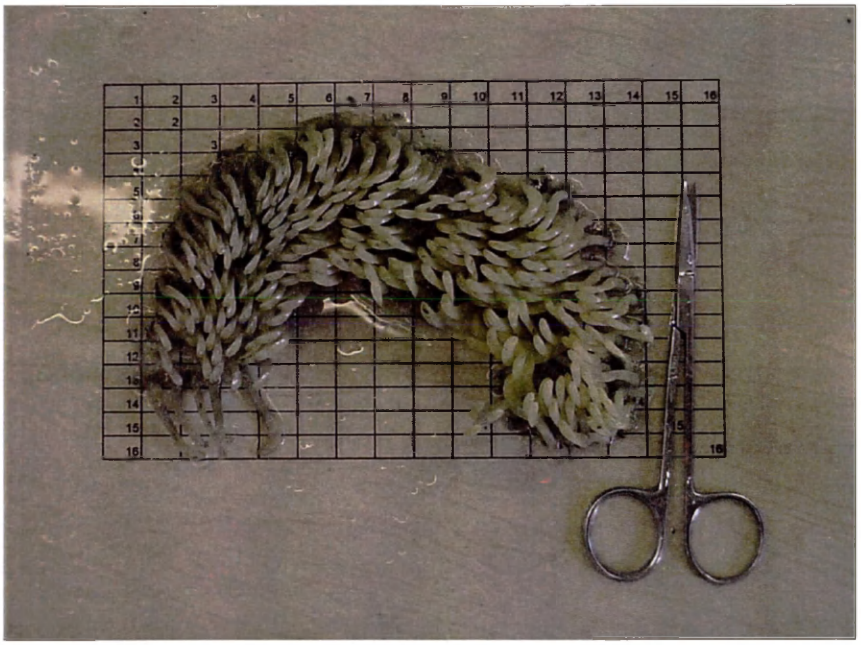


Figure 7.

Excess food (two chowder size clams each (*Mercenaria mercenaria*)) was provided to the rapa whelks and replenished as necessary. The length and height of the clams eaten by each whelk was recorded. The rapa whelks were weighed and shell length measured at the beginning and end of the experiment (May 6 and August 28, respectively). All egg masses were collected twice per week for image analysis and culture. Egg masses were not harvested if the adult was still in the process of laying them. Because the adults were observed to lay an egg mass over four to five days, dividing the reproductive season into weeks gave appropriate resolution to observe variation over the entire summer.

Egg Mass Culture

Once harvested from the buckets, egg masses were weighed, the number of egg cases counted, and a qualitative morphological description assigned (Figure 5). The egg mass was then placed on a 5 by 10 mm grid and 5 egg cases selected by random numbers based on grid coordinates (Figure 7). These 5 egg cases were set aside for image analysis. When 40 or more egg cases were in an egg mass, half of the egg mass (by mass) was frozen for archival purposes supporting other studies. Limiting the number of egg cases maintained in any one culture was important in order to minimize potential deleterious effects of culturing large egg masses in a limited-size jar (see Chaffee and Strathmann 1984). The remaining egg cases were dipped in a series of rinses akin to mariculture techniques for queen conch (Davis 1994): 1 L filtered seawater, 1L filtered seawater plus one capful of bleach, 1 L filtered seawater and 1 L filtered seawater. After rinsing, the egg mass was stored in an individual glass jar filled with filtered seawater (Figure 8). The water was changed every other day according to the

Figure 8. *Rapana venosa* egg masses in filtered seawater.

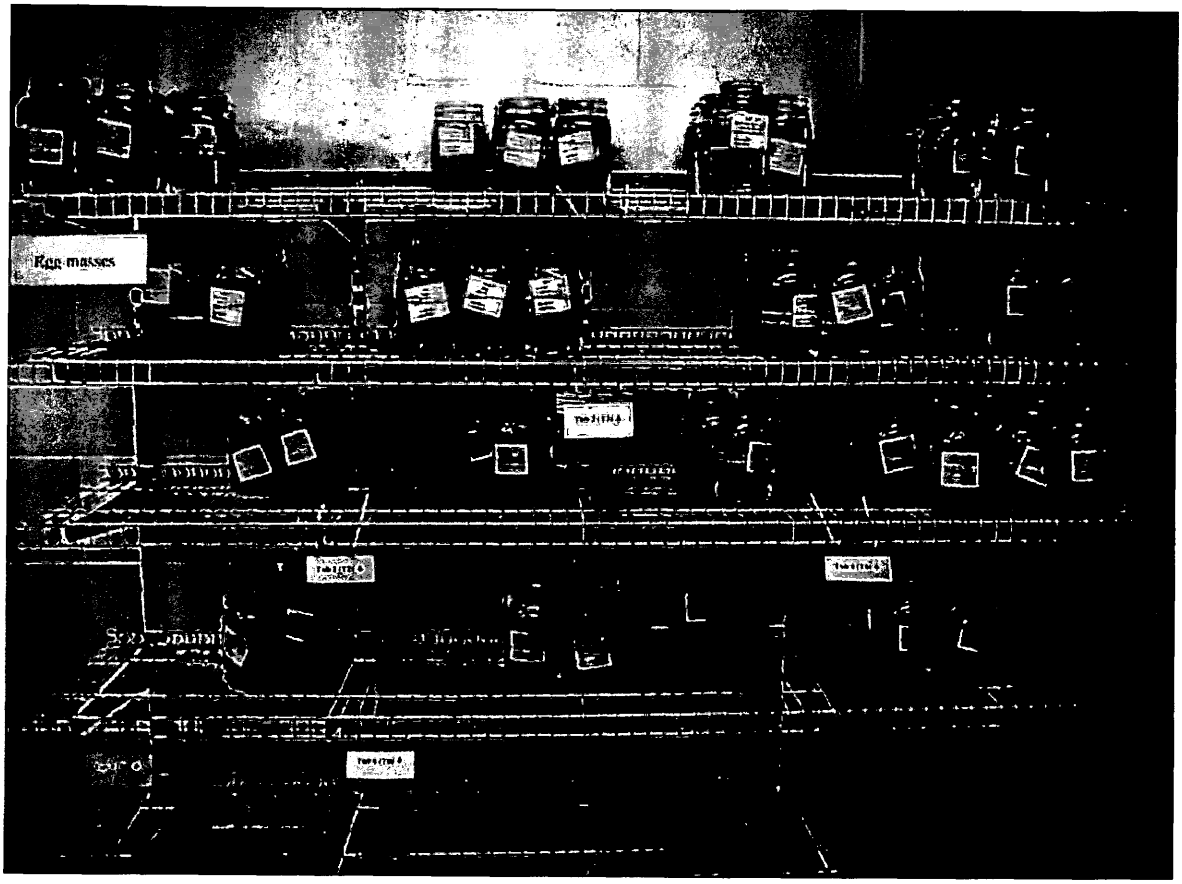


Figure 8.

protocol developed in 1999 (Harding and Mann unpub. data). During water changes, any cases with color other than pale to dark yellow were discarded.

Image Analysis

Once 5 egg cases had been randomly selected from the egg mass, digital images of the side view and the apical plate of each case were recorded (Figure 3b, (A) and (D)). From these images, height and area of the egg case side view and apical plate were measured using ImagePro® software.

In addition, four of these egg cases from six selected adults were dissected to count the eggs within. These six adults (two from each site) were chosen to represent the minimum and maximum weight and shell length within the size range of adults collected at each site. A dilution was performed to estimate the number of eggs in the egg case. Eggs were rinsed into 7 or 10 ml of seawater in a plastic centrifuge tube. The tube was gently mixed on a vortex mixer once (about 2 seconds) and three 1 ml aliquots successively withdrawn and placed on a Sedgewick Rafter counting cell.

To measure egg diameter in the above egg cases, digital images of the eggs in the counting cell were recorded. The eggs proved too fragile to withstand the shear forces exerted by the removal from the egg case and the pipette, so the shape of the egg was not sufficiently consistent to measure a representative diameter. For future study, photographing the eggs without removing them from the egg case may provide more accurate measurement, although the significance of egg size as a reliable indicator of parental investment and egg content has been challenged (McEdward and Carson 1987).

For the purposes of this study, counting the eggs accurately with the limited time and effort available took priority.

Image analysis was also used to measure 10 of the larvae released from the egg case. The larvae were preserved on the day they were released from the egg case in 95% EtOH for future photography and subsequent measurement of maximum shell length.

Data Collection and Archive

The sample size was dictated by the number of egg masses produced by the adults from each site. The five adults from Ocean View produced 30 egg masses, while the nine from Hampton Bar produced 100 egg masses, and the five adults from the James River produced 62 egg masses. For each egg mass the following information about the parent of the egg mass was recorded: the collection site of the parent, shell length and weight of the parent at the beginning and the end of the summer, and the number, height and length of the clams eaten by the parent.

Information relating to the morphometrics of the egg cases from an egg mass included the height and area of the egg case apical plate, and height and area of the cross-section of 5 egg cases from each egg mass at time of harvest. After 5 - 40 days post-harvest, when the egg mass had released larvae or was dead, measurements of the height and area of the egg case apical plate, and height and area of the cross-section of up to 6 egg cases from each egg mass were taken.

