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Egg Size as a Life History Character of Marine Invertebrates: Is It All It's Cracked Up to Be?

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Abstract. Egg size is one of the most important aspects of the life history of free-spawning marine organisms, and it is correlated with larval developmental mode and many other life-history characters. Egg size is simple to measure and data are available for a wide range of taxa, but we have a limited understanding of how large and small eggs differ in composition; size is not always the best measure of the characters under selection. Large eggs are generally considered to reflect increased maternal investment, but egg size alone can be a poor predictor of energetic content within and among taxa. We review techniques that have been used to measure the energetic content and biochemical makeup of invertebrate eggs and point out the strengths and difficulties associated with each. We also suggest a number of comparative and descriptive approaches to biochemical constituent analysis that would strengthen our understanding of how natural selection shapes oogenic strategies. Finally, we highlight recent empirical research on the intrinsic factors that drive intraspecific variation in egg size. We also highlight the relative paucity of these data in the literature and provide some suggestions for future research directions.

Introduction

Egg size is one of the most important and often-studied aspects of the life history of marine organisms, and much attention has focused on the ecological factors that drive changes in egg size (*e.g.,* Thorson, 1950; Vance, 1973a, b; Christiansen and Fenchel, 1979; Strathmann, 1985, 2000; Lessios, 1990; Moran, 2004; Levitan, 2000, 2006). Early interest in egg size evolution was spurred by the observation that egg size is strongly associated with developmental mode; species with small eggs generally have long-lived planktonic larvae that disperse widely and must feed and grow in the plankton prior to reaching metamorphosis (=planktotrophic), whereas species with large eggs tend to have short-lived, nonfeeding larvae or to lack dispersing planktonic stages altogether (=nonplanktotrophic) (Thorson, 1950, and much subsequent interest). Within and among developmental modes, egg size is also linked to a number of fundamental and adaptive traits such as length of larval development (Thorson, 1950; Vance, 1973a, b; Strathmann, 1985; Hadfield and Miller, 1987; Sinervo and McEdward, 1988; Wray and Raff, 1991), larval form (McEdward, 1986; Strathmann, 2000), length of the facultative feeding period (Miner *et al*., 2005), size at metamorphosis (Strathmann, 1985; Emlet *et al*., 1987), juvenile growth and survival (Marshall *et al*., 2003), resistance to starvation (Anger, 1995; Bridges and Heppell, 1996), and fertilization success (Levitan, 1993, 2000, 2006; Podolsky and Strathmann, 1996; Farley and Levitan, 2001; Luttikhuizen *et al*., 2004).

Despite wide interest in and focus on the selective, or top-down, factors that drive the evolution of egg size, considerably less is known about the bottom-up factors that underlie egg size at the physiological, biochemical, and ecological levels. One reason for this gap in knowledge is that egg size is, in and of itself, a simplistic and perhaps not always appropriate measure of the factors under selection. A large egg is generally considered to reflect increased maternal energy investment; in this scenario, the size of an egg directly affects larval physiological performance and success. However, among planktotrophic species, egg size is a poor predictor of energetic content when all data available from a broad range of echinoderms are considered (McEdward and Carson, 1987; McEdward and Coulter, 1987; McEdward and Morgan 2001). Despite this fact, large eggs are correlated with multiple life-history traits that, intuitively, should be reflections of increased egg energy. In

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particular, larger eggs produce larger larvae that develop more rapidly and reach metamorphosis sooner than larvae from smaller eggs. This presents an apparent contradiction which may be created in part by the diversity of methods used to measure or estimate "egg energy" (McEdward and Morgan, 2001), and in part by the fact that few studies have examined the biochemical correlates of egg size in a rigorous comparative framework. The identity and amount of different biochemical constituents that compose a "large egg," and the role that those constituents play during development, are poorly understood; thus, it has not yet been possible to draw clear, broad, functional connections between egg size and its many life-history correlates.

Another reason we know relatively little about the bottom-up forces influencing the evolution of egg size in marine invertebrates is that relatively few studies have focused on the genetic, morphological, physiological, or stochastic mechanisms that influence the size of eggs a mother produces. These mechanisms presumably drive much of the variation in egg size among individuals on which natural selection acts; likewise, they underlie the complex interactions between selective forces that act on egg size at different levels. Without a better understanding of these mechanisms, it will probably be difficult to improve or to empirically ground-truth models that have explored the evolution of egg size, or to predict how egg size is likely to play a role in species' adaptation to future environmental change.

A comprehensive review of many important egg-related ideas, "Echinoderm Larval Ecology Viewed from the Egg" (Emlet *et al.*), was published in 1987. Over the intervening two decades, considerable work has been done to advance the field. One such compilation of particular note was by Jaeckle (1995), who investigated correlates among size, biochemical composition, energy content, and developmental mode in invertebrate eggs (largely echinoderms); a few years later, Sewell and Manahan (2001) showed that adding data from 14 additional echinoderm species with a wide range of egg sizes to this dataset did not change the relationships described by Jaeckle (1995). The goal of our paper is to continue this conversation by examining the main trends and findings in egg composition and bottom-up factors regulating egg size, to point out remaining gaps in our understanding of how egg size functionally influences larval ecology and evolution, and to suggest some potentially valuable directions for future research.

We highlight two main types of questions and important directions for future work. First, under *Question 1* below, we discuss what constitutes an egg. More specifically, what are the biochemical constituents of an egg, and how is egg composition related to egg size? An egg is arguably the most influential cell in an animal's life history (Jaeckle, 1995), and the consequences of egg "size" for subsequent life-history characters appear to be many and strong. However, even standardized for size, eggs are clearly not all created equal among phyla, between developmental modes, between species, or even among individuals within a species. Biochemical compositions vary at all levels, and without quantifying this variation at as many levels as possible it is difficult to say what role size, as separate from composition, plays in larval ecology and life-history evolution. We review the main techniques that have been used to measure egg energy and biochemical composition, and suggest directions for future comparative research.

Second, from a bottom-up perspective, what determines egg size? What are the mechanisms, either genetic, physiological, or stochastic, that determine the size and composition of eggs that a mother produces under different environmental conditions? To what extent are these mechanisms shared among diverse and distantly related taxa? Under *Question 2* we give some examples of physiological and genetic mechanisms that may be at work in driving both innate variation in egg size and variation that occurs in response to environmental change. We also highlight the relative paucity of these data in the literature and provide some suggestions for future research directions.

Question 1: What Constitutes an Egg?

Or, what are the biochemical constituents of an egg, and how is egg composition related to egg size?

