FUNCTIONAL MORPHOLOGY OF EGG CAPSULES IN A MARINE GASTROPOD *NUCELLA EMARGINATA*

By

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ABSTRACT

Egg capsules of the marine whelk Nucella emarginata were examined with respect to intraspecific variation in capsule morphology and resistance to intertidal predators.

Capsules were collected from three intertidal populations separated along a wave-exposure gradient. Wall thickness, dry weight, and protective quality of capsules differed extensively among these populations; however, these capsular properties did not reflect differences in wave-exposure levels. Capsule size was found to increase from wave-exposed to wave-sheltered shores and paralleled differences in snail size among sites.

Laboratory experiments, and field tests incorporating predator-exclusion cages, were used to determine the extent of predation on Nucella emarginata capsules and to identify capsule predators. Capsule cases were regularly eaten by intertidal predators such as shore crabs, Hemigrapsus spp., and isopods, Idotea wosnesenskii. These invertebrates were responsible for opening a maximum of 32% of capsules present at two study sites. Despite differences in thickness and strength of capsules among populations, no capsules were completely resistant to these predators; however, thick capsule walls did appear to convey some protective advantage. Preliminary laboratory tests indicated that thin-walled capsules were preferentially opened by Idotea wosnesenskii.

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GENERAL INTRODUCTION

Marine gastropods exhibit a variety of reproductive and developmental patterns (Webber, 1977). An important advance in the reproductive ecology of these organisms has been the evolution of internal fertilization in the Meso- and Neogastropoda (Gallardo and Perron, 1982). Internal fertilization has allowed for the development of new reproductive patterns associated with a non-pelagic larval stage, such as the deposition of eggs into protective gelatinous envelopes or into elaborate egg capsules. In many meso- and neogastropods embryos are confined within benthic egg capsules for some, if not all, of their developmental period. The adaptive significance of such structures is generally thought to be associated with a reduction in the development time of embryos within the plankton and, consequently, an increase in the survivorship of embryos (Pechenik, 1979).

The confinement of embryos within benthic egg capsules, however, has its own risks. Embryos free of mortality associated with a planktonic existence are exposed to a variety of new environmental stresses within the benthic marine environment. Little is known about the importance of these stresses on the survivorship of encapsulated embryos. Recent studies, however, have shown that the wall strength of many neogastropod egg capsules is related to the development time of encapsulated embryos (Perron, 1981). These results

suggest that longer development times may be associated with a greater risk of embryonic mortality and, hence, differences in capsular structure may also be correlated with differences in the levels of specific environmental stresses.

The goals of the present study were: 1) to examine variation in the morphology of egg capsules among populations of the intertidal snail Nucella emarginata, and 2) to determine the effectiveness of these structures in protecting developing embryos from predators.

This thesis is divided into two chapters. Chapter 1 presents data on the morphology of Nucella emarginata egg capsules collected from three sites along a wave-exposure gradient. Differences in egg capsule wall thickness are examined within and among sites and correlated with measurements of capsule strength. The implications of intra- and interspecific differences in capsule wall thickness are discussed with reference to the possible function(s) of such extra-embryonic structures. Chapter 2 investigates the extent of predation on intertidal Nucella emarginata egg capsules. Potential intertidal predators are identified through a combination of field and laboratory studies, and evidence of species-specific predation in the field is described. Also, predator preferences for thick- or thin-walled egg capsules are examined. The extent of field predation on Nucella emarginata egg capsules is discussed in terms of the role that predation may have played in the evolution of capsule morphology.

CHAPTER 1

FUNCTIONAL MORPHOLOGY OF MARINE GASTROPOD EGG CAPSULES: INTRASPECIFIC VARIATION IN SIZE, WALL THICKNESS AND STRENGTH OF NUCELLA EMARGINATA EGG CAPSULES

INTRODUCTION

The confinement of developing embryos within egg capsules is a common phenomenon among marine invertebrates. Neogastropod molluscs enclose their embryos within structurally complex proteinaceous capsules, and embryos develop within these capsules for at least some, if not all, of their larval life. Gastropod egg capsules are frequently considered to be "protective" (Pechenik, 1979; Perron, 1981; Perron and Corpuz, 1982), but what they protect embryos against is unclear. Commonly, the egg capsules have been thought to reduce such environmental stresses as: predation (Pechenik, 1979; Perron, 1981), bacterial attack (Lord, 1986), osmotic changes (Pechenik, 1982; 1983, Hawkins and Hutchinson, 1988), desiccation (Spight, 1977; Pechenik, 1978), temperature shock (Spight, 1977; Pechenik, 1986), and wave action (Perron, 1981). However, only a few studies have specifically examined the resistance of encapsulated embryos to these factors (Spight, 1977; Pechenik, 1978; 1982; 1983; Brenchley, 1982; Davies, 1984; Lord, 1986; Hawkins and Hutchinson, 1988) and little is known about the survivorship of encapsulated embryos in actual field situations.

An examination of intra- and interspecific variation in the structure of egg capsule walls may be useful in assessing the role that capsules play in gastropod life-histories. Variation in capsule wall strength and the energy invested in capsular cases among species within the genus Conus has been found to be positively correlated with embryonic developmental time (Perron, 1981). Furthermore, capsule walls are known to be thicker in Conus species with long encapsulated development (Perron and Corpuz, 1982). As in some species capsular cases can account for almost 50% of the energy invested in intact capsules (Perron, 1981), strong, thick-walled capsules may have evolved for increased protection of encapsulated embryos with long-term exposure to environmental stresses (Perron, 1981; Perron and Corpuz, 1982). If selection has also responded to intensity of these environmental factors, then intraspecific comparisons of capsular structure across a variety of habitats could reveal differences associated with specific sources of mortality. As yet, no studies have specifically examined intra- or interspecific variation in the structure of gastropod capsules among different habitats.

Differences in the structure of egg capsules within a species may be most apparent among populations separated along sharp environmental gradients, such as wave-exposure. Stresses imposed on intertidal organisms are known to differ greatly along wave-exposure gradients (Menge, 1978a; 1978b; Menge and Sutherland, 1987). Studies have shown that increases in wave-exposure are matched by: 1) decreases in predation intensity, as wave action generally tends to disrupt

predator foraging activities (Kitching et al., 1959; Menge, 1978a; 1978b; Robles, 1987), and 2) decreases in desiccation stress, as areas splashed by waves suffer less drying (Dayton, 1971; Menge, 1978a). Wave-action itself is known to impose severe physical stresses upon intertidal organisms due to dislodgement (Denny, 1985, Denny et al., 1985; Etter, 1987), abrasion (Craik, 1980), and water-born rocks (Shanks and Wright, 1986). Assuming that benthic egg capsules are exposed to environmental stresses similar to sessile intertidal organisms, a wave-exposure gradient may provide a convenient means of comparing capsule structure across a range of environmental conditions.

In order that intraspecific differences in capsule structure can be interpreted, it is essential to understand certain constraints imposed on the morphology of gastropod egg capsules. For instance, it is known from the work of Spight et al.(1974), Spight and Emlen (1976) and Perron and Corpuz (1982) that the length, volume and wall thickness of gastropod egg capsules is related to female size. Constraints may also be placed on capsule structure by the requirements of developing embryos (Strathmann and Chaffee, 1984). For instance, thick capsule walls or capsule shapes with low surface area to volume ratios may restrict the diffusion of oxygen or waste products and thus reduce embryo survivorship. Hence, there may be tradeoffs in capsule design, such that thick-walled capsules protect embryos better from predation or physical stress, but may limit the number of embryos surviving. Therefore, a study of capsule function must

involve a knowledge of the interrelationships between capsule morphology, capsule wall thickness, and the packaging of embryos within capsules.

The marine intertidal snail, Nucella emarginata, is a common inhabitant of rocky shores from California to Alaska and ranges across wide extremes in wave-exposure. These snails deposit eggs within 6-10 mm-long vase-shaped capsules, which they attach directly to the substratum. Three to 35 embryos are enclosed with hundreds of nurse eggs, used as food by the developing larvae. After approximately 80 days (Emlen, 1966), embryos emerge from the egg capsules as juvenile snails.

In this study, I examined intraspecific variation in capsule morphology within and among populations of Nucella emarginata. Populations were chosen from three sites separated along a gradient of wave-exposure. Data were collected on: 1) the size and shape of snails within and among populations, 2) intraspecific differences in capsule size and wall thickness, 3) variation in the packaging of eggs and embryos within capsules, and 4) the relationship between capsule wall thickness and capsule strength, for each site.

STUDY SITE DESCRIPTIONS

This project was conducted at the Bamfield Marine Station on the west coast of Vancouver Island (125°10'N, 48°50'W; Fig.1). Study sites were established in Barkley Sound for the purpose of collecting Nucella emarginata adults and their egg capsules. Three locations were chosen to represent a gradient of wave-exposure from sheltered to exposed: Grappler Inlet, Ross Islets, and Seppings Island. Although no empirical studies have ranked these areas in Barkley Sound with respect to wave-exposure, my rating system, based on visual observations, corresponded with subjective exposure scales employed by others in the same geographic region (Austin, Druehl, and Haven, 1971; Kitching, 1976; Crothers, 1984).

1. Grappler Inlet

The Grappler Inlet site represented the least wave-exposed study area (Fig. 1). Although the actual site was too far up the inlet to receive oceanic swell, it was subject to a considerable tidal current due to the constricted width of the channel and large volume of tidal exchange. This site represented an extreme distributional limit for Nucella emarginata, which are normally absent from protected inlets (Crothers, 1984). A general search for N. emarginata along Grappler Inlet revealed that they were present only in two areas: at my study location and on rocky beaches around the mouth of the inlet.

The site was located in an intertidal channel, 1.6 m above zero chart datum, connecting the mainland and a small

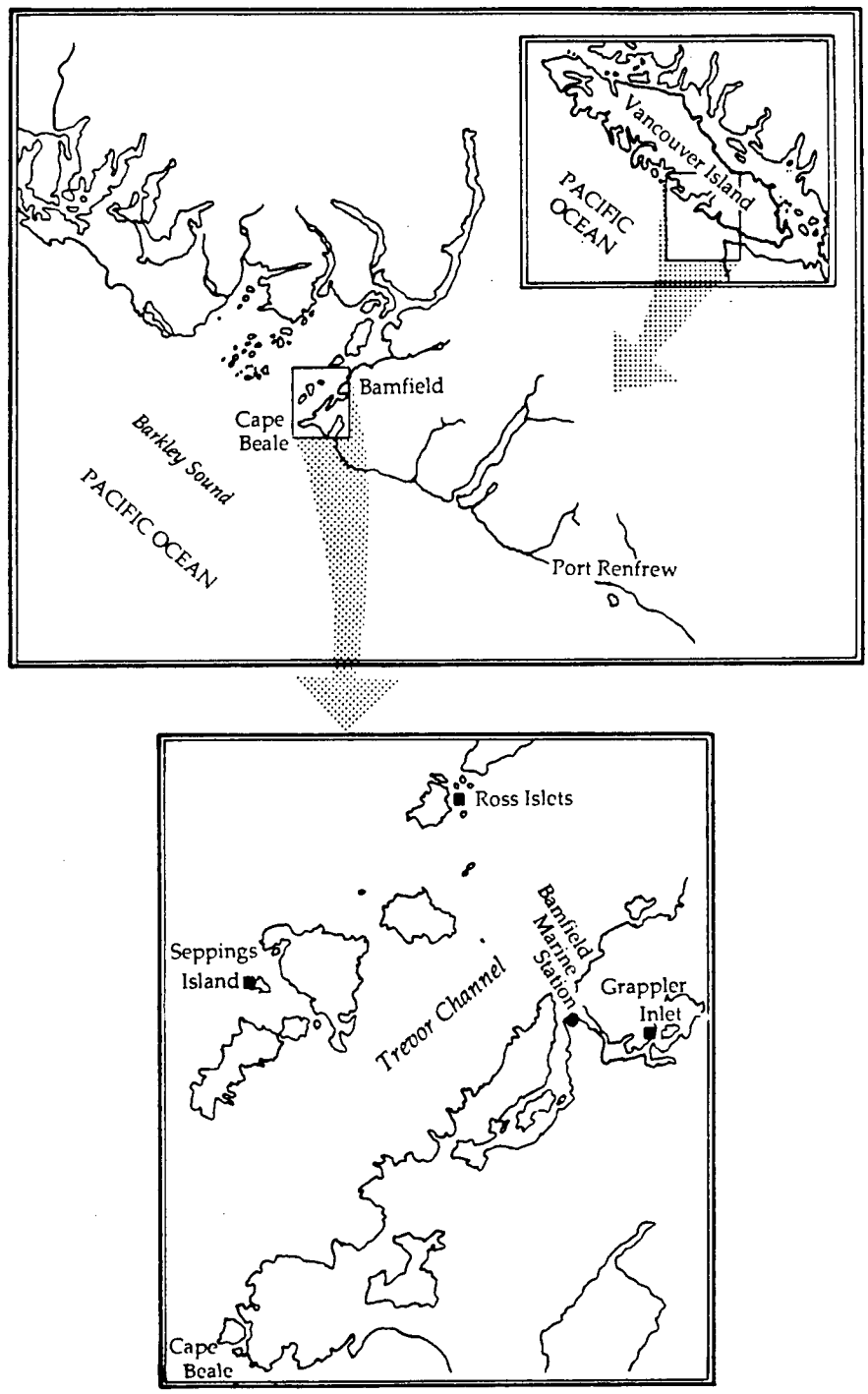


FIGURE 1. The location of study sites along the southern shores of Barkley Sound on the west coast of Vancouver Island, B.C. Study locations were chosen along a wave-exposure gradient. Wave-exposure levels were predicted to be lowest at Grappler Inlet, highest at Seppings Island, and intermediate at the Ross Islets site.

piece of land isolated at high tide. The intertidal substrate consisted of fine silty mud overlaid by clam shells and a meshwork of mussel- and barnacle-covered rocks (Mytilus edulis, Balanus glandula and Semibalanus cariosus). Nucella emarginata were present throughout the channel and ranged to a tidal height of approximately 2.6 m above zero chart datum. Other gastropods present were Nucella lamellosa, Searlesia dira, Onchidella borealis, and various limpet species. The chitons Mopalia spp. were also abundant throughout the channel. The polychaetes, Nereis vexillosa and several species of scale worms were associated with the mussel- and barnacle-covered rocks, while several nemertean species were ubiquitous. Three isopod species were present: Gnorimosphaeroma oregonense, Cirolana harfordi, and Idotea wosnesenskii. Hemigrapsus oregonensis burrows were evident in the compact mud found in higher intertidal regions; however, H. nudus were rarely seen. Hermit crabs (Pagurus spp.) could be found in amongst the meshwork of mussels, barnacles and rocks. Fucus and Ulva were the predominant intertidal algae.

2. Ross Islets

This site was chosen to represent a region with intermediate wave-exposure (Fig. 1). The collecting areas were located on both sides of a wave-protected channel. Normally these sites received only small swells resulting from waves breaking on the south-facing shores of the islets and on a reef between two neighbouring islands.

The intertidal substrate in this region consisted of granite bedrock, with a boulder beach covering some of the

lower regions. Three barnacle species were present intertidally: Balanus glandula, Semibalanus cariosus and Chthamalus dalli. Mytilus californianus were present in patchy clumps. Nucella emarginata were found in the mid- to high- intertidal zones between 1.8 - 3.1 m above zero chart datum, and were present occasionally in the lower boulder beach region. Other littoral gastropods present were Nucella lamellosa, Tegula funebralis, Searlesia dira and various limpet species. The chitons, Mopalia spp., were seen occasionally on granite promontories. Intertidal polychaetes and nemerteans were sparse, with Nereis vexillosa and Emplectonema gracile being the only obvious representatives of these groups. The purple shore crabs, Hemigrapsus nudus, and porcelain crabs, Petrolisthes spp. were abundant under rocks in the boulder beaches and in some higher intertidal crevices. Starfish, Pisaster ochraceus, were also common in the boulder beach area. The predominant high intertidal alga was Fucus distichus, distributed in a distinct vertical band at a tide height of approximately 2.6 - 2.9 m above zero chart datum.

3. Seppings Island

This was the most wave-exposed site (Fig. 1). The collecting area was located in a large boulder-lined channel, 10 m wide, facing a relatively unobstructed oceanic swell. The channel was bordered on one side by a high granite wall and on the other by a large rocky promontory covered with Mytilus californianus. Boulders in the channel ranged in size from 0.3 - 2.0 m diameter. During winter storms, boulders often were redistributed throughout the intertidal area by

wave action.

The collecting channel was characterized by a variety of barnacles (Balanus glandula, Semibalanus cariosus, Cthamalus dalli, and Policipes polymerus), whelks (Nucella emarginata, N. canaliculata, and Ceratostoma foliatum) and other gastropods (Ocenebra lurida, Tegula funebralis, Calliostoma ligatum and various limpet species). Idotea wosnesenskii were the only intertidal isopods present. Hemigrapsus nudus were present in large numbers underneath the rocky boulders, as were small hermit crabs (Pagurus spp.) and porcelain crabs (Petrolisthes cinctipes). Algae consisted of Laminaria and Iridaea species in the low intertidal area, and Pelvetiopsis limitata in the high intertidal regions.

MATERIALS AND METHODS

I. DIFFERENCES IN SNAIL MORPHOLOGY AMONG SITES

Shell-length to aperture-length ratios were used to compare shell morphology among sites, as these ratios are the quickest and simplest method of determining shell-shape variation in the genus Nucella (Crothers, 1984). Fifty snails were collected from each study area in the spring of 1988. Collections were made by removing all living Nucella emarginata from a given area to avoid any biases for shell colour, size, or shape. Snails considered to be juveniles (less than 10 mm in length) were replaced, as considerable variation in shell shape is known to occur during early juvenile development (Crothers, 1984). Shell and aperture-lengths were measured to the nearest 0.1mm with vernier calipers. Shell-length was defined as the maximum length between the shell apex and anterior tip of the siphonal canal, while aperture-length was the maximum distance between the posterior corner of the aperture lip and anterior tip of the siphonal canal.

II. CAPSULE MORPHOLOGY

1. Relationship between snail size and capsule size

In order to compare the size of egg capsules laid by snails from each site, approximately 50-100 snails were collected from each study area. Snails were brought into the laboratory, tagged for identification, and assigned to site-specific plastic containers (32x26x12cm, with 20x8cm 1mm-pore

mesh windows in each of the four sides). Approximately 40-50 snails were held in each container and were provided with barnacles for food. Containers were kept submerged in laboratory seawater tables and supplied with a continuous flow of fresh seawater. Each laboratory population was checked every two days over a six-week period to identify snails laying egg capsules. A snail was recognized to be laying an egg capsule only if it was found actually molding a new capsule with its ventral pedal gland. Such capsules could be identified readily by their soft walls and milky-yellow appearance. Other capsules were collected with the newly-laid ones only if they were considered to be part of the same clutch. A clutch was defined to be any number of capsules laid in within a few mm of one another that were similar in shape and orientation (Gallardo, 1979). A maximum of 5 capsules was collected from each female at any spawning check. Capsule-laying females were measured for shell length and returned to their containers. Capsules were preserved in 5% formalin for subsequent measurement.