Information describing the hatching success of the egg mass (the release of swimming larvae) includes the length of time the embryos were encapsulated and the

average size of larvae on the day of release from the egg case (maximum shell length of 10 preserved larvae).

To describe the eggs within the egg case, the egg masses of 6 adults were analyzed to find the number of eggs in 4 of the egg cases. The diameters of 10 of the eggs in those 4 egg cases were also measured.

Data Analysis

Variables

The variables used in the statistical analyses consist of the following:

Primary Variables	Description
Source	Nominal description of where the adult was collected (James River, Hampton Bar or Ocean View) to resolve spatial variation.
Sequence	For each individual *Rapana venosa*, this indicates whether the egg mass laid was the first, second, and so on in 2001 to resolve temporal variation.

Secondary Variables	Description
Adult ID	The adult that laid the egg mass.
Shell length	The shell length (mm) of the adult.

Weight	The average of the wet weights (g) of the adult, including shell, measured at the beginning and at the end of the experiment (May and August).
ECs	The number of egg cases in one egg mass.
HtA	The average height (cm) of the cross-sectional view (A) of 5 randomly selected egg cases from the egg mass.
AreaA	The average area (cm^2) of the cross-sectional view (A) of 5 randomly selected egg cases from the egg mass.
HtB	The average height (cm) of the apical plate (view B) of 5 randomly selected egg cases from the egg mass.
AreaB	The average area (cm^2) of the apical plate (view B) of 5 randomly selected egg cases from the egg mass.
Dev Time	Development time (days), or the time from the date the egg case was harvested until the release of veligers from the egg case.
Hatch Size	The average shell length (μm) of 10 veligers preserved on the day of release from the egg case.
No. eggs	The average number of eggs per case of 4 egg cases randomly selected from the egg mass, logarithmically transformed when used as an independent variable (but not when a response of a regression model).
Hatch?	Binary description of whether an egg mass released veligers or not (1 = yes, 2 = no).

Temp	The average for each week of daily mean water temperature (°C) measured at the VIMS ferry pier.
Salinity	The average for each week of daily salinity measurements (ppt) at the VIMS ferry pier.

Sample Size

The number of egg cases randomly selected for measurement was determined after examining a plot of the standard error of the mean versus added samples (Bros and Cowell 1987; e.g. Figure 9). Low standard error of the mean was balanced with the time constraints of processing fresh samples in order to eliminate the bias of preservation.

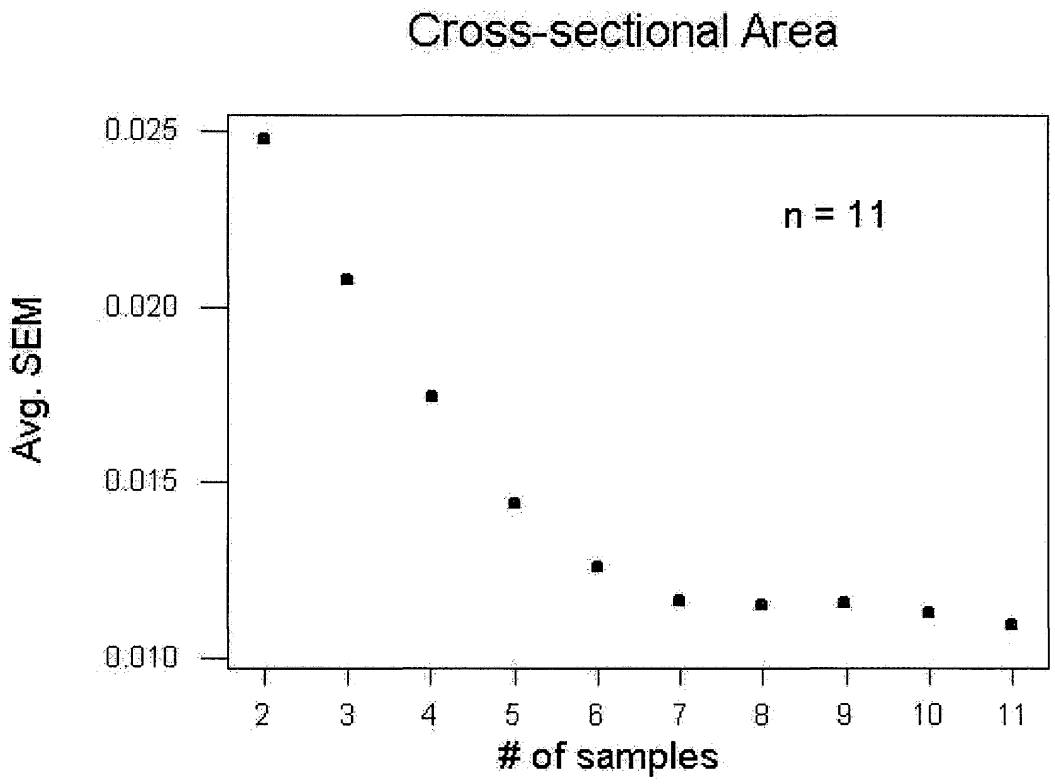
Data Transformation

As most variables were subject to natural variability and measurement error (violating the assumptions of Model I regression analysis), the variables were standardized to have a mean of zero and a standard deviation of one before the slope of a regression equation was computed, a technique necessary for Model II regression (Sokal and Rohlf 1981). This also allows the magnitudes of partial regression coefficients to be compared directly by eliminating the effect of differences in measurement scale. Except where otherwise specified, all statistical analyses were performed using Minitab®, version 12.1.

Description of Adult Specimens

Linear regression models were applied to the Shell length, Weight, and penis length (when present) to test the null hypothesis that no correlation among these variables

Figure 9. Change in standard error of the mean (SEM) in the measurement of cross-sectional area of egg cases from one egg mass with the addition of samples.


Cross-sectional Area
Avg. SEM
0.025
0.020
0.015
0.010
n = 11
2
3
4
5
6
7
8
9
10
11
# of samples

was present. To test whether a significant difference existed between the group of egg masses laid by imposex adults and the group laid by true females Analyses of Variance were performed on three estimates of fecundity using SYSTAT® version 10.2. The presence of a penis was converted into a binary descriptor (1 = yes, 2 = no) and entered as the factor variable. For the first estimate of fecundity, the dependent variable was No. eggs with Temperature and AreaA as covariates. A second ANOVA was run with the dependent variable Hatch Size (the second assay of fecundity) and Dev Time and ECs as covariates. To test whether the presence of a penis had an effect on the variable Hatch?, the third and only binary measure of fecundity, the Mann-Whitney test was used as a non-parametric analog of the two-sample t-test.

Change in Egg Case Size

A MANOVA compared the morphological measurements of egg cases, averaged for each egg mass, when harvested and at the release of veligers to test the hypothesis that egg cases change size while embryos are encapsulated.

Estimates of Fecundity

To test the null hypothesis that the primary spatial and temporal variables and the secondary variables do not affect fecundity, three metrics of fecundity were analyzed. These included the number of eggs per case, the hatch size of the veligers released from the egg case, and the ability of the egg mass to release veligers (a binary category). These three metrics were chosen to represent not only the number of offspring that an adult *R. venosa* may produce, but also the viability of these offspring. The number of egg

masses that an adult produces was not chosen as an estimate of fecundity because of the variable number of egg cases in egg masses. The number of egg cases per egg mass was similarly not chosen because of the variable number of egg masses produced, but was included as an independent variable in most regressions.

1. Number of eggs per case

The data set used to address the first estimate of fecundity consisted of 62 egg masses laid by 6 *R. venosa* (the maximum and minimum-sized adults from each site). A stepwise multiple regression was run with the number of eggs per case as the response and Adult ID, Source, Sequence, Weight, AreaA, ECs, Temp and Salinity as predictors. To determine significance of the variables an F-statistic value of 4.00 was used as the approximate significance level of 95% confidence in rejecting the null hypothesis of no effect of the variable on the number of eggs per case. The F-statistic determined whether to include the variable in the final regression model (the F-to-enter and F-to-remove). The T-value given is the square root of the F-statistic: informative because it is the same sign as the coefficient in the regression. Partial correlation coefficients (r) among the above variables were calculated using Pearson's method.

2. Veliger Hatch Size

The data set used to address the second estimate of fecundity consisted of 150 egg masses laid by 18 *R. venosa* adults. A stepwise multiple regression was run with hatch size as the response and 8 predictor variables: Source, Sequence, Weight, ECs, Temp, Salinity, AreaA, and Dev Time. Again, 4.00 was the F-statistic to enter and F-statistic to

remove to represent a 95% confidence level. Partial correlation coefficients (r) among the variables were calculated using Pearson's method.

3. Ability of egg mass to release veligers

To test whether there was a significant difference between the group of egg masses releasing veligers and the group of egg masses that did not, a mixed-design ANOVA was performed using SYSTAT® version 10.2. Source was a between-subject factor along with Hatch, due to their categorical nature. The within-subject dependent variables were Sequence, Adult ID, Weight, AreaA, AreaB, and No. eggs.