Egg size is a highly variable character, both between closely related species and among widely divergent taxa of marine invertebrates. For example, among species of echinoderms, which are perhaps the best-studied group in terms of egg size, egg volume varies by 5 orders of magnitude across taxa (Emlet *et al*., 1987). Variation in egg size among females of a single species is common as well, though intraspecific variation tends to occur on a much smaller scale (*e.g.,* McEdward and Carson, 1987; Jones e*t al.*, 1996; George, 1999; Marshall *et al*., 2000). Early and important models such as Vance's (1973a) time-fecundity hypothesis, which inspired a number of related models (*e.g.,* Podolsky and Strathmann, 1996; McEdward and Janies, 1997; Levitan, 2000; Miner *et al.,* 2005; and others reviewed in Strathmann, 1985; Havenhand, 1993), assume that a larger egg is energetically more costly for the parent to produce, and that the increased amount of energy in a larger egg functions to shorten the length of planktonic development by reducing the larva's dependence on exogenous food. The assumption that larger eggs contain more energy is largely borne out across species (*e.g.,* Emlet *et al.,* 1987; Jaeckle, 1995; McEdward and Morgan, 2001), although not always within species (McEdward and Carson, 1987; McEdward and Coulter, 1987). Although egg size appears to be at least a broadly accurate index for maternal investment, it is becoming increasingly clear that changes in size alone cannot account for all physiological, ecological, and evolutionary

correlates of egg size. To better understand the complex role of egg size in the life histories of marine invertebrates, we need to address more issues such as changes in biochemical composition of eggs with size; patterns of utilization of egg constituents by embryos, larvae, and juveniles; and energy expenditures and requirements of embryos and larvae.

One important issue for theoretical models that consider maternal investment, life-history evolution, and larval transport and performance in the field is to accurately describe the scaling of energy content with egg size for the given group of interest. Several methods, of varying complexity and difficulty, have been utilized to measure egg energy. Our goal here is to briefly review and differentiate between the more common methods, and to highlight some important considerations when selecting appropriate techniques.

The first methodological options to be considered, and sometimes the only options available, are the simplest and least expensive. One technique that has a very long history and has seen wide use for measuring energetic content of eggs and embryos is ash-free dry weight (AFDW or DOW, dry organic weight). Estimating AFDW is simple, but this method contains a number of potential sources of error. Sample preparation is one; salts are hygroscopic and, if not removed from the exterior of marine organisms prior to drying, can cause samples to gain weight after ashing (Moreno *et al.*, 2001), leading to an underestimate of AFDW. This phenomenon can be minimized by rinsing marine samples with ammonium formate (isotonic with seawater) or in some cases with distilled water to remove surface salts. Disturbingly, though, Moreno *et al.* (2001) found that some inorganic salts retain water at normal drying temperature (80 °C) but lose it at ashing temperatures (450 °C); thus, even in well-rinsed samples, water may be retained by intracellular salts in "dried" samples only to be burned away at 450 °C, leading to an overestimate of AFDW. Finally, AFDW measurements do not capture the differing energetic values of lipids, carbohydrates, and proteins (Podolsky, 2002), so AFDW may not be suitable for comparing samples in which biochemical composition is likely to differ. Despite these issues, the relative simplicity and low cost of this assay make it a valuable tool and have led to its widespread use in a number of systems.

Another method that has been employed by many authors (*e.g.,* McEdward *et al.,* 1988; McEdward and Chia, 1991; Moreno and Hoegh-Guldberg, 1999; Miner *et al.,* 2002; Zigler *et al.,* 2008) is the potassium dichromate wet oxidation method (PDWO) described in Parsons *et al.,* (1984). PDWO is a colorimetric method used to estimate total energetic content. Measurements of total energy *via* PDWO generally correlate tightly with AFDW measurements when the two methods are compared (Jaeckle, 1995; Moran, 1997). A recent study by Pernet and Jaeckle (2004) found a potentially serious drawback to using the PDWO method for comparative studies among species, though. These authors observed that the method gave consistently low estimates of AFDW-specific energy density in eggs of planktotrophic echinoderm and annelid species. This result may be due to the fact, pointed out by several other authors previously (*e.g.,* Paine, 1971; Crisp, 1984; Gosselin and Qian, 1999), that the PDWO method does not completely oxidize protein, and oxidizes some types of protein more completely than others. Because protein is a major biochemical constituent in all invertebrate eggs, PDWO will underestimate total energy content of any egg sample. This underestimation is a particular problem for comparing energy content across a broad range of egg sizes and taxa, because planktotrophic eggs tend to contain more protein per unit volume than lecithotrophic eggs (Jaeckle, 1995). Thus, the extent of this error is likely to be greater for species with small eggs and to vary with the type of protein present in different eggs (Pernet and Jaeckle, 2004).

A third option is direct calorimetry, in which samples are combusted in an enclosed chamber and the total energy contained in the samples is measured as heat production (bomb calorimetry). Because of the small amount of material generally available to larval biologists, either the Parr semi-microbomb (Paine, 1971) or the Phillipson microbomb (Phillipson, 1964) calorimeters are likely to be the most useful, and these are the most commonly used for the determination of caloric value in marine animals (Beukema, 1997). Both types operate on the same principles and are prone to several types of error. First, carbonate breakdown (an endothermic reaction forming CaO in the bomb chamber) can lead to substantial underestimates of caloric value in many types of marine tissue (Paine, 1964, 1971). The recommended way to avoid this issue is to separate calcium carbonate structures from soft tissues, which is feasible in adult organisms (Beukema, 1997) but substantially more difficult in marine larvae. Second, if energy content on a per-gram basis is desired, independent determinations of both ash content and dry weight must be obtained; errors in either will lead to over- or underestimating energy density of samples (discussed in Beukema, 1997). A third issue is that bomb calorimetry is somewhat exacting, requires fairly large tissue mass (generally >5 mg), and requires expensive and specialized equipment.

The strengths of both AFDW and PDWO are that they are relatively simple and inexpensive to perform (though the issues described above must be kept in mind), so despite potential drawbacks they can be used by a wide range of investigators under a wide range of circumstances. Bomb calorimetry provides direct information about caloric value of eggs, but requires specialized equipment and may not work well for later larval stages that contain difficult-to-remove calcium carbonate skeletons. In addition, investigators often want more information than simple egg energy alone, and neither AFDW, PDWO, nor bomb calorimetry

provides any direct information about the biochemical composition of eggs.

So how to accurately measure the energy content of eggs, while gaining more detailed information about energy storage and utilization? A fourth method is to individually measure the amounts of different biochemical constituents in eggs and sum their separate energetic values (*e.g.,* Turner and Lawrence, 1979; McClintock and Pearse, 1986; Moran and Manahan, 2003, 2004; Reitzel *et al.*, 2005; Byrne *et al.,* 2008). The three main constituents of interest in marine invertebrate eggs are protein, lipid, and carbohydrate, which are assayed separately using different suites of techniques. Numerous assays and measurement techniques are available for each type of biochemical constituent, and the choice of assay can be highly relevant. While we do not mean this to be a comprehensive review of the entire literature on proximal content analysis, we discuss below the most frequently utilized ways of measuring protein, lipid, and carbohydrate content of marine invertebrate eggs.

