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ABILITY OF SOME GASTROPOD EGG CAPSULES TO PROTECT AGAINST LOW-SALINITY STRESS

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Abstract: The ability of the egg capsules of three intertidal gastropod species to protect embryos against low-salinity stress was examined. Encapsulated embryos of *Ilyanassa obsoleta* (Say), *Nucella lamellosa* (Gmelin), and *N. lima* (Gmelin) were far more tolerant of transfer to water of reduced salinity than were embryos which had been prematurely removed from egg capsules and transferred to low-salinity sea water directly. However, the walls of *N. lamellosa* and *N. lima* capsules were found to be permeable to salts and at least to small carbohydrate molecules (glucose). Correspondingly, indirect evidence for all three species and direct evidence for *N. lamellosa* indicates that the osmotic concentration of intracapsular fluid declines to near ambient after transfer of egg capsules to dilute medium. Experiments conducted using embryos of *N. lamellosa* suggest that the egg capsules may protect embryos by reducing the rate at which the osmotic concentration of intracapsular fluid decreases rather than by reducing the magnitude of the decrease.

INTRODUCTION

Many marine gastropod species enclose their fertilized eggs within structurally and chemically complex encapsulating structures (Hunt, 1966; Bayne, 1968; Tamarin & Carriker, 1968; Flower *et al.*, 1969) which they affix to firm substrata and then abandon. The adaptive significance of encapsulation is uncertain (Pechenik, 1979; Caswell, 1981), although encapsulating structures are generally assumed to be protective (e.g., Carriker, 1955; Hunt, 1971; Mileikovsky, 1971; Perron, 1981). Intertidal prosobranch gastropods deposit capsules on a variety of substrata, including those subjected to high temperatures, desiccation, and low salinity during rainstorms (Carriker, 1955; Spight, 1977; Pechenik, 1978; Gallardo, 1979; Barnett *et al.*, 1980). To the extent that intertidal egg capsules fail to protect against environmental stresses, encapsulation represents a substantial cost to a species; a high proportion of reproductive calories are devoted to production of the capsules rather than to the production of offspring directly (Vance, 1973; Perron, 1981).

Surprisingly, the tolerances and requirements of encapsulated embryos have not been extensively studied. Some observations have been made on embryonic tolerances of a few species to desiccation stress and/or low-salinity stress (Feare, 1970; Fish & Fish, 1977; Spight, 1977; Pechenik, 1978), but the protective role of the egg capsule has generally not been evaluated. In fact, little is known about the physical and mechanical properties of the capsules, although it appears that such properties vary significantly among species (Perron, 1981; Daniel & Pechenik, in prep.). Detailed experimental

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work correlating capsule properties with embryonic tolerances to specific levels of environmental stress is lacking. This paper reports on the effectiveness of the egg capsules of three species of intertidal prosobranch gastropod as buffers against low salinity stress, and considers the mechanisms through which protection of developing embryos may be achieved. As defined by Giese & Pearse (1974), all developmental stages are considered to be "embryos" until they have left their capsules.

Materials and Methods

Source of capsules and embryos

Egg capsules of *Nucella lamellosa* (Gmelin) and *N. lima* (Gmelin) were deposited by snails held in sea tables at the Friday Harbor Laboratory, University of Washington, during May–June 1980. Adults of *N. lamellosa* had been collected near the laboratory. Adults of *N. lima* were obtained from Alaska and kindly made available to me by Dr. B. Rivest. Capsules of the mud snail, *Ilyanassa obsoleta* (Say), were deposited in the laboratory by adults collected from Woods Hole, Massachusetts. Capsules containing shelled, veliger-stage embryos were used in all experiments for all three species. Individuals of *I. obsoleta* emerge from egg capsules at uniform size as veliger larvae (Scheltema, 1965), whereas embryos of *Nucella lamellosa* and *N. lima* resorb the velum before leaving the egg capsule and emerge as juvenile snails (Spight, 1977). Mean shell lengths ($\pm$ SD) of individuals used in experiments were 272 ± 2.4 μm ($N = 35$) for *Ilyanassa obsoleta*, 967.8 ± 65 μm ($N = 198$) for *Nucella lamellosa*, and 1177 ± 83 μm ($N = 35$) for *N. lima*.