RESULTS

General Observations of Temporal and Spatial Variation of *Rapana venosa* egg case morphology and release of larvae (1999-2000)

In 1999 and 2000 combined, out of a total of 1587 egg masses, 24% hatched successfully (released swimming larvae).

At all sites, the short-flat morphology was the prevalent rare morphology type (Ware et al. 2001). Egg cases from Ocean View had the highest percentage of the common morphology but the lowest percentage (25%) of successful hatches. James River egg cases had the lowest percentage of common morphology type but also had the highest percentage (33%) of successful hatches. Egg cases from Hampton Bar fell between the two afore-mentioned sites in both percentage of rare morphology types and percentage of masses releasing swimming larvae.

Assuming that adult *R. venosa* are uniformly spread among these three sites in the lower Chesapeake Bay, the area that will produce the most viable larvae is the area that produces egg masses with the highest percentage of successful hatches. A slight difference in the percentage of egg masses releasing larvae, when multiplied by the number of females laying egg masses and the number of egg masses they lay in a season, can influence propagule pressure (the rate at which breeding individuals are released by adults; Williamson 1996) at a certain site. Based on this data from 1999 and 2000, the

James River broodstock adults produce the highest percentage of egg masses that release veligers, which translates into a significant advantage in the reproductive success of this population when all other factors are equal.

Quantifying the variability in reproductive output of *R. venosa* over time and space at the individual level

General Description

Size distribution of adults

Differences in body size (shell length or weight) were not significant among the three sites.

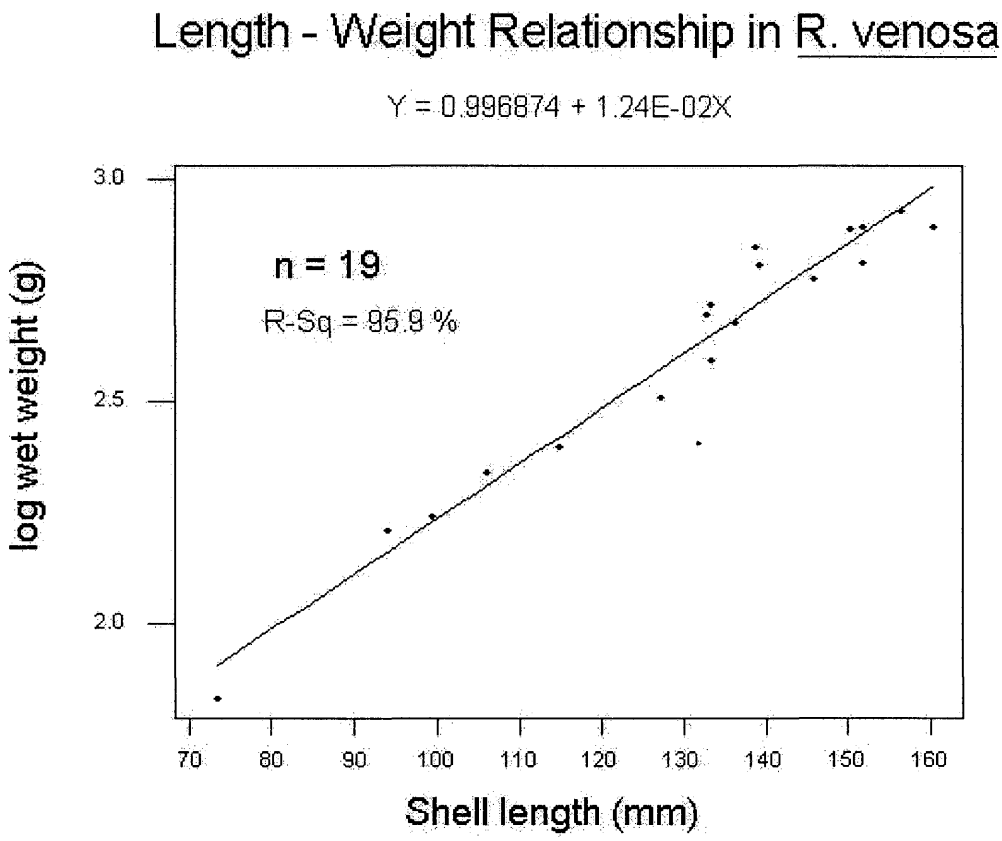
Table 2. Size of adults collected from three sites.

Source	N	Shell Length (mm)	Standard Deviation
James River	5	143.80	13.54
Hampton Bar	9	128.33	22.71
Ocean View	5	122.10	30.58

Length – Weight Relationship in Rapana venosa

Linear regression failed to reject the hypothesis that shell length is correlated with weight of adult *R. venosa*. Shell length is related exponentially to wet weight ($p < 0.005$, $R^2 = 0.959$, Figure 10). As weight and shell length are highly correlated, for further analyses only the weight of the animal was regressed with other variables. Weight of an

Figure 10. The linear regression plot of $\log_{10}$ transformed wet weight of *Rapana venosa* adults versus the shell length of the adults. The regression model explains 95.9% of the variance.


Length - Weight Relationship in R. venosa
Y = 0.996874 + 1.24E-02X
n = 19
R-Sq = 95.9 %
log wet weight (g)
3.0
2.5
2.0
70
80
90
100
110
120
130
140
150
160
Shell length (mm)

animal can fluctuate more than shell length, and is therefore more indicative of the condition of the animal.

Penis Length

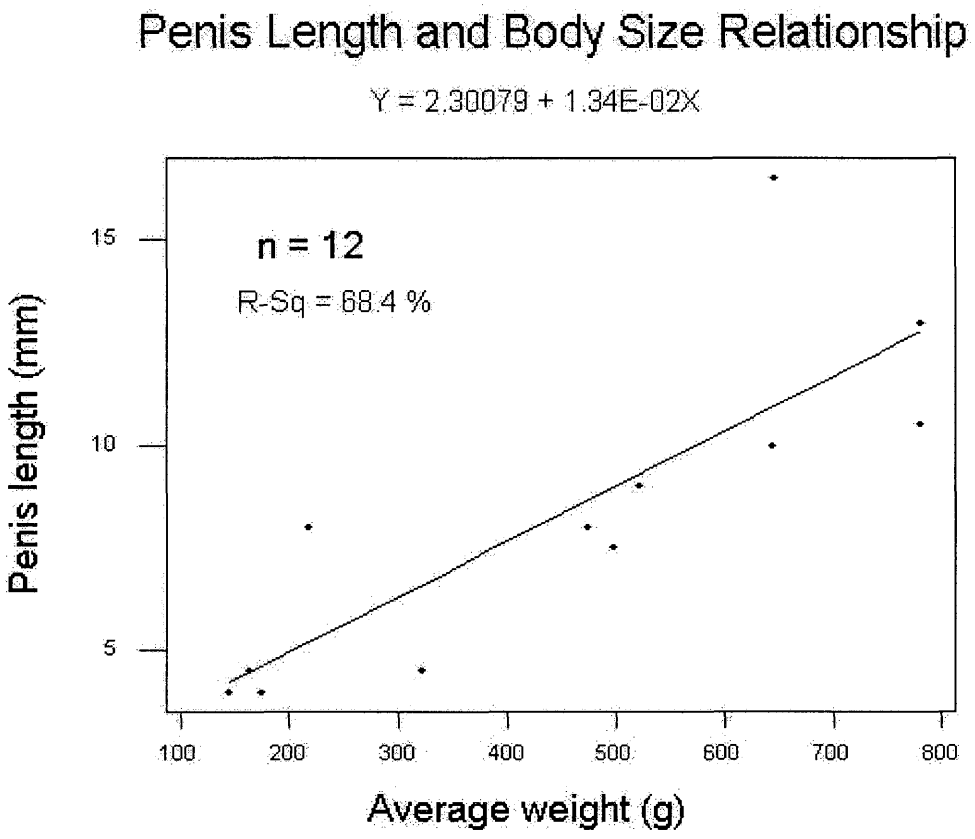
Out of the 19 adults in the experiment, 12 had penises (see Appendix I). Based on multiple regression analysis, there is a significant relationship between the weight of *R. venosa* and penis length in imposex individuals ($p < 0.005$, $R^2 = 0.684$; Figure 11), while Source, Adult ID and the number of egg cases laid per egg mass can not significantly predict penis length.

In the mixed-design Analysis of Variance, the presence of a penis had no significant effect on the total number of egg cases produced by an adult, the number of eggs in an egg case, the hatch size of the veligers, or whether the egg mass released veligers.

Change in Egg Case Size

To evaluate the potential for a change in size in the egg case morphology from the time of laying until release of veligers, the morphology measurements (HtA, AreaA, HtB, and AreaB) for each egg mass were compared at the day of harvest (within 48 hours post-deposition) and the day of release of veligers. The MANOVA ($n = 130$) indicated no significant difference between the two groups when the measurements were considered.

