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Role of Maternal Provisioning in Controlling Interpopulation Variation in Hatching Size in the Marine Snail *Nucella ostrina*

MICHELLE J. LLOYD[1] AND LOUIS A. GOSSELIN[1,2,*]

[1]*Department of Biological Sciences, Thompson Rivers University, Kamloops, British Columbia V2C 5N3, Canada; and Bamfield Marine Sciences Centre, Bamfield, British Columbia VOR 1BO, Canada; and* [2]*Department of Biology, University of Victoria, Victoria, British Columbia V8W 3N3, Canada*

Abstract. This study examined the role of maternal provisioning in controlling interpopulation variation in hatching size in nine isolated populations of the intertidal gastropod *Nucella ostrina*, in which development to the early juvenile stage takes place within an egg capsule. Variation among populations was almost entirely due to the ratio of nurse eggs to embryo, which explained 65% of the variation in hatching size. Egg size was not a significant predictor of hatching size. Differences among seven of these populations in the nurse egg/embryo ratio were entirely due to the number of nurse eggs allocated per capsule; these populations allocated different numbers of nurse eggs per capsule but allocated the same number of embryos. Intriguingly, the two most wave-sheltered populations allocated significantly more nurse eggs and more embryos to each capsule than did the seven other populations, but they maintained nurse egg/embryo ratios consistent with patterns observed in the other populations. Inter- and intrapopulation variation in hatching size appears to be controlled largely by different mechanisms: within-population variation being controlled mainly by differences in allocation of embryos per capsule, whereas most among-population variation being due to differences in allocation of nurse eggs per capsule.

Introduction

The amount of organic material provided to an embryo in the form of yolk can be important in determining offspring size in benthic marine invertebrates (Spight, 1976a, b; Rivest, 1983; Sinervo and McEdward, 1988; Emlet and Hoegh-Guldberg, 1997; MacKay and Gibson, 1999). In addition, since large offspring can grow more rapidly than small offspring (Moran and Emlet, 2001; Marshall *et al.,* 2003), initial size differences among offspring may persist well into juvenile life or even up to the adult stage (Marshall, 2005). Maternal provisioning at the time of reproduction can therefore have a lasting influence on the size and fitness of the offspring. In species with planktotrophic larvae, the provision of yolk to the embryo by the female parent can be a major determinant of offspring size up to the point at which the larva begins feeding (Sinervo and McEdward, 1988); beyond that point, growth, and thus offspring size, become increasingly influenced by the feeding success of the larva (Sinervo and McEdward, 1988; Pechenik *et al.,* 1998; Phillips, 2002; Emlet and Sadro, 2006). In species without planktotrophic larvae, however, the influence of maternal provisioning can extend further into the life of the offspring (Emlet and Hoegh-Guldberg, 1997). For instance, in several species of marine gastropods, the female parent deposits embryos into egg capsules or egg masses in which full embryonic development takes place and individuals eventually hatch as early juveniles (Spight, 1976a; Collin, 2003). Since there is no planktonic larval feeding stage in these species, maternal provisioning to the embryos at the time of spawning is the primary source of organic matter for the offspring through to the beginning of juvenile life. Correspondingly, variation in maternal provisioning may largely control variation in initial juvenile size (Spight, 1976b; Rivest, 1983).

Initial juvenile size varies substantially among siblings, clutches, or populations in several species of marine prosobranch gastropods such as *Acanthina spirata* (Spight,

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* To whom correspondence should be addressed. E-mail: lgosselin@tru.ca

1976b), *Crepidula dilata* (Charparro *et al.,* 1999), *Lirabuccinum dirum* (previously known as *Searlesia dira*) (Rivest, 1983), *Nucella crassilabrum* (Gallardo, 1979), *N. lapillus* (Etter, 1989), *and N. ostrina* (Spight, 1976b; Gosselin and Rehak, 2007). These species all have encapsulated development and add unfertilized nurse eggs to the capsules as a food source for the embryos—a common trait among prosobranch gastropods (Spight, 1976a; Hadfield, 1989; Collin, 2003) and other groups of marine invertebrates (Rivest, 1983; MacKay and Gibson, 1999). In such species, the number of nurse eggs consumed by a developing embryo can be an important determinant of hatching size (Spight, 1976a, b; Gallardo, 1979; Rivest, 1983; Hadfield, 1989; Chaparro *et al.,* 1999). The reproductive mechanisms controlling variation in initial juvenile size within populations have been explored in some detail (Spight,1976b; Gallardo, 1979; Rivest, 1983; Pechenik *et al.,* 1984; Chaparro *et al.,* 1999; MacKay and Gibson, 1999). The mechanisms controlling variation among populations, however, have received much less attention and are not well understood.