Three measurements were taken for each capsule (Fig. 2): 1)"capsule body length", which excluded the stem length as this was known to be extremely variable (Spight and Emlen, 1976); 2)"capsule chamber length", representing the length of chamber housing the embryos and nurse eggs; and, 3) the "maximum capsule width", which was the maximum width of the capsule-chamber. The "capsule chamber volume" was estimated using the formula for a prolate ellipsoid, $V=4/3\pi(a/2)(b/2)^2$, where a = the capsule chamber length and b = the maximum

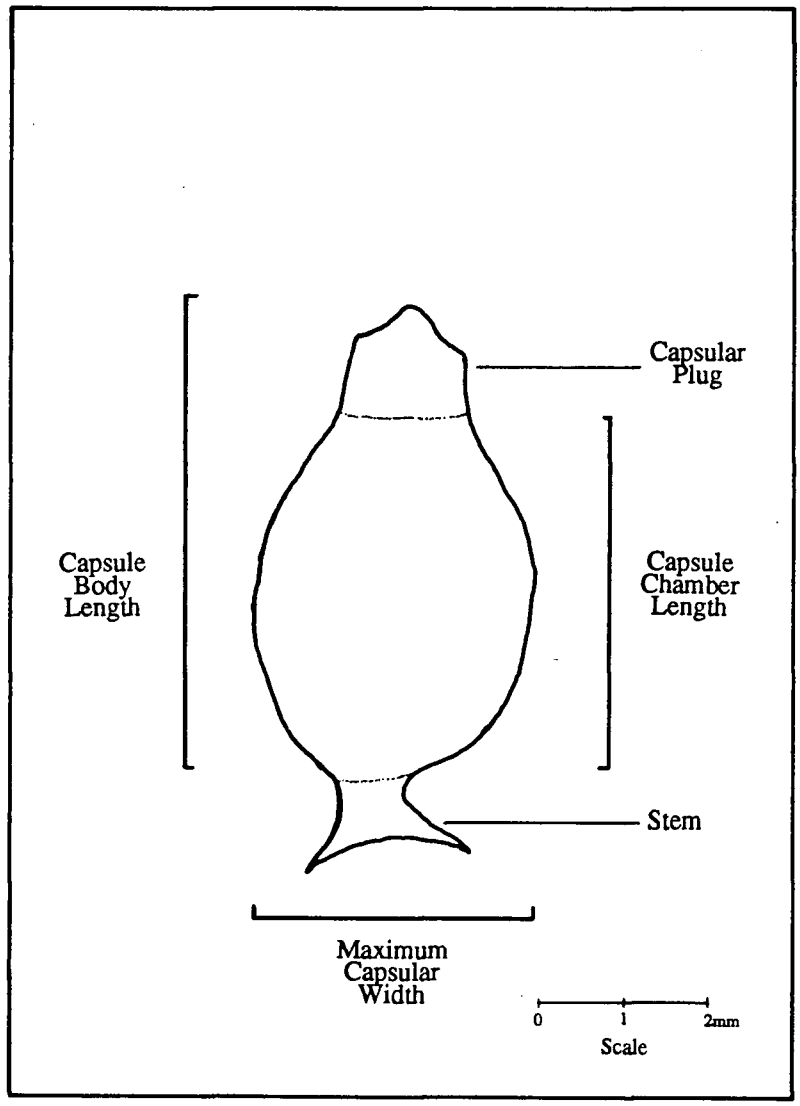


FIGURE 2. Nucella emarginata egg capsule, illustrating the three measurements taken for each capsule: capsule-body length, capsule-chamber length, and maximum capsule width. The capsular-plug region and the stem are also indicated.

capsule width (Pechenik, 1982).

2. Micromorphology of Nucella emarginata egg capsules

Representative egg capsules of Nucella emarginata were sectioned using a freeze microtome. The wall microstructure of each capsule was examined using a compound light microscope and interpreted with respect to previous histological studies on the egg capsules of N. lapillus and other muricids (Bayne, 1968; Sullivan and Maugel, 1984; D'Asaro, 1988).

3. Intracapsular variation in wall thickness

Intracapsular variation in the wall thickness of Nucella emarginata capsules was examined for each site. Capsules were collected from different clutches within a site to minimize the chance of obtaining capsules from any one female. In the laboratory, these were then placed in cylindrical vials (3.5x3.5x6cm with two 2.0x2.5cm 1mm-pore mesh panels) and maintained in flowing seawater. Capsules were measured as before, emptied of all contents by removing the capsular plug, and then individually frozen on a freeze microtome. Sections, each 10-12μm thick, were taken at 10 percentile intervals along the length of the capsule chamber. The first sections (0%) were taken at the opening of the plug region into the capsule-chamber. The last section (100%) was taken close to the junction between the capsule-chamber and stem. Sections were mounted in a seawater-soluble mounting medium and measurements taken using a compound microscope with calibrated ocular micrometer. Eight measurements of wall thickness were

taken at approximately equal intervals around each capsule section. The average wall thickness within each section was used in all subsequent data analyses.

4. Variation in capsule wall thickness among populations.

In order to examine wall thickness differences among a large number of egg capsules, a laboratory population of 60 snails was established from each site. Snails were sexed by allowing each animal to attach to a rock surface and then gently pulling the shell away from the body mass. A male could be identifed by the presence of a penis larger than its right tentacle (rather than the smaller penis of the female; Palmer, 1984). Five male and five female snails from each site were allocated to each of six replicate cages (26x16.5x13cm, with 8x16cm 1mm-pore mesh panels in each side) and were supplied with barnacles for food. Female snails were selected so that each replicate cage had a similar mean snail size (Grappler: $\overline{X}$=3.0 cm; Ross Islets: $\overline{X}$=2.3 cm; Seppings: $\overline{X}$=2.0 cm). All cages were kept completely immersed in flowing seawater.

Every two weeks freshly laid capsules were collected and placed in small mesh-panelled vials. Vials were labelled, dated, and immersed in flowing seawater until capsules were needed. Capsules contained in these "capsule vials" were used in several experiments as follows.

To examine variation in capsule wall thickness within and among populations, five capsules were selected randomly from each replicate vial (n=30 capsules/site). Capsules were

measured individually and marked at a point 70% along the length of the capsule chamber with a fine Staedtler Lumocolour pen. Previous data on wall thickness variation within a capsule indicated that capsules were thinnest and wall thickness least variable in this region. Capsules were emptied by removing the plug and then sectioned on the freeze-microtome. Sections were collected only when the blade reached the portion of the capsule chamber marked with ink. Five sections, each 10-12μm thick, were taken for each capsule and one section was chosen for measurement.

5. Variation in capsule wall thickness within and among clutches laid by different females

Intra- and interclutch variability in wall thickness was examined to determine if females within a population laid capsules of different wall thicknesses. One clutch of egg capsules was collected from each of five replicate cages for each of the three sites to ensure that all clutches were laid by different females. Each clutch was collected and kept in its own labelled mesh-panelled container until capsules were needed for sectioning. Representative capsules were selected randomly from each clutch, measured under a dissecting microscope, and then sectioned at a point 70 % along the length of the capsule chamber. Wall thickness measurements were made as described previously.

6. Variation in dry weight of capsules among populations.

Dry weight measurements of capsules were taken for each

population to determine if differences among sites corresponded to differences in capsule wall thickness. For each population, representative capsules were collected from each of six replicate capsule vials. Capsules were measured and then opened to remove all contents. Stems were removed from capsules to minimize variability in weight among capsules. Capsules were rinsed twice in distilled water and then dried for 48 hrs at 75°C. After drying, capsules were weighed to 0.01mg.

III. VARIATION IN CAPSULE CONTENTS AMONG SITES

1. Egg number and volume

Egg capsule contents were examined to determine if the number or size of eggs per capsule differed with population differences in capsule structure. Three newly laid capsules were collected from each of six replicate capsule vials for each site (18 capsules/site). Capsules were measured, sliced open, and their contents washed with filtered seawater into a small petri dish. As nurse eggs tended to clump together, eggs were gently agitated apart with a pipette. All nurse eggs and embryos within a capsule were counted. Before these counts were made, however, embryos were examined to ensure that they had not advanced past the second veliger stage. LeBoeuf (1971) and Lyons and Spight (1973) have noted that once past this developmental stage, embryos become capable of feeding on encapsulated nurse eggs.

As egg size within a capsule was relatively constant, only five eggs were sampled from each capsule. Length and

width measurements were taken for each egg. Egg volume was estimated by using the formula for a prolate ellipsoid as described previously.

2. Number and size of hatching snails.

To determine if there was a difference in the number of embryos per capsule from each site, embryos were allowed to hatch in the laboratory. Encapsulated larvae developed within their capsule vials until development neared completion. At this point, individual capsules from each site were placed in their own mesh-panelled vials (600 μm mesh pore) and submerged in seawater until the embryos hatched. Capsules were checked every two days and as soon as an embryo had emerged, the capsule was measured and emptied of all remaining embryos. All live embryos were counted and their shell-lengths measured.

IV. CAPSULE STRENGTH MEASUREMENTS

1. Resistance to puncturing

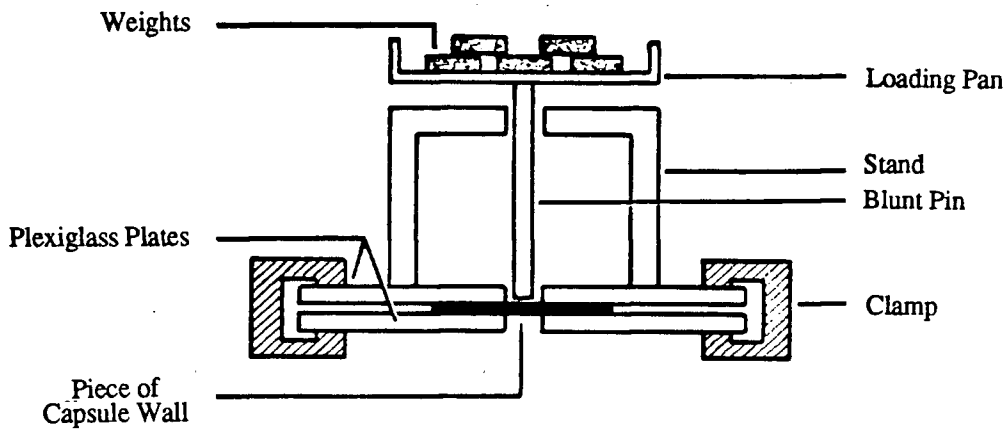
This index determined the resistance of capsule walls to puncturing and was based on Perron's (1981) procedure for Conus egg capsules. Newly laid Nucella emarginata capsules were taken from their respective capsule vials, measured, and then marked at a point 70% along the length of the capsule chamber. Capsules were then bisected by cutting along the two seams of the capsule chamber and their contents discarded. Each capsule half was mounted individually between two pieces of plexiglass (8.5x5cm) and orientated such that a 1mm dia.

hole in each piece of plexiglass was positioned directly over the marked region of the capsule chamber (Fig. 3a). Care was taken to insure that there were no folds or creases in the capsule section and that it remained moist. A puncturing device, consisting of a blunt-ended needle (0.36 mm^2 area) mounted beneath a flat weighing pan, was positioned over the plexiglass such that the needle rested at right angles to the exposed capsule wall. Five-gram weights were added to the weighing pan until the needle punctured the capsule wall. Each capsule piece was only punctured once.

2. Resistance to squeezing

This index of capsule strength measured the force needed to squeeze the plug out of intact capsules. Shore crabs were often seen to rupture Nucella emarginata egg capsules by squeezing them in their chelae. Laboratory capsules were collected from replicate capsule vials and measured. Individual capsules were mounted with "Superglue" onto a metal plate, which was then bolted to a vertical piece of plexiglass (Fig. 3b). Strain gauges, glued to the plexiglass, were connected to an amplifier and chart recorder. A second metal plate was attached to a spindle so that this plate could be hand-cranked towards the mounted capsule. A chart recorder provided a record of the force required to rupture the egg capsule. This system was calibrated with known weights.

a)



b)

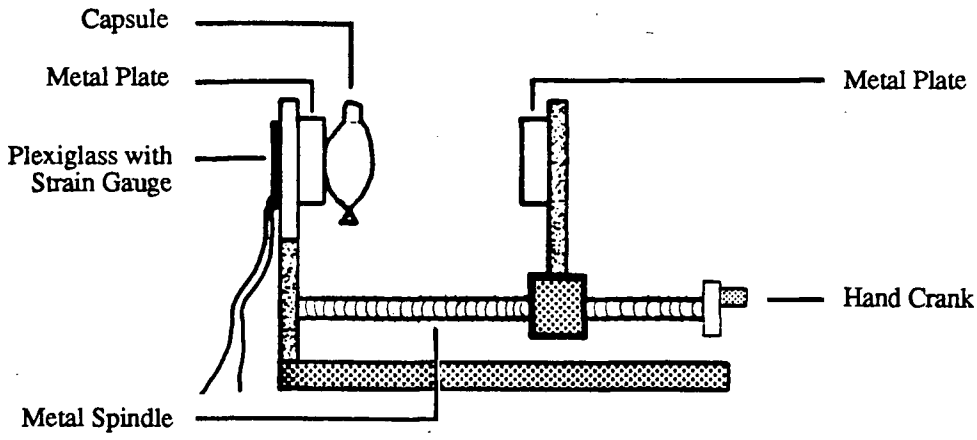


FIGURE 3. Techniques used to compare the strength of egg capsules: a) puncturing method used to calculate the force required to pierce capsule walls, and b) squeezing method used to determine the resistance of intact capsules to squeezing forces.

RESULTS.

I. DIFFERENCES IN SNAIL MORPHOLOGY AMONG SITES

The size and shape of snails differed extensively among study sites (Fig. 4). Nucella emarginata from Seppings Island, the most wave-exposed area, had the smallest mean size ($\bar{X}$=1.9cm) and the lowest length/aperture ratio ($\bar{X}_{L/A}\pm$S.D.=1.42$\pm$0.05). Snails collected from the less exposed Ross Islets site had a greater range of shell lengths, a larger mean size, ($\bar{X}$=2.1cm), and proportionally larger length/aperture ratios than snails from Seppings ($\bar{X}_{L/A}\pm$S.D.=1.53$\pm$0.07). Wave-sheltered snails from Grappler Inlet were intermediate in body shape between Seppings and Ross Islets snails ($\bar{X}_{L/A}\pm$S.D.=1.49$\pm$0.06), but had the largest shell lengths of all three populations ($\bar{X}$=2.7cm). These differences in the average shell length of snails showed an obvious relationship with wave-exposure, with shell size increasing from wave-exposed to wave-sheltered shores. Changes in length/aperture ratios among sites did not appear to reflect changes in wave-exposure, as Grappler snails did not follow the same trend in body shape as Seppings and Ross Islet snails.

II. CAPSULE MORPHOLOGY

1. Relationship between snail size and capsule size

Figure 5 shows the relationship between capsule size and snail shell-length within and among populations. Within each population larger females produced significantly longer egg

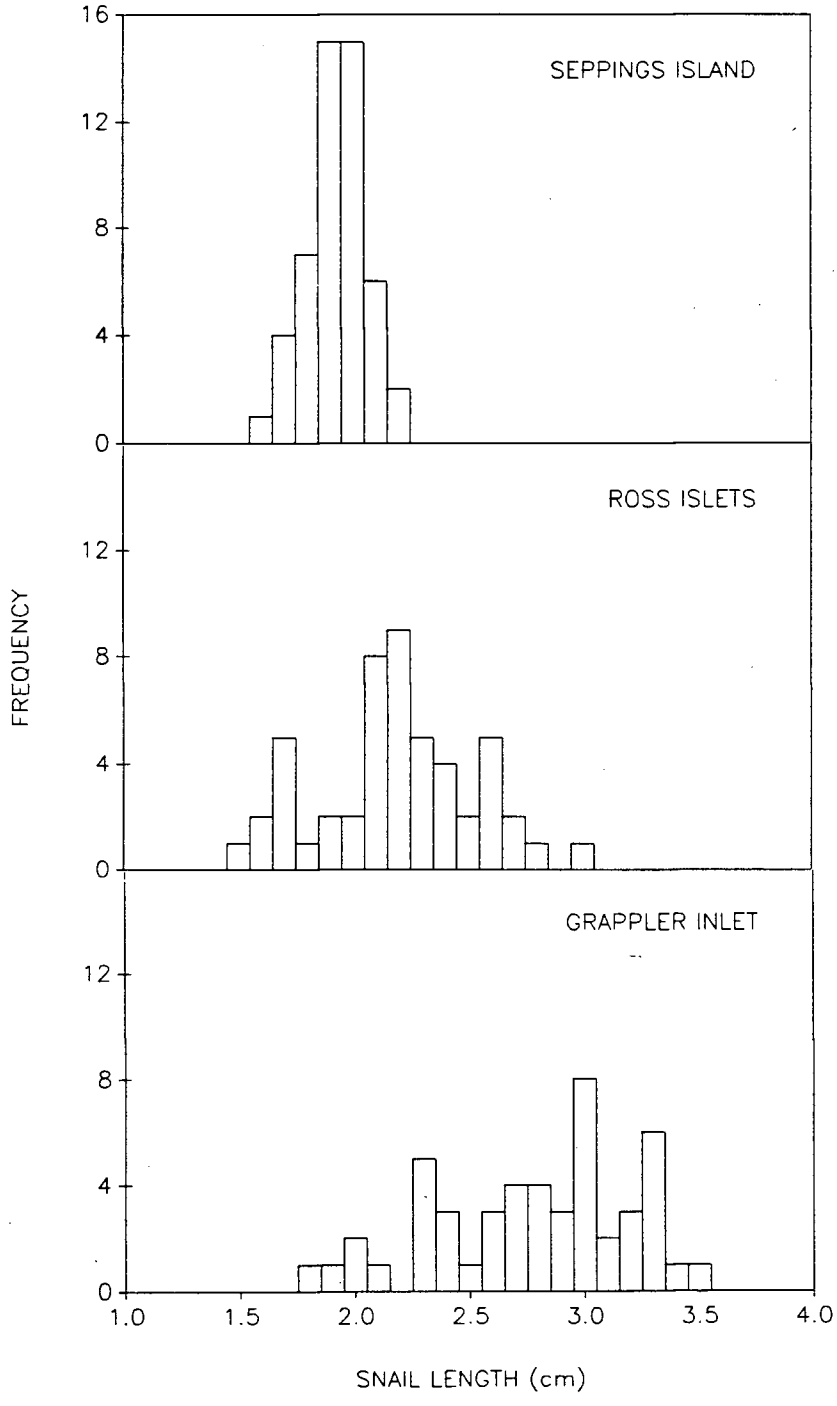


FIGURE 4. Size-frequency histograms of 50 snails collected from each study site in March, 1988. Snails smaller than 10 mm in shell length were not included. Wave-exposure levels increased from Grappler Inlet to Seppings Island. The mean shell lengths of snails were: 1.9, 2.1 and 2.7 cm for Seppings, Ross Islets, and Grappler Inlet snails, respectively.