Figure 11. The linear regression plot of the penis length of imposex *Rapana venosa* adults versus the weight of the adults, averaged over the May to August time period. The regression model explains 68.4% of the variance.


Penis Length and Body Size Relationship
Y = 2.30079 + 1.34E-02X
n = 12
R-Sq = 68.4 %
Penis length (mm)
15
10
5
100
200
300
400
500
600
700
800
Average weight (g)

Estimates of Fecundity

General Description of Fecundity

The 19 *R. venosa* adults produced egg masses from May 15 until August 6, 2001. This period was characterized by temperatures ranging from 19.2 °C to 27.7 °C and salinities from 16.8 ppt to 19.9 ppt. The adults laid between 2 and 18 egg masses each over the summer. The origin of the adult significantly affected the number of egg masses that the adults laid ($p = 0.014$; Appendix II), but the weight of the adult did not. The adults from Ocean View laid significantly fewer egg masses (mean = 6.0, std dev = 3.08) than either James River or Hampton Bar adults (which were not significantly different from each other, laying on average 12.6 and 11.1 egg masses, respectively) based on Tukey's pairwise comparisons test. The number of egg masses produced per week was not significantly correlated with either temperature or salinity.

The adult *R. venosa* laid between 4 and 599 egg cases in each egg mass, with a mean of 149 egg cases per mass and a standard deviation of 104.9. The average number of egg cases laid in each egg mass did not differ significantly among sources, nor was it significantly correlated with weight of the adult. The total number of egg cases, summed over all egg masses, per adult, however, differed significantly among source ($p = 0.004$; Appendix II). James River *R. venosa* produced significantly more egg cases than the adults from Hampton Bar and the adults from Ocean View based on Tukey's pairwise comparisons, but there was no significant difference between the latter two sites.

Number of Eggs per Case

The average number of eggs per case was 1440 eggs (ranging from 113 to 3258, averaged within the egg mass; Figure 12).

The magnitude of correlation between the predictor variables (Adult ID, Source, Sequence, Weight, ECs, Temp, Salinity, HtA, AreaA, HtB, and AreaB) was calculated by the Pearson partial correlation coefficients shown in Table 3. Temperature was significantly correlated to both Salinity and Sequence (the ordinal number assigned for each individual's egg masses)(Figures 13 and 14). Source, the area from which the adults were collected, and Sequence were significantly correlated, most likely because the higher values of Sequence occur solely within those Sources whose adults laid more egg masses. Temperature was correlated negatively with the cross-sectional area of the egg cases and the apical plate area, as was Sequence, although the regression coefficients for these correlations were not large (-0.30 or lower). The measurements of the apical plate (both the area and height measurement) both correlated significantly with the variable AdultID, a categorical variable assigned for each individual *R. venosa.*

All egg case measurements were positively correlated with weight of the adult. The correlation coefficients between the morphological measurements of HtA, AreaA, HtB, and AreaB are large in magnitude and highly significant (Table 3). AreaA, the cross-sectional area, alone was used in regression analysis and the other measurements of case morphology omitted because of the high correlation. AreaA was chosen because of the high magnitude of correlation not only with HtA, but also with HtB and Area B.

Figure 12. Distribution of the number of eggs (in increments of 200 eggs) per egg case averaged for each egg mass, with normal curve. Four randomly selected egg cases were dissected from 62 egg masses.

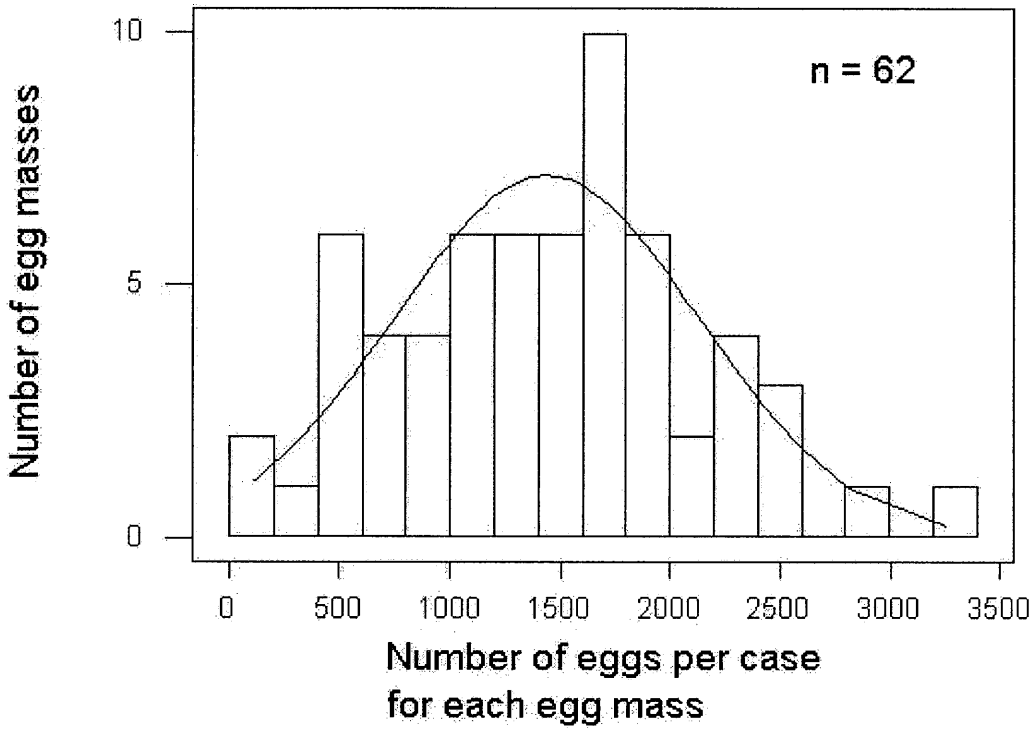

n = 62
Number of egg masses
10
5
0
0
500
1000
1500
2000
2500
3000
3500
Number of eggs per case
for each egg mass

Table 3. Partial Correlation Coefficient Matrix (Pearson). Correlations of variables describing egg masses (n = 193). The cells contain the partial correlation coefficient (*r*) above the P-value for the coefficient. * denotes $p < 0.05$.

	Adult ID	Source	Sequence	Weight	ECs	Temp	Salinity	HtA	AreaA	HtB
Source	0.029 0.694									
Sequence	-0.061 0.401	-0.237 0.001*								
Weight	0.043 0.549	-0.102 0.156	0.008 0.916							
ECs	0.027 0.712	-0.039 0.590	-0.131 0.070	0.061 0.402						
Temp	-0.026 0.719	-0.026 0.721	0.605 0.000*	-0.029 0.693	0.024 0.743					
Salinity	-0.058 0.425	0.082 0.257	0.102 0.159	0.006 0.934	-0.089 0.218	-0.277 0.000*				
HtA	0.035 0.632	-0.167 0.020*	-0.011 0.878	0.755 0.000*	0.226 0.002*	-0.036 0.619	0.052 0.475			
AreaA	0.044 0.543	-0.090 0.214	-0.174 0.015*	0.765 0.000*	0.250 0.000*	-0.153 0.034*	-0.061 0.403	0.929 0.000*		
HtB	-0.243 0.001*	-0.196 0.006*	-0.085 0.239	0.566 0.000*	0.128 0.076	-0.161 0.025*	0.067 0.357	0.702 0.000*	0.739 0.000*	
AreaB	-0.210 0.003*	-0.186 0.010*	-0.200 0.005*	0.578 0.000*	0.129 0.075	-0.302 0.000*	0.076 0.291	0.668 0.000*	0.747 0.000*	0.944 0.000*

Figure 13. A plot of mean water temperature, as measured at the Virginia Institute of Marine Science's Ferry Pier, versus the week over which measurements were taken.