In a recent study of the intertidal snail *Nucella ostrina* (Gould, 1852), Gosselin and Rehak (2007) found substantial variation in initial juvenile size among 10 populations that were 10 km or less apart, with the average hatching size of the populations tending to increase with the degree of wave exposure at the site. The physiological mechanisms controlling this interpopulation variation, however, are not known. In this study, we therefore examined the role of maternal provisioning in controlling interpopulation variation in *N. ostrina* hatching size. Spight (1976b) suggested that variation in hatching size among capsules within a population might be controlled primarily by the number of embryos allocated per capsule by the female parent, a hypothesis later supported by Rivest (1983). We examined whether this mechanism might also control variation in hatching size among populations. Our first goal was to determine whether differences in average hatching size among populations are controlled by the amount of yolk allocated per embryo. Having confirmed this, our second goal was to determine how the yolk volume/embryo ratio is controlled. We therefore documented three reproductive parameters for each of nine populations of *N. ostrina*: (1) the number of embryos deposited per capsule, (2) the number of nurse eggs deposited per capsule, and (3) egg size. Finally, we also examined the relationship between these traits and the degree of wave exposure to identify reproductive traits that vary across this gradient in the same way as hatching size.

Materials and Methods

Study organism

Nucella ostrina (previously known as *N. emarginata* [northern], see Marko *et al.,* 2003) is found in the midintertidal zone of rocky shorelines from Alaska to California (Palmer *et al.,* 1990; Marko, 1998; Marko *et al.,* 2003). These snails can spawn all year round (Houston, 1971; Rawlings, 1996), but in our study area most spawning generally takes place from early spring to mid-summer (Gosselin, 1994). Female *N. ostrina* lay clutches of 3–15 egg capsules on rock or shell surfaces (Houston, 1971; Rawlings, 1996; Moran and Emlet, 2001). The female inserts embryos and nurse eggs into each capsule and seals the top with a plug that dissolves once embryonic development is complete (Houston, 1971; LeBoeuf, 1971; Rawlings, 1995). After the female has attached the capsules to a surface, the embryos are left to develop on their own. The period of encapsulated development lasts 72–140 d (Palmer, 1994), after which the young snails emerge and crawl away from the capsule as early juveniles. Hatching size in *N. ostrina* is positively correlated with energy content (Moran and Emlet, 2001). Since *N. ostrina* lacks a dispersing planktonic stage, migration among populations is mostly limited to how far juveniles and adults can crawl (Gosselin and Chia, 1995a). *Nucella ostrina* populations separated by open water or by distances of a few hundred meters or more are therefore likely to experience limited gene flow (Palmer, 1984; Gosselin and Chia, 1995a), potentially allowing evolutionary divergence among groups of snails within a small geographical area.

Study sites

The present study was conducted at sites near the Bamfield Marine Sciences Centre on the west coast of Vancouver Island, British Columbia, Canada. Egg capsules were collected during the period of 1 June to 2 August 2004, from nine *Nucella ostrina* populations for which average hatching sizes are known (Gosselin and Rehak, 2007). These nine sites are separated from each other by at least 500 m, and the two most distant sites, Ross Islets and Cape Beale, are 10.4 km apart. Most are separated by open water or by soft-sediment (sand or mud) intertidal areas that act as barriers to dispersal of *N. ostrina*. The nine sites cover a broad gradient of exposure to wave action, from very sheltered to fully exposed to open-ocean surge and waves. The ranking of seven of these sites with regard to level of wave exposure was based on Rawlings (1994, 1995): Rawlings ranked the sites on the basis of the maximum height of the *Balanus glandula* zone and the lowest height of vascular plants. Gosselin and Rehak (2007) used similar parameters to rank the remaining two sites (Dixon Island sheltered and Scotts Bay) relative to the others. Gosselin and Rehak (2007) then used this information to group the sites into four categories of wave exposure: full exposure to the open ocean (full exposure: Cape Beale); partial shelter from open-ocean swell (high exposure: Voss Point, Prasiola Point, Kirby Point); intermediate shelter from open-ocean swell (moderate exposure: Dixon Island exposed, Wizard Islet, Scotts