Resistance to osmotic stress

Sea-water dilutions were made using double-distilled (in glass) water and sea water freshly filtered through Whatman filter paper. The salinity of full strength sea water used in control experiments was $\approx 30‰$ as determined with a refractometer. Salinities of 2 to 5‰ were used to examine the ability of capsules to protect against low-salinity stress. Comparable salinities have been recorded in intertidal areas during heavy rains (Gibbs, 1968; Pechenik, unpubl.). Determinations of the osmotic concentration of all solutions used in experiments were made using a freezing-point depression osmometer (Advanced Instruments, Inc.) with a precision of ± 1 mOsm.

To test embryonic tolerances to low salinity and to examine the protective value of encapsulation, some capsules were drawn at random from a large pool of capsules and were distributed among the experimental treatments. Three to five dishes of capsules were used per treatment with three to five egg capsules per dish. Other capsules from the same pool of capsules were carefully cut open with a razor blade to remove the developing embryos. Some of these "excapsulated" embryos were then transferred to dishes of full-strength filtered sea water (controls) and the rest were distributed among

dishes containing sea-water dilutions. Each dish received 8 to 15 excapsulated embryos of *N. lamellosa* and *N. lima*, and 12 to 15 embryos of *Ilyanassa obsoleta*. Two to four replicates were run per treatment. Exposures were made at the sea-water temperatures at which capsules had been deposited: 10 to 12 °C for *Nucella lamellosa* and *N. lima*, and 18 to 20 °C for *Ilyanassa obsoleta*.

All individuals and egg capsules were returned to full-strength sea water after predetermined exposure periods. Stressed, excapsulated embryos showed no movement immediately following exposures to low salinity lasting 3 h or more. However, at least some individuals recovered strikingly within 48 h following their return to full strength sea water. The recovery period was therefore standardized at 48 h for all experiments. The water in each dish was changed twice during this interval. The effects of osmotic stress were then evaluated by inspecting embryos at a magnification of 25 × under a dissecting microscope. In several experiments with the *Nucella* species, the number of excapsulated embryos that had acquired purple coloration was also noted, since this characteristic has been reported to indicate mortality of muricid embryos (Spight, 1975; Gallardo, 1979). In one experiment, the subsequent fate of such tinted individuals of *N. lamellosa* was followed for an additional 4 days. Survival of embryos exposed to test conditions while still encapsulated was also assessed 48 h after the return of capsules to full-strength sea water. In addition, the effect of reduced salinity on time subsequently required for escape of veliger larvae from the capsules of *Ilyanassa obsoleta* was monitored.

An additional experiment was conducted, using excapsulated embryos of *Nucella lamellosa*, to distinguish between the consequences of abrupt versus gradual salinity changes upon subsequent embryonic survival. Some excapsulated embryos were subjected to an abrupt shift from full strength sea water to ≈5‰. The salinity of the medium surrounding other embryos drawn from the same pool of embryos was also decreased to 5‰, but gradually, over 1.5 h. Both groups of embryos were held at the final low salinity for 8 h, after which time the salinity was increased to that of full strength sea water. This was done abruptly for the first group of embryos, and gradually (over 1.5 h) for the second group. Three replicates were conducted for each of the two treatments plus control, with 20 embryos in each dish.

CAPSULE WALL PERMEABILITY

Capsule wall permeability was assessed for capsules of *N. lamellosa* and *N. lima* using diffusion chambers constructed from polystyrene disposable spectrophotometer cuvettes. Holes of 1.32 mm mean diameter (SD = 0.08 mm, $N = 8$) were drilled 4 mm above the bottom of each cuvette, in the midline. A 2 to 2.5 mm^2 piece of egg capsule wall was cut from an egg capsule, adhering debris was removed with a fine paint brush, and the piece of capsule was then placed over the hole in one of the cuvettes. A second cuvette was then placed atop the first, such that the openings of the two cuvettes were superimposed, with the egg capsule wall material sandwiched in between. The cuvettes

were fastened together in the upright position. Four ml of distilled water were pipetted into one cuvette, and 4 ml of either salt water, NaCl solution, or glucose solution were pipetted into the other cuvette of the pair. Air bubbles occluding the openings between cuvettes were removed by pipette and the chambers were covered to limit evaporation. Because the egg capsule pieces had a distinct curvature, the inside and outside surfaces were easily distinguished during assembly of the diffusion chambers. Movement of solute in each direction could thus be determined in separate experiments.