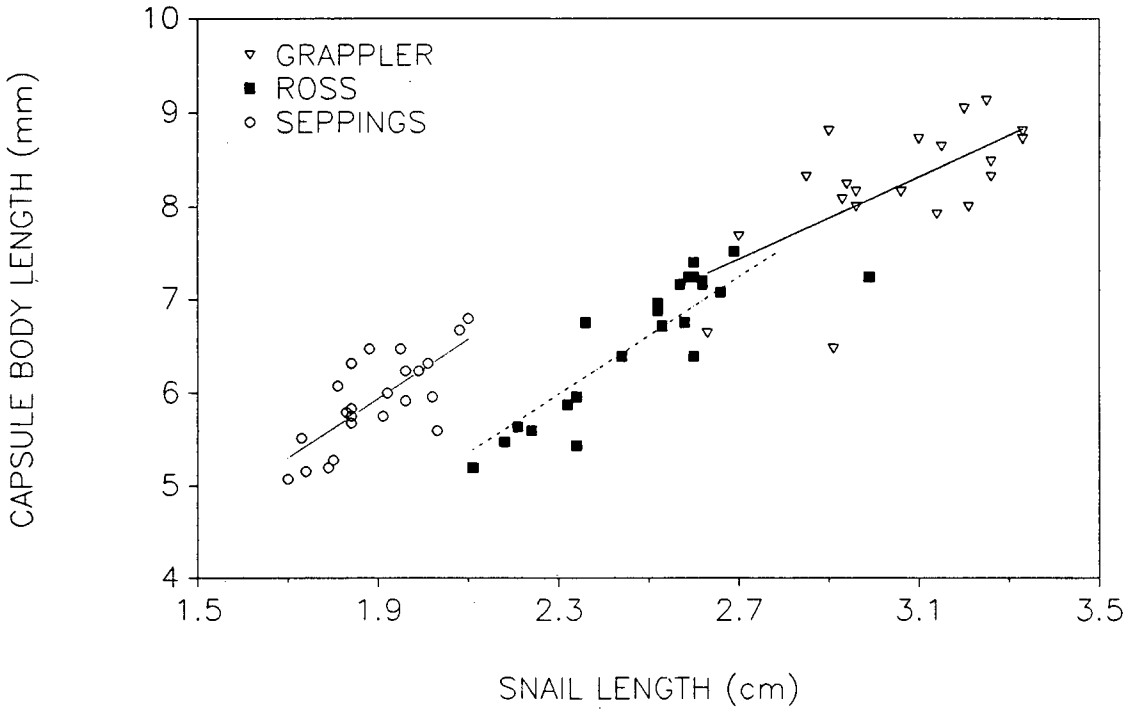


FIGURE 5. Relationship between capsule body length and snail length for laboratory laid capsules for each study site. Snails smaller than 1.7, 2.1 and 2.7 cm from Seppings, Ross Islets and Grappler populations did not spawn in the laboratory. Regression equations for each site are: Seppings: $Y = 3.185X - 0.111$, $r = 0.740$, $n = 23$; Ross Islets: $Y = 3.139X - 1.232$, $r = 0.867$, $n = 23$; Grappler Inlet: $Y = 2.206 + 1.481$, $r = 0.650$, $n = 20$.

capsules than smaller females. The slopes of these relationships did not vary significantly among sites (ANCOVA for slopes; $F=1.20$, $p>0.25$), however, slope elevations differed significantly between the Seppings and Ross Islets populations (ANCOVA for elevations; $F=14.84$, $p<0.001$), with Seppings snails producing disproportionately large capsules. Substantial differences were also evident in the size of spawning snails from each laboratory population, as shown in Figure 5. Although laboratory snail populations greatly overlapped in size range (Seppings: 1.4-2.2cm; Ross Islets: 1.4-2.9cm; Grappler: 1.8-3.5cm), the range in size of spawning females from each population barely coincided. The smallest snails to spawn in the laboratory were 1.7, 2.1, and 2.7 cm in shell length for Seppings, Ross Islets, and Grappler populations, respectively. This suggests that the onset of reproductive maturity varied among sites, with wave-exposed snails maturing at a much smaller size than wave-sheltered snails.

Snail size was also reflected in other capsular dimensions, as larger snails produced capsules with greater chamber volumes than smaller snails (Fig. 6). Comparisons among sites showed that, although the slopes of these relationships did not differ significantly (ANCOVA for slopes; $F=0.57$, $p>0.25$), Seppings and Ross Islets snails laid capsules with larger chamber volumes than similar-sized snails from Grappler Inlet (ANCOVA; $F=5.56$, $p<0.01$). As chamber volumes are known to reflect the number of eggs contained within the capsules (Spight and Emlen, 1976), such differences in capsule

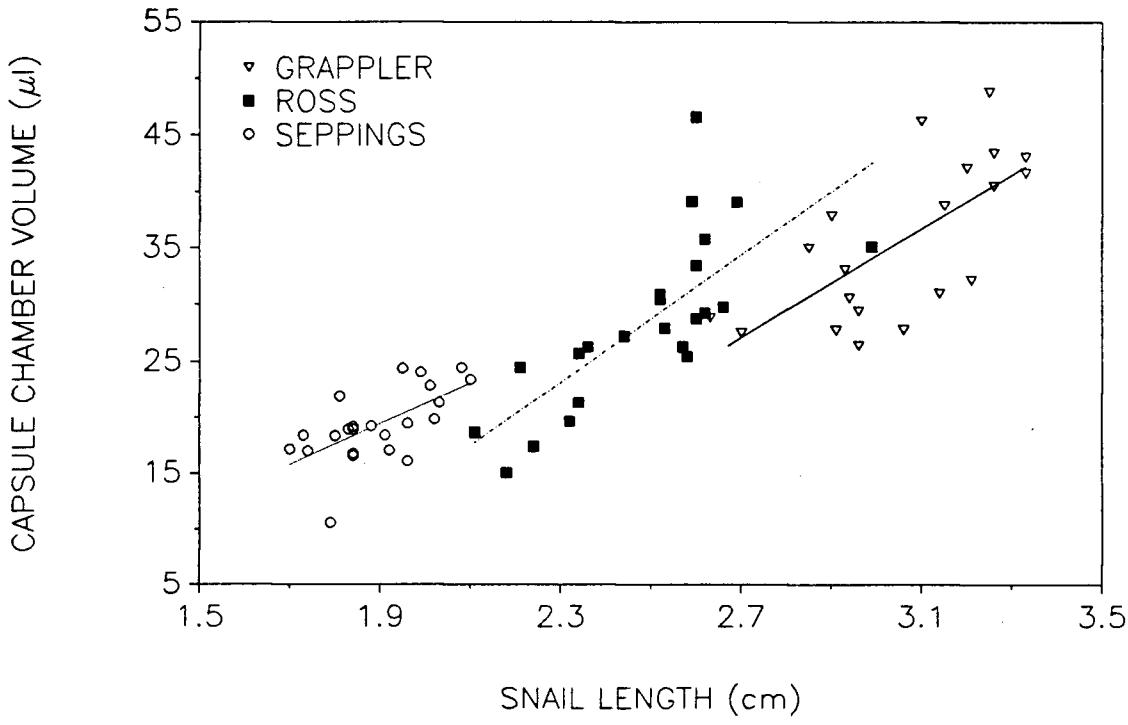


FIGURE 6. Relationship between capsule-chamber volume and snail length for laboratory populations established from each study site. Regression equations for each site are: Seppings: Y = 18.245X - 15.257, r = 0.628, n = 23; Ross Islets: Y = 28.193X - 41.722, r = 0.749, n = 23; Grappler, Y = 24.006X - 37.710, r = 0.690, n = 20.

size can also indicate important differences in the fecundity of these populations.

2. Micromorphology of Nucella emarginata egg capsules

The microstructure of N. emarginata egg capsules is shown in Figure 7. Capsule walls of N. emarginata consisted of three discrete laminae (L_1, L_2 and L_3) and were similar in structure to the capsule walls of other muricids (Sullivan and Maugel, 1984; D'Asaro, 1988; Fig. 7A,B). All measurements of capsule wall thickness were taken solely from the middle lamina (L_2), as this was the thickest part of the capsule wall. This lamina consisted a dense, fibrous middle layer (L_{2b}), which was sandwiched by two transparent, homogeneous layers (L_{2a} and L_{2c}). The innermost capsule lamina (L_3) lined the capsule chamber and enclosed developing embryos, nurse eggs, and intracapsular fluid. Sections in the apical region of the capsule indicated that this lamina was actually connected to the capsule plug and appeared to be composed of a similar material (Fig. 7D). The structure of these capsule walls was not homogeneous throughout the capsule, as is shown by longitudinal sections through the stem and capsular-plug regions (Fig. 7C,D).

3. Intracapsular variation in wall thickness.

Serial sections along the capsule chamber of Nucella emarginata showed considerable variation in wall thickness (Fig.8). Within individual capsules, walls tended to be thickest in the plug and stem regions and thinnest about 75%

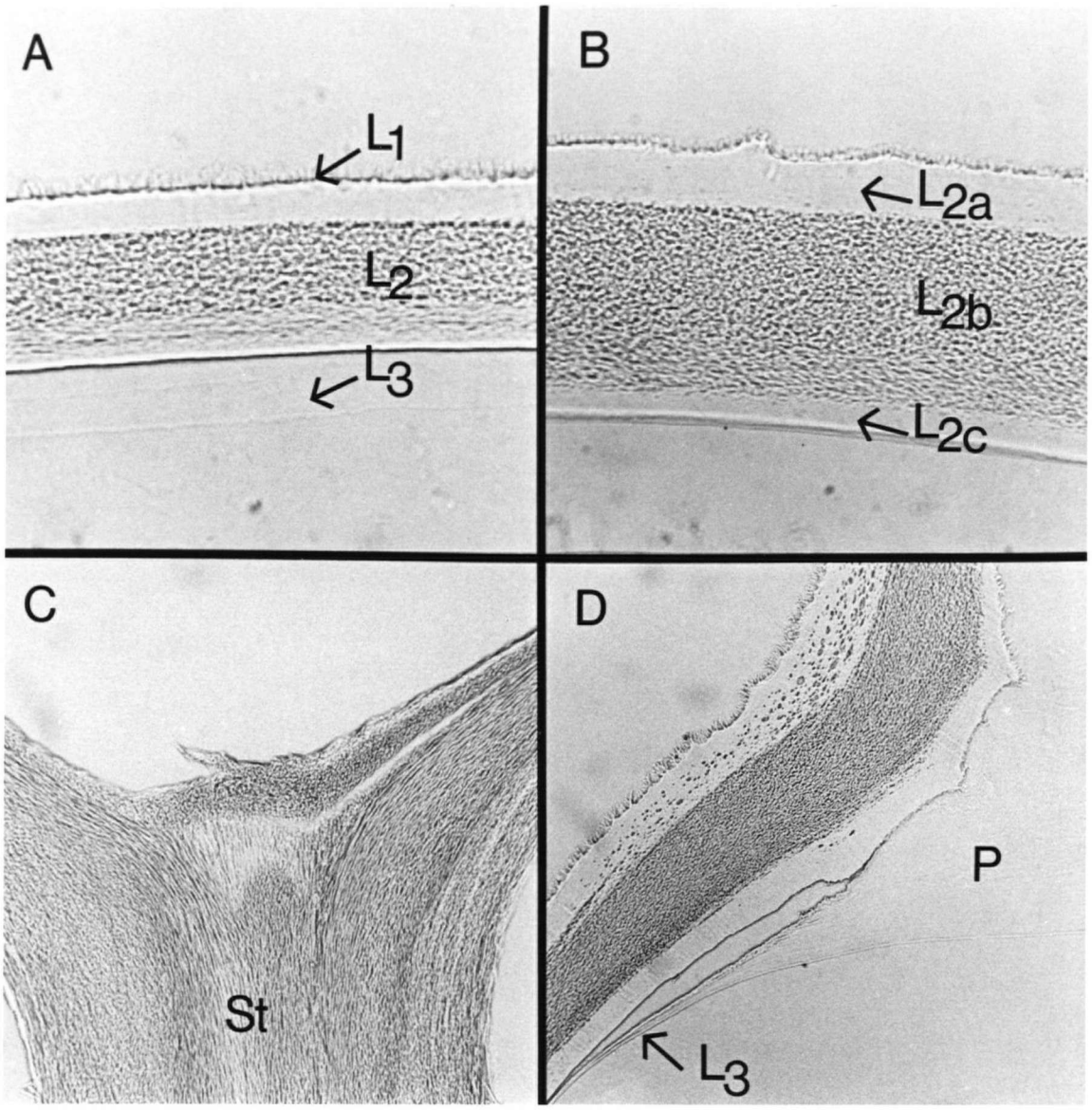


FIGURE 7. Microstructure of Nucella emarginata egg capsules: a) transverse section 70 % along the chamber of a capsule from Ross Islets (mean thickness = 60μm), b) transverse section 70% along the chamber of a capsule from Grappler Inlet (mean thickness = 90μm), c) longitudinal section through the stem region of a N. emarginata capsule, d) longitudinal section through the plug region of a N. emarginata capsule. Outer (L_1), middle (L_2) and inner (L_3) capsule wall laminae are indicated, as are the three component layers (L_{2a}, L_{2b}, L_{2c}) of the middle lamina. The capsule plug (P) and stem (St) are also shown.

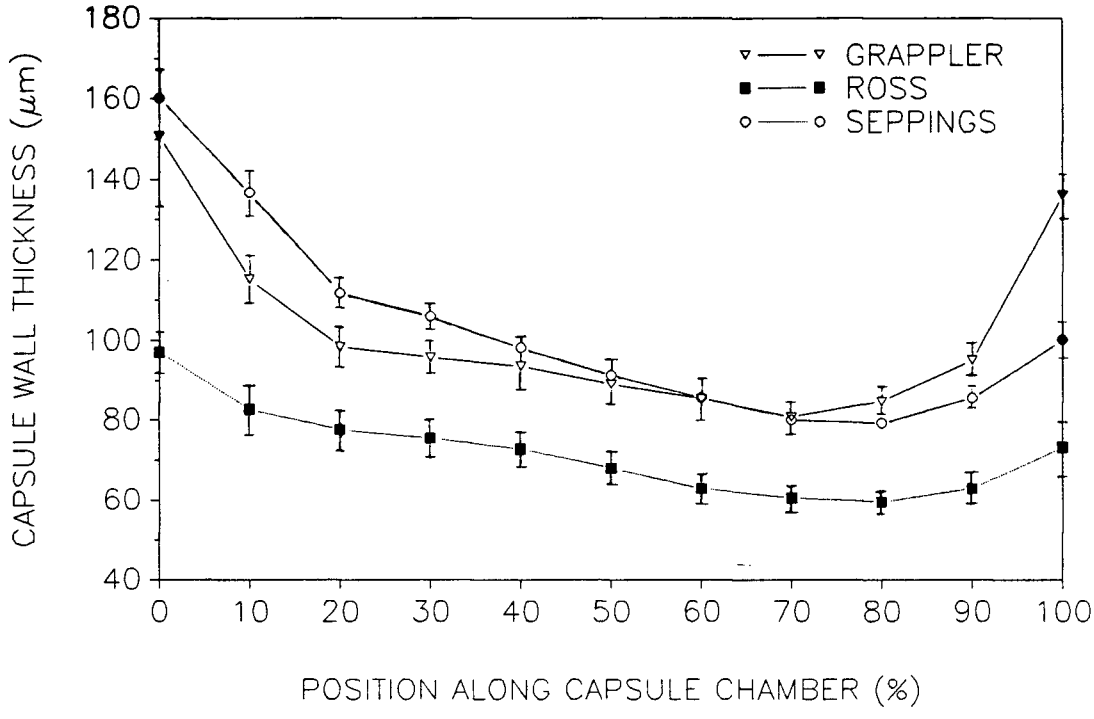


FIGURE 8. Variation in wall thickness along the capsule chamber of field-collected Nucella emarginata capsules from Grappler Inlet, Ross Islets, and Seppings Island. Values are expressed as X ± 1 S.E. for n = 8 capsules from each population.

along the capsule chamber. Although changes in capsule width also occurred along the length of the chamber, these did not appear to correspond with variation in wall thickness (Fig.9).

Sections through the walls of field-collected Nucella emarginata capsules revealed obvious differences in thickness among the Grappler Inlet, Ross Islets, and Seppings Island populations. Comparisons of wall thickness data from a point 70% along the chamber chamber indicated that capsules from Ross Islets were significantly thinner than capsules from Seppings and Grappler (means of 61, 80 and 81μm, respectively; ANOVA: $F=29.9$, $p<0.001$; Fig. 7A,B). Capsules collected from Grappler and Seppings did not show any significant differences in wall thickness. Therefore, wall-thickness variation among sites did not appear to reflect differences in wave-exposure, as capsule walls were most similar between wave-sheltered and wave-exposed populations.

4. Variation in capsule wall thickness among laboratory populations

Capsule wall thickness also varied among laboratory populations with Ross Islet capsules being the thinnest, and Seppings and Grappler Inlet capsules the thickest ($\bar{X}$=60, 78, and 83μm, for Ross, Seppings, and Grappler, respectively; Fig. 10). These differences were significant among all three laboratory populations (ANOVA; $F=81.32$, $p<0.001$) and corresponded well with previous results for field-collected capsules. Hence, there was no a posteriori reason to suspect that snails produced different thicknesses of capsules in the

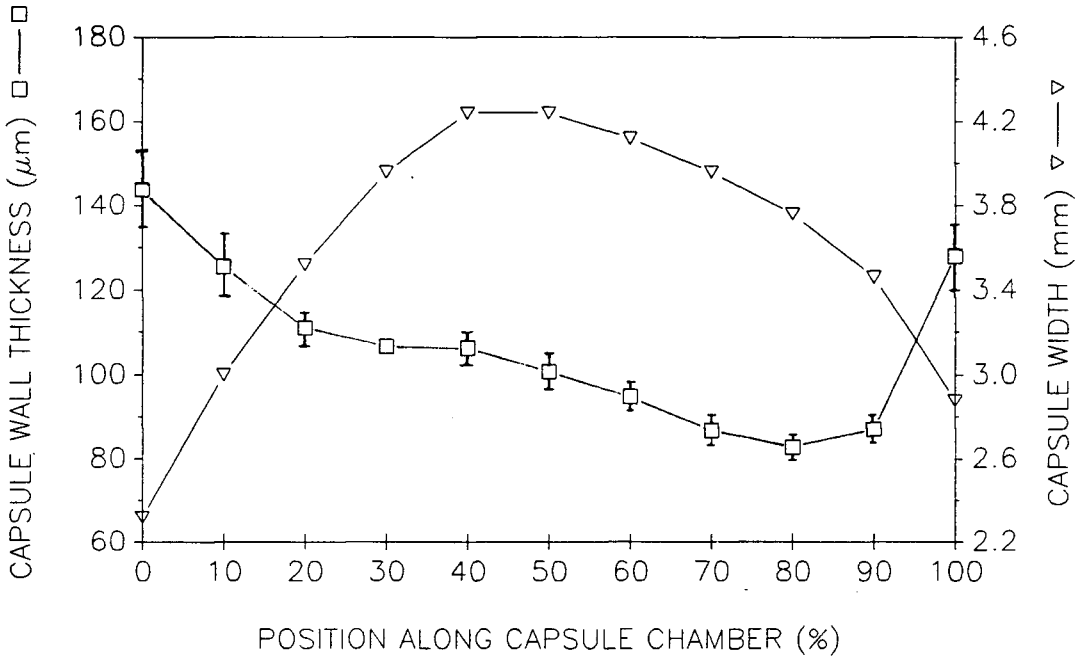


FIGURE 9. Variation in wall thickness and capsule width within one Nucella emarginata capsule from Grappler Inlet. Wall thickness values are the mean ± 1 S.E. of 8 measurements taken from within each section. Capsule width values represent the maximum width at each section along the capsule chamber.