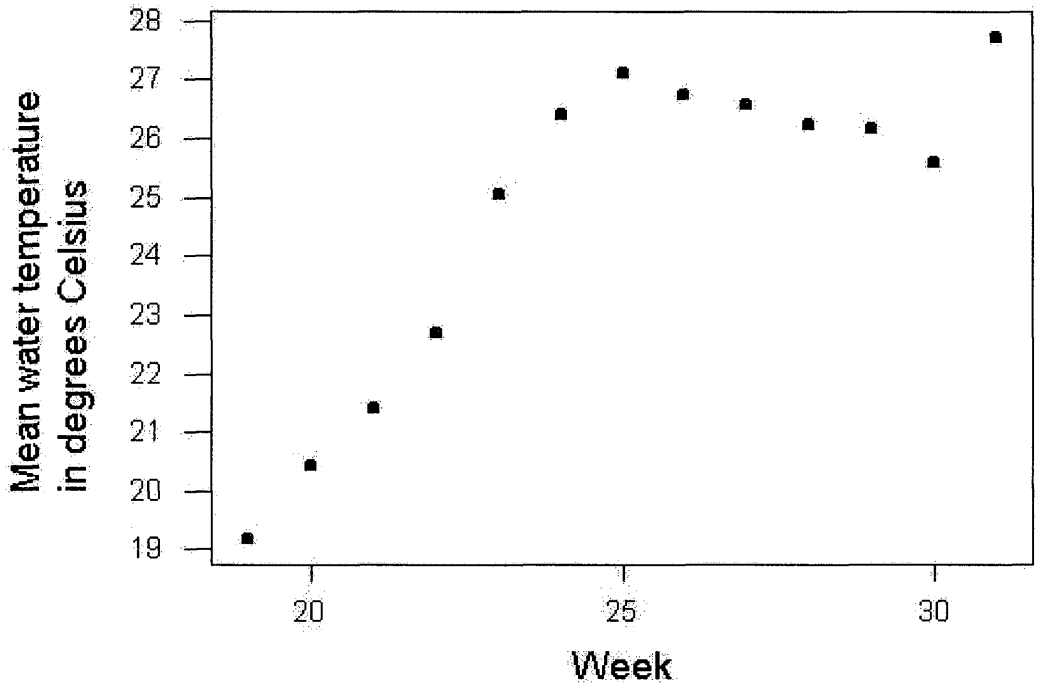
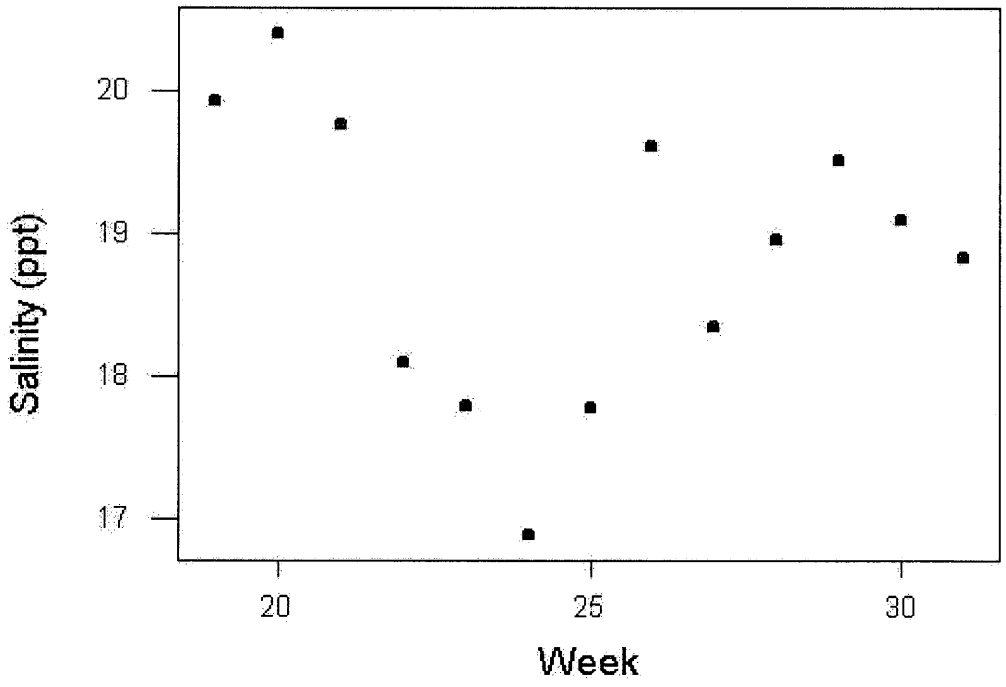

Mean water temperature
in degrees Celsius
28
27
26
25
24
23
22
21
20
19
20
25
30
Week

Figure 14. A plot of mean salinity, as measured at the Virginia Institute of Marine Science's Ferry Pier, versus the week over which measurements were taken.


Salinity (ppt)
20
19
18
17
20
25
30
Week

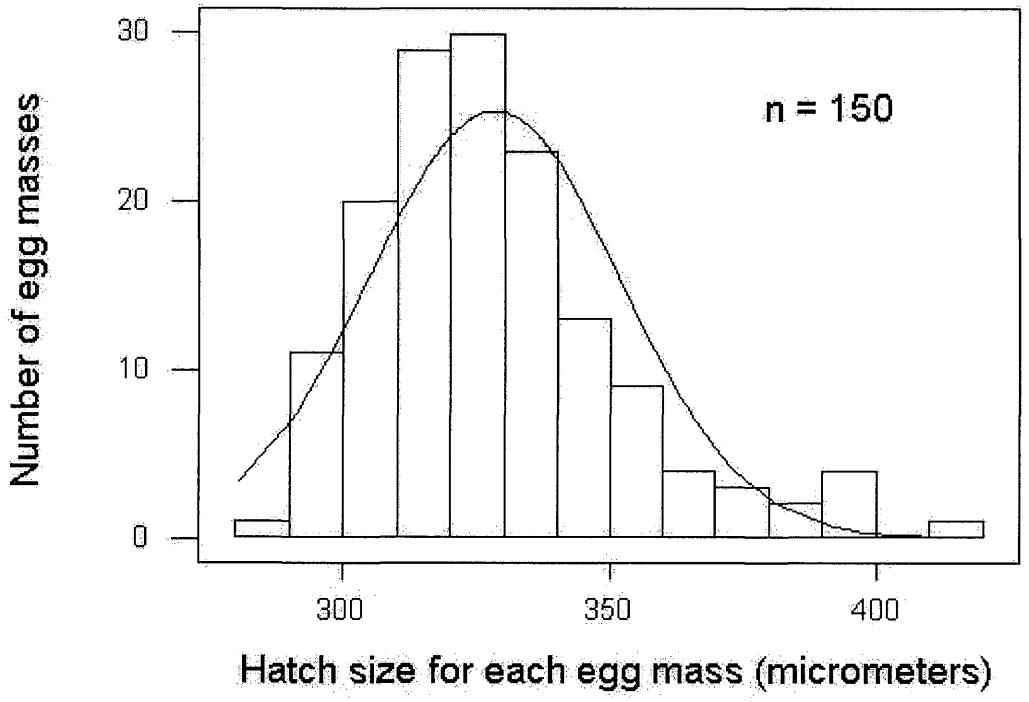
The stepwise regression showed a statistical dependence of the number of eggs per case on Temperature and AreaA (the egg case cross-sectional area). When the response No. eggs was regressed with 8 predictors (Adult ID, Source, Sequence, Weight, ECs, Temp, Salinity, AreaA), AreaA and Temp were significant predictors ($R^2 = 0.568$, T-value of 8.65 and 4.55, respectively). These two variables regress positively with the numbers of eggs per case. Sequence and Source, the temporal and spatial variables in the regression, as well as Adult ID, Weight, ECs, and Salinity have no significant statistical effect on the number of eggs per case.

In order to analyze whether or not the effect of temperature on the number of eggs per case was a temporal index, the null hypothesis that there would be no change in the significance of temperature between the weeks before and including week 25 (when the temperature increases linearly with week) and the weeks after week 25 (when a temporal trend is less apparent), these data were separated into two groups and the multiple regression repeated. The null hypothesis could not be rejected, as temperature was not a significant predictor of the number of eggs in either data set.

Hatch Size

Egg cases releasing veligers appear to release all of the veligers within the egg case – a healthy egg case (i.e. those with normally developing veligers) is completely empty after release. Before release, the veligers can be seen swimming in the egg case. The average size distribution of hatching larvae ranged from 280 to 412 micrometers, with an average shell length of 328 ± 23.6 micrometers (Figure 15). The time until release from the egg case (Dev Time) varied from 6 to 68 days, with an average of 30

Figure 15. Distribution of shell length at its maximum (micrometers) of shelled larvae released from egg masses (in 10 micrometer increments), with normal curve. For each of 158 egg masses, the shell length of 10 larvae was averaged.


30
20
10
0
n = 150
300
350
400
Number of egg masses
Hatch size for each egg mass (micrometers)

days in the egg case (Figure 16).

The null hypothesis that the predictor variables (Adult ID, Source, Sequence, Weight, AreaA, ECs, Dev Time, Temp, Salinity) are independent of each other was refuted by the partial correlation coefficients shown in Table 4. As for the numbers of eggs per case, the partial correlation coefficients of greatest magnitude were those between the measurements of egg case morphology so all but one, AreaA, were omitted from the analysis. This analysis was performed on 150 egg masses (those that released veligers) and took into account 9 variables: Adult ID, Source, Sequence, Weight, ECs, Temp, Salinity, AreaA, and Dev Time.

Based on the low R^2 value, the stepwise multiple regression (n = 150) showed that hatch size was not dependent on the variables tested (Adult ID, Source, Sequence, Weight, ECs, Temp, Salinity, AreaA, and Dev Time), thus failing to reject the null hypothesis. Dev Time, or the time from the laying of the egg mass until it released veligers, was the only significant predictor of hatch size (T-value = -5.25, R^2 = 0.157) and regresses negatively with hatch size.

The time that the larvae remain in the egg case (Dev Time) can be predicted by a regression model including the number of egg cases in the egg mass (ECs), temperature, and the weight of the adult (R^2 = 0.498; T-value = 8.45, T-value = -7.81, and T-value = 3.18 respectively). Thus, the length of time that the embryos are encapsulated increases both as the number of egg cases in the egg mass increases, as temperature decreases, and as weight increases (magnitude of effect in that order). The other variables tested (Source, Sequence, Salinity, AreaA and AdultID) did not contribute significantly to the regression equation.