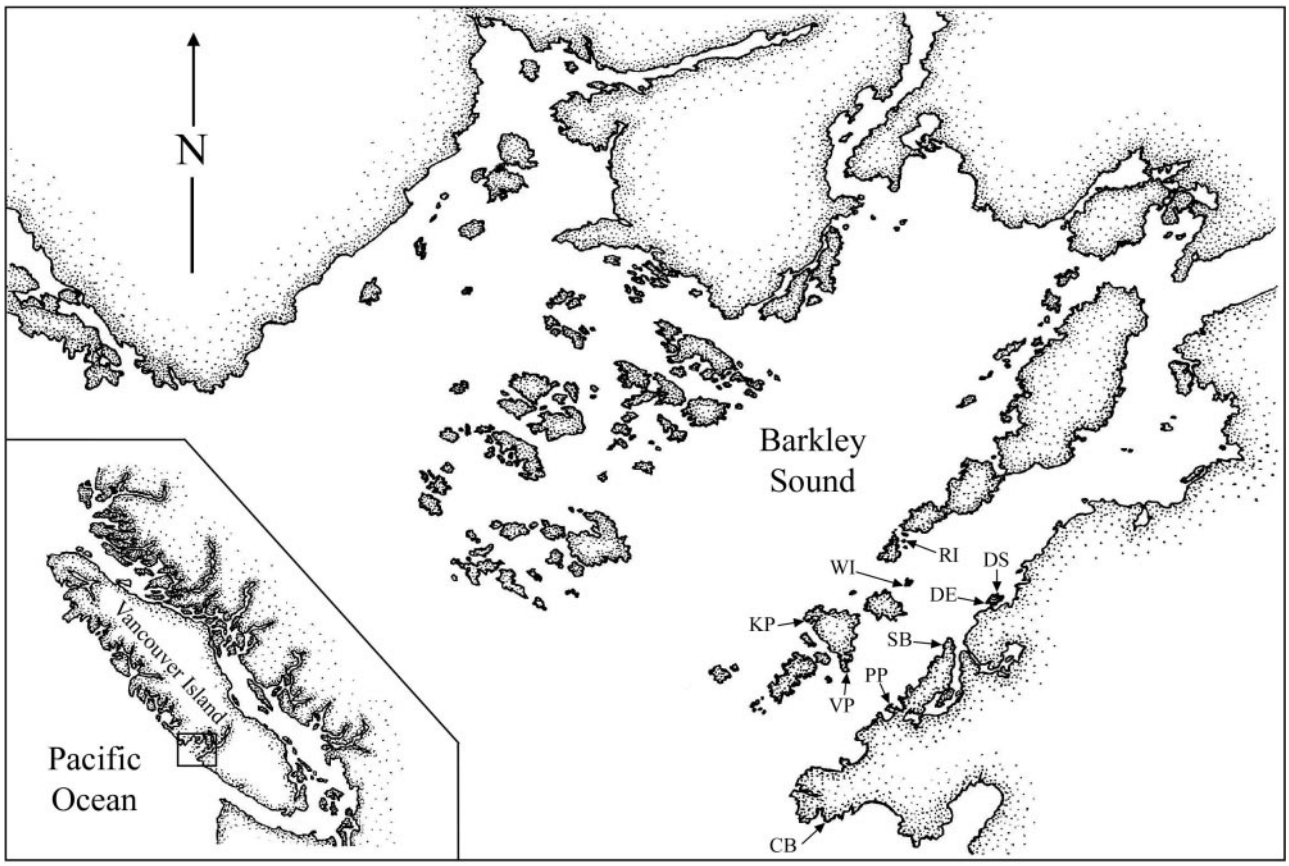

Figure 1. Map of Barkley Sound showing the nine study sites where *Nucella ostrina* egg capsules were collected: Dixon Island sheltered (DS), Ross Islets (RI), Dixon Island exposed (DE), Wizard Islet (WI), Scotts Bay (SB), Voss Point (VP), Prasiola Point (PP), Kirby Point (KP), and Cape Beale (CB). Map modified from Gosselin and Chia (1995b).

Bay); and extensive shelter from open-ocean swell (low exposure: Dixon Island sheltered, Ross Islets) (Fig. 1). Gosselin and Rehak (2007) quantified hatching size for five of the populations in 1999 (Dixon Island sheltered, Ross Islets, Dixon Island exposed, Wizard Islet, and Kirby Point), and then for all of the populations in 2000 and 2001. Of the 10 sites studied by Gosselin and Rehak, only Folger Island, the most wave-exposed site, is not included in the present study; it could not be accessed in 2004 owing to rough seas.

Collection and analysis of egg capsules

To quantify variation in reproductive traits among these populations, we collected freshly spawned capsules, recognized by their light color and smooth surface texture, from each of the nine sites. Forceps were used to gently detach clutches (a group of capsules laid by one female or an aggregation of capsules produced by many females [Rawlings, 1996]) from rock or shell surfaces, and each clutch was placed in a separate cage. These capsules were returned to the laboratory and placed in trays with flowing seawater. Capsules were then individually examined: after the plug was removed with a scalpel, the contents were gently rinsed out for analysis. Capsule walls in *Nucella ostrina* are only slightly translucent, so the capsules must be opened and the contents removed to analyze the eggs and embryos, precluding subsequent determination of hatching size for the same capsules.