Protein

For measuring protein, many researchers turn to one of two colorimetric assays: the Bradford assay (Bradford, 1976; *e.g.,* Moran and Manahan, 2003; Bryan, 2004; Falkner *et al*., 2006) or a modified Lowry assay (Lowry *et al*., 1951; *e.g.,* Schiopu *et al.,* 2006; Prowse *et al*., 2008). Both assays are commercially available as kits, are relatively simple to use, and do not require highly specialized equipment beyond heating blocks and a spectrophotometer. They are also sensitive to microgram-level concentrations of protein, which can be essential when only a limited amount of tissue is available, as in many situations working with eggs of marine invertebrates. However, there are well-known artifacts associated with both methods (the Bradford in particular) that investigators should be aware of. When direct comparisons of the two assays are made on the same material, the Bradford assay generally gives substantially lower estimates of total protein; in marine invertebrates, this has been demonstrated in eggs and larvae of oysters, in which the difference in protein estimated by the Lowry *versus* Bradford methods reached almost 400% (Chu and Casey, 1986). The differences in protein detection between the two methods are likely attributable to underestimation of protein by the Bradford assay (Chu and Casey, 1986). The Bradford dye reagent does not detect all proteins equally but reacts primarily with arginine residues, and is less sensitive for basic or acidic proteins; this may cause an underestimation of quantity in complex combinations of proteins (Stoschek, 1990). In addition, choice of standard inevitably affects estimates of protein, particularly if the standard reacts differently with the dye reagent than with sample proteins. One very commonly used standard is bovine serum albumen (BSA). The Bradford assay is considerably more sensitive to BSA than to "average" proteins (Stoschek, 1990), so using this common standard in assaying complex mixes of egg, embryo, and larval proteins with the Bradford assay may add to underestimates of total protein. Further, if protein composition changes during development from egg to larva to juvenile, the sensitivity of the Bradford assay may change correspondingly.

Ideally, the standard for any protein assay should be purified protein from the samples of interest; this point has been made many times in different invertebrate groups (*e.g.,* Zamer *et al*., 1989), but is rarely feasible in studies involving invertebrate eggs and larvae. It may be advisable to compare results from multiple methods and standards (such as using both bovine gamma globulin (BGG) and BSA as standards for the Bradford assay; Stoschek, 1990) to detect bias. Of the two assays discussed here, the Lowry method may be preferable to the Bradford for invertebrate egg and larval protein assays because it provides higher (and likely more accurate) estimates of total protein and is not as greatly affected by protein composition or choice of standard (Chiappelli *et al*., 1979; Chu and Casey, 1986). However, both methods may be insensitive to small peptides and amino acids (Mayer *et al*., 1986; Crossman *et al.*, 2000). Depending on the concentration of these small molecules in experimental samples, total protein may be underestimated regardless of which method is used.

If strong bias or inaccuracy is suspected, this should provide motivation to change methods, or to proceed to more complex quantitative techniques such as the stoichiometric elemental CHN method (Gnaiger and Bitterlich, 1984; *e.g.,* Chaporro *et al*., 2006) or quantitative amino acid analysis (*e.g.,* Crossman *et al.,* 2000). These latter methods are considerably more time-consuming, expensive, difficult, and equipment-intensive than colorimetric assays such as the Bradford or Lowry. Appropriate choices of standards and assays can greatly reduce the degree of error in the simpler colorimetric methods and conserve their value as tools for understanding the composition of eggs and energetics of embryonic and larval development.

Lipid

There has been a long discussion in the literature about how best to estimate total lipids in marine samples (*e.g.,* Lovern, 1964; Giese, 1966; Marsh and Weinstein, 1966; Knight *et al*., 1972; Barnes and Blackstock, 1973; Smedes, 1999; Iverson *et al*., 2001). Our goal here is not to review this extensive literature but to briefly describe the most commonly used methods for studying the role of lipids in marine invertebrate larval life histories, and to call attention once again to the important caveats that must be considered with each method.

The simplest, and on the surface most straightforward, method for estimating total lipids in a sample is to extract

and weigh them. In these gravimetric analyses, lipids are extracted from the total sample, usually with a water-methanol-chloroform mixture in a particular ratio (*e.g.,* Folch *et al*., 1957; Bligh and Dyer, 1959), followed by drying and weighing on a microbalance (*e.g.,* Jones *et al.* 1996). Gravimetry is relatively simple, but it is only as precise and accurate as the equipment available for weighing, and can be affected by the composition of samples (Inouye and Lotufo, 2006). Overall the gravimetric method appears to be most accurate when large amounts of tissue are available for analysis (>5 g; Honeycutt *et al.*, 1995), which reduces the potential impact of error in either direction. With marine invertebrate eggs and larvae, large masses of tissue may not be available, so more sensitive colorimetric methods are often preferred.

Two colorimetric methods that have been used for measuring invertebrate egg and larval lipids are the sulfuric acid charring method of Marsh and Weinstein (1966) (*e.g.,* Holland and Gabbott, 1971; Thiyagarajan and Qian, 2003) and the sulphophosphovanillin method of Chabrol and Charonnat (1937) with subsequent modifications (*e.g.,* Barnes and Blackstock, 1973; Achituv *et al.*, 1980). Both are relatively simple, highly sensitive, and inexpensive to perform; but, as with colorimetric protein assays, the composition of samples and the choice of standard will affect the quantitative results. The sulfuric acid charring method, for example, is slightly more sensitive to saturated than to unsaturated lipids, though this is not generally a major concern (Marsh and Weinstein, 1966).

The sulphophosphovanillin method has been used mostly in the vertebrate and insect literature. It too can be reasonably accurate if the correct standards are used, but the colorimetric reaction in this assay requires a carbon-carbon double bond, so lipid classes that lack these bonds, such as triacylglycerols and saturated fatty acids, do not cause a color change (Knight *et al*., 1972). If the relative proportions of different lipids in a sample are known, then an appropriate correction factor can be calculated and applied; however, when lipid composition is unknown, the sulphophosphovanillin method will likely provide inaccurate estimates of total lipid. A case in point: triacylglycerol appears to be the primary storage lipid in most marine invertebrate eggs and embryos and in many cases serves as the primary source of energy during morphogenesis (Holland, 1978; Moran and Manahan, 2003, 2004; Byrne *et al.*, 2008). An assay that is not sensitive to fully saturated lipid classes may fail to detect important physiological and evolutionary changes in egg and larval energetics. Another issue with this assay is that its efficacy will vary for animals that are acclimated to different environmental temperatures, because lipid saturation levels in ectotherms change with temperature (Weber *et al.*, 2003). Thus, the sulphophosphovanillin technique should be approached with caution for use with seasonal or latitudinal comparative studies.