Figure 16. Distribution of the days between egg mass collection and the release of shelled larvae (in increments of 5 days), with normal curve.

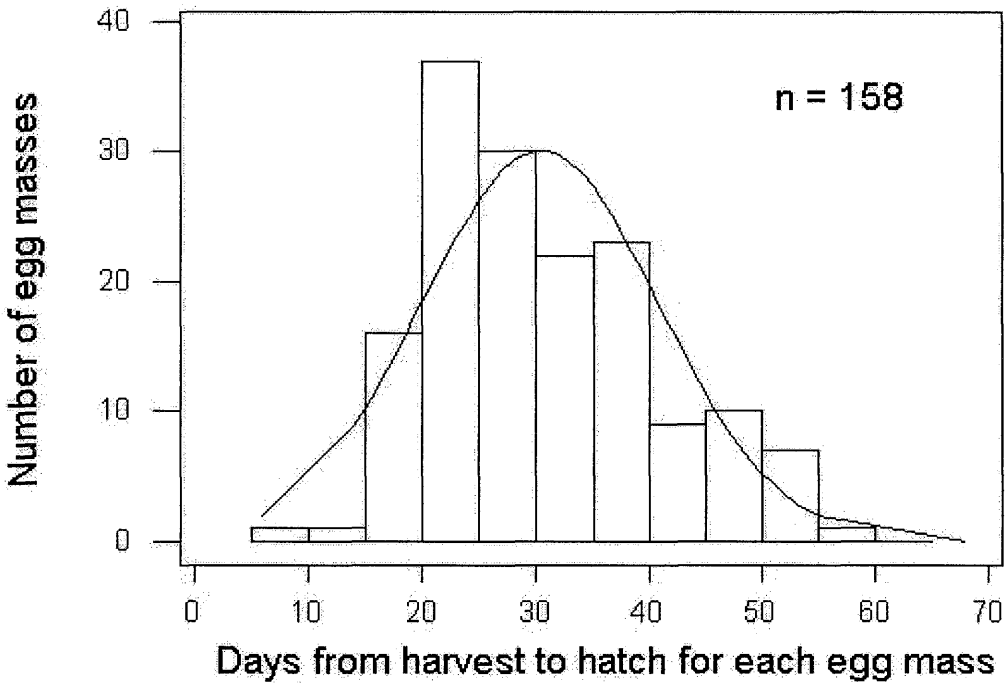

n = 158
Number of egg masses
Days from harvest to hatch for each egg mass
0
10
20
30
40
50
60
70

Table 4. Partial Correlation Coefficient Matrix (Pearson). Correlations of variables describing the egg masses that released veligers (n = 150). The cells contain the partial correlation coefficient (*r*) above the P-value for the coefficient. * denotes $p < 0.01$.

	Adult ID	Source	Sequence	Weight	ECs	Temp	Salinity	AreaA
Source	-0.030 0.715							
Sequence	-0.102 0.215	-0.213 0.009*						
Weight	0.030 0.712	-0.138 0.093	0.046 0.572					
ECs	-0.023 0.783	-0.001 0.987	-0.157 0.054	0.097 0.239				
Temp	-0.106 0.195	-0.013 0.878	0.564 0.000*	0.067 0.412	0.004 0.958			
Salinity	0.019 0.822	0.041 0.617	0.142 0.083	-0.018 0.825	-0.008 0.919	-0.244 0.003*		
AreaA	0.014 0.863	-0.142 0.084	-0.142 0.083	0.763 0.000*	0.291 0.000*	-0.094 0.252	-0.090 0.273	
Dev Time	0.080 0.333	0.088 0.282	-0.361 0.000*	0.205 0.012*	0.514 0.000*	-0.444 0.000*	0.081 0.324	0.341 0.000*

Ability of Egg Mass to Release Veligers

Out of the 188 egg masses cultured, 158 (84%) released swimming larvae. Based on the variables tested (Source, Sequence, Adult ID, Weight, AreaA, AreaB, and No. Eggs) in the mixed-design ANOVA, there was no significant difference between the group that released larvae and the group that did not release larvae.

DISCUSSION

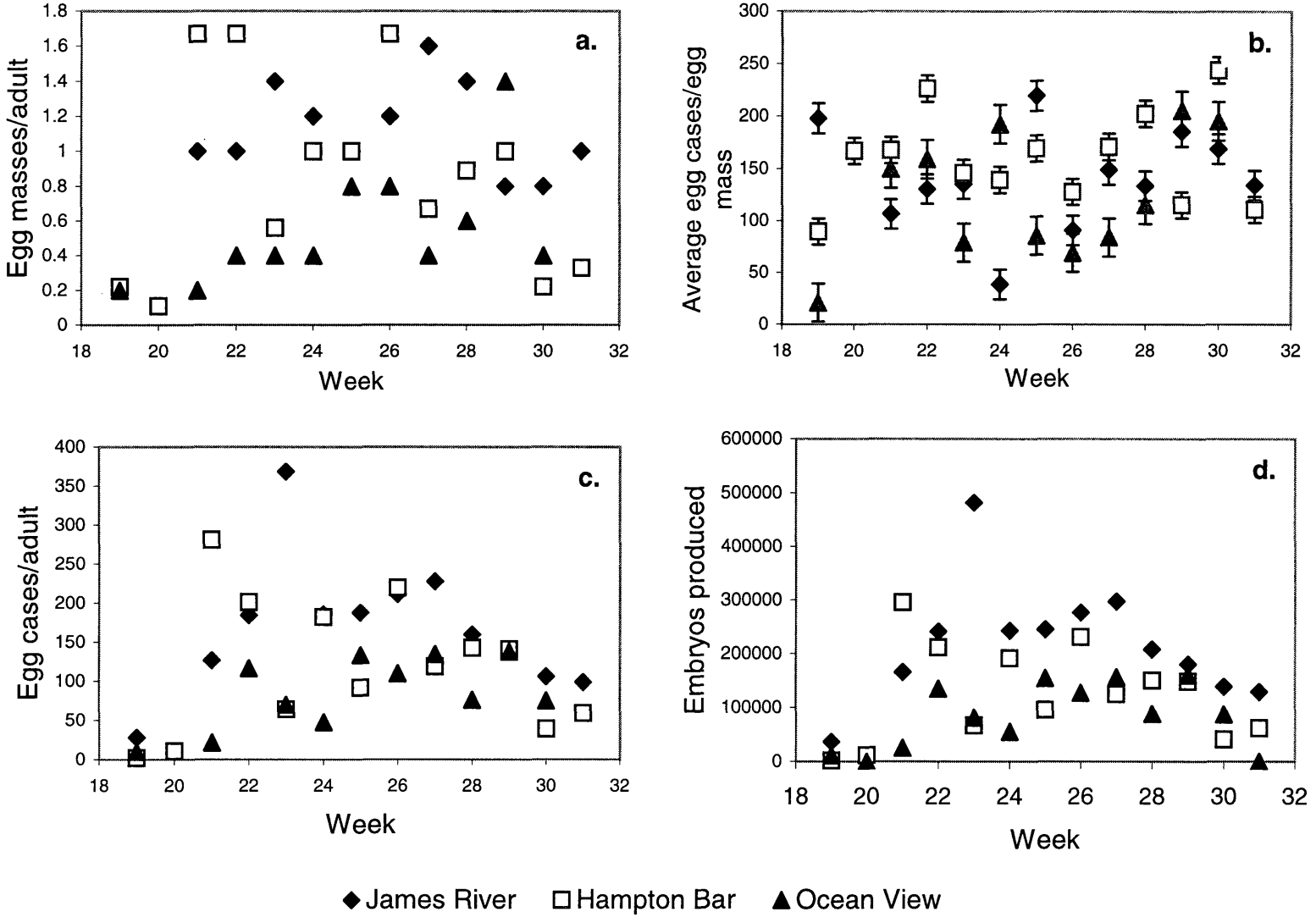
General Description of Reproductive Biology of *Rapana venosa* in the Chesapeake Bay

Spatial variation at the adult level

The spatial gradient from which the adults were collected appears to have an effect on the number of egg masses and egg cases that adult *R. venosa* produce. The general trends of spatial and temporal variation are shown in Figure 17. The James River adults produced significantly more egg cases than did those from either of the other sites, and, along with Hampton Bar adults, produced more egg masses than did Ocean View adults. Because there were no effects of the source of the adults on the three estimates of fecundity of the egg mass (number of eggs per egg case, hatch size of veligers, or the release of veligers), this difference in the number of egg cases produced will translate into greater propagule pressure in the James River area of the lower Chesapeake Bay.