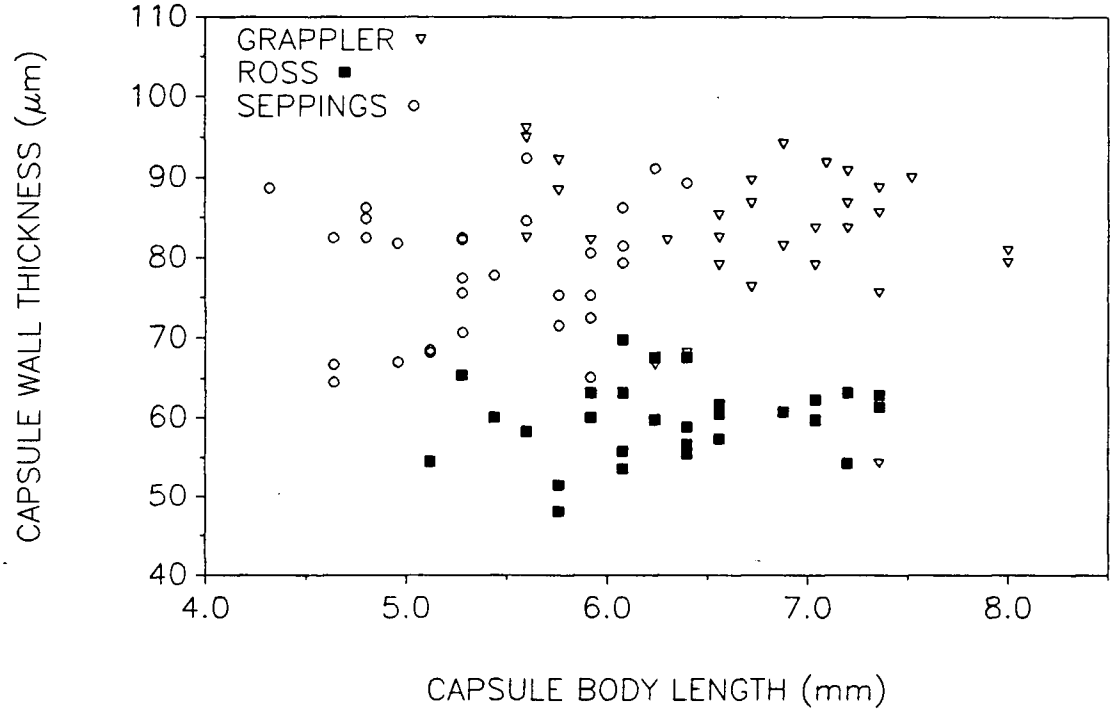


FIGURE 10 Variation in capsule wall thickness with capsule body length for 30 Nucella emarginata capsules from each laboratory population. Each value represents a mean of 8 measurements taken from one representative section at a point 70% along the capsule-chamber.

laboratory as compared with the field. In fact, snails still continued to produce their thick- or thin-walled capsules even after 5 months in the laboratory. These differences in capsule wall thickness appeared to result from variation in the thickness of all three component layers of the middle lamina, rather than in one component alone. Component layers varied in the proportion of 2:7:1 for L_{2a}, L_{2b} and L_{2c}, respectively, regardless of the absolute thickness of capsule walls.

No relationship was evident between wall thickness and capsule length within each population (Fig. 10). Since capsule length was related to female shell length (Fig. 5), differences in capsule wall thickness within each population were probably not related to female size. Site differences in capsule wall thickness also did not appear to reflect variation in snail size, as small Seppings snails ($\bar{X}$=1.9 cm) and large Grappler snails ($\bar{X}$=2.7cm) both produced thick-walled capsules.

5. Variation in capsule wall thickness within and between clutches laid by different females

Figure 11 shows the variation in wall thickness within and among clutches for each laboratory population. Capsule wall thickness was found to vary significantly among clutches within each population (ANOVA; Grappler, $F=3.44$, $P=0.02$; Seppings, $F=47.52$, $P<0.001$; Ross, $F=32.05$, $P<0.001$). Variability in capsule wall thickness among clutches, however, did not obscure the site differences in wall thickness found

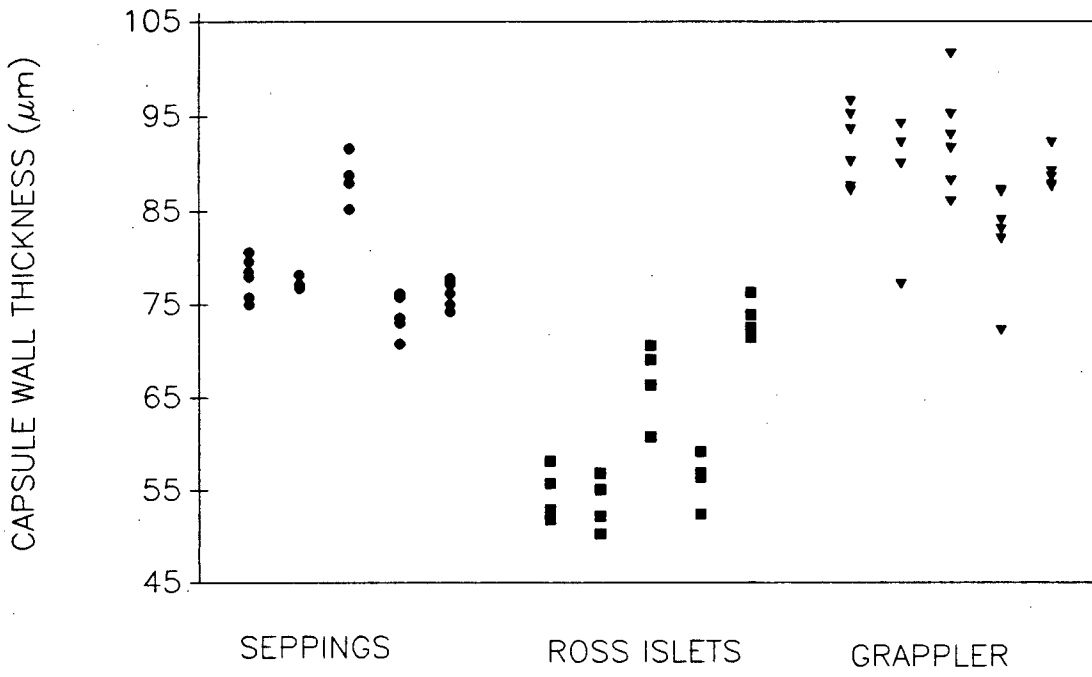


FIGURE 11. Variation in wall thickness within and between clutches of Nucella emarginata capsules. Five clutches were sampled from each laboratory population with n = 6 capsules/clutch, n = 4 capsules/clutch, and n = 6 capsules/clutch for Seppings, Ross Islets, and Grappler populations, respectively. Each data point represents the mean of 8 measurements taken from one representative section at a point 70 % along the capsule chamber. Each vertical group of points represents one clutch of capsules.

previously. Ross Islet capsules ranged in wall thickness from 50-76μm, while Seppings and Grappler capsules ranged from 71-92μm and 72-102μm, respectively.

6. Variation in dry weight of capsules among sites

A significant relationship was found between dry weight of the capsular case and capsule body length for each laboratory population of snails (Fig. 12). The slopes of these relationships were not significantly different (ANCOVA for slopes; $F=1.20$, $p>0.25$), but the elevations did vary significantly (ANCOVA for elevations; $F=50.52$; $p<0.001$). These data corresponded well with the already known differences in capsule wall thicknesses among populations, as thin-walled Ross Islet capsules were found to weigh significantly less than thicker-walled capsules from the other two sites. For example, a 6.5 mm-long egg capsule from the Ross Islets weighed 24% less than a capsule of the same length from Grappler Inlet and 16% less than one from Seppings (Fig. 12). Grappler capsules were also found to be significantly heavier than Seppings capsules, which again reflected the differences found previously in capsule wall thickness between these populations.

In summary, there were obvious differences in the size and wall structure of capsules among the three populations of Nucella emarginata. Variation in capsular size was largely accounted for by differences in snail shell length, although, snails from Seppings Island were found to produce proportionally larger capsules per unit shell length than

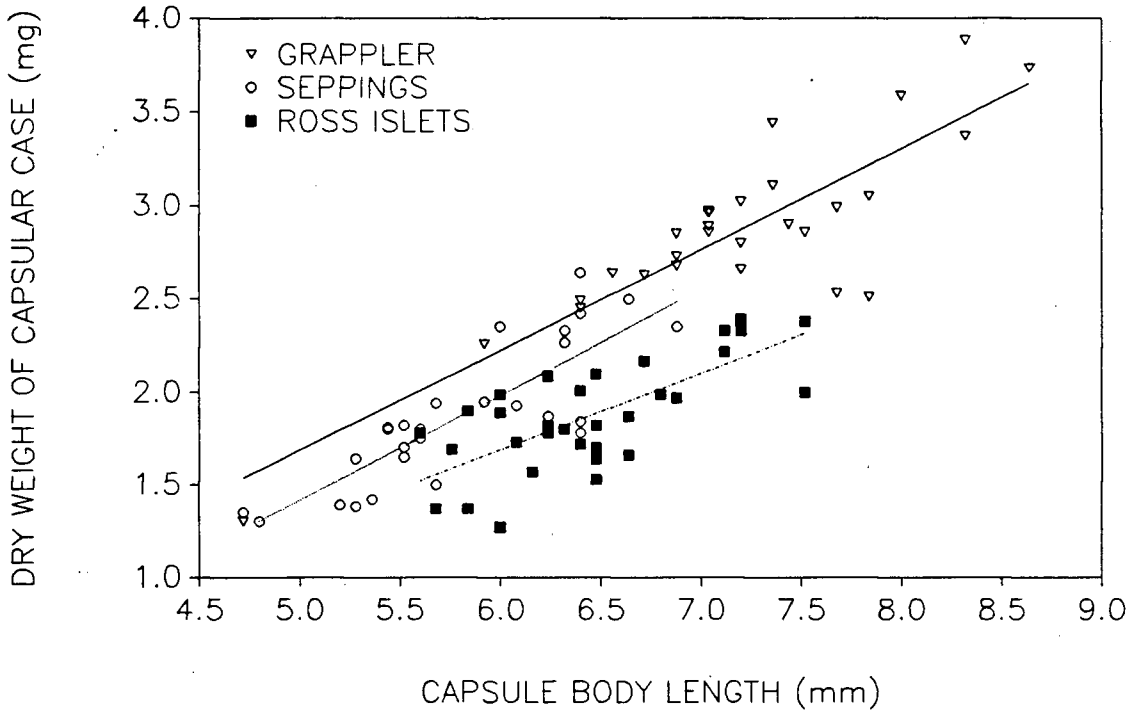


FIGURE 12. Dry weight of empty capsular cases as a function of capsule body length for each laboratory population. Each data point represents one capsule. Regression equations for each site are: Seppings: Y = 0.569X - 1.429, r = 0.837, n = 27; Ross Islets, Y = 0.414X - 0.792, r = 0.723, n = 33; Grappler Inlet, Y = 0.539X - 1.007, r = 0.860, n = 28.

snails from Grappler Inlet or Ross Islets. The structure of capsule walls also differed among sites, with Ross Islets snails producing significantly thinner-walled capsules than Grappler and Seppings snails. Variation in capsule wall thickness did not appear to reflect differences in capsule size, female size, or increasing wave-exposure levels. However, the dry weight of capsular cases differed among populations and corresponded with differences in wall thickness. Hence, for a given capsule size, snails from Ross Islets allocated significantly less material to their egg capsules than did snails from Seppings Island or Grappler Inlet.

III. VARIATION IN CAPSULE CONTENTS AMONG SITES

1. Egg number and egg volume

The relationship between egg number (including both nurse eggs and embryos) per capsule and capsule volume is shown for each population in Figure 13. Comparisons among sites indicated that there were no significant differences in the total number of eggs allocated to Seppings, Ross, and Grappler capsules. Neither the slopes (ANCOVA for slopes: $F=0.65$, $p>0.50$) nor elevations (ANCOVA for elevations: $F=0.36$; $p>0.50$) of these relationships differed significantly among populations.

The size of eggs did not differ significantly among sites either. Although Ross Islet capsules contained slightly larger eggs than Grappler or Seppings capsules (X egg volume $=40.5\times10^{-4}mm^3$, $39.6\times10^{-4}mm^3$, and 38.1×10^{-4} mm^3, respectively),

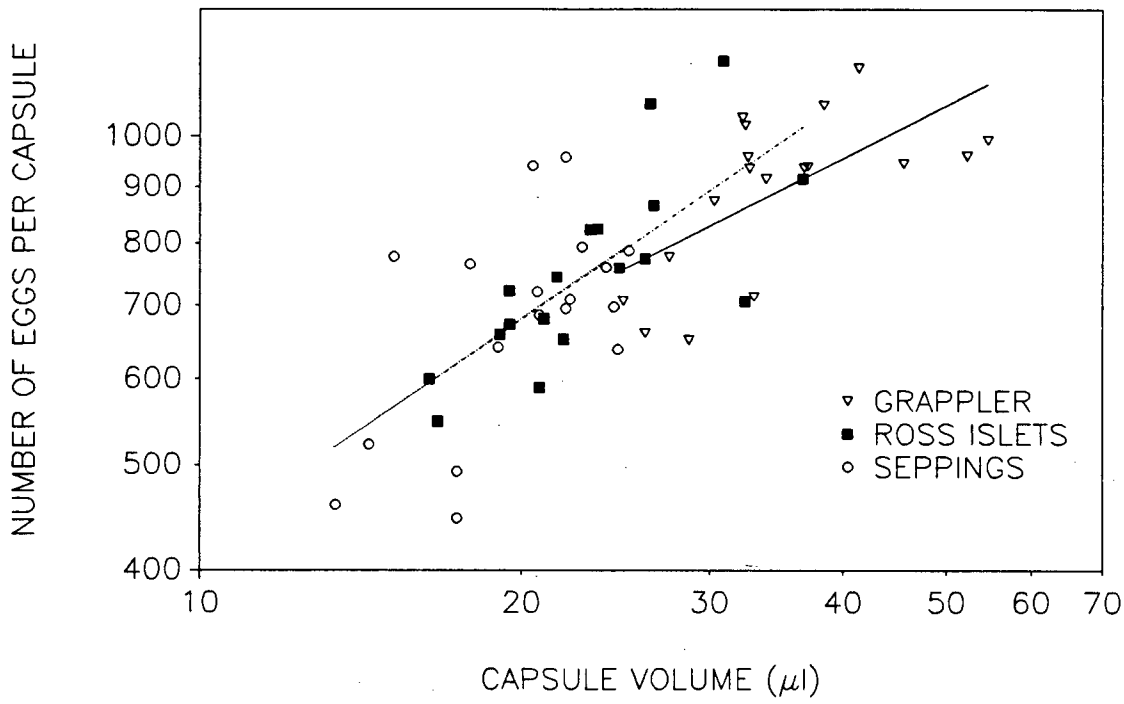


FIGURE 13. Relationship between the number of eggs per capsule and the volume of the capsule-chamber for Seppings, Ross Islets, and Grappler capsules. Counts of eggs include both developing embryos and non-developing nurse eggs. Regression equations for each population are: Seppings: Log Y = 0.676 Log X + 1.954, r = 0.742, n = 18; Ross Islets: Log Y = 0.669 Log X + 1.962, r = 0.583, n = 18; Grappler: Log Y = 0.492 Log X + 2.192, r = 0.640, n = 18.

these differences were not significant (ANOVA: F=1.55, P>0.22). Therefore, there was no evidence of independent selection for egg number or egg size in these populations.

2. Number and size of hatching snails

Grappler Inlet capsules were found to contain slightly fewer embryos per unit volume than similar-sized capsules from Seppings Island and Ross Islets (means of 14, 17 and 19 embryos for a 30μl capsule from Grappler, Seppings and Ross Islets, respectively). However, the relationships between embryo number and capsule volume did not differ significantly among sites (ANCOVA for slopes, F=0.76, p>0.25; and for elevations, F=0.82, p>0.25).

The mean size of juveniles hatching from capsules was inversely proportional to the number of embryos contained within each capsule (Fig. 14). Thus, large juveniles emerged from capsules containing relatively few embryos, while small juveniles emerged from capsules containing many embryos. Because embryos are known to compete for nurse eggs during their development (Spight, 1976; Rivest, 1983), differences in these relationships among populations could indicate variation in either: 1) the number of nurse eggs apportioned to embryos within capsules, or 2) the number of embryos allocated to each capsule (Etter, 1987). However, such differences were not found (comparisons among populations indicated that slopes (ANCOVA for slopes: F=0.93, P>0.5) and elevations (ANCOVA for elevations: F=3.2, P=0.01) were not significantly different). In summary, therefore, the number of eggs and embryos per unit

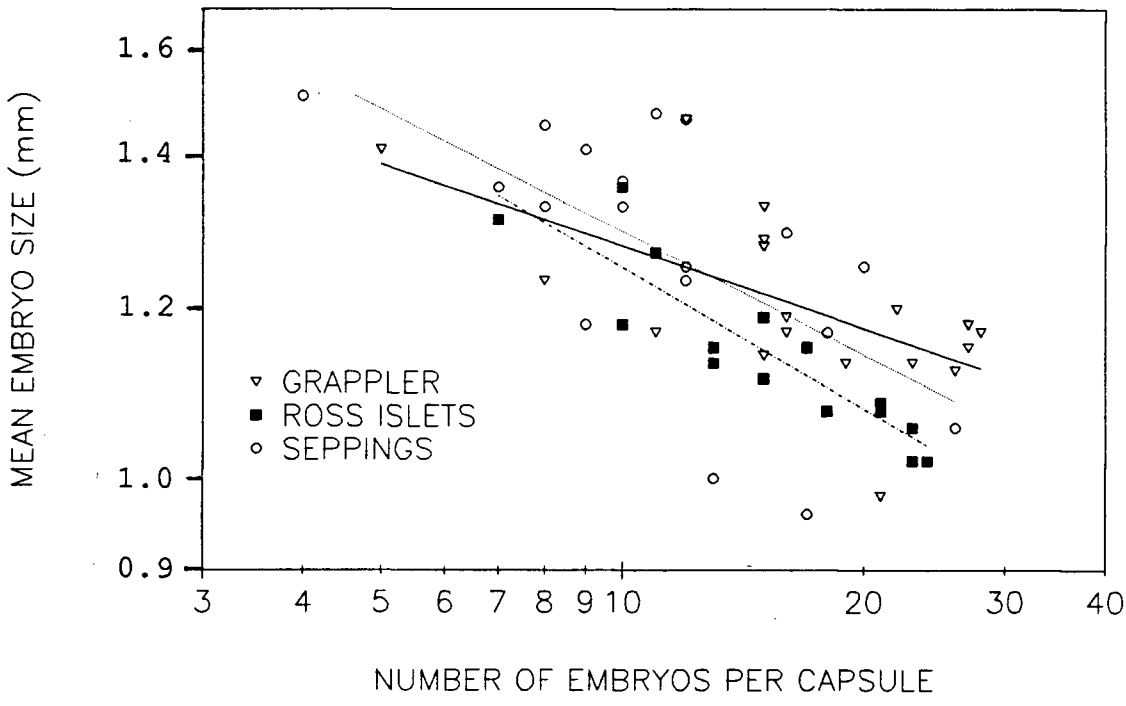


FIGURE 14. Mean hatching size of embryos as a function of the number of embryos contained within each capsule for Seppings, Ross Islets and Grappler populations. Regression equations for each population are: Seppings: Log Y = -0.199 Log X + 0.321, r=0.636, n=19; Ross Islets: Log Y = -0.228 Log X + 0.322, r=0.895, n=16; Grappler Inlet: Log Y = -0.134 Log X + 0.248, r=0.614, n=18.

volume did not vary among capsules from Seppings Island, Ross Islets and Grappler Inlet.