The thin, innermost layer of the egg capsules of *N. lamellosa* and *N. lima* was carefully separated from the rest of the capsule in some experiments. Permeability of the capsule wall to salts and to glucose could therefore be assessed with and without this layer, and the permeability of the inner layer itself could be determined. No tears in this thin inner layer were ever observed upon microscopic examination following experiments. Four replicates (four pairs of cuvettes) were run simultaneously in all experiments.

Determinations of osmotic concentration were made in each cuvette every 1–2 h for up to 5 h, using 0.25-ml samples. The fluid in each chamber was thoroughly mixed before each sample was taken. No net change in volume of fluid in diffusion chambers was apparent in these experiments. The rate at which osmotic concentration of the fluid in the distilled-water chambers increased reflects permeability of the capsule wall to solute.

To test for the possibility of leakage between chambers, a 2.5-mm^2 piece of plastic wrap was placed over the aperture of each of three diffusion chamber assemblies. One chamber of each pair then was filled with 4 ml of distilled water and the other was filled with an equal volume of full strength sea water. No detectable changes in osmotic concentration occurred during the 8-h test period.

Changes in osmotic concentrations of intracapsular fluid were determined directly in an experiment using capsules of *N. lamellosa*. Fluid was withdrawn from egg capsules using a 0.25-ml tuberculin syringe equipped with a No. 27 gauge needle, before and at hourly intervals after transfer of capsules to 5‰ salinity sea water. Between 17 and 31 capsules were used to obtain the 0.2-ml sample volume required for each analysis. The smaller size of the *Ilyanassa obsoleta* egg capsule, and the limited supply of capsules of *Nucella lima* prevented such measurements from being made for capsules of these two species.

The role of capsule wall thickness in contributing to interspecific differences in wall permeability to salts and glucose was considered for capsules of *N. lamellosa* and *N. lima*. Average capsule wall thickness was determined from cross-sectional slices taken from at least 15 capsules of each species. Measurements were made at a magnification of 63 × using a dissecting microscope. Capsule surface area estimates were obtained from five measurements of capsule heights and widths for each species, assuming that the capsules approximate the shape of prolate ellipsoids. Capsule volumes were estimated as $V = 4/3\ \pi a^2 b$, where $a = 1/2$ capsule width and $b = 1/2$ capsule height (neglecting stalk and operculum).

RESULTS

Mortality of encapsulated or excapsulated embryos held under control conditions was low in all experiments for all three species (Tables I, III).

Survival of encapsulated veligers of *Ilyanassa obsoleta* at 48 h after exposure (Table I)

TABLE I

Protection of developing embryos by egg capsules of *Ilyanassa obsoleta*: mortality was assessed 48 h after capsules or individuals were returned to full strength sea water; 16 capsules were used in each treatment of encapsulated embryos, and 60 individuals were used in each treatment of excapsulated embryos.

Expt.	Osmotic concentration (mOsm)	Exposure time (h)	Percent mortality	
			Excapsulated	Encapsulated
I	140	2	70	6
		4	100	0
	952 control	2	10	0
		4	10	0
II	135	1	35	6
		2	67	12
		3	97	12
		4	100	19
		6	100	12
		8	100	25
		10	100	12
	948 control	4	8	0
		10	3	12
III	140	6	85	0
	966 control	6	0	0

and survival of encapsulated veligers of this species to hatching (Table II) were not affected by immersion of capsules in low salinity sea water in any experiment ($P < 0.05$ in all experiments as tested by one-way analysis of variance). In contrast, exposure of excapsulated veligers of *I. obsoleta* to the identical level of stress for as little as 1 h increased developmental mortality significantly (Table I) ($P < 0.05$). Although exposure to low-salinity stress for as long as 10 h failed to increase mortality of encapsulated veligers above control levels, escape of veligers was delayed relative to controls for those capsules exposed to low-salinity sea water for even a few hours (Table II) ($P < 0.05$).

All of the excapsulated *Nucella lamellosa* embryos died following exposures to 5‰ salinity ($\approx$ 150 mOsm) for at least 5 h (Table III). In contrast, encapsulated embryos of this species withstood exposures of up to 9 h with 0% mortality (Table III).