While both gravimetric and colorimetric analyses are useful for determining total lipid (with appropriate precautions for each method), they cannot separate and quantify individual lipid classes such as neutral lipids and phospholipids. Because different lipid classes play different biochemical and energetic roles in embryogenesis and larval development, many researchers are interested in quantifying these separate classes in eggs and tracking them over development. Lipid classes and fatty acids can be identified using chromatography (*e.g.,* thin layer chromatography (TLC), gas liquid chromatography (GLC)). Examples from the marine invertebrate egg and larval literature include Villinski *et al.* (2002) for TLC, and Schiopu *et al.* (2006) for GLC. Unless a mechanism for quantification is integrated into the chromatography system, however, lipids must still be independently quantified, for example by gravimetry (*e.g.,* Villinski *et al.* 2002). A system that simultaneously identifies and quantifies separate lipid classes, and one that has been used with increasing frequency in the marine literature (*e.g.,* Moran and Manahan, 2004; Steer *et al.,* 2004; Sewell, 2005; Prowse *et al.*, 2008), is flame ionization detection thin layer chromatography (FID-TLC) (methods summarized in Parrish and Ackman, 1983; Parrish, 1987, 1999). These chromatographic methods require considerably more specialized equipment and expertise than gravimetric or colorimetric methods alone, and are liable to their own types of error. However, they also provide considerably more detail on lipid composition and the roles of different lipid classes in developmental energetics than colorimetric or gravimetric methods for estimating total lipid.

Carbohydrate

Carbohydrate makes up the smallest percentage by mass of the three major biochemical constituents, generally constituting less than 3% of eggs and larvae (Jaeckle, 1995). Because carbohydrate is such a minor component of total energy, many studies focus only on quantifying lipid and protein (*e.g.,* Prowse *et al.*, 2008). When information on total carbohydrate is desired, varied techniques have been employed, including the ferricyanate reduction reaction of Folin and Malmros (1929) as modified by Holland and Gabbott (1971) (*e.g.,* Moran and Manahan, 2003), and the phenol-sulphuric acid procedure of Dubois *et al.* (1956) (*e.g.,* Chaporro *et al.*, 2006). Many of the same issues discussed for protein and lipid, such as differential sensitivity to specific biochemical-constituent classes and appropriate choice of standards, also apply to colorimetric assays of carbohydrate. Because carbohydrate is generally considered a minor energetic constituent, however, even relatively large errors in calculating total carbohydrate are unlikely to substantially affect calculations of total energy budgets, so we will not go into these in detail.

Extraction issues

The accuracy of all these methods for assessing biochemical composition also hinges, in part, on how effectively the constituent of interest is first extracted and purified. Again, we do not aim to review the extensive biochemical literature on protein, lipid, and carbohydrate extraction and purification, but we would like to call attention to one example of how lipid extraction techniques may affect quantitative estimates of lipid through development. Multiple studies have compared the extraction efficiencies of different solvent concentrations for marine (and other) samples (*e.g.,* Smedes and Thomasen, 1996; Smedes and Askland, 1999; Smedes, 1999; Iverson *et al.*, 2001). The extraction protocols of Bligh and Dyer (1959), often as modified by Holland and Gabbott (1971), are widely used for analyzing samples of marine eggs and larvae (*e.g.,* George, 1999; Morais *et al.*, 2002; Moran and Manahan, 2003; Ritar *et al.,* 2003; Lee *et al*., 2005). One topic of particular interest to scientists working with marine egg and larval samples was raised by Iverson *et al.* (2001), who found that the extraction method of Bligh and Dyer (1959) underestimated lipid content in samples containing more than 2% lipid compared to the extraction methods of Folch *et al.* (1957). The difference was substantial; total lipids extracted *via* the Bligh and Dyer method were 50% lower than those measured by the Folch *et al.* method in marine samples containing 26% lipid (the highest lipid percentage tested). This difference was attributed to under-extraction by the Bligh and Dyer method (Iverson *et al*., 2001).

Because marine invertebrate eggs contain energy necessary for morphogenesis and development, even small eggs of planktotrophic species tend to contain around 20% lipid (*e.g.,* for oysters, Moran and Manahan, 2004). Large eggs of lecithotrophic echinoids can be well over half lipid by weight (*e.g.,* McClintock and Pearse, 1986; Hoegh-Guldberg and Emlet, 1997; Byrne *et al*., 2008). If the findings of Iverson *et al.* (2001) are broadly applicable to marine invertebrate samples, there seems to be potential for substantially underestimating lipid content through using the Bligh and Dyer (1959) method, unmodified, for assaying marine eggs. It is often difficult to determine precise extraction methods from the literature because modifications to either Bligh and Dyer (1959) or Folch *et al.* (1957), though invoked in methods sections, are often not described in detail (Iverson *et al*., 2001). Hypothetically, however, this type of systematic underestimation due to poor extraction efficiency could go far toward explaining the average 20% of dry mass that is not recovered by biochemical constituent analysis—the "remainder fraction" of Jaeckle and Manahan (1989) and Jaeckle (1995)—and could potentially confound energetic analyses as well.

General patterns

Overall, the use of biochemical constituent analysis has expanded our understanding of egg composition and energy utilization during development of marine invertebrate larvae. As we have pointed out, the methods utilized in the literature are many and diverse, and prone to various types of error. In many instances, despite established drawbacks, a particular method (*e.g.,* AFDW) may be the most desirable because it allows for direct comparisons with previous work on the same questions or taxa. Ideally, however, selection of a "best" method for extraction and quantification in any project will involve not only comparative considerations and the availability of expertise, supplies, and equipment for a particular method, but also an understanding of the accuracy and limitations of different methods, including—but not limited to—those laid out above.

The previous section was intended to highlight some of the more widely used methods and to summarize their strengths and weaknesses, with the goal of providing a general guide for those considering biochemical analysis. But drawing broad comparative inferences about egg size and composition from the literature is a difficult venture, for several reasons. First, biochemical studies relating egg size to composition are relatively rare; second, the majority of studies have focused on a single group, echinoderms; and third, the techniques that have been employed are varied and numerous. Despite these issues, some patterns have begun to emerge. In his 1995 review, Jaeckle combined information from studies that utilized DOM, PDWO, and biochemical constituent analysis to pull out broad patterns for echinoderms, and found a common scaling pattern of egg energy with size across five orders of magnitude in size and among diverse developmental modes. Eggs of lecithotrophic species had greater mass-specific energy contents than eggs of planktotrophic species (see fig. 5 in Jaeckle, 1995), which was attributed to the proportionally higher lipid content of lecithotrophic eggs.

More recent studies, for example by Sewell and Manahan (2001) and Prowse *et al.* (2008), have tended to uphold these general patterns. In a comparative study of asterinid sea stars, Prowse *et al.* (2008) found that the evolution of lecithotrophic development was associated with a reduction in protein deposition and an increase in triacylglycerol (an energetic lipid class). The one exception to this pattern was a lecithotrophic species with benthic development, which had a protein/lipid ratio similar to that of planktotrophs (presumably to reduce buoyancy; Prowse *et al.,* 2008). Thus, at least in this group, the evolutionary loss of planktotrophy was apparently accompanied by an increase in egg size that was in most cases largely attributable to vitellogenic processes that increased deposition of triacylglycerol. Comparative data for taxa other than echinoderms are largely lacking, so whether this is a general phenomenon

that holds across many invertebrate groups remains to be seen.