IV. CAPSULE STRENGTH MEASUREMENTS

1. Resistance to puncturing

The force required to puncture capsules differed among the three laboratory populations ($\bar{X}\pm$S.D.=6.18$\pm$0.66 Mega Newtons/m^2 (n=10), 5.17$\pm$0.70 MN/m^2 (n=15), and 4.92$\pm$0.46 MN/m^2 (n=10), for Grappler Inlet, Ross Islet and Seppings capsules, respectively). Grappler Inlet capsule walls appeared to be the strongest, as they required significantly greater puncturing forces than did Ross Islet or Seppings capsules (ANOVA, F=11.77, P<0.001; p<0.05, Tukey Multiple Comparison Test). However, Ross Islet and Seppings capsules did not differ significantly in puncturing resistance (p>0.05, Tukey M.C.T.). Hence, thick-walled Grappler Inlet capsules were stronger than both thick-walled Seppings capsules and thin-walled Ross Islet capsules.

2. Resistance to squeezing

The force needed to rupture capsules by squeezing also differed among laboratory populations and followed a trend similar to the previous results ($\bar{X}\pm$S.D.= 14.5$\pm$4.3 N (n=19), 7.5$\pm$2.7 N (n=21), and 9.7$\pm$3.9 N (n=15), for Grappler, Ross Islets and Seppings capsules, respectively). Grappler capsules required significantly larger forces to rupture the capsular plug than did Ross Islet and Seppings capsules (ANOVA, F=18.67, P<0.001; p<0.05, Tukey M.C.T.). Although

Seppings capsules were slightly more resistant to squeezing than Ross Islet capsules, this difference was not significant ($p>0.05$, Tukey M.C.T.).

In summary, Grappler Inlet capsules were significantly more resistant to puncturing and squeezing forces than capsules from Seppings Island and Ross Islets. This corresponded to the fact that Grappler capsules also had the thickest capsule walls. Seppings capsules, however, which were also thick-walled, were not significantly more resistant to puncturing or squeezing forces than were thin-walled Ross Islet capsules.

DISCUSSION

Advantages of thick, strong capsule walls

Although numerous studies have documented interspecific differences in the thickness and strength of capsule walls in gastropods (Perron, 1981; Perron and Corpuz, 1982; Pechenik, 1983), as yet there is little evidence to indicate that thick-walled capsules actually protect developing embryos better than thin-walled capsules. In fact, Pechenik (1983) found that rate of salt movement across the walls of Nucella lamellosa, N. lapillus and N. lima capsules did not differ in accordance with capsule wall thickness. Thus, these results suggest that rate of osmotic change and desiccation might not differ among thick- or thin-walled capsular structures in the related species Nucella emarginata.

The only evidence to support the hypothesis that thick-walled capsules are more protective than thin-walled capsules comes from positive correlations found between capsule strength (as measured by the puncture-resistance of capsule walls), capsule wall thickness, and developmental time of encapsulated embryos among Conus species (Perron, 1981; Perron and Corpuz, 1982). Perron (1981) also found that the proportion of reproductive energy invested in capsule walls increased from 20 to 47% with an increase in encapsulated development time from 11 to 26 days in Conus species. These studies by Perron (1981) and Perron and Corpuz (1982) have provided at least some data to suggest that strong, thick-

walled capsules may reflect selection for increased protection of embryos when exposure to environmental stresses is long.

The relationship between capsule strength and embryonic development time found by Perron (1981) may be general for all marine gastropods. If so, then Perron's data on puncture resistance in Conus spp. can be extrapolated to Nucella emarginata. Capsules containing embryos with an 80 day development period, such as N. emarginata (Emlen, 1966), should require 6.11 MN/m^2 of force to puncture their walls. In the present study, the mean force needed to puncture Nucella emarginata capsules walls ranged from 4.92-6.18 MN/m^2, thus closely approximating Perron's (1981) predictions.

Intraspecific differences in capsule structure

Although differences in capsular structure may be more apparent between species, studies of variation within a species are likely to be more useful in elucidating the importance of 1) physical constraints of body size, 2) phenotypic responses to variation in environmental parameters, and 3) genetic divergence resulting from selection, in accounting for differences in capsular structure (Brown, 1983). Surprisingly, no previous studies on gastropods have examined intraspecific variation in capsule wall structure among populations. In the present study, such comparisons indicated that the thickness and strength of capsule walls varied among intertidal locations. Capsules collected from Grappler Inlet had the thickest walls and were most resistant

to puncture and squeezing tests, whereas capsules from Seppings Island were also thick-walled, but were significantly less resistant to these tests. Ross Islet snails produced comparatively weak thin-walled capsules. What is the cause of intraspecific differences in capsular structure among these populations?

Differences in capsule wall thickness among populations could simply reflect constraints associated with female size. Although the morphology of neogastropod egg capsules is governed by the size of the capsule gland which, in turn, is restricted by female shell-length (Spight et al., 1974; Spight and Emlen, 1976; Perron and Corpuz, 1982; present study), little is known about the effect of female size on capsule wall thickness. Previously, Perron and Corpuz (1982) reported that wall thickness and strength of Conus pennaceus capsules increased with capsule size and snail shell-length. Their results suggest that the structure of capsule walls may be limited by the size of the capsule gland. In the present study, capsule size and snail shell-length varied markedly within and among the Grappler, Ross Islets and Seppings populations. However, the thickness and strength of capsule walls did not vary as predicted with capsule length or snail shell-length. Hence, intraspecific variation in the structure of capsule walls among Nucella emarginata populations did not reflect allometric contraints associated with female size.

Variation in the structure of Nucella emarginata capsule walls was not phenotypically plastic. This was indicated by

two different results. First, variation in capsule wall thickness within a clutch was low compared to variation among clutches produced by different individuals. Hence, individual snails appeared to be restricted in the thickness of capsule walls they produced. Second, snails from each site continued to produce their respective thick- or thin-walled capsules even after 5 months in the laboratory during which all snails were kept under similar environmental conditions. This indicated that differences in the structure of egg capsules were not phenotypic responses to site differences in diet, food abundance, or levels of environmental stress.

The production of thick capsule walls must have adaptive value, as thick-walled capsules incur a greater energetic cost than thin-walled capsules. For instance, thin-walled capsular cases from the Ross Islets (6.5 mm in length) weighed 24 % less than thick-walled capsules from Grappler Inlet, and 16% less than thick-walled capsules from Seppings Island. As capsular cases can account for more than 50% of the dry weight of intact capsules (ie., including the eggs; this study; Roller and Stickle, 1988) and as Nucella emarginata capsular material has almost the same energy content per unit weight as the eggs (22.6 KJ per ash-free g^{-1} compared to 25.1 KJ per ash-free g^{-1} of embryos; Davies, 1984), the energy spent in producing thicker capsule walls must represent either a substantial decrease in the energy available for egg production or an increase in the reproductive effort of an individual.

Perron (1982) found that the production of puncture-resistant, thick capsule walls among Conus spp. was associated with a higher annual reproductive effort than the production of weak, thin-walled capsules. The reproductive effort of snails was not compared among populations in the present study. However, the production of thick-walled capsules did not result in a reduction of the number of eggs contained per unit capsule volume. Hence, on a per capsule basis, there was no evidence of a tradeoff between the amount of energy invested in extraembryonic products versus eggs.

Effects of environmental stresses on encapsulated embryos

It seems likely that the thickness of capsule walls reflects selection for increased protection of embryos against intense environmental stresses. Desiccation (Feare, 1970; Spight, 1977; Pechenik, 1978), osmotic stress (Pechenik, 1982; 1983), wave-action (Perron, 1981), bacterial attack (Lord, 1986), predation (Spight, 1977; Perron, 1981; Brenchley, 1982), and thermal stress (Spight, 1977) are all potentially important sources of mortality for encapsulated embryos. In the present study, although the structure of capsules was compared among sites separated along a gradient of wave-exposure, the thickness of capsule walls did not vary as predicted by this gradient.

The thickness of capsule walls may be selected for by more than one factor. For example, capsule walls were thick at the wave-exposed site, where wave-action and associated abrasion

would be predicted to be most extreme and were also thick at the wave-sheltered site, where desiccation, predation, and osmotic stresses should be most intense. In this way, capsule wall thickness might be a good biological indicator of the intensity of environmental stresses acting upon developing embryos in various intertidal localities. Furthermore, different environmental stresses may have selected independently for capsule wall thickness and capsule strength, as the strength of capsules from Grappler Inlet, Seppings Island, and Ross Islets did not correspond to differences in capsule wall thickness among these sites. Hence, an examination of both components of capsule structure in future studies might be necessary to differentiate between selective pressures acting upon gastropod egg capsules.

Tradeoffs in capsule structure

Thick capsule walls may increase the survivorship of developing embryos exposed to severe environmental stresses, but such structures could also act to limit embryo survivorship by restricting the diffusion of oxygen and nitrogenous wastes across capsule walls. In this regard, Perron and Corpuz (1982) found that large, thick-walled Conus pennaceus capsules contained fewer eggs per unit volume than did small, thin-walled capsules. In contrast, the wall thickness of Nucella emarginata capsules did not appear to affect the number of eggs and embryos allocated to capsule-chambers in the present study. For instance, the density of

eggs and embryos contained within Grappler Inlet capsules, which had the thickest capsule walls and lowest surface area to volume ratios of capsules from all three study sites, was not significantly different from the density in Seppings and Ross capsules. Although large-volumed Nucella emarginata capsules did contain lower densities of eggs than smaller-volumed capsules (cf. Conus pennaceus; Perron and Corpuz, 1982), larger capsules were not found to be thicker-walled than smaller capsules. Hence, egg and embryo density did not reflect differences in capsule wall thickness.

Effects of environmental stresses acting upon adult snails

Differences in the intensity of environmental stresses acting upon the adult snails at different sites may make interpretations of site differences in capsule wall structure even more difficult. Environmental stresses such as wave-exposure are known to affect the reproductive effort of gastropods profoundly. For instance, wave-exposed snails typically mature at smaller sizes and exhibit higher reproductive efforts over shorter lifespans than longer-lived wave-sheltered snails (Lymnaea peregra, Calow, 1981; Littorina rudis, Roberts and Hughes, 1980; Nucella lapillus, Etter, 1987). There was some evidence for this in the present study, as Seppings snails matured at smaller sizes and produced proportionally larger capsules than Grappler Inlet snails. It is not known how such differences in reproductive effort might be reflected in the partitioning of energy

between eggs and extraembryonic products.

Snail populations expending a low reproductive effort might allocate proportionally more energy into protective capsular cases versus eggs than snails exhibiting a high reproductive effort. The explanation for this is that with less energy to expend, snails should adopt a "bet-hedging" strategy - one of ensuring maximal protection for a few eggs rather than minimal protection for many. Therefore, although the thickness and strength of capsule walls undoubtedly must reflect the primary effects of stresses acting upon encapsulated embryos, secondary effects acting upon adult snails might also influence the thickness and strength of capsule walls produced.

The results of the present study have shown the first evidence of intraspecific variation in capsule wall thickness and strength among populations of a marine gastropod. These findings are a necessary basis for further investigation into the intensity of specific environmental stresses acting upon egg capsules in situ, such as desiccation (Spight, 1977; Pechenik, 1978), predation (Spight, 1977; Perron, 1981; Brenchley, 1982) and osmotic stress (Pechenik, 1982; 1983). The second chapter of this thesis will examine the effect of one of these stresses, predation, on survival of encapsulated embryos of Nucella emarginata.

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CHAPTER 2

FUNCTIONAL MORPHOLOGY OF MARINE GASTROPOD EGG CAPSULES: LABORATORY AND FIELD TESTS OF PREDATION

INTRODUCTION

Despite frequent speculation about the "protective" role that egg capsules play in gastropod life-histories (Pechenik, 1979; Perron, 1981; Pechenik, 1986), little is known about the survivorship of encapsulated embryos in the marine environment. Gastropod embryos may spend from a few days (Perron, 1981) to more than 6 months within benthic egg caspules (West, 1973) before emerging as larvae or juvenile snails. During this period of encapsulation embryos are susceptible to: predation (Spight, 1977; Brenchley, 1982, Abe, 1983), bacterial attack (Lord, 1986), desiccation (Feare, 1970; Spight, 1977; Pechenik, 1978), osmotic stress (Feare, 1970; Pechenik, 1982; 1983), thermal stress (Spight, 1977), and wave shock (Perron, 1981). As yet, few studies have examined the relative importance of these stresses in the survivorship of encapsulated embryos.

Predation appears to be an important source of mortality for encapsulated gastropod embryos (Emlen, 1966; Spight, 1977; Brenchley, 1982). Capsular cases are opened in the laboratory and field by a variety of organisms, such as: crustaceans (MacKenzie, 1961; Emlen, 1966; Phillips, 1969; Spight, 1977; Perron, 1981; Brenchley, 1982), polychaetes (Feare, 1970), chitons (Emlen, 1966; Eaton, 1972), prosobranchs (Phillips,

1969; West, 1973; Eaton, 1972; McKillup and Butler, 1979; Brenchley, 1982; Race, 1982; Abe, 1983), echinoderms (Spight, 1977; Martel and Larrivee, 1986) and possibly fish (Spight, 1977). However, relatively few studies have directly examined the intensity of predation on gastropod egg capsules in the marine environment (Spight, 1977; Brenchley, 1982; Abe, 1983).

Although some gastropod species actively protect their egg capsules from predators until embryonic development is complete (Ostergaard, 1950; D'Asaro, 1970; Eaton, 1972), in the majority of species parental care is passive - limited only to the production of capsular cases. Recently, differences have been noted in the structure of capsules within and among gastropod species (Perron, 1981; Perron and Corpuz, 1982; Chapter 1). For example, Perron (1981) noted that the strength of Conus capsules, as measured by wall puncturing tests, was directly correlated with the developmental time of the encapsulated embryos. From these results he suggested that thick-walled capsules might deter predators more effectively than would thin-walled capsules. Comparative studies of the resistance of thick- and thin-walled capsules to predators have not yet been undertaken.

The marine intertidal snail, Nucella emarginata, is a common inhabitant of rocky shores along the west coast of North America, ranging in distribution from California to Alaska. These snails deposit eggs within tough proteinaceous capsules which are attached to hard intertidal substrates. Embryos develop within capsules for a period of up to 80 days (Emlen, 1966), after which they hatch as juvenile snails.

Nucella emarginata egg capsules are known to vary in wall thickness and strength among sites, with capsules from some populations having walls up to 25% thicker than capsules from other populations (Chapter 1).

The aim of this study was to examine predation on the egg capsules of the intertidal whelk Nucella emarginata in both laboratory and field environments. Laboratory experiments were conducted to identify predators of the capsules and to determine the effectiveness of thick-walled versus thin-walled capsules in protecting developing embryos from specific predators. Field studies were undertaken to estimate the extent of predation on naturally deposited egg capsules and to identify invertebrates responsible for opening egg capsules at two different intertidal locations.

MATERIALS AND METHODS

The project was conducted at the Bamfield Marine Station on the west coast of Vancouver Island from May-November 1988. Experimental study sites were established at intertidal locations in Grappler Inlet and Ross Islets, Barkley Sound. Observations were also made at a third site at Seppings Island. Descriptions of these habitats are given in Chapter 1.

I. LABORATORY EXPERIMENTS

Several species of intertidal invertebrates were collected from field sites to determine which might prey on Nucella emarginata egg capsules in the laboratory. Groups of individuals of each species were placed in appropriate sizes of mesh-panelled vials (3x3x6 cm) and mesh-panelled containers (8x8x10 cm), and were provided with intertidal shells or bare rocks for shelter. Containers were then partially immersed in seawater tanks and provided with a continuous flow of fresh seawater. Test animals were starved for 24 h before being presented with 8 intact N. emarginata egg capsules. Capsules were mounted on small flat rocks using "Superglue" and arranged in a standardized circular configuration. A predator was defined to have "opened" an egg capsule only if it ruptured or ate through the capsule chamber which contained the developing embryos. Thus, an animal was not required to eat embryos or nurse eggs to be termed a predator. Capsules were checked every 1-2 days for evidence of predation and

experiments were continued for at least two weeks or until all capsules had been opened, whichever occurred first. A summary of the species tested is given in Table I. Five to ten replicates, including controls consisting of cages with no predators, were conducted for each species.

Laboratory experiments were also conducted to determine the maximum rate at which predators could open egg capsules. Predators of various sizes were caged individually in mesh containers (8x8x10 cm) and placed in seawater tables. Animals were starved for 24 h and then each was presented with 8 intact Nucella emarginata egg capsules. Capsules were checked at least once a day to determine the number that had been opened. Experiments were continued for five days or until all capsules had been opened.

II. FIELD CENSUSES OF PREDATION

1. Grappler Inlet

All field work was conducted in an intertidal channel located along Grappler Inlet. The substrate consisted of a fine silty mud overlaid by clam shells and a meshwork of mussel- and barnacle-covered rocks (Mytilus edulis, Balanus glandula and Semibalanus cariosus). Large boulders (1.0 m dia.) occurred throughout this region. In May 1988 two transects (10 m in length) were established parallel to the shoreline on the east-facing slope of the channel at 1.2 and 2.2 m above zero chart datum. Eight quadrats ($0.25m^2$) were sampled at 0.5-1.0 m intervals along these transects to determine: a) the abundance and size of snails, b) the

abundance of egg capsules, and c) the number of egg capsules that had been preyed upon. Egg capsules were collected from each quadrat and categorized on the basis of whether the capsule chambers were intact or ruptured. The age of intact capsules was estimated by noting the developmental stage of the embryos. New capsules were identified by the presence of nurse eggs, while older capsules contained well developed embryos. Opened capsules were examined to determine whether they had been attacked by predators or whether developing embryos had hatched naturally. If capsules were empty, but had been chewed into the capsule chamber, they were considered to have been opened by predators. Such capsules were described by distinctive "bite marks" on the capsule walls. The abundance of potential predators (identified from laboratory work) was also censused along these transects.

