OCCURRENCE AND SIGNIFICANCE OF OFFSPRING SIZE VARIATIONS: INSIGHTS FROM BROODING MARINE INVERTEBRATES

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by

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ABSTRACT

This thesis starts by summarizing what is known to date on the occurrence of offspring size variations in marine invertebrates, critically assessing and redefining related methodologies and definitions, and illustrating the main gaps and consequent weaknesses in current knowledge. This review makes recommendations to orient future research in this field and forms the framework on which subsequent chapters are built. The experimental work integrates studies on offspring size variation, its underlying mechanism and the effects of offspring size on offspring performance in two species of brooding (viviparous) sea anemones; one that releases competent lecithotrophic larvae (*Urticina felina*) and one that releases fully-developed juveniles (*Aulactinia stella*). The main findings highlight previously neglected mechanisms that can generate important offspring size variation. More precisely, the co-occurrence of morphologically-aberrant (sectorial) and fully homogeneous chimeras (mega-larvae) that form at the embryonic stage cause increased offspring size and size variations in *U. felina.* The long non-fixed brooding period, the co-existence of different cohorts of juveniles and intra-brood feeding and competition cause the marked offspring size variation in *A. stella.* Thus, I propose that brooding species exhibit strategies that increase offspring size significantly during the period of parental care, and that the occurrences of offspring size variation should be investigated more thoroughly in viviparous taxa before formulating general theories. In addition, results indicate that size advantage in offspring seems confined to pre-metamorphic stages in *U. felina*, whereas the post-metamorphic stages exhibit species-

specific size-performance relationships determined by interactions between offspring and predator phenotypes. Thus, the relationship between offspring size and performance appears to vary ontogenetically and inter-specifically, depending on the complex suite of environmental and biotic factors encountered at different life stages, e.g. the presence of optimal substratum during settlement and the level and type of predation at the juvenile stage. Future studies on the offspring size-performance relationship should more explicitly take parent-offspring and sibling conflicts as well as external factors into consideration.

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我亲爱的父亲母亲

慈爱的师长

和一路同行的朋友们

感谢你们为我撑开一片天空

让我能够无忧无虑

充满力量的奔跑

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CO-AUTHORSHIP STATEMENT

The research described in this thesis was carried out by Zhao Sun, with guidance from Annie Mercier, Jean-François Hamel and Chris Parrish. Zhao Sun was responsible for data collection and analysis. Manuscripts resulting from this thesis were prepared by Zhao Sun, with editing assistance and intellectual input from co-authors as follows:

Authorship for publications arising from **Chapter 2, Chapter 3** and **Chapter 5** will be Z. Sun, J.-F. Hamel and A. Mercier.

Authorship for publication arising from **Chapter 4** will be Z. Sun, J.-F. Hamel, C. Parrish and A. Mercier.

The manuscripts in chapters 2 to 5 have been prepared for publication in different scientific journals, and the format of chapters varies according to the specific journal requirements.

CHAPTER 1 : **Introduction**

Variation in offspring size is a central concept in ecology and evolution. The study of offspring size variation has been conducted at different levels in vertebrates (Sinervo 1990, Krist 2011) and invertebrates (Marshall & Keough 2007, Allen et al. 2008). Initial work concentrated on comparison among different species (especially between species with different reproductive strategies, i.e. free spawning *vs* brooding), and among populations (comparing size variations in the same species but under different environmental conditions). Although inter-specific offspring size variation is impressive, intra-specific offspring size variation is more important for understanding its influence on performance in every life-history stage, including survival, dispersal, settlement, growth, resistance to predation, etc. Only a limited number of recent studies have focused on the offspring size variation among individuals of a population, and within clutches in marine invertebrates (Marshall et al. 2008).

Before going any further, it is worthwhile to clarify some of the terminology, as *offspring* is often very loosely defined in the literature. In this research, I base the definition of offspring on the review of Marshall and Keough (2007) who define it as a "propagule[1] that becomes independent of maternal nutritional investment", thus including freely spawned eggs, embryos, larvae and juveniles. *Embryo* generally refers to the early morphological stages, including egg cleavage, blastula and gastrula, before subsequent transition to larva (Benitez-Villalobos 2005). *Larva* generally refers to the stage between an embryo and a juvenile. Although it is hard to adopt a clear definition, I will use the one provided by Pechenik (1999) of a *larva* being the developmental stage before the

[1] The term *propagule* refers to any of the various structures that can give rise to a new individual organism.

juvenile stage is reached through metamorphosis (transitional stage). The larval stage is an important segment in the life history of benthic marine invertebrates, especially for free-spawning (broadcasting) species and species that brood to the larval stages, because of its role in increasing the chance of finding congenial substrata, favouring dispersal, and decreasing competition for resources with adults (Pechenik 1999). *Juvenile* is the stage that exhibits the same symmetry and general body shape as the adult when major systems, especially locomotion and feeding, become functional and it excludes the transitional period of metamorphosis (McEdward & Janies 1993). While similar looking, juveniles are smaller in size than adults, and are not sexually mature.