To correctly quantify the number of embryos and nurse eggs present in a capsule, the embryos must have developed sufficiently to be distinguishable from nurse eggs, yet not to the point where they begin consuming nurse eggs. Embryos younger than veliger stage 1 could not be reliably distinguished from nurse eggs. By veliger stage 2, however, the larvae have active oral ciliations (LeBoeuf, 1971) that allow them to ingest nurse eggs (Lyons and Spight, 1973). We therefore only analyzed the contents of capsules in which the embryos were at veliger stage 1. Stage 1 veligers can be distinguished from nurse eggs in that the veligers are ovoid rather than spherical and small lobes have begun to form (LeBoeuf, 1971). For each clutch, we collected data from

the first two capsules that were found to contain embryos at veliger stage 1; in several cases, however, only one capsule of a clutch contained stage 1 veligers, in which case we obtained data from only one capsule. The number of clutches and egg capsules for which data were obtained for each population are listed in Table 1. Prior to analyzing data obtained from these capsules, values from pairs of capsules of a same clutch were averaged to obtain a single datum per clutch. For each of the capsules documented in this study, three parameters were quantified: the number of nurse eggs, the number of stage 1 veligers (*i.e.,* embryos), and egg size. Egg size was determined by measuring the diameter of 10 haphazardly selected nurse eggs per capsule, using the ocular micrometer of a compound microscope. Fertilized eggs are indistinguishable from nurse eggs, precluding separate measurements of the two egg types. If any difference in size does exist, it is not noticeable and would be modest. In addition, nurse eggs provide 95%–99% of the yolk allocated per embryo; thus a modest size difference between nurse eggs and fertilized eggs, if such difference does exist, would constitute an insignificant portion of the total amount of yolk obtained by the embryo. Finally, we examined the relationship between reproductive parameters and (1) the degree of wave exposure of the site, (2) average hatching shell length (SL) for these populations, and (3) average capsule volumes of the populations (as reported by Rawlings, 1995).

Results

Embryos per capsule

The number of embryos per capsule varied significantly among *Nucella ostrina* populations (ANOVA: $F = 4.20$, df $= 8$, $P < 0.001$). This difference, however, was almost entirely due to the two most sheltered populations, Dixon Island sheltered and Ross Islets, which allocated considerably more embryos to each capsule than the seven other populations (Fig. 2a). The number of embryos per capsule was not significantly different among the seven other populations, as determined by a Tukey HSD multiple comparisons test. The correlation between the average number of embryos per capsule and the ranking of sites in terms of wave exposure was determined using a Spearman rank-order correlation analysis, with all sites within an exposure category being given the same rank; this analysis revealed no significant correlation for the seven populations (Spearman rank-order correlation: $r_s = -0.077$, $n = 7$, $P = 0.869$) or more broadly among the nine populations ($r_s = -0.581$, $n = 9$, $P = 0.101$).

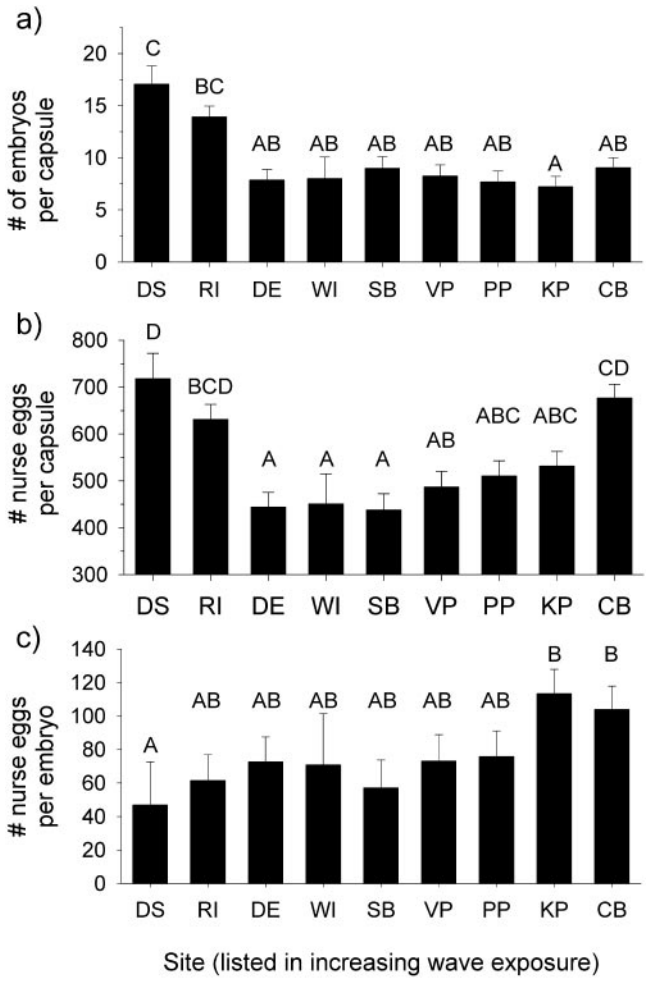

Figure 2. Contents of egg capsules from nine *Nucella ostrina* populations in Barkley Sound: (a) number of embryos per egg capsule, (b) number of nurse eggs per egg capsule, and (c) number of nurse eggs per embryo. Values represent the mean ± SE.

Table 1

Egg capsule sample sizes

Site	Number of clutches	Total number of egg capsules
Dixon Island sheltered	7	9
Ross Islets	21	30
Dixon Island exposed	17	30
Wizard Islet	5	9
Scotts Bay	17	30
Voss Point	19	31
Prasiola Point	20	31
Kirby Point	19	29
Cape Beale	24	30

Number of clutches and egg capsules for which data were obtained from each of the 9 populations of *Nucella ostrina*.