While the data indicate that size matters in the sense that lecithotrophic species appear to have eggs that are compositionally different from those of planktotrophs, we have yet to understand how evolutionary changes in egg size that occur within developmental modes (*e.g.,* within planktotrophy) are associated with changes in biochemical composition. Additional comparative biochemical studies of nonechinoderm taxa that contain species with a wide range of egg sizes and are well-understood phylogenetically are necessary; examples of candidate taxa for whom phylogeny, developmental mode, and egg size have already been largely determined are the calyptraeid gastropods (Collin, 2004) and arcid bivalves (Marko and Moran, 2002). Further insights could also be gained from comparative biochemical work on closely related species that inhabit different habitats, such as geminate species pairs in tropical America. In many invertebrate taxa the Pacific member of planktotrophic species pairs has smaller eggs than its Caribbean geminate species (*e.g.,* Lessios, 1990; Moran, 2004), but it is not known whether these evolutionary changes in egg size are also associated with changes in biochemical composition that might reflect differing energetic strategies in the two oceans.

Finally, one additional avenue that has yet to be explored is comparing egg composition in those rare species that show flexibility in developmental mode, a life-history mode known as poecilogony. One example of a poecilogonous species is the gastropod *Alderia willowi,* which produces either small planktotrophic larvae or large larvae that do not need to feed in order to metamorphose. Nonfeeding larvae develop from eggs that are 105 $\pm$ 5 μm in diameter while planktotrophic larvae develop from much smaller eggs (68 $\pm$ 4 μm diameter), and individuals can vary the developmental mode of offspring they produce (Krug, 2007). Because *A. willowi* shows such strong and unusual plasticity in egg size and developmental mode, working with this species could shed substantial light on how a change in developmental mode is, or is not, accompanied by changes to egg biochemical composition, larval energetics, and vitellogenic processes.

Question 2: What Determines Egg Size?

Or, what environmental and genetic factors affect egg size, and what physiological mechanisms underlie egg size variation?

Variation in phenotypic traits such as egg size is generally attributed to one or a combination of three factors: environmental influences (environment), genetic differences among organisms (genotype), and stochastic developmental traits (Vogt *et al*., 2008). As we have discussed, egg size is a highly variable trait both within and among species of marine invertebrates; however, the relative importance of these three factors in determining egg size, and thus in shaping the evolution of larval life histories, has not, to our knowledge, been thoroughly explored in any marine invertebrate. We have some understanding of how environmental factors affect egg size in a handful of taxa, but these findings are often not consistent across taxa. The genetic basis for egg size variation has rarely been explored in marine animals, and therefore we have very little understanding of how egg size is inherited or how genotype and environment interact to determine egg size at a particular spawning event. Finally, while stochastic developmental events can play a large role in determining variation of many phenotypic traits (*e.g.,* Lajus and Alekseev, 2004), we know virtually nothing about the extent to which egg size variation in marine invertebrates may be underlain by such "developmental noise," if at all. Our goal here is to highlight cases-in-point from the recent literature on determinants of egg size, and to point to areas where further work would be of great value.

Temperature, salinity, and toxicants

One of the most frequently noted environmental factors that affects egg size in marine invertebrates is temperature. Temperature has a well-documented negative correlation with egg size in many marine taxa, such that both within and among species, animals that live at colder temperatures often produce larger eggs (Rass, 1941; Clark, 1992; Hoegh-Guldberg and Pearse, 1995; Levitan, 2000; Peck *et al.,* 2007). Some of these temperature-size relationships can be attributed to evolutionary shifts in developmental mode or to different adaptive strategies associated with latitude (*e.g.,* Rass, 1941; Thorson, 1950; Marshall, 1953; Mileykovsky, 1971; Alekseev, 1981; Laptikhovsky, 2006; Lardies *et al*., 2008). However, a substantial component may also be due to a direct physiological response in which the temperature experienced by a female during oogenesis influences the size of eggs she produces. This phenomenon has been observed for cell (including egg) size (see Azevedo *et al.*, 1997, for review) and for body size as well in numerous ectotherm model systems; thus, when egg size changes inversely with temperature, this can be considered part of the size-temperature rule of Rass (1941; Laptikhovsky, 2006). The physiological bases for this pattern are not understood but may include, for egg size, lower temperature sensitivity of vitellogenesis compared to oocyte production rate and egg maturation time (Steigenga and Fischer, 2007). This hypothesis is based on a biophysical model that assumes different temperature constraints on growth (*i.e.,* vitellogenesis), which depends on the rate of protein synthesis, and on differentiation (*i.e.,* oocyte production), which depends on the rate of DNA replication (van der Have and de Jong, 1996).

A handful of authors have experimentally examined the effects of temperature on egg size in marine invertebrates (Dugan *et al.*, 1991; Simonini and Prevedelli, 2003; Steer *et al.*, 2004). Simonini and Prevedelli (2003) reared individuals from two geographically separated populations of the polychaete *Dinophilus gyrociliatus* at a broad range of temperatures and found that the smallest eggs were produced by individuals reared at the highest temperatures; their analysis revealed a significant effect of temperature on egg size. However, in the case of the cephalopod *Euprymna tasmanica* (Steer *et al*., 2004), laboratory rearing temperatures representative of tidal sandflat temperatures in austral summer (18 °C) and winter (11 °C) did not appear to affect egg size. Similarly, Dugan *et al.* (1991) found no correlation between egg size and water temperature among northern and southern populations of a sand crab, *Emerita analoga*, collected from the California coast. More experimental work is necessary to determine how broadly distributed the temperature-size pattern is in a wider variety of marine invertebrate taxa, and whether the effects of temperature on egg size are underlain by similar mechanisms in disparate groups. These are important questions: if physiologically driven plasticity in egg size is heritable, and if temperature-driven, plastic changes to egg size impact larval life histories in predictable ways, then a change of environmental temperature could represent a tipping point that drives or facilitates an evolutionary change to a different developmental mode (Laptikhovsky, 2006).

Salinity is a second environmental factor that has been linked to changes in egg size, though a clear adaptive function of such salinity-driven changes is lacking. Gimenez and Anger (2001) demonstrated that estuarine crabs, *Chasmagnathus granulata*, held at a pre-hatching salinity of 15 ppt laid eggs that were larger than those from crabs held at 32 ppt. A similar result was obtained by Skadsheim (1989) for the amphipod *Gammarus salinus*; adults reared in lower salinities produced eggs with greater volumes than did individuals reared in higher salinities. This salinity-driven plasticity in egg size is likely to be due simply to osmotic water uptake by eggs in low-salinity water, since the brooded eggs of amphipods osmoconform (Vlasbloom and Bolier, 1971). In free-spawners, however, an osmotically driven increase in egg size might provide an energetically inexpensive strategy for increasing the sperm target size of eggs, though we know of no studies that have examined this question.