Offspring size plays an important role in performance at pre- and post-metamorphic stages. For example, egg or larval size may influence the competency period, settlement choice and survival at pre-metamorphic stages or during metamorphosis, and may translate into "carry-over" effects on post-metamorphic performance, including survival, growth and reproduction (Marshall et al. 2006, Phillips 2006, Allen et al. 2008). The relationship between offspring size and performance is context-dependent and is strongly affected by external factors; however, only a few empirical examinations of offspring size carry-over effects have considered the effect of external factors (Marshall et al. 2006, Allen et al. 2008). Furthermore, the ecological and biochemical mechanisms underlying the relationship between offspring size and performance are generally unexplored in marine invertebrates (except Harii et al. 2007).

Offspring size variation is a significant dynamic and adaptive characteristic in marine invertebrates, which could be mediated by several factors, including parental

genotype, environmental factors, and the interaction between them (Dalsgaard et al. 2003). Although marked intra-specific offspring size variations have been reported in marine invertebrates (Marshall et al. 2008, Jacobs & Podolsky 2010), there is no clear explanation of how this variation is partitioned within and among clutches, females, or at the population level. Theories have suggested that parental investment into offspring of variable size in marine invertebrates may either be the outcome of physiological constraints or of an adaptive strategy that ensures the survival of certain sized offspring under unpredictable environmental conditions (i.e. bet-hedging). However, these assumptions have not yet been tested in the context of a brooding strategy in marine invertebrates, which shares similarities with viviparity and live-bearing in vertebrates.

Brooding in marine invertebrates could be defined as "the retention of offspring by a parent through the embryonic stages usually passed in the plankton, thereby shortening or entirely eliminating the dispersal stage" (Allen et al. 2008). Internally brooding mothers can predict the environment in which the eggs/embryos/larvae develop (inside the body cavity) before releasing them into the presumably less predictable external milieu. Offspring size variation in brooding species may be more complex than in broadcasting species, because there is a closer relationship between the parent and the offspring that may favour the evolution of conflicts. Thus, the study of brooding species may provide significant insight in developing general concepts of offspring size variations.

The main goal of the present study was to: (1) summarize what is known to date about the occurrence of offspring size variations in different taxa at various scales and on

factors capable of mediating offspring size; (2) illustrate the main gaps and consequent weaknesses in current knowledge, (3) provide novel data and reassess previous studies to fill those gaps and orient future research in this field. The study involved a thorough review of the literature and in-depth examination of offspring size variation in two internally-brooding sea anemones; one that releases lecithotrophic larvae (*Urticina felina*) and one that releases fully-developed juveniles (*Aulactinia stella*). The underlying mechanisms that cause offspring size variation in the two species were explored. Furthermore, the effects of offspring size on offspring performance at pre- and post-metamorphic stages were investigated.

The chapters of the thesis include: a reassessment of offspring size variations measured in marine invertebrates with important considerations and new insights from innovative data (Chapter 2); examination of fusion among offspring (chimerism) in a sea anemone (*Urticina felina*) and its effect on offspring size variation (Chapter 3); an in-depth study of the brooding strategy in the cold-water sea anemone *Aulactinia stella* and of its effect on offspring phenotype (Chapter 4); a study of the effect of offspring size on pre- and post-metamorphic performance in the two internally-brooding sea anemones (Chapter 5).

In Chapter 2, I review the data on offspring size variations in marine invertebrates, as well as factors capable of mediating offspring size, and redefine the current methodologies and definitions, i.e., classification of developmental modes. Also, I reassess published data together with novel empirical data from poorly studied development modes in light of this unified classification. I illustrate the main gaps and

the consequent weaknesses in current knowledge, and make recommendations for the study of offspring size and orient future research in this field.

In Chapter 3, I investigate the size structures and size shifts at various ontogenetic stages in the brooding sea anemone *Urticina felina.* I propose that co-occurrence of morphologically-aberrant (sectorial) and fully homogeneous chimeras (mega-larvae) formed at the embryonic stage causes increased offspring size and size variations. Through an analysis of lipid composition in sectorial chimeras and singleton juveniles, I show that the latter exhibit greater fitness and propose that fusion among maternal siblings may be a form of kin cooperation integral to the reproductive success of *U. felina.*

In Chapter 4, I provide new data on the brooding process and size structure of brooded juveniles in the sea anemone *Aulactinia stella.* I also provide new data on and compare lipid composition and fatty acids in adult tissues and juveniles of various sizes to elucidate phenotype plasticity and detect any shift from maternal to dietary nutritional resource during early ontogeny. I suggest the prolong non-fixed brooding period, the co-existence of different cohorts of juveniles and intra-brood feeding and competition, instead of the factors currently proposed to explain offspring size variations (i.e. unpredictable environments and maternal phenotypes) cause the marked offspring size variation in *A. stella.*

In Chapter 5, I investigate the effects of size on the survival, time to settlement and lipid composition of *U. felina* larvae. I also provide new data on size-related survival of juveniles of *U. felina* and *A. stella* in the presence of specialized predators, and support the previous assumption that the relationship between offspring size and performance is

highly variable and context-dependent. Finally, in Chapter 6, I present a summary of the main conclusions and their significance and I identify areas in which future research is particularly needed.