Nurse eggs per capsule

Nucella ostrina populations differed significantly in their allocation of nurse eggs per capsule (ANOVA: $F = 8.045$,

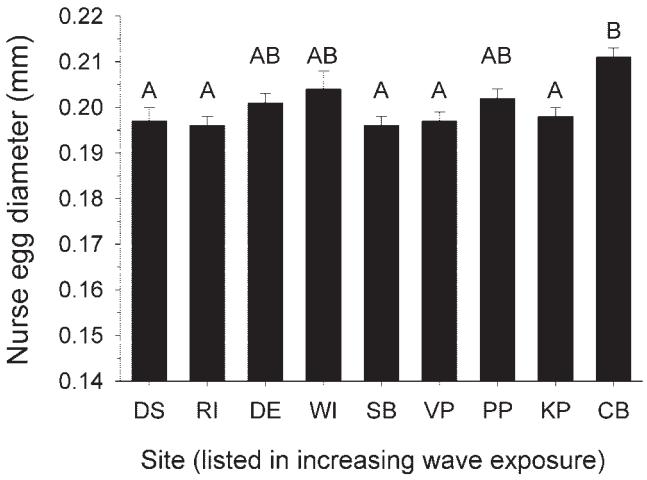

Figure 3. Nurse egg diameter for nine populations of *Nucella ostrina*. Values represent the mean ± SE based on measurements of 10 nurse eggs per capsule.

df = 8, $P < 0.001$). As was the case for the number of embryos per capsule, the Dixon Island sheltered and Ross Islets populations did not fit the trend observed in the seven other populations in terms of number of nurse eggs per capsule: Dixon Island sheltered and Ross Islets each had a large number of nurse eggs per capsule, comparable to the Cape Beale population at the other end of the gradient of wave exposure (Fig. 2b). There were, however, significant differences in this trait among the seven other populations as well, and a pronounced trend of increasing average number of nurse eggs per capsule with wave exposure among those seven populations (Spearman rank-order correlation: $r_s = 0.926$, $n = 7$, $P = 0.003$). The correlation was not significant when values for Dixon Island sheltered and Ross Islets were included in the analysis ($r_s < 0.001$, $n = 9$, $P = 0.999$).

Nurse eggs per embryo

The nurse egg/embryo ratio varied significantly among the nine populations of *Nucella ostrina* (ANOVA: $F = 3.058$, df = 8, $P = 0.003$). The ratio increased with wave exposure (Fig. 2c) (Spearman rank-order correlation: $r_s = 0.884$, $n = 9$, $P = 0.002$), and even the two most sheltered populations, Dixon Island sheltered and Ross Islets, conformed to this trend.

Egg size

To determine if *Nucella ostrina* populations differ in the size of the eggs they produce, measurements (10 per capsule) of nurse-egg diameter were averaged per capsule, and these values were compared among populations by ANOVA, an approach equivalent to a nested ANOVA (Hurlbert, 1984). There was significant variation in nurse-egg diameter among the populations (ANOVA: $F = 6.700$, df = 8, $P < 0.001$). The variation was modest, however, with only a 7.5% difference between the largest and smallest average egg diameters (Fig. 3). When expressed in terms of volume (diameter measurements converted to volume using the equation for the volume of a sphere), this corresponds to a difference of 24.3% in average egg volume, considerably less than the 97%–210% difference among these populations in the average hatchling body volume (Gosselin and Rehak, 2007). Variation in egg diameter was not related to wave action (Spearman rank-order correlation: $r_s = 0.615$, $n = 9$, $P = 0.078$).

Yolk volume per embryo

For each capsule, the total volume of yolk provided per embryo was calculated as follows:

total yolk volume per embryo = # nurse eggs per embryo
× average volume of yolk per nurse egg

The nine populations differed significantly in volume of yolk allocated per embryo (ANOVA: $F = 3.042$, df = 8, $P = 0.003$), and differences among these populations were considerable (Fig. 4). Females in the Cape Beale population allocated on average 2.8 times more yolk per embryo than did females in the Dixon Island sheltered population. There was also a significant correlation between volume of yolk per embryo and wave exposure, with yolk volume per embryo increasing with wave exposure (Spearman rank-order correlation: $r_s = 0.814$, $n = 9$, $P = 0.008$).