Although there was a significant difference in the number of egg masses and total egg cases produced by adults from the three sites, there was no effect of source on the number of egg cases in each egg mass. This suggests a reproductive strategy of the adults to increase total production not through bigger egg masses, but more frequent laying of variably sized egg masses. There are several potential explanations for this strategy. In an evolutionary sense, frequently producing smaller egg masses can be advantageous by increasing the chances that the appropriate environmental conditions are

Figure 17. Temporal and spatial variation in reproductive output: a. the number of egg masses that were laid each week by the adults of each source, divided by the number of adults from that source laying egg masses; b. the average number of egg cases in each egg mass laid each week; c. the number of egg cases that were laid each week divided by the number of adults from that source laying egg masses; and d. the number of larvae produced by adults from each of the three sources over one summer. Calculated by multiplying the number of cases per adult by the average number of eggs adults from each source laid in an egg case and the percentage of egg masses that released larvae.


a.
Egg masses/adult
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
18
20
22
24
26
28
30
32
Week
b.
Average egg cases/egg mass
300
250
200
150
100
50
0
Week
c.
Egg cases/adult
400
350
300
250
200
150
100
50
0
Week
d.
Embryos produced
600000
500000
400000
300000
200000
100000
0
Week
James River
Hampton Bar
Ocean View

present for larval development. Alternatively, anatomy may limit the size of the egg mass that an adult can produce. Although the timing of egg production and egg case production is not precisely known, the adult may have a limited capacity for storing eggs before processing them into egg cases. This could necessitate a more fecund animal to produce more egg masses with lower number of egg cases per mass rather than egg masses with more egg cases. A third possibility is that the size of the egg mass is affected by external causes. Interruption of the adult by physical disturbance, a limit of suitable substrate, or contact with other animals during egg laying may prematurely terminate a large percentage of egg masses. Prosobranch molluscs spawning in the laboratory were found to produce egg masses with a smaller number of egg cases than those molluscs spawning in the field (D'Asaro 1970), most likely because of the higher density of snails present resulting in greater disturbance. Also observed in the laboratory was that the shape of the mass as a whole reflected the contours of the substrate (D'Asaro 1970). Limited areas of suitable substrate (in this study, limited by the holes cut in the experimental buckets) may also haphazardly terminate egg mass production.

The James River adults may produce more egg cases due to favorable environmental conditions. Conditions in the first months of a mollusc's life appear to affect growth rates as adults (M. Harasewych, Smithsonian Institution, and J. Harding, VIMS, pers. comm.). This, in turn, may dictate at an early stage the potential for reproductive output as an adult. Differences in the substrate among the three collection sites reflect physical differences that may influence the early development of *R. venosa*. Because veligers settle on hard substrate, the detrimental effect of increased physical

energy at the Hampton Bar and the Ocean View areas may be magnified compared to later life stages where the adults spend more time in the sediments.

Biotic factors are important to consider in describing the habitat conducive to the growth of *R. venosa*. The prey availability at the three sites is difficult to compare as *R. venosa* will eat many species of shellfish, not just those that are commercially valuable and therefore assessed for management. Hard clam (*Mercenaria mercenaria*) stock assessments in the early 1970s of the three areas where rapa whelks were collected show a fairly high abundance of clams in the limited area sampled in Ocean View and patchy but high densities of clams along the Newport News half of the Hampton Bar area (Haven et al., unpub. data). Stock assessments performed in the summer and fall of 2001 show similar relative distributions of lower densities in the James River and along the southern half of the Hampton Bar area (Mann, unpub. data). The known distribution of this prey species does not therefore explain the spatial variation in reproduction of *R. venosa* in the lower Chesapeake Bay, but suggests that alternative prey sources are available in the James River. Data on bivalve distribution is currently being collected and analyzed in conjunction with the previously mentioned 2001 stock assessments (Mann, unpub. data).

The high reproductive potential of the adults from the James River is somewhat surprising due to the contaminants found in this area of the lower Chesapeake Bay due to heavy commercial and military ship traffic, as well as recreational boats. In the Hampton Bar collection area, tributyltin (TBT) concentrations greater than 100 µg/kg dry weight have been found in sediments in the Hampton Roads marina and between 20 and 30 µg/kg west of the James River Bridge (Espourteille 1986). In populations of *Nassarius*

obsoletus in the York River region of the Chesapeake Bay, concentrations of TBT in seawater around 2 ng/l are considered adequate to initiate imposex (Bryan et al. 1989). In the United Kingdom, populations of *Urosalpinx cinerea* have developed imposex to an advanced state in which the oviduct is malformed, and copulation and egg case formation inhibited (Gibbs et al. 1991). Current collections of 127 adult female *R. venosa* from the lower Chesapeake Bay show a higher proportion of imposex females to true females in the James River collection area than either the Hampton Bar or Ocean View sites (Harding and Mann, unpub. data). The proportions of imposex females to true females at the latter two sites do not significantly differ from each other (Harding and Mann, unpub. data). Although the conditions in the lower Chesapeake Bay may be conducive to inhibiting the reproduction of muricid whelks, the imposex exhibited by *R. venosa* does not appear to affect the population's viability, as all of the imposex females were reproductively functional.

Variation Among Egg Masses

Primary Variables

In spite of the larger-scale spatial differences discussed above, there were no significant differences in fecundity (number of eggs per case, hatch size of veligers, and release of veligers) among egg masses laid with respect to the origin of the adult or the order in which the egg mass was produced (Source and Sequence). These components of fecundity were chosen because of their fundamental importance to the propagation of the species, but as such, may be highly conserved characteristics. Because the frequency of

egg mass production and total egg case production is variable among individuals, these aspects of fecundity may be more affected by short-term population-scale differences in the environment rather than the number of eggs per case, the hatch size of veligers and release of veligers.

Secondary Variables

Temperature and the cross-sectional area of the egg case (AreaA) were both highly correlated with each other and significant predictors of the number of eggs per case. As temperature increased, both the area of the cross-section of the egg case and the numbers of eggs within the case increased. There was no correlation between the height measurement of the egg case cross-section and temperature, however, which suggests that with the increase in temperature and then number of eggs per case, the egg cases increase in dimensions other than height (Table 3). Because the period of egg mass laying appears to be water temperature related (based on observations in 1999, 2000 and 2001; J. Harding, Virginia Institute of Marine Science, Gloucester Point, pers. comm.), this data supports the idea that the production of eggs both requires and may be enhanced by warmer temperatures.

AreaA, in addition to being a significant predictor of the number of eggs per egg case, was also significantly correlated (Table 3) with the number of egg cases in the egg mass, the Sequence of the egg mass, and the weight of the adult. The significant correlation of AreaA with the number of cases per egg mass has a low correlation coefficient (0.250), which makes interpretation difficult but suggests that perhaps the number of cases in an egg mass may not be entirely due to external disturbance, as earlier

suggested, but is positively related to egg case size. The Sequence also had a low, yet significant, partial correlation coefficient with AreaA (-0.174), which suggests a slight trend of egg masses that were laid later having a smaller cross-sectional area.

The weight of the egg mass had the highest partial correlation coefficient with AreaA (0.765), suggesting clearly that the larger the adult, the larger the egg case (and, by extrapolation, the higher the number of eggs in the egg case). Larger adults producing larger egg cases which consequently hold a larger number of eggs has been observed inter-specifically among muricid whelks (Spight et al. 1974) and is most likely explained by the capacity of the reproductive anatomy increasing as body size increases. Assuming the larger rapa whelks are older than smaller individuals (size-age parameters for this species are currently unknown), the older whelks in the population will be the most fecund age class.

Another interesting correlation was that between AdultID and the measurements of the apical plate (both height and area). Because the variable AdultID is a categorical value with no quantitative value (unlike Sequence), no trends can be discerned from the coefficient, but the presence of significance merits further investigation. This significance could suggest that the size and shape of the apical plate is unique to individuals, although the data from this experiment cannot be used to test this hypothesis conclusively.

Development time, the time that the embryos spend in the egg case before being released as veligers, was highly dependent on temperature, the number of egg cases per egg mass, and the weight of the adult. The significance of temperature suggests that the release of the larvae may depend on the rate of their development, as controlled by

physiological processes. Increasing temperature lowered the time the embryos spent in the egg case, possibly because the higher temperatures raises the metabolism of the embryos (Dorit et al. 1991), increasing their growth and consumption of the yolk material the adults provided. A second possibility is that release of larvae could be a function of dissolution of the egg case plug by an enzyme that is inhibited by lower temperatures (as in *U. cinerea*; Hancock 1956) and enhanced at warmer temperatures.

Mann and Harding (2000b) speculated that the variability in the length of encapsulation time of *R. venosa* might be a demonstration of phenotypic plasticity allowing exploitation of optimal hatching conditions within a variable environment. This idea, also applicable to recruitment in fish stocks, spiny lobsters, and Dungeness crabs, is similar to Cushing's (1990) match/mismatch hypothesis: that the release of larvae corresponds to peaks in plankton production. The correlation of the development time with water temperature may synchronize the release of *R. venosa* larvae to facilitate eventual recruitment to the benthos at a time when potential prey, e.g. newly recruited bivalves, are also present. There is no evidence, however, that any correlation between the settlement of *R. venosa* veligers and the settlement of other invertebrate planktotrophs is an evolutionary adaptation rather than simple result of temperature-driven physiology.