A third environmental factor that has been demonstrated to influence egg size is the presence of certain chemicals in the environment during oogenesis. Of particular note is the effect of maternal exposure to natural or anthropogenic toxicants. Lardies *et al*. (2008) demonstrated an association between maternal exposure to copper mine tailings and significantly lower than expected egg volumes in a Chilean population of the snapping shrimp *Betaeus emarginatus*. As another example, Cox and Ward (2002) showed that individuals of the broadcast-spawning coral *Montipora capitata* reared in ammonium-enriched experimental tanks produced eggs that were significantly smaller in diameter than control individuals, whereas total fecundity, egg number, chlorophyll *a* content, and fertilization success were not affected. In both of these examples, egg size decreased in response to the presence of anthropogenic pollution, likely reflecting an organismal stress response. While no mechanistic explanation was proposed for the decrease in egg size seen in response to ammonia, Lardies *et al*. (2008) proposed that an oxidative stress condition, which develops in some marine species when exposed to excesses of copper, may have forced individuals of *B. emarginatus* to redirect energetic investment from activities such as oogenesis towards antioxidant mechanisms. Is there a general stress response that results in the production of smaller, fewer, or lower quality eggs in females that are exposed to toxicants or other stressors? If so, we may be able to make broad predictions about how environmental quality (from the perspective of the female) drives carryover effects that reduce the fitness of offspring across marine taxa. Knowing more about the mechanistic bases for the relationship between particular anthropogenic toxicants and how these toxicants affect egg size in a wider variety of taxa would be valuable information for environmental management.

Maternal age, size, and condition

Environmental factors that are experienced by the mother and that have resultant effects on the phenotype and performance of her offspring are termed maternal effects (see Marshall *et al.*, 2008, for a recent review of this topic). Egg size has been demonstrated to be affected by several maternal effects, including maternal age, maternal size, and maternal nutrition, which we highlight individually below.

Egg size has been demonstrated to be affected by maternal age in a wide range of taxa (Wiklund and Persson, 1983; Cavers and Steel, 1984; Kane and Cavers, 1992; Ruohomaki *et al.,* 1993; Braby, 1994). In at least some groups of marine invertebrates, egg size decreases with advancing maternal age (Qian and Chia, 1992; Ito, 1997). For example, Qian and Chia (1992) investigated the effects of aging on multiple correlates of reproduction in the marine polychaete *Capitella* sp. In laboratory-reared individuals, egg size, as well as fecundity, egg energy content, total egg volume, and total energy investment, decreased significantly with advancing maternal age and in successive spawns. The mechanisms underlying a decrease in egg size with maternal age are not known, but this pattern is more prevalent in animals with short life spans, determinate growth, and a single reproductive season and may be attributable to a decrease in maternal condition with age. For example, Ito (1997) showed that egg size and the number of eggs spawned by

the sea slug *Haloa japonica* decreased over the course of the breeding season under laboratory conditions. Similar patterns have been found in other molluscs (Macginitie, 1934; Thompson, 1958; Gibson and Chia, 1995; Steer *et al*., 2004). With respect to *H. japonica*, the decrease in egg size and number may reflect the fact that this species feeds very little after the onset of the reproductive season. If egg size reflects maternal investment per ovum, then females likely have decreasing amounts of organic material to package into eggs with each successive spawn. Similarly, decreases in egg size with successive spawns have also been noted for the nudibranch mollusc *Adalaria proxima* (Jones *et al*., 1996), which dies after its single spawning season. In contrast, in a long-lived animal, *Haliotis laevigata*, the greenlip abalone, successive spawns did not lead to differences in the average egg diameters, although egg diameter was more variable with each successive spawn in a hatchery system (Graham *et al*., 2006). Despite no change in egg size, the density of protein and lipid in eggs of *H. laevigata* increased throughout the spawning season (Fukuzawa *et al*., 2005): although eggs were of the same size, later-spawned eggs represented higher maternal investment per offspring. This result may reflect either (1) a differential release of eggs throughout the spawning season, such that some eggs are held for longer periods and subsequently packaged more densely with protein and lipid, or (2) a ramping-up in the efficiency of egg production mechanisms over time, such that later spawned eggs are produced at higher quality than earlier spawned eggs. Comparative studies that examine temporal changes in oogenic mechanisms among wild populations will help us to understand the relationship between egg size and maternal age.

Egg size can in some cases be positively correlated with maternal size, with larger mothers producing larger eggs (George, 1994; Chester, 1996; Ito, 1997; Marshall *et al*., 2000; Bingham *et al*., 2004). For example, Marshall *et al*. (2000) demonstrated that large *Pyura stolonifera* tunicates produced larger eggs than smaller individuals. This pattern held for tunicates from two different populations, although the relationship between maternal body size and egg size differed between sites—that is, the slopes of the regression of egg to body size was different for the two populations. Egg size may be affected by maternal size for the relatively straightforward reason that maternal size reflects the amount of resources a given mother has available for reproduction (George, 1994); this assumes, however, that egg size is determined by maternal resource availability, which may not always be the case (see Meidel *et al.*, 1999; Diaz *et al.*, 2003).

Egg size and maternal investment per egg can also be affected by the availability and quality of the maternal food supply (George, 1990, 1994; George *et al*., 1990, 1991; de Jong-Westman *et al*., 1995; Guisande and Harris, 1995; Pond *et al.*, 1996; Bertram and Strathmann, 1998; Steer *et al*., 2004). As one example, starved individuals of the estuarine nudibranch *Tenellia adspersa* produced eggs that were as much as 26% smaller in volume and 40% lighter in weight than eggs of well-fed individuals (Chester, 1996). Additionally, seastars from sites of low food quality produced larger eggs when food ration was experimentally increased, whereas stars from sites of high food quality did not (reviewed by George, 1996), suggesting that in the field, mothers were physiologically responding to low food by producing smaller eggs. These changes in egg size likely represent maternal nutritional stress rather than an adaptive alteration in size (Bertram and Strathmann, 1998), though the physiological mechanisms underlying stress-related changes in egg size are not known. Lastly, data collected from the eggs of multiple fish species indicate that the nutritional state of females affects the water content, buoyancy, and hence size of eggs (Craik and Harvey, 1987; Kjesbu *et al*., 1991; Nissling *et al*., 1994). It seems possible that similar patterns may exist among marine invertebrates, though this has yet to be tested.

Although these studies demonstrated that egg size can be affected by maternal nutrition, few studies have separated the effects of maternal nutrition on egg size *versus* on egg composition, and then followed by linking these to larval performance. Some exceptional studies in the aquaculture literature have put all of these pieces together, but the emerging picture does not necessarily indicate that egg size is a highly relevant parameter in predicting larval performance. Buchal *et al.* (1998) reared adult red abalone (*Haliotis rufescens*) on two diets and found that on the "superior" diet, mothers produced eggs that had higher dry weight and greater lipid and protein contents than did mothers kept on the "inferior" diet. Larvae from eggs produced by mothers on the superior diet went on to have higher hatching and metamorphic success. However, there was no difference in egg size between maternal food treatments. Thus, in this case at least, egg size was not a good indicator of either egg energy content or the subsequent performance of embryos and larvae.