Relationship of reproductive parameters with hatching size and capsule volume

In regression analyses between hatching size and reproductive parameters, we used (average hatching SL)^3 to

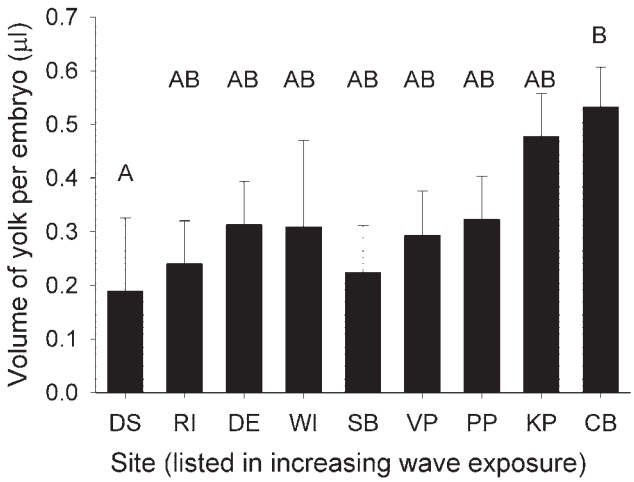

Figure 4. Yolk volume per embryo for nine populations of *Nucella ostrina*. Values represent the mean ± SE.

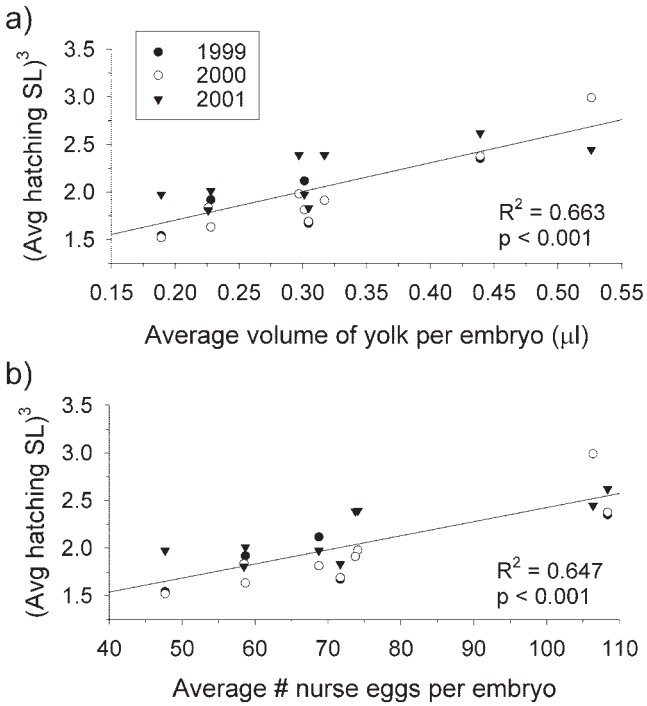

Figure 5. Relationship between (average hatching SL)^3 of nine *Nucella ostrina* populations and (a) yolk volume per embryo and (b) number of nurse eggs per embryo. Average hatching shell length (SL) values for 1999, 2000, and 2001 included in the analyses; $n = 23$ for each analysis. Hatching SL data obtained from Gosselin and Rehak (2007).

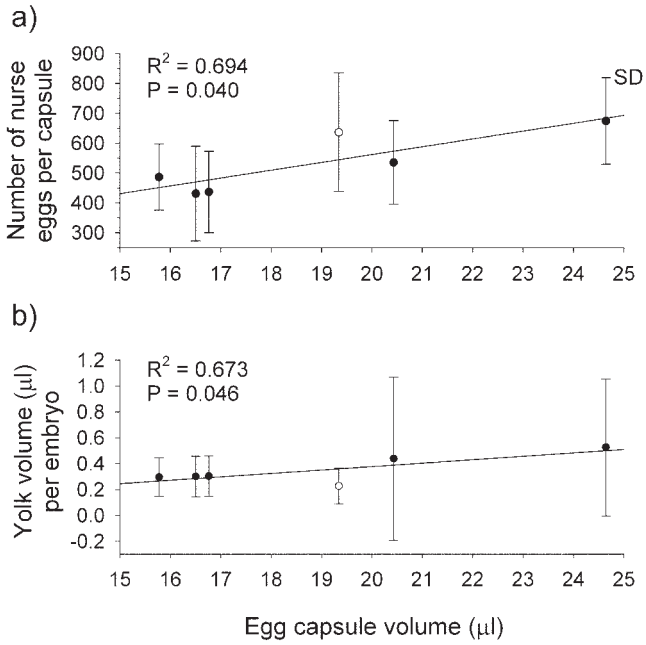

Figure 6. Relationship between average egg capsule volume of six *Nucella ostrina* populations and (a) number of nurse eggs per egg capsule and (b) yolk volume per embryo. In each of these graphs the value represented by an empty circle is from the Ross Islets area (see text).

provide a best fit with yolk volume and number of nurse eggs. The average volume of yolk per embryo explained 66% of the variation among populations in average hatching SL (Fig. 5a). Since yolk volume per embryo is determined by both the nurse egg/embryo ratio and the average egg volume, a stepwise forward multiple regression model was used to examine the role of each parameter in controlling hatching size variation among populations. The nurse egg/embryo ratio was a significant predictor of hatching size (Table 2), explaining 65% of the variation in hatching size (Fig. 5b), whereas egg volume had no significant influence.