2. Ross Islets

All field work was conducted on a north-facing beach in the Ross Islets. In June 1988 three transects were established parallel to the rocky shoreline at tidal heights of 1.9, 2.3 and, 2.6 m above zero chart datum. The two highest transects were positioned along a steeply sloping granite wall which was sparsely covered with Fucus distichus, Balanus glandula, and Semibalanus cariosus. The lowest transect was set along a boulder-covered beach directly below the higher transects. Data were collected as described previously for the Grappler Inlet site. A large rocky outcropping, adjacent to the study site, was also censused in

August 1988. The top of this promontory (16 m^2) ranged from 2.8-3.1 m above zero chart datum and was densely covered with Fucus distichus, Balanus glandula and Semibalanus cariosus. For censusing, this area was divided into 6 equal-sized grids and a quadrat was thrown haphazardly into each region. Snail density, egg capsule density, and predator abundance were recorded as described previously.

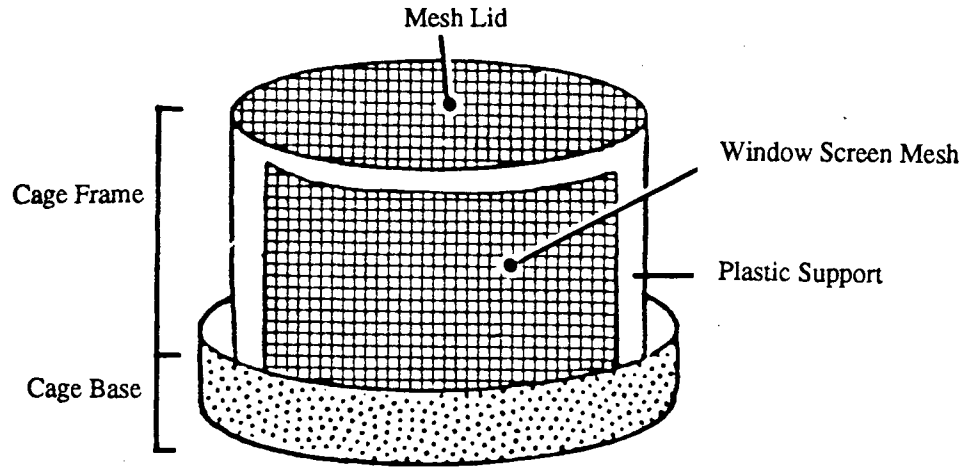
3. Seppings Island.

The study area was located in a large boulder-lined channel, approximately 10 m wide. Capsules were regularly collected to determine if the capsular remains were similar to those found at the other sites. Censuses of predation intensity or predator abundance were not made at this site.

III. FIELD EXPERIMENTS

Experimental cages were used to assess the importance of predation on Nucella egg capsules and to identify the invertebrates responsible for opening capsules at the Grappler and Ross Islet study sites. Cages also served to exclude different size-classes of predators from capsules. Cages were constructed from round plastic containers (9 cm dia.). Each cage consisted of a base, mounted to a heavy slate plate, and a frame that screwed into the base. Each frame consisted of two plastic supports, two large openings covered to varying degrees by window screen mesh (0.01 cm^2), and a mesh lid (Fig. 1a). Egg capsules obtained from laboratory populations of Nucella emarginata were glued onto small plastic saucers that

a)



b)

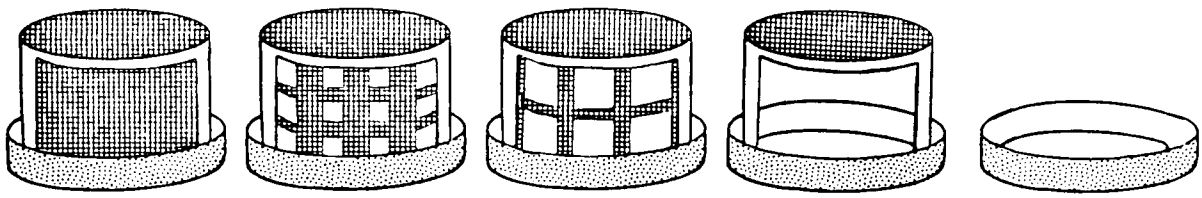


FIGURE 1. Predator exclusion cages, ilustrating: a) the cage base and detachable frame and, b) the five cage types used to exclude various size-classes of predators: the small-hole cage (holes: 0.1x0.1cm), medium-hole cage (holes: 0.5x0.5cm), large-hole cage (holes: 1.5x1.5cm), open cage (holes: 2.5x11.5cm) and the frameless cage.

screwed into the base of each cage. Four types of cage frames were made to provide differing degrees of predator-access to egg capsules (Fig. 1b). Sizes of entry holes were 0.1x0.1 cm (small-hole cage), 0.5x0.5 cm (medium-hole cage), 1.5x1.5 cm (large-hole cage) and 11.0x2.5 cm (open cage). To determine the predation intensity on unprotected capsules, capsules were mounted on cage bases without a protective frame (hereafter referred to as frameless cages).

1. Grappler Inlet

At the Grappler Inlet site cages were placed along established transects (1.2 m and 2.2 m above zero chart datum) in June 1988. Twenty-five cage bases were placed in five groups of five along both high and low transects. Each group of five represented a sampling block. In each block, cage bases were placed approximately 5 cm away from one another in order to minimize physical and biological differences among cages. Different blocks were placed 0.5-1.0 m apart along the transect line depending on where suitable habitats were available.

To determine if different predation rates occurred within and among cage blocks due to a heterogeneous distribution of predators or cage effects, a pilot experiment was performed using frameless cages. This gave an estimate of the natural predation rate on egg capsules in the field. Eight capsules were glued to each plastic saucer, which, in turn, was screwed into each cage base. To prevent capsules from drying, barnacle- and mussel-covered rocks were carefully placed over

each cage base, covering capsules from direct sunlight. Capsules were checked for signs of predation on alternate days (except when tide levels were not low enough). Capsules that disappeared from their saucers during the experiment were assumed to have been lost due to wave-action or currents, unless there was evidence that they had been removed by predators. Lost capsules were replaced with fresh capsules from the laboratory. This experiment was terminated after 19 days, when a sufficent number of capsules had been opened by predators.

Following this pilot experiment, I then used cage frames to exclude certain size-classes of predators. Four frames, one of each type, were randomly assigned to cage bases within each block, and one cage base was left without a frame. Eight capsules were placed within each cage. These were checked every second day for signs of predation. At each check, all intact capsules were replaced with the same number of fresh capsules from the laboratory. Field-collected capsules were examined thoroughly for signs of predation and, if developing embryos were still healthy and capsules still securely glued, these capsules were reused.

2. Ross Islets

At the Ross Islet site, cages were placed along the low intertidal boulder beach (1.9 m above zero chart datum) and higher intertidal rocky outcropping (2.9 - 3.1 m above zero chart datum), during August and September 1988. Because of the uneven substrate along the rocky outcropping, cages were

not placed along a transect. Instead, cage blocks were placed wherever the substratum was relatively flat and Fucus cover abundant. Pilot and predator-exclusion experiments were carried out as described previously for the Grappler Inlet site. As these experiments were conducted over a much shorter period at Ross Islets than at Grappler Inlet, capsules were only replaced if they had dried out. Experiments lasted for 6-8 days.

IV SUSCEPTIBILITY OF CAPSULES TO PREDATORS

Laboratory experiments were conducted to determine if thick- and thin-walled capsules were equally susceptible to predation. Capsules differed substantially among sites in wall thickness (Chapter 1). Thick-walled capsules were found at the Grappler Inlet and Seppings Island sites, while thin-walled capsules were present at Ross Islets. Predatory isopods, Idotea wosnesenskii, were tested to determine if they exhibited preferences for thick- or thin-walled capsules. My field observations and also those by Emlen (1966) indicated that these might be important predators of Nucella emarginata egg capsules.

Idotea ($\bar{X}$ = 2.1 cm in length) were collected from rocks in Grappler Inlet during November 1988. Groups of three Idotea were placed in mesh-panelled cages (8x8x10 cm), which were then partially immersed in trays of fresh seawater. These predators were starved for 24 h and then given five capsules from each of two snail populations (10 capsules in total). Predator preferences were tested for: a) thick vs

thin-walled capsules (Grappler vs. Ross, and Seppings vs Ross), and b) thick- vs thick-walled capsules (Grappler vs Seppings). Capsules were arranged in a circular configuration such that capsules from each population were interspersed. Cages were checked daily and the number of capsules opened was recorded. Experiments were terminated when 4-6 out of 10 capsules had been opened. Five to ten replicate cages were used for each combination of capsule types.

RESULTS

I. LABORATORY EXPERIMENTS

Only three types of invertebrates tested opened Nucella emarginata egg capsules in the laboratory: isopods (Idotea wosnesenskii), shore crabs (Hemigrapsus nudus and Hemigrapsus oregonensis), and chitons (Mopalia spp.) (Table I).

Idotea wosnesenskii regularly preyed upon egg capsules in laboratory experiments. Caged Idotea (1.6 - 3.2 cm in body length; $\bar{X}$ = 2.2 cm) opened a mean of 4.5 Nucella capsules over a five-day period (Fig. 2a). Feeding rates varied among individuals, but not in relation to size or sex. Newly hatched Idotea (5 mm in length) did not prey on egg capsules in the laboratory. Other intertidal isopods (eg., Gnorimosphaeroma oregonense ($\bar{X}$ = 0.9cm) and Cirolana harfordi ($\bar{X}$ = 1.4cm)), nibbled extensively around the capsular plug, but never chewed through capsule walls.

Idotea readily consumed both the contents of egg capsules, as well as the capsule walls. Of 51 capsules opened by Idotea in laboratory tests, 16% were chewed to the stem, 53% were partially chewed and emptied of all eggs, and 31% were partially chewed but still contained developing embryos. Although the isopods often ate large portions of the capsular cases, they did not digest this material extensively. Pieces of capsular wall passed through their guts apparently intact.

The shore crabs Hemigrapsus nudus and Hemigrapsus oregonensis frequently opened capsules in the laboratory. Larger crabs opened considerably more capsules over the five

TABLE I. Summary of the species tested for predatory activity on *Nucella emarginata* egg capsules in the laboratory. Groups of individuals of each species were presented with 8 intact egg capsules for a two-week period, or until all capsules had been opened. Five to ten replicates (with controls) were conducted for each species. Species that were found to open capsules on a consistent basis are indicated with an "X".

PHYLUM	SPECIES TESTED	PREDATORS
NEMERTINEA	*Emplectonema gracile*	
ANNELIDA	*Nereis vexillosa*	
MOLLUSCA	*Mopalia* spp.	X
	Littorina scutulata	
	Onchidella borealis	
	Searlesia dira	
	Tegula funebralis	
	Nucella emarginata	
	juveniles	
	adults	
ARTHROPODA	*Pagurus granosimanus*	
	Pagurus hirsutiusculus	
	Hemigrapsus nudus	X
	Hemigrapsus oregonensis	X
	Idotea wosnesenskii	X
	Gnorimosphaeroma oregonense	
	Cirolana harfordi	
ECHINODERMATA	*Leptasterias hexactis*	
	Pisaster ochraceus	
CHORDATA	*Oligocottus maculosus*	
	Anoplarchus purpurescens	

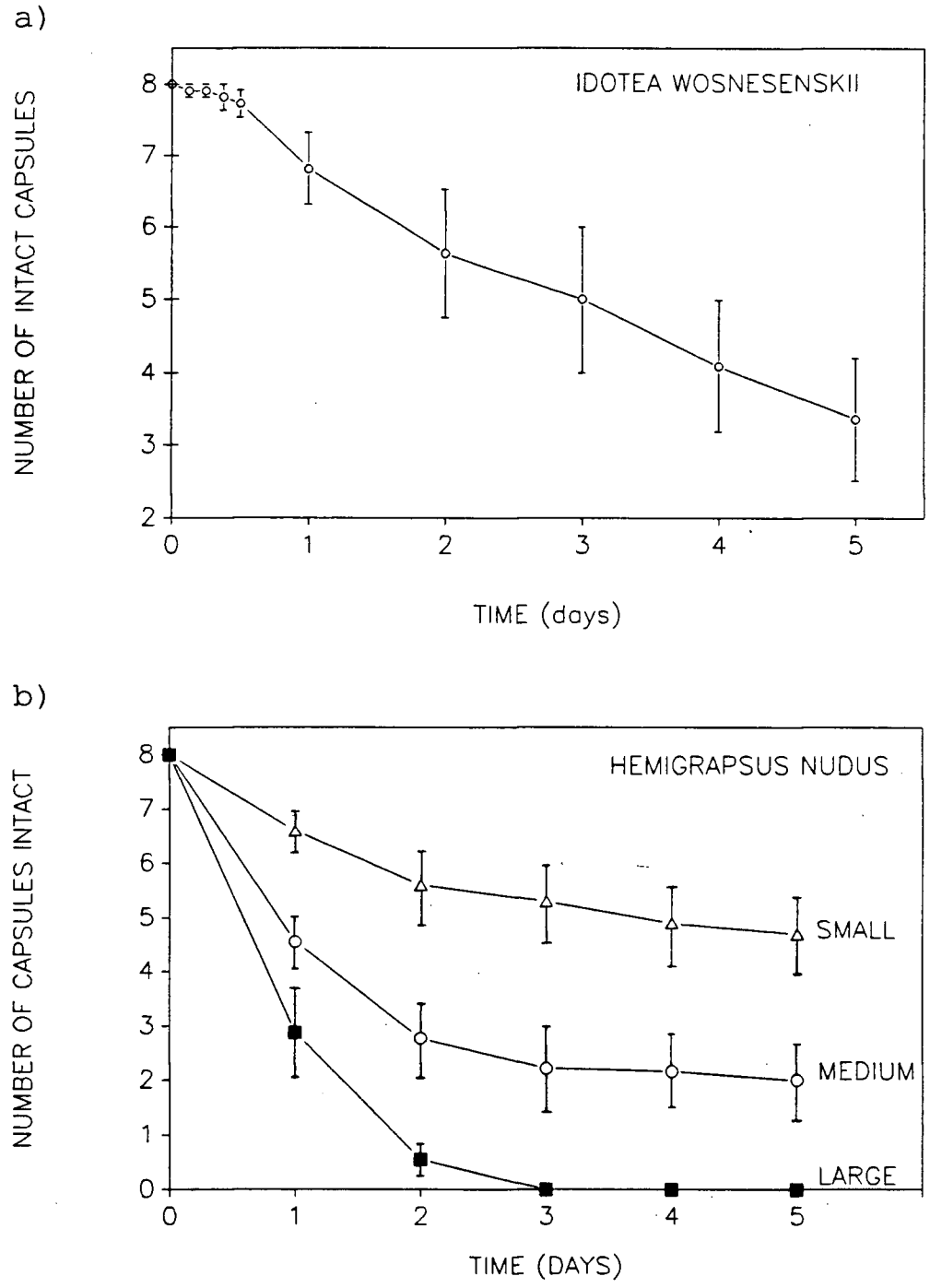


FIGURE 2. Mean number (± 1 S.E.) of Nucella emarginata egg capsules remaining intact after 5 days exposure to: a) isopods, Idotea wosnesenskii (n = 11) and b) three size-classes of Hemigrapsus nudus (small: <1.5 cm carapace width (n=10); medium: 1.5-2.5 cm carapace width (n=18); and large: >2.5 cm carapace width (n=9)). All animals were confined individually with 8 intact egg capsules on Day 0, and the number of egg capsules remaining intact was recorded daily.

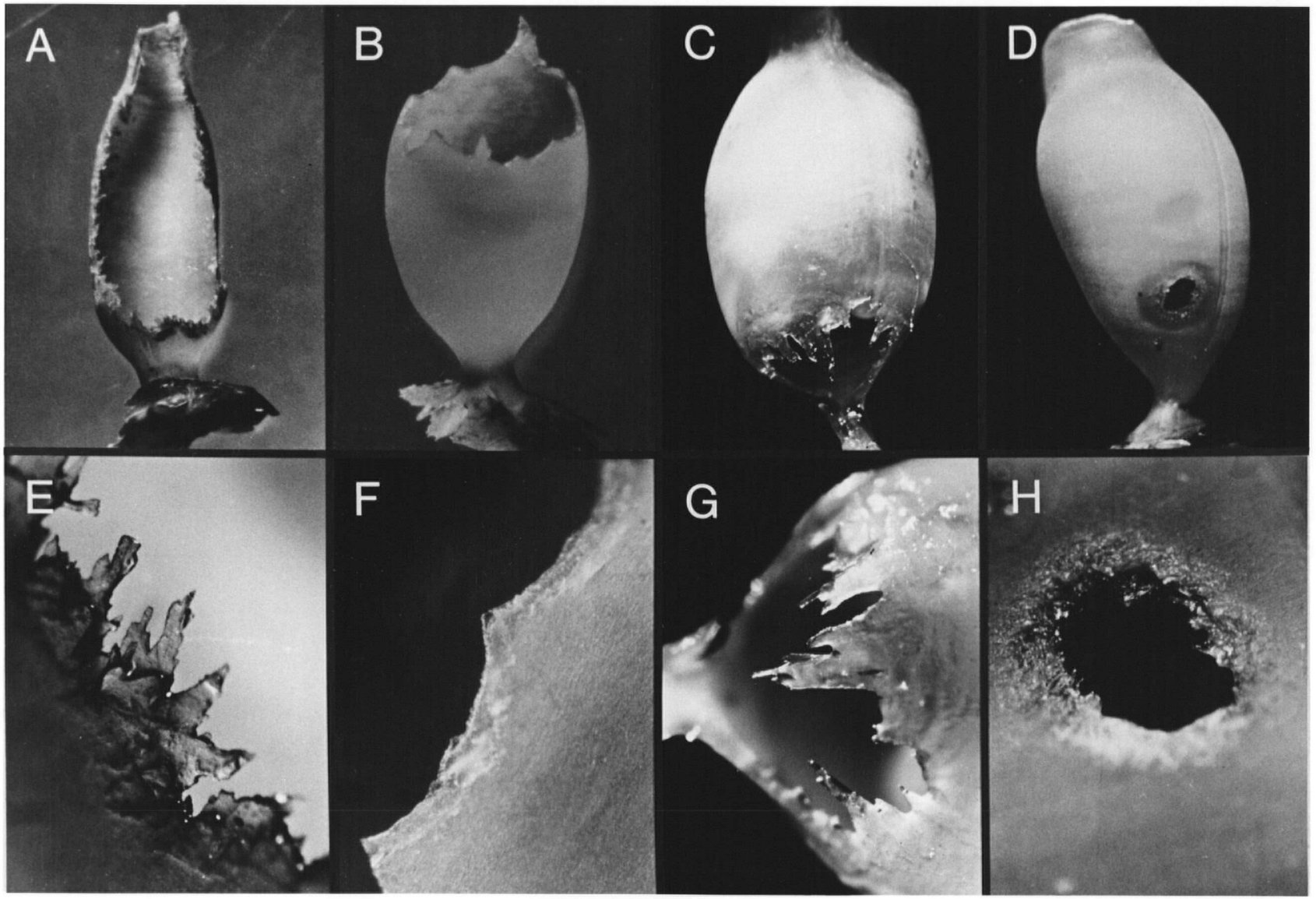
-day period than smaller crabs (Fig. 2b). Hemigrapsus spp. exhibited two methods of opening the capsules. Larger crabs were able to rupture the plug by squeezing the capsule body with their chelae. However, crabs usually chewed through the plug region directly into the capsule chamber. Although Hemigrapsus spp. often consumed large portions of the capsular case in this process, like Idotea, they did not seem to digest this material.