Control embryos of *N. lamellosa* and *N. lima* never acquired purple pigmentation (Table III). Purple (i.e., "stressed") embryos were encountered most frequently among excapsulated individuals (Table III), providing additional evidence that encapsulated

embryos were stressed less by exposure to low salinity sea water. After a 6-h exposure of *N. lamellosa* embryos to water of 149 mOsm, for example, 65% of the excapsulated individuals became purple within 24 h, but only ≈18% of the encapsulated veligers

TABLE II

Effect of exposure to reduced salinity on the escape of veligers from egg capsules of *Ilyanassa obsoleta*: four replicates of four capsules each were used in Expt. A, and three replicates of three capsules each were used in Expt. B; the last column shows the percentage (mean ± SD) of egg capsules empty by Day 5 after initiation of the experiment, of those capsules which eventually emptied.

Expt.	Exposure time (h)	Capsules eventually emptying (%)	Percentage empty by Day 5
A	1	69	54 ± 16
	2	75	58 ± 10
	3	81	54 ± 16
	4	69	62 ± 25
	6	69	44 ± 10
	8	69	64 ± 24
	10	75	50 ± 14
	4 control	88	73 ± 26
	10 control	81	75 ± 16
B	6	100	11 ± 19
	6 control	100	56 ± 19

transferred to the same level of stress evidence this coloration. Of 27 purple *N. lamellosa* embryos whose fates were monitored over an additional 86 h in full strength sea water, 37% lost the purple pigmentation and survived. Of 36 non-purple excapsulated individuals drawn from the same experiment, 89% survived the additional 86 h after return to full strength sea water. Acquisition of purple coloration is therefore a suggestive, but imperfect indicator of impending mortality.

Excapsulated embryos of *N. lamellosa* subjected to gradual salinity changes were far more tolerant of the stress than were those individuals subjected to abrupt salinity transfers (Table III, Expt. V), although both groups of embryos spent 8 h at the final test salinity. Encapsulated embryos suffered essentially no mortality from either abrupt or gradual salinity changes, but the appearance of purple coloration was more pronounced following the abrupt shift (Table III). This, too, indicates that gradual salinity changes are less stressful to these embryos than sudden changes.

In contrast to results obtained for *N. lamellosa*, few excapsulated embryos of *N. lima* died at 142.5–144 mOsm even after 8 h exposures (Table III). Acquisition of purple coloration was seen in only a few embryos exposed to this treatment (Table III), providing further evidence that the embryos of this species are more tolerant of low salinity than are the embryos of *N. lamellosa* and *I. obsoleta*. An 8-h exposure of excapsulated *N. lima* veligers to 58 mOsm (≈2‰ salinity) resulted in high embryonic mortality, however, and a high proportion of purple individuals. In contrast, there was

TABLE III

Protection of developing embryos by egg capsules of *Nucella lamellosa* and *N. lima*: mortality (all experiments) and pigmentation (Expts. II, III, V–VII) were assessed 48 h after return of animals or capsules to full strength sea water; *, **, and *** indicate that 16–20, 30–59, or 60–130 embryos were used in each treatment, respectively.

Expt.	Species	Concentration (mOsm)	Time (h)	Percent mortality		Percent purple	
				Excapsulated	Encapsulated	Excapsulated	Encapsulated
I	*N. lamellosa*	151	0.5	0**	0***		
			1	0**	0***		
			3	0**	0***		
		874 control	3	0**	0**		
II	*N. lamellosa*	144	3	0*	0***	0*	3***
			4	0*	0**	5*	8**
			5	0*	0***	45*	29***
		881 control	5	0*	0**	0*	0**
III	*N. lamellosa*	149	6	100*	0***	65*	18***
			8	100*	0**	80*	22**
			9	100*	0***	70*	47***
		894 control	9	0*	0**	0*	0**
IV	*N. lamellosa*	154	5	100*	0***		
			6	100*	0***		
			8	100*	28**		
		968 control	8	0*	0***		
V	*N. lamellosa*	145	8	100***	1***	77***	21***
		145 mOsm gradual	8	8***	0***	60***	2***
		903 control	8	12***	0***	0***	0***
VI	*N. lima*	142	4.5	0*	0**	13*	0**
			8.5	0*	0**	13*	0**
		873 control	8.5	0*	0**	0*	0**
VII	*N. lima*	58	8	97**	0**	64**	0**
		144	8	6**	0**	0**	0**
		866 control	8	0**	0**	0**	0**

no mortality of encapsulated *N. lima* embryos exposed to 58 mOsm, and none of these encapsulated embryos evidenced any purple pigmentation following the exposure (Table III, Expt. VII).