The second significant predictor of development time, the number of egg cases in an egg mass, may have artificially enhanced effects in the laboratory when the egg masses develop in static seawater. With many egg cases in the container, the limited amount of oxygen and a potential increase in excretion products (neither of which were measured in this experiment) may artificially restrict the rate of embryo development, causing the time until release from the egg mass to be prolonged. In the field this

problem may be alleviated by greater water flow. Removal of the dead egg cases from the container most likely alleviated the problems of both consuming and disposing of dissolved gases in a restricted environment, and may have limited the spread of protozoans among egg cases. This procedure may have been critical in increasing the successful release of larvae from egg masses from around 25% in 2000 to 84% in 2001 (158 of the 189 egg masses cultured).

The weight of the adult has a lower F-statistic and explains less of the variance in the regression than either of the previous two variables that also significantly predict the development time of larvae within the egg case. Larger adults may be capable of better provisioning their embryos with yolk to increase the time of development within the egg case. Because weight was not a predictor of hatch size, however, the advantages conferred by remaining in the egg case longer are dubious.

The independence of hatch size from the variables measured supports the idea that release of the veligers depends on larvae development. If release were not possible until the larvae reached a certain stage of development, development time should vary in response to environmental factors, but not the size of the released veligers, as was the case.

Imposex

Although not a factor that was incorporated into the design of the experiment, the paucity of true females in the group of adults selected seemed to have no effect on any aspect of reproduction observed. This is in agreement with observations at the gametogenic level, where no gonadal abnormalities differentiated the imposex females

from true females in *R. venosa* from the lower Chesapeake Bay (Westcott 2001). The presence of the male external reproductive anatomy does not appear to affect the reproductive processes of *R. venosa* collected in the Chesapeake Bay.

Reproductive Strategy of Rapana venosa

Investment in reproduction at the sacrifice of somatic growth is a phenomenon especially common in short-lived species with a single reproductive event each year or each lifetime. The converse of this situation is that species with longer lives and increasing fecundity, such as *R. venosa*, should have a lower level of reproductive effort in any given breeding season (Williams 1966). Few other invertebrate species within the Chesapeake Bay exhibit the magnitude of perennial iteroparity that *R. venosa* does, laying on average 10 egg masses per summer regardless of age (as measured by weight). An individual oyster, *Crassostrea virginica,* may spawn millions of eggs at once, but does so only three to five times a year. *Rapana venosa* produces, on average, 209,643 embryos per egg mass, which results in over 2 million embryos released per summer. In addition, in the laboratory *R. venosa* have begun to produce egg masses at less than one year of age (J. Harding, unpub. data). By the numbers, *R. venosa* appears to have many characteristics of an r-selected species as defined by Pianka (1970), yet also has a life-span that enables multiple spawning seasons and each with increasing fecundity.

The question of whether the reproductive strategy of *R. venosa* (in terms of mixed development) or that of the native whelks (crawl-away young) is the more recently derived life history has yet to be answered. Planktotrophic young are thought to be the primitive evolutionary form for gastropod molluscs (Strathmann 1978, Hadfield and Iaea

1989). The development of ciliated velum in embryos that do not go through a planktotrophic life stage, but rather ingest nurse eggs, supports this idea. All reproductive patterns should involve adaptations to maximize the number of offspring that survive and, consequently, their reproductive fitness: these adaptations should include an appropriate developmental time and hatching size (Gallardo 1979). If this is the case then the species of whelks native to the Chesapeake Bay may have an advantage in that their adaptations may be better suited to the environment than an invader's.

Many invasive species thrive in a foreign environment despite their not having evolved in that habitat. Potential explanations for their success at the expense of the populations of native species (competitive superiority) could be the increasing amount of disturbance of the natural environment, which alters the existing parameters (Chesson and Warner 1981), or the vulnerability of evolutionarily "young" estuaries (Leppakoski 1993). In addition, organisms are limited by ancestry and evolutionary time lag, so the optimal reproductive strategy may not be represented in any given environment (Grant 1983). *Rapana venosa*'s unique life history allows it to exploit a niche (that of planktotrophy) either evolutionarily unavailable or unexplored for other reasons by gastropods in the Chesapeake Bay. Therefore, even in an environment with a diverse endemic community, native species may not have an intrinsic advantage over those species from foreign environments.

Further Study

One of the keys to the exceptional reproductive capability of *R. venosa* is the species' mixed development strategy. Like the oyster, rapa whelks eventually release millions of veligers into the water for planktonic development. The adult animal,

however, has a size and life span comparable to that of the large native whelks, *Busycotypus canaliculatum* and *Busycon carica*. Whether or not this life history strategy is best suited to the environment of the Chesapeake Bay is, however, more complex than a tally of the numbers of embryos produced. Assuming that reproduction represents a large part of an organism's energy budget (Stickle 1973), selection will favor the reproductive pattern with the greatest efficiency, or the one that results in the greatest number of reproducing offspring per calorie devoted to reproduction (Vance 1973). A caloric cost-benefit analysis comparing the lecithotrophic strategies of the native whelks to the mixed development of *R. venosa* would both help to predict which species will be more successful in the Chesapeake Bay and other potentially invasible habitats, and also shed light on the origin and evolution of the diversity of life history strategies.

Further research on the predictors of the amount of time veligers are encapsulated would be augmented by reliable data on the size of the eggs at deposition in the egg cases. The study of the reproductive plasticity of *Rapana venosa* across spatial and temporal clines lends insight into the reproductive strategies of invertebrate species in general. The energetic costs and benefits behind producing a few large eggs versus many small eggs can be applied to many species. Encapsulation of eggs for any length of time requires a balance between physiological and energetic constraints. Variation in development time within species may be dependent on temperature (Spight 1975) or could be dictated at the time of deposition (Perron 1981b). Smaller eggs that quickly develop to a planktonic form will be encapsulated for a shorter time than larger, more slowly developing eggs (Perron 1981b). A correlation between egg size within a species and the periods of feeding and prefeeding development would have to be shown in order

to support the idea that the size and energy budget of the developing larvae dictates time of encapsulation (Grant 1983).

CONCLUSIONS

1. There is a significant spatial gradient in the number of egg masses and egg cases produced among the populations of *R. venosa* in the lower Chesapeake Bay.

2. Viability of the egg masses produced in terms of the size of released veligers and successful release of veligers did not vary over space or time.

3. The temperature of the water, the size of the egg cases produced, and the number of eggs in the egg case are highly correlated.

4. No variance in reproduction due to the presence of external male appendages on females producing egg masses was seen.

APPENDIX I.

Description of Experimental Adults

	13-May		28-Aug			
Origin	**SL(mm)**	**Weight (g)**	**SL (mm)**	**Weight (g)**	**External Sex**	**Penis Length (mm)**
OV	74	60	73	75	Female	0
OV	133	380	134	405	Female	0
OV	112	250	118	250	Female	0
OV	152	840	152	721	Imposex	13
OV	138	445	135	505	Imposex	8
HB	100	160	99	188	Imposex	4
HB	105	210	107	225	Imposex	8
HB	95	140	94	150	Imposex	4
HB	149	790	152	760	Female	0
HB	154	885	159	811	Female	0
HB	134	525	133	520	Imposex	9
HB	138	690	140	715	Female	0
HB	93	145	95	180	Imposex	4.5
HB	136	650	143	640	Imposex	10
JR	133	470	133	525	Imposex	7.5
JR	150	610	154	686	Imposex	16.5
JR	147	565	145	635	Female	0
JR	127	300	128	342	Imposex	4.5
JR	158	755	163	808	Imposex	10.5

Key

Origins

OV = Ocean View Site

HB = Hampton Bar Site

JR = James River Site

Measurements

SL = Shell length

External Sex

Female = female gonad + no penis

Imposex = female gonad + penis

Appendix II.

	df	SS	*MS*	F_s	*p*
Source	2	124.4	62.2	5.65	0.014
Error	16	176.1	11		
Total	18	300.5			

Table I. Analysis of variance for the number of egg masses laid over the spawning period compared among sites where *Rapana venosa* adults were collected (Source).

	Ocean View	**Hampton Bar**
Hampton Bar	-3.287 : 6.265	
James River	1.185 : 12.015	0.335 : 9.887

Table II. Tukey's pairwise comparisons of the number of egg masses laid over the spawning period. The null hypothesis of no difference between means is rejected if zero is not contained in the confidence interval shown above.

	df	SS	*MS*	F_s	*p*
Source	2	2990783	1495391	8.09	0.004
Error	16	2958120	184882		
Total	18	5948903			

Table III. Analysis of variance for the number of egg cases each adult laid over the spawning period compared among collection sites (Source).

	Ocean View	**Hampton Bar**
Hampton Bar	-152 : 1086	
James River	387 : 1790	2 : 1240

Table IV. Tukey's pairwise comparisons of the number of egg cases each adult laid over the spawning period. The null hypothesis of no difference between means is rejected if zero is not contained in the confidence interval shown above.

LITERATURE CITED

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