Rawlings (1995) documented *Nucella ostrina* capsule morphology, including capsule volume, for 10 sites in Barkley Sound; five of his study sites were identical to sites examined in the present study (Dixon Island exposed, Wizard Island, Voss Point, Kirby Point, and Cape Beale), and another of his sites was located about 200 m from our Ross Islets site. To examine the relationship between the average capsule volumes documented by Rawlings (1995) and reproductive parameters measured in our study, we included data from all six sites in the analysis, but we have labeled values from Ross Islets differently in the figure, given that the locations were nearby but not identical in the two studies (Fig. 6). Average capsule volume for a population was significantly correlated with average number of nurse eggs

Table 2

Multiple regression analysis between hatching size and reproductive parameters

Variable	β	R_{mult}	R^2_{mult}	t	P
Dependent variable: (Avg hatching shell length)^3					
Independent variables:					
Avg nurse egg/embryo ratio	**0.15 (0.002)**	**0.805**	**0.647**	**6.21**	**<0.001**
Avg egg volume	156.66 (204.86)	0.811	0.657	0.765	0.453

Results of multiple regression analysis, using the stepwise forward addition of variables, of (average hatching shell length)^3 as a function of the average nurse egg/embryo ratio and average egg volume ($n = 23$). Shown are standardized partial regression coefficients β (their standard errors in parentheses), multiple correlation coefficients R_{mult}, multiple coefficients of determination R^2_{mult}, and the significance level of each variable to be included in the model. Significant predictors ($P < 0.05$) are printed in bold.

per capsule (Fig. 6a) and average yolk volume per embryo (Fig. 6b), but not with average number of embryos per capsule ($R^2 = 0.003$, $n = 6$, $P = 0.912$) or with average egg volume ($R^2 = 0.394$, $n = 6$, $P = 0.182$).

Discussion

Role of yolk volume/embryo ratio

Despite the year-to-year variation in hatching size for each population and the fact that yolk volume and hatching size were measured in different years, the yolk volume/embryo ratio nevertheless explained two-thirds of the variation among populations in hatching size. In addition, the yolk volume/embryo ratio was correlated with wave exposure, matching the increase in hatching size with wave action reported by Gosselin and Rehak (2007): at sites experiencing intense wave action, more yolk was allocated per embryo, and hatching sizes were larger, than at sites where wave action was less intense. These results indicate that the volume of yolk available to the embryo is the primary mechanism responsible for differences in hatching size among *Nucella ostrina* populations in Barkley Sound.

Mechanism controlling the yolk/embryo ratio

Although differences in energy allocation per embryo could in principle be achieved in several different ways, such as altering the nurse egg/embryo ratio (Spight, 1976b; Rivest, 1983), the egg diameter (Spight, 1976a; Jaeckle, 1995), or the energy density of the eggs (Jaeckle, 1995) or intracapsular fluid (Pechenik *et al.,* 1984), differences among most of these *Nucella ostrina* populations appear to be achieved primarily by controlling the nurse egg/embryo ratio. Egg size did not correlate with wave exposure and was not a significant predictor of interpopulation variation in hatching SL. The nurse egg/embryo ratio, on the other hand, was strongly correlated with wave exposure and with hatching SL. In fact, the amount of variation in hatching SL explained by the nurse egg/embryo ratio ($R^2 = 0.647$) was almost identical to that explained by the yolk volume/embryo ratio ($R^2 = 0.663$). These findings are consistent with previous reports of significant differences in nurse egg/embryo ratios and little difference in egg size, among geographically isolated populations of prosobranch gastropods, and support the hypothesis that variation among populations in allocation of nurse eggs per embryo is responsible for differences in hatching size (Rivest, 1983; Hadfield, 1989).

Variation among most *N. ostrina* populations in the nurse egg/embryo ratio was controlled by differences in the number of nurse eggs allocated per capsule rather than by differences in the allocation of embryos per capsule. There were no differences in the number of embryos per capsule among seven of the nine populations, nor was this parameter related to wave action. On the other hand, numbers of nurse eggs per capsule varied significantly among populations and increased with wave exposure, with the exception of the two most sheltered populations. The role of nurse egg allocation per capsule as the most common mechanism controlling interpopulation variation in the yolk/embryo ratio, and thus hatching size, contrasts with earlier observations that variation within a population in nurse egg/embryo ratios is largely determined by the allocation of embryos per capsule (Spight, 1976b; Rivest, 1983). Consequently, although both inter- and intrapopulation variation in hatching size would be controlled by the yolk volume/embryo ratio, that ratio itself appears to be controlled by different mechanisms depending on the scale being considered (within or among populations). Variation in hatching size within a population may result mainly from variation in number of embryos per capsule (Spight, 1976b; Rivest, 1983), whereas variation in hatching size among most populations would be a consequence of differences in nurse egg allocation per capsule (this study).