Few Nucella emarginata capsules were opened by Mopalia spp. in the laboratory tests. Over a two week period, 21 chitons only opened 6 of 56 capsules.

Idotea, Hemigrapsus, and Mopalia spp. left very distinctive capsular remains (Fig. 3). Idotea tended to chew through the sides of capsule chambers and left characteristic "bite marks". Hemigrapsus spp, in contrast, usually chewed capsules from the plug downwards and left broad "U-shaped" marks and sometimes small tears in the capsule wall. Capsules squeezed open by crabs showed no evidence of predation other than the absence of a capsular plug. Chitons exhibited the most variable type of predation on capsules. They usually rasped capsules near the base of the capsule chamber, sometimes completely severing the capsule body from the stem. However, they could also open capsules by drilling small holes (approx. $0.03mm^2$) in the capsule wall with their radulae.

In summary, only four of 17 invertebrate species tested in the laboratory were found to open Nucella emarginata egg capsules. Of these, crabs and isopods appeared to be the most potentially dominant predators. As the capsular remains of

FIGURE 3. Characteristics of species-specific predation on Nucella emarginata capsules by: Idotea wosenesenskii (A, E), Hemigrapsus spp. (B, F), Mopalia spp. (C, G), and an unknown predator (D, H). For each type of predator, a whole mount of the opened capsule is shown (Mag: 8X), with a close-up below illustrating the characteristic "bite-marks" (Mag: 25X-50X).


A
B
C
D
E
F
G
H

each predator was distinct, capsules opened by these invertebrates were readily identifiable in the field. Although fish species were not broadly tested as predators of capsules, tidepool sculpins (Oligocottus maculosus) and cockscomb pricklebacks (Anoplarchus purpurescens) did not appear to eat capsules in the laboratory.

II. FIELD CENSUSES OF PREDATION

1. Grappler Inlet

Nucella emarginata and their egg capsules were most abundant in the lower regions of the intertidal channel (Table II), with egg capsules being deposited deep within the dense meshwork of mussel- and barnacle-covered rocks. Three known predators of Nucella capsules, Hemigrapsus oregonensis (1.0 - 2.2 cm in carapace width), Idotea wosnesenskii (2.1 - 2.9 cm in body length) and Mopalia spp. (2 - 6 cm in body length), were present in this region, with Mopalia spp. being the most numerous. Approximately 18% of capsules collected along the lower transect had been opened by predators (Tables II and III). The majority of these capsules appeared to have been eaten by isopods, even though they were scarce at the time of censusing. Only 2 capsules showed evidence of predation by Hemigrapsus spp; however, these animals may have been responsible for a number of torn and chewed capsules found along this transect. Some capsules had been emptied by means of one or two bevelled holes (0.4x0.2mm; Fig.3). The predators of these capsules were probably intertidal gastropods, since they have been reported to make similar

TABLE II. Density ($\bar{X} \pm 1$ S.D.) of Nucella emarginata, their egg capsules, and egg capsule-predators at study sites in Grappler Inlet and Ross Islets. Estimates were based on censuses of 8 quadrats (0.25 m^2) for snails and egg capsules and 9 quadrats for predators along each transect. Two transects, 1.2 m and 2.0 m above zero chart datum, were sampled at Grappler Inlet in May 1988. Three intertidal transects, 1.9, 2.3, and 2.6 m above zero chart datum, were sampled at Ross Islets in June 1988, and a fourth area, 2.8-3.1 m above zero chart datum, was sampled in August 1988. The percentage of capsules opened by predators was determined by dividing the number of torn and chewed capsules by the total number of egg capsules collected along each transect.

		Density/m^2 of *Nucella emarginata*	Density/m^2 of Egg Capsules	%Capsules Opened By Predators	Density/m^2 of *Hemigrapsus* spp.	Density/m^2 of *Idotea wosnesenskii*	Density/m^2 of *Mopalia* spp.
GRAPPLER	LOW (1.2 m)	29.6 ± 26.0	126.0 ± 144.0	18%	4.9 ± 6.3	0.4 ± 1.3	9.3 ± 6.6
	HIGH (2.0 m)	14.0 ± 18.0	9.6 ± 16.0	32%	10.2 ± 10.0	8.0 ± 11.8	0
ROSS ISLETS	LOW (1.9 m)	26.0 ± 28.4	24.8 ± 51.2	24%	360.8 ± 96.6	0	0
	MID (2.3 m)	6.4 ± 13.6	70.0 ± 91.6	22%	0	0	0
	HIGH (2.6 m)	289.6 ± 295.6	5.2 ± 14.0	0%	0	0	0
	ROCKY OUTCROPPING (2.8-3.1 m)	203.3 ± 81.3	744.6 ± 46.3	22%	24.0 + 17.2	0	0

			CAPSULE CHAMBER INTACT		CAPSULE CHAMBER OPENED		CATEGORIZATION OF CAPSULAR REMAINS				
		Total # of Capsules	Contained Ova	Contained Embryos	Hatched Naturally	Opened by Predators	Hemigrapsus Predation	Idotea Predation	Mopalia Predation	Bevelled Holes	Misc. Predation
GRAPPLER	LOW (1.2 m)	255	88(35)	55(22)	55(22)	47(18)	2(4)	20(43)	1(2)	8(17)	16(34)
	HIGH (2.0 m)	19	13(68)	-	-	6(32)	-	6(100)	-	-	-
	LOW INTERTIDAL BOULDERS (1.2 m)	167	43(26)	75(45)	2(1)	43(26)	-	36(84)	-	1(2)	6(14)
		140	26(19)	86(61)	-	24(17)	-	22(92)	-	-	2(8)
		187	52(28)	83(44)	-	20(11)	2(10)	14(70)	-	-	4(20)
ROSS ISLETS	LOW (1.8 m)	37	17(46)	2(5)	9(24)	9(24)	9(100)	-	-	-	-
	MID (2.3 m)	139	27(19)	18(13)	63(45)	31(22)	24(77)	-	-	-	7(23)
	HIGH (2.6 m)	10	10(100)	-	-	-	-	-	-	-	-
	ROCKY OUTCROPPING (2.8-3.1 m)	1117	2(0)	75(7)	779(70)	242(22)	177(73)	-	-	1(0)	64(26)

drill holes in other gastropod egg capsules (Abe, 1983).

The density of snails and egg capsules was low along the high transect at Grappler Inlet (Table II). In contrast, egg capsule predators (eg. Hemigrapsus and Idotea) were notably more abundant than in the lower parts of the intertidal channel. The percentage of capsules opened in this region was high, with all chewed capsules showing evidence of predation by Idotea (Table III).

Table III also shows the extent of predation on capsules collected from three boulders along the lower intertidal transect. Idotea were often found amongst torn egg capsules and appeared to be responsible for the majority of predation on these capsules. The percentage of capsules opened by predators ranged from 17 - 26 % on two of the boulders, although they were less than 0.5 m apart. On the third boulder, approximately 10 m away, only 11% of capsules had been opened.

2. Ross Islets

Densities of snails and egg capsules varied markedly among transects at the Ross Islets site (Table II). All capsules at this site were attached to vertical surfaces or overhangs. The extent of predation on capsules decreased with increased tidal height, and corresponded well with the density of Hemigrapsus nudus (0.6 - 2.4 cm in carapace width), the only known predators of Nucella egg capsules at this site. Although crabs were not present along the higher transects when censuses were taken, on subsequent visits crabs were

occasionally found in small crevices along the 2.3 and 2.6 m transects. The majority of opened egg capsules appeared to have been eaten by Hemigrapsus spp. (Table III). Only 3 capsules had bite marks similar to those associated with Idotea, and only one of these isopods was ever found in the study area.

Censuses of the high intertidal outcropping at Ross Islets in August 1988 also indicated that Hemigrapsus spp. were important predators. Over 22% of these capsules appeared to have been opened by predators, with the majority showing evidence of attack by Hemigrapsus.

3. Seppings Island

Egg capsules from Seppings Island showed evidence of bite-marks by both Idotea and Hemigrapsus. Despite the greater levels of wave action at this site, Idotea wosnesenskii and Hemigrapsus nudus were abundant, especially within the thick beds of Mytilus californianus. Capsules were also found with bevelled holes identical to those collected from Grappler Inlet (Fig. 3).

In summary, predators were found to have opened up to 32% and 24% of egg capsules present at the Grappler Inlet and Ross Islets sites, respectively. Observations of capsular remains indicated that the intertidal isopods, (Idotea wosnesenskii), crabs (Hemigrapsus oregonensis), and unknown gastropods were responsible for the majority of predation at Grappler Inlet, whereas crabs, (Hemigrapsus nudus), were the only numerous predators at the Ross Islets site.

III. FIELD EXPERIMENTS

1. Grappler Inlet

a) Pilot experiments

Capsules were left within frameless cages for a period of 19 days. Over this period a mean of 2.0 $\pm$ 0.3 ($\bar{X}$ $\pm$ 1S.E., n=25 cages) and 1.8 $\pm$ 0.3 capsules (n=25 cages) were opened in the low and high intertidal cages, respectively (out of a possible 8 capsules/cage). The number of capsules opened by predators did not differ significantly with cage position within or among blocks along each transect (low transect: ANOVA; $F_{WITHIN} = 0.73$, $p>0.25$; $F_{AMONG} = 1.53$, $p>0.10$; high transect: ANOVA; $F_{WITHIN} = 1.34$, $p>0.25$; $F_{AMONG} = 2.17$, $p>0.10$). Hence, predation levels were relatively constant at each tidal level.

A mean of 6.1 capsules were "lost" in total from all cages (n=50) over a two day period. Although the cause of lost capsules was unknown, I suspected that capsules became detached due to a failure of the glue.

Table IV shows the type of capsular remains found in the frameless cages after 19 days. Along the low intertidal transect shore crabs (Hemigrapsus oregonensis) appeared to be responsible for the majority of predation; however, in some cases the predators of egg capsules could not be identified. Along the higher intertidal transect, isopods (Idotea) were the chief predators and were frequently found eating capsules. Fewer capsules were opened by Hemigrapsus oregonensis or drilled by other predators in this region.

TABLE IV. Summary of Nucella emarginata egg capsules opened by predators in the pilot experiment in Grappler Inlet, June 1988. Capsules were classified by the bite and chew marks left on the capsular chamber.

	CHARACTERISTICS OF CAPSULAR REMAINS					
	Hemigrapsus Chew Marks	Idotea Chew Marks	Bevelled Holes	Mopalia rasping	Misc. Predation	Total # Eaten
LOW TRANSECT (1.2 m)	17	-	14	1	19	51
HIGH TRANSECT (2.0 m)	4	22	2	-	16	44

b) Predator-exclusion experiments

Predator-exclusion experiments revealed that the major predators of capsules at the Grappler site were larger than the 2.25 cm^2 entry holes of the large-hole cages (Fig. 4a,b). The number of capsules opened after 20 days varied significantly among cage types (Kruskal-Wallis One-Way ANOVA: low transect, $K=10.41$, $P<0.05$; high transect, $K=8.37$, $P<0.05$) with capsules in the open and frameless cages experiencing the most predation.

During this experiment, crabs (Pagurus granosimanus, Pagurus hirsuitisculus, Hemigrapsus oregonensis), isopods (Idotea wosnesenskii) and whelks (Nucella lamellosa, Nucella emarginata) were often found within the open and frameless cages. Other intertidal snails (Searlesia dira and Littorina spp.) regularly entered cages with entry holes as small as 2.25 cm^2. Of these organisms, Hemigrapsus and Idotea were the only animals that opened Nucella emarginata capsules in the laboratory and, in fact, the majority of opened capsules appeared to have been chewed by Hemigrapsus. As no capsules were opened in the small-hole cages, smaller organisms often found in these cages such as nemerteans, nematodes, and polychaetes were probably not important predators.

2. Ross Islets

a) Pilot experiments

Capsules were placed in frameless cages at Ross Islets for a period of 6 days. During this period the intensity of predation on capsules was severe compared with the Grappler

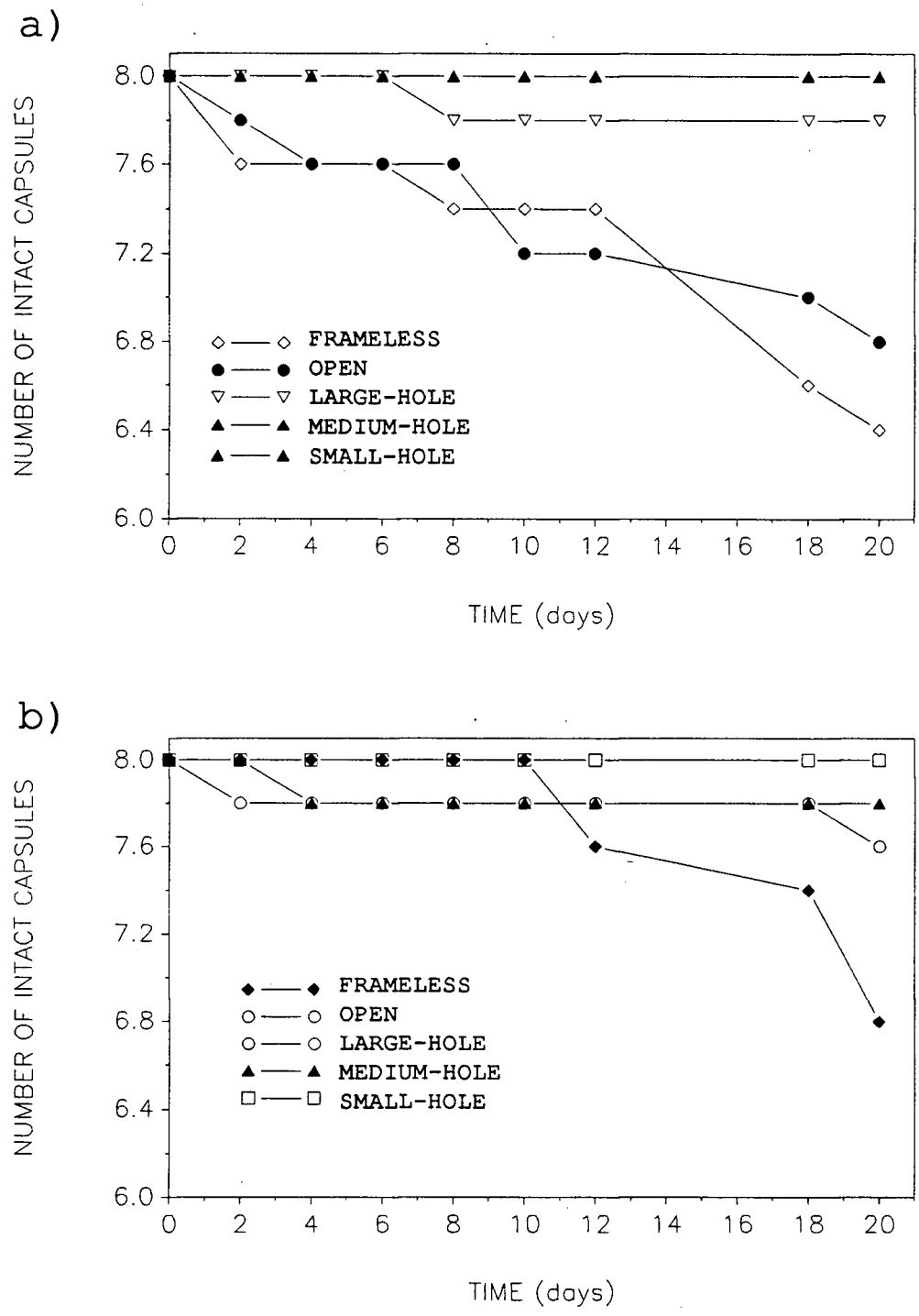


FIGURE 4. Results of the predator-exclusion experiments in Grappler Inlet, July 1988 for: a) the low intertidal transect (1.2 m above zero chart datum) and b) the high transect (2.0 m above zero chart datum). Each point represents the mean number of capsules remaining intact in five replicate cages (ie. one cage from each block) for each cage type .

Inlet site. A mean of 7.7 $\pm$ 0.3 ($\bar{X} \pm 1$ S.E., n=25 cages) and 4.8 $\pm$ 0.5 (n=25 cages) capsules were opened in the low and high intertidal cages, respectively. The difference in predation rate between these tidal heights corresponded well with differences in the abundance of shore crabs (Table II).

Predators of approximately 80% of the low intertidal capsules and 50% of the high intertidal capsules could not be determined. Such capsules had either been completely chewed, leaving only a stem, or totally removed from cage bases. Missing capsules were assumed to have been removed by predators, since: 1) remains of chewed capsules were occasionally found nearby and, 2) in saucers where capsules were missing, remaining capsules had identifiable bite marks indicating that predators had been active. As large Hemigrapsus were the only predators able to remove glued capsules from rocks in the laboratory, these crabs were believed to be responsible for most capsule losses in the field. Of the remaining chewed capsules, 30 of 32 low intertidal capsules showed evidence of Hemigrapsus bite marks, while 53 of 63 high intertidal capsules appeared to have been attacked by these crabs.

The number of capsules eaten by predators did not differ with respect to the position of cages within or among blocks along the low intertidal transect. After 6 days, all capsules had been opened, except for 7 capsules remaining in one cage. Hence, predation levels were relatively constant in this region. Along the high intertidal region a few capsules still remained in some cages after this period. Although the number

of capsules opened by predators did not differ significantly among cage positions within blocks (ANOVA: $F_{WITHIN}=0.74$, $p>0.25$), the number of capsules eaten did differ among blocks (ANOVA; $F_{AMONG}=3.25$, $p<0.05$). Hence, predation was patchy along the high intertidal area. This may have been because cage blocks were not placed along a transect. Sites were originally selected for habitat cover, which may have inadvertently limited the accessibility of certain cage blocks to predators.

b) Predator-exclusion experiments

The results of the predator-exclusion experiments confirmed that crabs were the most important predators at the Ross Islet study site (Fig. 5a,b). The number of capsules opened differed significantly among cage types (Kruskal-Wallis test: low transect, $H=22.55$, $P<0.05$; high transect, $H=17.23$; $P<0.05$), with most capsules being eaten in open and frameless cages. Crabs were often found within the large-hole, open and frameless cages, and capsular remains in these cages were indicative of predation by Hemigrapsus. Other invertebrates found regularly in cages were amphipods, present in all except the small-hole cages, and snails (Searlesia dira), found only in the large-hole, open and frameless cages.