CAPSULE WALL PERMEABILITY

Average capsule wall thickness was 56.2 μm (SD = 7.5 μm, $N = 24$) and 108.5 μm (SD = 25.5 μm, $N = 15$) for capsules of *N. lamellosa* and *N. lima*, respectively. Approximate capsule volumes, based upon measurements of capsule dimensions, were 0.030 cm^3 and 0.034 cm^3 for capsules of *N. lamellosa* and *N. lima*, respectively. Average egg capsule surface area was calculated to be 56.4 mm^2 and 52.5 mm^2 for the two respective species. Thus the ratio of surface area to volume was 1.9 for capsules of *N. lamellosa* and 1.5 for capsules of *N. lima*.

The osmotic concentration of distilled water in the diffusion chambers increased at a constant rate over time, as expected in the absence of pronounced changes in the osmotic gradient across the capsule walls during the experiments (Fig. 1). Osmotic concentration

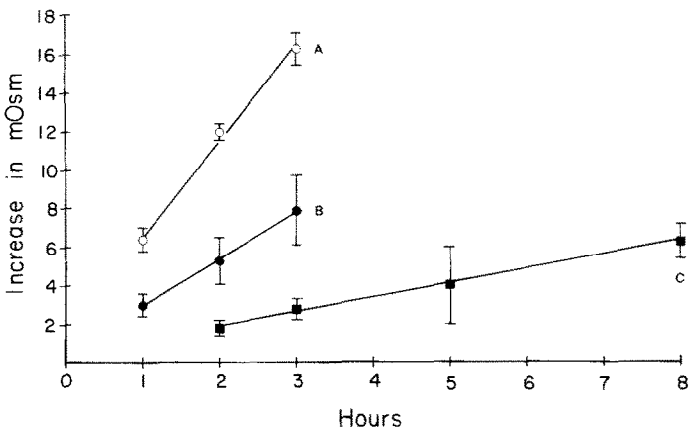

Fig. 1. Diffusion of solutes across 1.35-mm^2 pieces of egg capsule wall of *Nucella lamellosa* over time: each point represents the increase in osmotic concentration in the distilled-water chambers, and is the mean of three to four replicates sampled at each time period; error bars show 1 SD about the mean; regression lines were calculated by the method of least squares; Expt. A, inner membrane only; Expts. B and C, entire portions of capsule wall; Expts. A and B used sea water of 900 mOsm vs. distilled water; Expt. C used glucose solution of 900 mOsm vs. distilled water.

in adjacent chambers decreased at the same rates. The permeability of the thin, inner layers of the egg capsules to salts and glucose is at least double that determined for the complete capsule wall or the wall minus the inner layer for both species ($P < 0.05$) (Table IV). Moreover, one-way analysis of variance revealed no statistically significant differences in rates of movement of salts and glucose across the capsule wall with and without the inner membrane being present ($P > 0.10$). The permeability properties of

TABLE IV

Permeability of *Nucella lamellosa* and *N. lima* egg capsules to salts and glucose: Series A, B, and C experiments were conducted using solutions of sea water, NaCl, and glucose, respectively, against distilled water; data given in the most right-hand column are the rates of increase in osmotic concentration of fluid in the distilled water chambers.

Species	Series	Capsule portion studied	Initial osmotic concentration (mOsm)		Sample times (h)	Mean increase (mOsm/h ± SD)
			Left	Right		
N. lamellosa	A	entire wall	1.7	847.6	1, 2, 3	2.76 ± 0.58 (N = 12)
		entire wall	846.5	1.5	1, 3	2.25 ± 0.60 (N = 8)
		outer wall	1.0	880.8	3	2.89 ± 0.52 (N = 4)
		outer wall	883.1	0.5	3, 5	3.04 ± 0.20 (N = 6)
		inner layer	892.6	0.8	3	5.44 ± 0.27 (N = 3)
		inner layer	891.3	2.7	3	6.64 ± 1.24 (N = 3)
	B	entire wall	880.8	1.5	1, 2, 3	2.64 ± 0.48 (N = 12)
	C	entire wall	844.8	1.9	3, 5, 8	0.84 ± 0.16 (N = 11)
		inner layer	801.7	2.1	1, 2, 3.5, 5.5	2.27 ± 1.17 (N = 16)
N. lima	A	entire wall	3.4	903.1	1, 2, 3	1.81 ± 0.35 (N = 11)
		outer wall	883.3	5.9	1, 3	2.44 ± 0.32 (N = 8)
		inner layer	884.0	7.5	3	6.14 ± 0.48 (N = 4)
	C	entire wall	876.1	2.5	2, 3, 4	0.63 ± 0.30 (N = 12)
		inner layer	903.5	3.4	2, 5	2.52 ± 0.38 (N = 8)

the capsule with respect to these solutes seems to be determined primarily by the outer wall layers.