Clearly the size and age of the mother, her nutritional history, and the number of successive spawning events she experiences in a given season are all interrelated factors that can have some bearing on the size of eggs she can produce. What is unknown is whether there are interactive effects of two or more of these factors on egg size. Take, for example, the question of whether egg size is distinctly correlated with the interaction of maternal size and nutritional condition: George (1994) found a positive relationship between maternal size and egg size in the sea star *Leptasterias epichlora*, but speculated that the effects of maternal size in this case may have been confounded with maternal nutrition because the larger mothers were from an environment with a higher food supply. Similarly, Chester (1996) demonstrated a correlation between starvation and a decrease in

adult size that was associated with a decrease in egg size. Starved individuals of the nudibranch *Tenellia adspersa* produced smaller eggs than fed individuals produced. In these two studies it is not clear whether maternal size, maternal nutritional condition, or some combination of the two was the primary determinant of changes in egg size. The interrelatedness of these maternal effects suggests that there may be common mechanistic underpinnings for their effects on egg size. Because maternal size, maternal age, and maternal condition are often confounded both in nature and in experimental work, it may be difficult to tease apart any independent effects these factors have on egg size. What does seem clear is that females that are "stressed," either by limited resources, advancing age, or physical and chemical stressors in the environment, in some cases respond by producing smaller eggs. What is not clear is how egg size is *mechanistically* affected by these stressors, and to what extent different stressors affect egg size by the same or different mechanisms. For these reasons, future studies that can effectively isolate the effects of multiple maternal factors on egg size in a single system would be valuable.

Population density

In a single instance, egg size amongst females has been shown to differ depending on adult population density. Crean and Marshall (2008) demonstrated in field experiments that eggs produced by *Styela plicata* females reared at low densities were 5% smaller in area than eggs produced by females reared at high density. However, these smaller eggs had a larger area of accessory structures (*i.e.,* follicle cells), such that the overall "target size" for fertilization by sperm was 9% larger for low-density individuals. This small proportional change may represent an energetic tradeoff between investment in eggs and investment in accessory structures such as follicle cells (Crean and Marshall, 2008), suggesting that in this species, plastic responses that increase sperm target size in sperm-limiting environments may limit the resources available to females for producing large eggs. This phenomenon, while currently noted in only one species of ascidian, might also be found in other taxa that utilize egg accessory structures, such as jelly coats, which can increase the effective target size for sperm.

Genetic factors

Evolution by natural selection requires both variation in a trait and genetic heritability of that variation. As we have discussed above, a considerable amount of variation in egg size can be attributed at least in part to the environment, but the component of variation that is underlain by genetic (and hence heritable) factors has been examined in only a few marine invertebrates. Phenotypic variation (V_P) in a character such as egg size can be partitioned into variation due to genetics (V_G), environment (V_E), and their interaction (V_{GxE}). One component of V_G accounts for parent-offspring resemblance and is known as additive genetic variance (V_A). Narrow-sense heritability (h^2) is a measure of the proportion of V_P that is due to V_A where $h^2 = V_A/V_P$, and is a predictor of the short-term response to selection (Falconer and Mackay, 1996). A recent study by Miles *et al.* (2007) showed that egg size of the polychaete *Hydroides elegans* was a heritable character: in response to artificial selection, egg size increased significantly in as little as four generations in their experiments ($h^2 = 0.58$). A half-sib breeding design conducted on the same population showed a similar estimate of narrow-sense heritability ($h^2 = 0.45$). Similarly high heritability estimates were obtained by Levin *et al.* (1991) for egg diameter ($h^2 = 0.75$) in the poecilogonous polychaete *Streblospio benedicti*, and by Arcos *et al.* (2005) for oocyte diameter ($h^2 = 0.57$) in the shrimp *Litopenaeus vannamei*. Also, a study by Hilbish *et al.* (1993) found a comparable heritability estimate of $h^2 = 0.58$ for the shell length of prodissoconch I in the hard clam *Mercenaria mercenaria*. Prodissoconch I length is tightly correlated with egg size in *M. mercenaria* because it is formed early in development directly around the egg yolk mass (Goodsell and Eversole, 1992). Additionally, a recent study by Diz *et al.* (2009), which utilized proteomic and bioinformatic techniques to assess genetic variation underlying protein expression in eggs of the bivalve *Mytilus edulis*, found a broad-sense heritability ($H^2 = V_G/V_P$) of gene expression for the egg proteome at about 50% or greater. Although the results of Diz *et al.* (2009) do not pertain specifically to egg size, their study does highlight the possibility that new suites of techniques may be useful in investigating the bottom-up mechanisms underlying egg size variation on many levels of inquiry. The results from these studies suggest that a significant portion of the variability in egg size can be attributed to genetic differences between individuals, and that egg size may be able to evolve rapidly under the appropriate selective regime. Aside from polychaetes and commercially important shellfish, though, few marine invertebrates are tractable model systems for narrow-sense heritability studies due to long generation times and complex rearing requirements.

Even in the presence of strongly heritable variation in egg size among individuals, the evolution of egg size may be limited by phylogenetic constraints (Derrickson and Ricklefs, 1988; McKitrick, 1993); these are life-history tradeoffs, physiological characteristics, and selective pressures unique to different taxonomic groups that may result in limitations on the extent of evolutionary changes in egg size (Lessios, 1990). Such phylogenetic effects were invoked by Lessios (1990), who measured egg diameters and calculated egg volume of 24 echinoderm species found in coastal waters off the Pacific and Atlantic (Caribbean) coasts of Panama. Of these species, 14 belong to geminate pairs. In 6 of 7 geminate pairs, the eggs of the Pacific species were

smaller than the eggs of its Atlantic counterpart, demonstrating six independent evolutionary changes in egg size in the same direction. Despite the fact that the eggs from Pacific species within any given pairing were smaller than their Atlantic geminates, there was no common egg size for a given ocean; rather, in all cases egg sizes of closely related animals were more similar than those of distantly related ones. This result suggests that the optimal egg size for a given environment is not independent of phylogenetic history. In contrast to the similarity in egg sizes found by Lessios (1990) for closely related geminate pair echinoids, Levitan (2006) noted that, in fact, egg size often varies considerably among closely related species of echinoids.

The degree to which egg size is free to evolve in response to natural selection is likely to depend largely on both the genetic and physiological mechanisms by which eggs are made. Research concerning specific genes or gene networks that affect egg size are limited. We do know that in echinoid echinoderms, oogenesis is largely a two-step process consisting of an initial deposition of yolk protein (vitellogenesis), followed by deposition of lipids (lipogenesis) and non-vitellogenic proteins (Byrne *et al*., 1999). Yolk protein is deposited during vitellogenesis by activation of the vitellogenin gene (Shyu *et al*., 1986; Scott *et al*., 1990), and the levels of activation appear to be conserved at least between congeneric species of *Heliocidaris* that vary in egg size and developmental mode (Byrne *et al*., 1999).