Capsules were also removed by predators in this experiment. Forty-five and 57% of opened capsules disappeared along the low and high tidal cages, respectively. No capsules were removed in cages inaccessible to Hemigrapsus.

In summary, the predator-exclusion experiments indicated

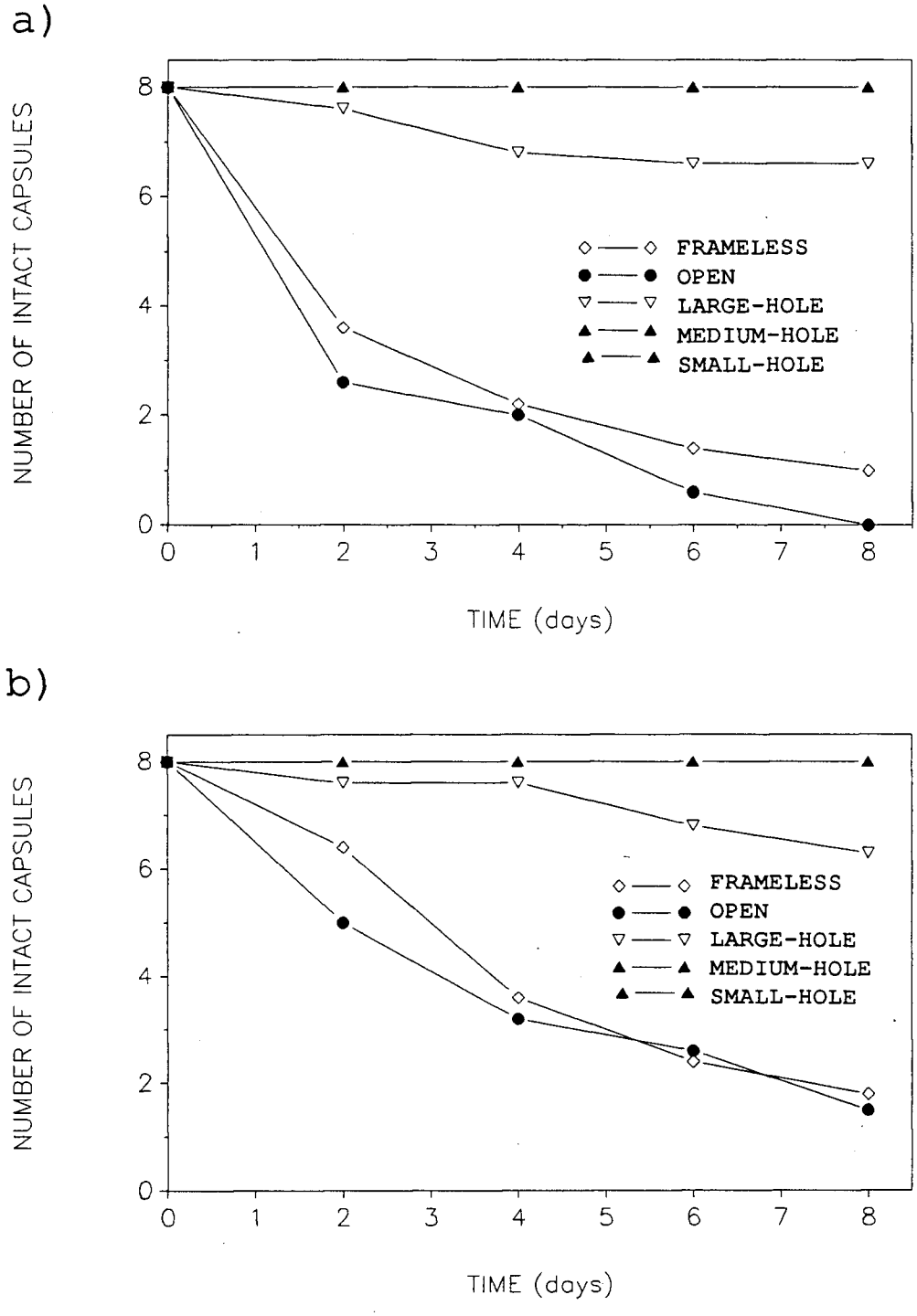


FIGURE 5. Results of the predator-exclusion experiments in Ross Islets, September 1988 for: a) the low intertidal region (1.8 m above zero chart datum) and b) the high intertidal region (2.8-3.1 m above zero chart datum). Each data point represents the mean number of capsules still intact in five replicate cages (ie. one cage frome each block) for each cage type.

that relatively large organisms, such as Idotea and Hemigrapsus spp., were important predators of capsules in the field. In contrast, small organisms that could enter the cages with entry holes smaller than 0.25 cm^2 did not often prey on encapsulated eggs. Caging experiments also indicated that predation on egg capsules was more intense at the Ross Islets compared with Grappler Inlet (Figs. 4, 5). These results were contrary to results of the field censuses, which indicated similar percentages of capsules opened by predators at the two sites.

IV. SUSCEPTIBILITY OF CAPSULES TO PREDATORS

Idotea opened thin-walled capsules from Ross Islets more frequently than thick-walled capsules from either Grappler Inlet or Seppings Island (Fig. 6). In ten trials comparing the relative susceptibility of Ross Islet or Grappler capsules to these isopods, 35 Ross Islet capsules were opened compared with 15 Grappler capsules (Fisher's Exact Test, P=0.0001). Comparisons between the number of Seppings versus Ross Islet capsules opened by Idotea showed a similar trend, with more Ross Islet capsules being opened (16 Ross Islet capsules versus 10 Seppings capsules; Fisher's Test, P=0.08). In contrast, isopods did not exhibit any preferences for Seppings versus Grappler capsules (10 Grappler versus 9 Seppings capsules; Fisher's Test, P=0.50). In 2 of 6 trials, none of these thick-walled capsules were eaten during a ten day period. Hence, these results were consistent with the prediction that thick-walled capsules are more resistant to predation than thin-walled capsules.

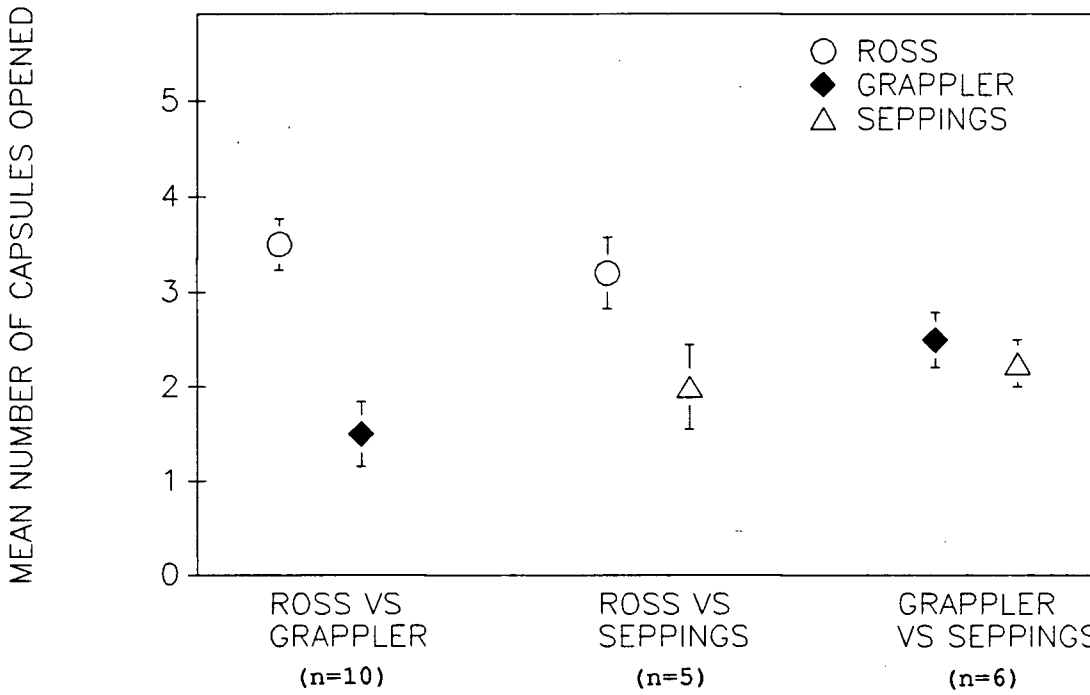


FIGURE 6. Mean number of the first five capsules opened by Idotea wosnesenskii when given a choice of feeding on capsules from two different study sites. Predators were placed in cages with 10 capsules (5 from each site) and the first five capsules to be opened were recorded. Capsules from Grappler and Seppings Island had walls 20 - 25 % thicker than ones from Ross Islets.

DISCUSSION.

Prosobranch egg capsules have been suggested to protect developing embryos from predation in the benthic marine environment (Pechenik, 1979; Perron, 1981). However, few tests have been made of this assumption. If capsules do protect embryos from predation, then one would expect to find direct evidence of this, in that: 1) capsule walls should physically limit access by predators to developing embryos and, 2) these structures should not serve as an energy source that might attract predators. Hence, there should be little evidence of predation on egg capsules in their natural habitat. Furthermore, if predation is an important selective pressure acting upon the structure of capsule walls, intra- and interspecific differences in capsule wall thickness may actually reflect differences in abundance of intertidal predators.

Resistance of intact capsules to predators

In this study, egg capsules of Nucella emarginata were found to be resistant to some intertidal predators. Nemerteans (Emplectonema gracile) are capable of eating Nucella emarginata embryos contained within ruptured capsules (Glynn, 1965), but these did not open intact Nucella egg capsules in either laboratory or field experiments. Hermit crabs (Pagurus spp.) and starfish (Pisaster ochraceus and Leptasterias hexactis) are also known to feed on juvenile

Nucella spp. (Spight, 1976; Rivest, 1983). However, these predators did not prey upon encapsulated embryos in this or other studies (Spight, 1977). Capsular cases therefore do protect developing embryos from some intertidal predators.

Encapsulated Nucella emarginata embryos were not safe from all intertidal predators. Shore crabs, Hemigrapsus spp., and isopods, Idotea wosnesenskii opened capsules in both my laboratory and field tests (see also Emlen, 1966; Spight, 1977). Capsules were also opened by chitons, Mopalia spp., and an unknown predator, which made small bevelled holes in capsule walls. In studies by Emlen (1966), Feare, (1970), and Spight (1977), egg capsules of Nucella spp. were also eaten by crabs (Cancer oregonensis), sea urchins (Strongylocentrotus droebachiensis), polychaetes, (Eulalia viridis), sculpins (Enophrys bison) and snails, (Nucella emarginata). Although capsule walls are obviously not resistant to these organisms, such results do not necessarily refute the idea that capsules have evolved to protect embryos from predation. Obviously, if the structure and strength of capsules represent a selective response to specific predatory species, then the resistance of capsule walls should depend on the frequency that predators encounter these capsules. Hence, the protective nature of capsules can only be interpreted by examining the resistance of capsule walls to animals that regularly encounter these capsules in the field.

Digestibility and palatability of egg capsule walls

As noted, if capsular cases have evolved to protect embryos from predation, then these structures might not only be impervious to predators, but also indigestible or unpalatable. Capsular cases can contain up to 47% of the total energy invested in reproductive products in some prosobranchs (Perron, 1981), and thus, represent an energy source almost equivalent to that of the encapsulated eggs. In my study, although Idotea and Hemigrapsus spp. readily ate large portions of the capsule walls, this material did not appear to be digested. Similar results have been reported by Brenchley (1982) for the predators, Littorina littorea. Other predators have been shown to feed exclusively on the contents of egg capsules, rather than on the capsule walls (MacKenzie, 1961; Abe, 1983).

Some predators have been found to digest capsule walls. Brenchley (1982) found that crabs, Carcinus maenas and Pagurus longicarpus, can digest capsular cases of the snail Ilyanassa obsoleta. Capsules of this gastropod species are typically thin-walled (<10 μm, Sullivan and Maugel, 1984) and contain embryos with short developmental periods compared with the thick-walled capsules (60-120 μm, Chapter 1) and long embryonic development times of Nucella emarginata. Hence, as Perron (1981) found for Conus spp., the selective pressures acting upon the structure of gastropod egg capsules may differ depending on embryonic developmental time. Therefore, while the majority of results would appear to support the prediction

that capsular cases are not utilized as a source of energy, generalizations about the protective quality of egg capsules among all gastropod species may be unjustified. Clearly the exposure time of embryos to predatory species must also play a large role in selection for specific types of capsule structures.

Predation on egg capsules in the field

The notion that all egg capsules are effective at protecting developing embryos has also been challenged in studies showing extensive predation on egg capsules in the field. For example, Brenchley (1982) found that 52% of the capsules of the mud snail, Ilyanassa obsoleta, were opened by crabs or snails during 10 days of a development period lasting up to 3 weeks. Spight (1977) noted that predators opened 77% of Nucella lamellosa capsules in some spawning aggregations. Other studies, such as those by MacKenzie (1961), Haydock (1964), and Emlen (1966) have also documented high levels of predation on gastropod egg capsules. These findings thus are contrary to the prediction that predation on egg capsules in the field should be minimal.

In my study, field estimates of predation on Nucella emarginata egg capsules were comparatively low. For example, one-time censuses of predation revealed that 18-32% of capsules collected at the wave-sheltered Grappler Inlet site had been opened by predators, whereas 0-24% of capsules along transects at Ross Islets had been opened. These results

resembled those of Spight (1972), who found that 22-25% of Nucella emarginata capsules were opened during their 80 day embryonic developmental period. The extent of predation on egg capsules in the present study was not as severe as expected given that: 1) laboratory-identified predators Idotea wosnesenskii, and Hemigrapsus spp. were abundant and, 2) field observations indicated that these predators and others did eat capsules in the field. Nevertheless, both laboratory and field results indicated that capsules actually offered little structural resistance to these isopod and crab predators.

The positioning of capsules in the field by Nucella emarginata may help to protect embryos from predators. This was evident at the Ross Islets, where snails always laid capsules on vertical surfaces and overhangs, rather than on horizontal surfaces. Predation on these capsules ranged from 0 - 24%. In caging experiments where capsules were glued onto horizontal surfaces, predation was much more intense. As crabs were abundant at this site, capsules positioned on vertical surfaces were likely to be less accessible to predatory crabs than capsules glued to horizontal surfaces. In support of this, few crabs were seen along the 2.3 and 2.6 m transects at Ross Islets where the granite wall sloped steeply; however, crabs were abundant along a horizontal boulder beach and a high rocky outcropping (1.8 and 2.8-3.1 m above zero chart datum, respectively) in the same area. At Grappler Inlet, the placement of capsules appeared to be less critical. Both field censuses and caging experiments

indicated that the rate of predation was slow, perhaps because the density of crabs was generally lower at this site.

Susceptibility of thick- and thin-walled capsules to predators

Thick capsules may be more difficult to open or require longer handling times by predators than thinner capsules. Hence, thick-walled capsules might be selected for in areas where predators are abundant. The wall thickness of Nucella emarginata capsules has been shown to vary intraspecifically (Chapter 1), with thick-walled capsules being laid at Grappler Inlet and Seppings Island, and thin-walled capsules being laid at Ross Islets. Since the density of crabs was highest at the Ross Islets where capsules were thin-walled, differences in wall thicknesses did not correspond to the density of these predators. In contrast Idotea wosenesenskii were only found at the Grappler and Seppings sites where capsules were thick-walled.

Idotea opened thin-walled capsules more readily than thick-walled capsules in my laboratory experiments. However, thick-walled capsules were not totally resistant to predation, as all capsules regardless of thickness were eventually eaten. Nevertheless, these results suggest that thicker capsule walls may protect developing embryos better than thin capsule walls against certain predators. Although thick-walled capsules may have been selected for through the action of any number of environmental stresses (Chapter 1), the presence of predatory isopods would obviously be expected to reinforce selection for

such structures.

Despite some encouraging results from this study, it was not possible to assess the importance of predation precisely in the evolution of capsular structures. Although predation on egg capsules can be intense (Spight, 1977; Brenchley, 1982), it is not always severe. This raises a number of questions. If the intensity of predation on egg capsules is low in an area, is this because capsular structures have been selected to protect embryos from predators, or are capsules simply laid in areas where the abundance of predators is limited? Alternatively, if predation on egg capsules is intense, is this because snails are unable to produce capsules resistant to predation, or have snails responded to different selective pressures? The key to such questions may lie in a better understanding of how the intensity of predation on egg capsules relates to the developmental time of encapsulated embryos. Predation should not be expected to exert the same selective pressure on species with short encapsulated developmental times as it does on species with long developmental times (cf. Perron, 1981).

It was apparent from this study that Nucella emarginata embryos were not resistant to all marine predators. Crabs and isopods were important predators of these capsules in the field; however, embryos were somewhat protected from these by being deposited in thicker capsular structures and by being laid in areas inaccessible to predators. Attempts to generalize about the protective role of all gastropod egg

capsules may prove to be extremely difficult. Conflicting results in the literature suggest that the protective role of prosobranch egg capsules differs among species, and elucidation of any general trends among gastropod species may be impossible.

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GENERAL CONCLUSIONS

The two major objectives of this study were: 1) to examine intraspecific variation in morphology of egg capsules of the marine intertidal whelk Nucella emarginata and, 2) to examine the resistance of these structures to intertidal predators.

I showed in Chapter 1 that the size, strength, and wall thickness of Nucella emarginata capsules varied among populations separated along a wave-exposure gradient. Capsules were thick-walled at wave-sheltered Grappler Inlet and wave-exposed Seppings Island, and were significantly thinner at Ross Islets, an area exposed to intermediate levels of wave-action. Capsule wall strength did not directly reflect differences in wall thickness, as capsules were strongest at Grappler Inlet, and were relatively weak at both at Seppings Island and Ross Islet sites. As differences in capsular morphology did not correspond to wave-exposure levels, I have suggested that the structure and strength of capsule walls may reflect a number of different environmental stresses acting upon developing embryos. Further studies will be needed to examine the physical resistance of thick and thin capsule walls to such stresses.

In the second chapter I showed that predation may be an important source of mortality among Nucella emarginata embryos. Capsule walls provided little resistance to a number of intertidal predators such as shore crabs, Hemigrapsus spp, isopods, Idotea wosnesenskii, and chitons, Mopalia spp..

However, I did find some evidence to suggest that differences in capsule wall thickness may affect the susceptibility of encapsulated embryos to predators. With respect to the isopod predators, Idotea wosnesenskii, embryos contained within thick capsule walls were found to be less preferred prey than those contained within thin-walled capsules. This is the first study to show a selective advantage for the production of thick-walled capsules in gastropods.

The results of this study provide the groundwork for further investigations of the functional significance of neogastropod egg capsules. It is apparent that subtle differences occur in the structural properties of egg capsules even among populations of one species. Associations between intraspecific variation in capsule morphology and specific environmental stresses should ultimately enhance our understanding of the protective role of prosobranch egg capsules.