The capsule wall of both *N. lamellosa* and *N. lima* is clearly less permeable to glucose than to salts (Fig. 1 and Table IV). Also, the permeability of the capsule walls to salts and glucose differed significantly ($P < 0.05$) between the two species, as tested by one-way analysis of variance.

In keeping with the findings that the capsule wall of *N. lamellosa* is permeable to at least small solute molecules, and presumably to water as well, the osmotic concentration of the fluid within intact egg capsules was found to decline significantly after transfer of capsules to water of reduced salinity (Fig. 2). The osmotic concentration of the

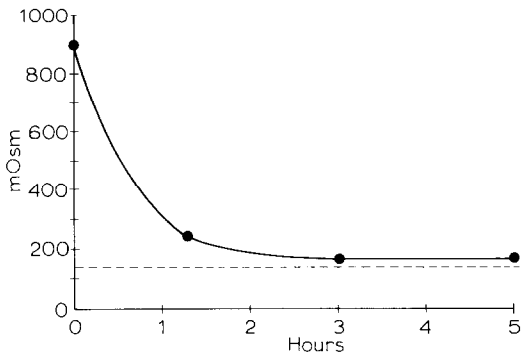

Fig. 2. Rate of decline of osmotic concentration within intact egg capsules of *Nucella lamellosa* transferred to sea water of ≈ 5‰ salinity (– – – –): fluid from 17–30 egg capsules was withdrawn for each determination.

intracapsular fluid of *N. lamellosa* closely approximated that of full strength sea water surrounding the capsules at the start of the experiment, but declined to ≈ 28% of the initial value within ≈ 1 h after transfer of the capsules to dilute sea water. Within 3 h, osmotic pressure of the intracapsular fluid had declined to within ≈ 35 mOsm of the osmotic pressure of the test medium.

Discussion

Encapsulation offers significant protection against low-salinity stress to developing embryos of *Ilyanassa obsoleta*, *Nucella lamellosa*, and *N. lima*. Yet, the walls of *N. lamellosa* capsules are permeable to at least small solute molecules and, presumably, to water. The osmotic concentration of the intracapsular fluid surrounding the embryos of all three species declines after capsules are transferred to low salinity medium.

The evidence for such a decline in the osmotic concentration of intracapsular fluid following transfer to low-salinity media is direct for *N. lamellosa*, and indirect for the other two species. Exposing capsules of *Ilyanassa obsoleta* to water of ≈ 5‰ salinity

delays subsequent release of veligers from the capsules, probably by delaying overall developmental rate or at least delaying the time of release of the hatching substance (Pechenik, 1975). Encapsulated embryos of *Nucella lima* and *N. lamellosa* show a pronounced incidence of purple pigmentation after intact capsules are transferred to reduced salinity, indicating a clear response to environmental stress (Spight, 1975; Gallardo, 1979). The egg capsules of the several pulmonate species which have been examined are also permeable to water and ions (Raven, 1972).

One factor contributing to the protective value of encapsulation in the face of low salinity stress seems to be the effect of the egg capsule on the rate of change of osmotic concentration of the fluid surrounding the embryos. This is clearly the case for *N. lamellosa*; intracapsular osmotic concentration required 1–3 h to reach ambient levels after transfer of the capsule from full strength sea water to sea water of 5‰ salinity, and the differential effects of an abrupt versus a gradual salinity change to the same final concentration were demonstrated directly for embryos of this species. The reduced incidence of purple pigmentation for encapsulated embryos of *N. lamellosa* subjected to a gradual rather than to an abrupt decline in salinity of the surrounding medium (Table III, Expt. V) also shows the importance of rate of change of salinity in determining the degree of stress experienced. Similarly, veligers of the scallop *Pecten maximus* and nauplii of three barnacle species suffered significantly less mortality at reduced salinity when the change occurred gradually rather than abruptly (Davenport *et al.*, 1975; Cawthorne, 1978).