The differences in egg size between *Heliocidaris* spp. are largely attributable, therefore, to the amount of lipid and non-vitellogenic proteins that are deposited subsequent to vitellogenesis. The specific genes that are activated to provision eggs with these components are unknown; however, the temporal separation between vitellogenesis and lipogenesis suggests that these processes may represent separate developmental modules (Raff, 1996; Byrne *et al*., 1999). Prowse *et al.* (2008) suggest that elaboration of this aspect of oogenesis may be associated with the evolution of developmental mode, and that increases in egg size due to hypertrophic lipogenesis may transform a species that develops as a facultative planktotroph into a lecithotroph. Similar comparative approaches using taxa beyond echinoderms would contribute substantially to our understanding both of the similarities and differences among oogenic mechanisms in disparate invertebrate taxa and their relationship to egg size, and of the extent to which evolutionary changes in developmental mode (and egg size) are underlain by particular aspects of the oogenic pathway. In addition to comparative approaches, examining oogenesis and gene expression in poecilogenous species, such as the polychaete *Streblospio benedicti* (Levin *et al.* 1991) or molluscs in the genus *Alderia* (Krug, 2007), would also be likely to provide valuable additional insights into the mechanisms underlying egg size evolution as it relates to changes in larval developmental mode.

Conclusions

Egg size is one of the most often-studied aspects of the life history of marine organisms, and considerable comparative and theoretical work has explored its many life-history correlates (see references in Introduction). Egg size is a simple character to measure, has a long history in the marine literature, and is closely related to developmental mode in many phyla. Because of its strong correlation with developmental mode (at least within large taxonomic groupings), at a large scale egg size can be a powerful predictor of dispersal potential, and even of geographic range and geological longevity of species (Hansen, 1978, 1980; Jablonski and Lutz, 1983; Bhaud, 1993; Emlet, 1995; Jeffery and Emlet, 2003). Egg size is also broadly correlated with energy content (Jaeckle, 1995) as well as with many energy-related larval life-history characters such as length of larval development (Thorson, 1950; Vance, 1973a, b; Strathmann, 1985; Hadfield and Miller, 1987; Sinervo and McEdward, 1988; Wray and Raff, 1991), larval form (McEdward, 1986; Strathmann, 2000), length of the facultative feeding period (Miner *et al*., 2005), juvenile growth and survival (Marshall *et al*., 2003), and dependence on exogenous food (Anger, 1995; Bridges and Heppell, 1996). For these important reasons, egg size has long been, and will continue to be, a valuable tool for understanding the life histories of marine taxa.

However, there are two ways in which egg size may not be all it's cracked up to be. First, it is important to keep in mind that among most life-history studies that examine egg size evolution, the character that is measured—egg diameter or volume— is likely not the actual target of selection. In the majority of studies dealing with larval development and energetics, differences in "egg size" serve as a convenient, though coarse (McEdward and Morgan, 2001), surrogate for energy reserves provided by the mother to the embryo, larva, or juvenile. A finer-scale understanding of not just the size but the composition of eggs can provide considerably more insight into the selective forces acting on eggs and early life-history stages. For example, Prowse *et al.* (2008) found that loss of planktotrophy in asterinid sea stars was associated with a reduction in protein deposition (protein density was greater in planktotrophic than lecithotrophic species) and an increase of energetic lipids in the egg, presumably reflecting selection for rapid, nonfeeding development and reduction in unneeded larval feeding structures. One lecithotrophic species, however, had a protein/lipid ratio similar to those of planktotrophs, and this species was also unique among lecithotrophs in exhibiting demersal development. Since proteins are heavier than lipids and increase the weight/volume ratio of eggs, Prowse *et al.* (2008) speculated that maternal provisioning had in this case been influenced by selection for reduced buoyancy to retain larvae in their benthic habitat. Without knowledge of

the biochemical composition of eggs, adaptive evolutionary scenarios like this one would be missed. Additional comprehensive, comparative studies of egg composition, larval energy utilization, the processes of oogenesis, and the correlation of all of these with egg size on a finer phylogenetic scale are vital to shedding light on the selective forces that have shaped the evolution of oogenic strategies in marine invertebrates.

One conceptual framework under which egg size *per se* is in fact the focus of natural selection is the argument that larger eggs are favored under some conditions because they make better targets for sperm (*e.g.,* Levitan, 1993, 2000, 2006). In that case, too, we argue that a better understanding of the biochemical composition of eggs, relative to size, can shed light on how egg size evolves. Under sperm-limiting conditions, empirical evidence and theoretical models both suggest natural selection will favor a physical increase in egg size. This selective force, because it acts on sperm target size alone, will likely be independent of selection for increased energy investment. Can mothers who experience selection for increased sperm target size in fact optimize their fitness by increasing egg size *without* a concurrent increase in per-egg energy and subsequent decrease in fecundity? This result might be achieved by producing eggs that are larger target areas, not by increasing ovum size but by adding relatively inexpensive accessory structures such as jelly coats (Podolsky, 2001, 2004) or follicle cells (Crean and Marshall, 2008) to the outside of eggs. Another solution might be for mothers to increase egg size itself *via* hydration or through the addition of less energy-rich organic constituents (*e.g.,* protein instead of lipid). Are these oogenic strategies physiologically possible, and if so, has this optimization scenario played out? Without a better grasp of egg biochemistry and mechanisms of oogenesis, these sorts of fundamental questions must remain largely an intellectual exercise.

A second area of egg size biology that lacks resolution is the description of relationships between egg size and egg energy at medium-to-fine scales. While there appears to be a strong correlation between egg size and egg energy when these two characters are examined across the entire range of egg volumes seen in echinoderms (Jaeckle, 1995; Sewell and Manahan, 2001), at finer scales this correlation is much weaker, such that within and even among species egg size can be a very poor predictor of egg energy content (McEdward and Carson, 1987; McEdward and Coulter, 1987; McEdward and Morgan, 2001). Egg size and egg energy can even be entirely uncoupled: Buchal *et al.* (1998) demonstrated that a superior maternal diet in abalone resulted in the production of eggs that had higher amounts of per-egg protein and lipid and developed into more successful larvae, yet egg size was entirely unchanged between the two treatments. When intraspecific, between-female variation in egg size is poorly correlated (or not correlated at all) with egg energy content, how do we explain the broad-scale patterns in egg size that *are* related to energy content, and that have evolved in multiple phyla?

One factor that complicates the detection of intraspecific relationships between egg size and egg energy is that researchers have used diverse methods to measure biochemical composition, and these methods do not always produce comparable results. A second issue is that most of these biochemical methods are not sensitive enough to accurately measure the biochemical content of single eggs. Yet another set of difficulties lies in the many environmental, genetic, and stochastic factors that may affect egg size at the organismal level. While these factors appear to be numerous, we have a poor understanding of (1) the mechanisms by which these factors generate variation in egg size and egg energy, and how these characters are evolutionarily coupled; (2) whether and to what extent different factors interact with each other (for example, genotype by environment interactions) in generating this variation; and (3) how broadly applicable patterns and trends seen in a particular taxon are across other groups. These are substantial gaps in our knowledge; without a better understanding of the mechanisms that underlie intraspecific variation in egg size and egg composition among individuals, it is difficult to delve more deeply into how natural selection has shaped the life histories of marine invertebrates. A stronger focus on identifying egg constituents and more experimental and comparative studies that identify the underlying mechanisms that generate intraspecific variation in egg size are fundamental to generating new insights into the evolution of egg size and life histories of marine invertebrates.

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