Intriguingly, the two most wave-sheltered populations did not follow the broader trends in numbers of nurse eggs and embryos per capsule observed in the seven other populations. The Dixon Island sheltered and Ross Islets populations allocated as many nurse eggs per capsule as did Cape Beale, our most wave-exposed site. However, females in these two sheltered populations also deposited almost twice as many embryos per capsule as did females in the seven other *N. ostrina* populations. In doing so, the Dixon Island sheltered and Ross Islets populations used a different allocation strategy for nurse eggs and embryos than the other populations, but did so in a way that the nurse egg/embryo ratio remained consistent with the broader trend of increasing number of nurse eggs per embryo along a gradient of wave exposure observed among all nine populations.

Although the significance of the high numbers of embryos and nurse eggs per capsule in the Dixon Island sheltered and Ross Islets populations remains unclear, these parameters can nevertheless be linked to capsule size. Sheltered populations of *N. ostrina*, such as these, do produce relatively large capsules (Rawlings, 1995), possibly as a way to decrease the surface-to-volume ratio and thus better protect embryos against physical stresses such as desiccation or osmotic shock (Rawlings, 1999) that are relatively high in wave-sheltered intertidal habitats. Correspondingly, large gastropod egg capsules generally hold more eggs than small capsules (Spight *et al.,* 1974; Gallardo, 1979; Chaparro *et al.,* 1999). The average capsule volumes reported by Rawlings (1995) for six of our populations were significantly and quite strongly correlated with our data on average number of nurse eggs per capsule and yolk volume per capsule, but not with number of embryos per capsule. These correlations based on population averages (interpopulation variation) are consistent with correlations reported by

Spight (1976b) and Geller (1990) among these same parameters but based on counts or estimates from individual capsules (intrapopulation variation) in *N. ostrina.* Capsule size and nurse egg allocation per capsule are therefore probably interdependent parameters. The number of embryos deposited per capsule, however, appears to be controlled by females in a way that is independent of capsule volume.

Ecological implications

The findings of this study suggest three general conclusions regarding reproductive strategy in *Nucella ostrina*: (1) Interpopulation variation in average hatching size is primarily a result of differences in the yolk volume/embryo ratio, which in all but the most wave-sheltered populations is controlled by adjusting the average number of nurse eggs allocated per capsule. (2) Although the number of embryos per capsule may vary haphazardly around an average value among capsules of a clutch or population (Spight, 1976b; Rivest, 1983), the average number of embryos per capsule of a population does not vary haphazardly among populations. At the population level, the average numbers of nurse eggs and embryos per capsule each appear to be controlled rather than haphazard; this results in population-specific average nurse egg/embryo ratios, which in turn produces average hatching sizes specific to each population. In addition, although differences in egg size seem to contribute little to interpopulation variation in hatching size, egg diameter did differ significantly among populations, indicating that even this trait may be responding to local environmental conditions, although more work on this is needed. (3) At least at the population level, several reproductive parameters, including the allocation of nurse eggs and embryos per capsule, egg size, and perhaps capsule size, appear to be controlled independently. For instance, the production of larger capsules does not necessarily result in larger eggs or more embryos per capsule. This is an important finding, as an independent control of reproductive parameters would allow fine-tuned responses to local conditions affecting different stages of offspring development, such as the period of encapsulated development and that of early juvenile life, as well as constraints on the female.

Why are some of these reproductive parameters, in particular the yolk volume/embryo ratio and the number of nurse eggs per capsule, related to wave exposure? This relationship may result from a phenotypic response of spawning females to the environmental conditions they experience—for example, to wave exposure itself or to other parameters that covary with wave exposure, such as desiccation, thermal stress, or food availability. Alternatively, the traits might be adaptive for the offspring, enhancing the performance of the embryos or early juveniles in each environment. Rawlings (1995) found that capsule size and number of embryos allocated per unit of capsule volume in *N. ostrina* exhibit phenotypic plasticity. When he provided snails with greater food rations in the laboratory prior to spawning, the snails produced larger capsules containing more embryos per unit volume than did snails receiving smaller rations. It is therefore possible that interpopulation variation in yolk allocation and thus hatching size are largely plastic responses of spawning females to local environmental conditions. These traits in turn would be correlated with wave exposure if food availability or quality also varied along a gradient of wave exposure. Evidence to date nevertheless suggests that the allocation of nurse eggs and embryos to capsules in *N. ostrina* and other invertebrates is at least partly genetically determined (Rivest, 1983; Rawlings, 1995; MacKay and Gibson, 1999). A logical next step is to decipher the relative roles of phenotypic plasticity and genetic control in determining interpopulation variation in this life-history trait. In addition, the role of other traits and factors in determining initial juvenile size in *N. ostrina* and other marine invertebrates has yet to be determined. These traits and factors include the uptake of dissolved organic matter by developing embryos (Manahan, 1990), and the effects of environmental conditions such as temperature, salinity, oxygen levels, desiccation, wave action, or bacterial infections on the rate of uptake and assimilation of available energy and the amount of energy metabolized prior to hatching.

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