The fact that an abrupt salinity change is more stressful to embryos of *Nucella lamellosa*, and presumably to embryos of *N. lima* and *Ilyanassa obsoleta* as well, suggests that these embryos may have osmoregulatory or at least volume regulatory capabilities (Gainey & Greenberg, 1977; Oglesby, 1981). Volume regulation has been reported for larvae of several polychaetes (Krishnamoorthi, 1963; Lyster, 1965), but seems not to have been investigated for gastropod larvae. Some crustacean larvae have been shown capable of osmoregulation (Kalber, 1970; Foskett, 1977; Young, 1979), but again, osmoregulatory capability seems not to have been investigated for larval gastropods. Adults of both *I. obsoleta* and *Nucella lamellosa* appear capable of volume regulation (Kasschau, 1975; Stickle & Ahokas, 1975).

It should be noted that, although 48 h mortality was low, encapsulated embryos of *N. lamellosa* subjected to a gradual change of salinity evidenced clear signs of stress (acquisition of purple pigmentation). Such signs of stress were rarely observed in encapsulated embryos after transfer of capsules to low-salinity medium. The precise rate of change of fluid osmotic concentration may be of critical importance to embryonic survival, especially once the osmotic concentration declines below some particular level. Rate of salinity change may also be only part of the explanation of increased tolerance to lowered salinity shown by encapsulated embryos. Encapsulated embryos may be somehow protected by the presence of large molecular weight (non-diffusible) components of the intracapsular fluid. More detailed determinations of the time-course of the change in intracapsular osmotic concentration must be obtained, and further

experiments must be conducted with excapsulated embryos using a range of salinity-change rates to resolve this issue.

The rate of decline in osmotic concentration of the fluid within an intact egg capsule will depend upon the amount of surface are available for diffusion of solutes and water, the volume of the capsule in relation to the amount of exposed surface area, the magnitude of the concentration gradients across the wall of the capsule, the chemical composition of the intracapsular fluid, the degree to which capsule volume can increase to accommodate influx of water, and the permeability of the capsule wall to solutes and water. Several factors may be operating to reduce rates of decrease in intracapsular fluid osmotic pressure. As osmotic concentration within the capsule declines, the magnitude of the osmotic concentration gradient across the capsule wall will be reduced and the rate of further decline will be slowed. In addition, the capsule walls of all three species resist substantial deformation. Mean capsule wall stiffness ($\pm$ SE) has been determined to be 15.0 ± 0.46 MN/m^2 ($N = 8$ capsules studied) and 12.3 ± 0.57 MN/m^2 ($N = 6$) for capsules of *N. lamellosa* and *N. lima*, respectively (Daniel & Pechenik, in prep.). The capsules cannot swell to accommodate a significant influx of water when transferred to dilute medium. In addition, the capsules of the few gastropod species which have been examined are filled not with sea water, but rather with a complex mixture of carbohydrates and proteins (Hunt, 1966; Bayne, 1968; Raven, 1972; Harasewych, 1978). Since the capsule walls of *N. lamellosa* and *N. lima* are far less permeable to even as small a molecule as glucose relative to the capsule wall permeability for salts (Table IV), the rate at which osmotic concentration within intact egg capsules decreases may be at least partly limited by the rate at which the large organic molecules of the intracapsular fluid can diffuse out of the capsules. Detailed studies of the actual movement of water and solutes into and out of intact egg capsules transferred to low salinity medium are needed, in conjunction with studies of capsule wall permeability to solutes representing the range of molecular weights found in intracapsular fluids.

It should be noted that excapsulated embryos of *N. lamellosa* were less tolerant of reduced salinity than were those of *N. lima*, as indicated by differences in survivorship of stressed individuals and by differences in incidence of purple pigmentation following exposure to water of reduced salinity (Table III). Nevertheless, the capsule wall of *N. lamellosa* appears to be more permeable, at least with respect to salts and glucose, than the capsule wall of *N. lima* (Table IV). The interspecific difference in permeability to salts is only $\approx 22\%$, however, despite the difference of $\approx 48\%$ in capsule wall thickness. This suggests that properties of the capsule walls may differ significantly between the two species. The larger ratio of surface area : volume for capsules of *N. lamellosa* with respect to the ratio for capsules of *N. lima* is also not in keeping with the differences in salinity tolerance for embryos of the two species. Interspecific comparisons of actual rates of change of osmotic concentration within intact capsules of both species will be interesting in this regard.

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