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CHAPTER 2 : **Offspring size variations in marine invertebrates: reassessment of the framework and new insights**

The manuscript in this chapter is in preparation for Biological Reviews

Abstract

Offspring size, together with its influence on offspring performance, is a central concept in ecology and evolution. In recent years, marine invertebrates have been increasingly used as model organisms in studies of offspring size because of the diversity of species available, their complex life histories and wide range of reproductive strategies. Here we offer a new outlook on the occurrence and mediating factors of offspring size variations within different taxa and at various scales. A preamble draws attention to problems inherent to studies of marine invertebrate phenotypes, highlighting limitations and suggesting alternative approaches. We argue that a multi-factorial classification of reproductive modes must evolve to allow the identification of variables acting as selective pressures on offspring phenotype plasticity. By reassessing previously published and new data, we also identify the most adequate statistical analysis for comparing offspring size variations at the inter-specific level. Based on current gaps in knowledge, future studies should not only investigate offspring size variation inter-specifically, but also examine intra-specific mechanisms responsible for offspring size variation. Of particular relevance is the fact that offspring size variation in species with post-zygotic parental care (e.g. brooders, live-bearers) displays a more complex scheme than free-spawning species, due to increased opportunities for conflicts between parent and offspring and among siblings. More comparisons at the finest scales, e.g. inside clutches and/or at different ontogenetic stages, are particularly needed to clarify our understanding of the function and evolution of offspring size plasticity. In addition, we found that

structural organization has so far been overlooked and show that offspring size variation is significantly greater in unitary than in colonial species. Thus, future studies should consider structural organization when comparing offspring size variations among taxa. By drawing from recent literature and novel data and from principles of evolutionary and reproductive biology, this work highlights the main gaps and consequent weaknesses in current knowledge, and makes recommendations for the study of offspring phenotype to orient future research in this field.

Introduction

Investigations of the interplay between parental and offspring phenotypes have a long tradition in ecology and evolutionary biology. The contribution of invertebrates to the current conceptual framework is surprisingly small relative to their contribution to our planet's biodiversity (they comprise > 90% of animal species). Indeed, hypotheses surrounding offspring phenotype plasticity largely derive from studies on species belonging to phylum Chordata, which includes the most familiar/charismatic taxa (mammals, birds, reptiles, amphibians, fishes) as well as some lesser known ones (urochordates). The other ~30 phyla of invertebrates are comparatively understudied, with the possible exception of Arthropoda (i.e. insects). Despite this obvious imbalance, the rich diversity of invertebrate taxa that thrive in marine ecosystems (all except 3 phyla) has prompted a number of empirical and theoretical investigations of offspring size variations.

Thorson (1950) was among the first to explore the pervasive inter-specific variation in offspring size in marine invertebrates. Since then, investigations of offspring size have been conducted at various levels. Initial work concentrated on comparison among species (especially between species with different developmental modes, i.e. lecithotrophic *vs* planktotrophic), and among populations (comparing size in the same species but under different environmental conditions). Although inter-specific offspring size variation is impressive, intra-specific offspring size variation is perhaps more important for understanding the influence of this trait on performance in life-history

stages. Recently, a limited number of studies (Marshall, Bonduriansky & Bussiere, 2008b) have focused on the offspring size variations among individuals of a population, and within clutches. While the latter expression is often synonymized with "within broods" or "intra-brood" in the literature (irrespective of reproductive modes), we will avoid those terms to prevent any confusion with a reference to brood-protecting parental care.

The most recent review of the evolutionary ecology of offspring size variation in marine invertebrates was published by Marshall and Keough (2007). It focused on the influence of developmental modes on offspring size variations at the inter-specific level and presented different offspring-size models. The analysis of variance in offspring size revealed interesting trends; however, it also highlighted shortcomings in the study as well as important gaps in the current knowledge. In particular, the classification of offspring into three simplified development modes (planktotrophs, lecithotrophs and direct developers) appears problematic for reasons discussed in the present review. While models or theories including the size-number trade-off (Smith & Fretwell, 1974), the safe-harbour hypothesis (Shine, 1978), and the more recent bet-hedging hypothesis are proposed to explain and predict parental investment into offspring (frequently estimated through offspring size), empirical testing of these theories in marine invertebrates is still relatively scarce. It is also apparent that offspring evolving from different reproductive and development patterns are not equally well studied, greatly hampering our ability to obtain a comprehensive overview. In addition, the appropriate use of models to explain or predict offspring size variation obviously requires a greater understanding of reproductive

processes and the selection for offspring size, especially of the factors that mediate offspring size variability within clutches.

The goal of the present contribution is to put forward the argument that a clear understanding of offspring size variation will require a more coherent and unified framework. Our approach is threefold: (1) A critical assessment of current methodologies and definitions, using concrete examples to illustrate limitations in common measurement techniques, and proposing standardized definitions to favour comparability. (2) A thorough re-analysis of published data together with novel empirical data from poorly studied taxa and development modes in light of this unified classification. (3) A comprehensive review of the factors proposed to mediate offspring size and offspring size variations, examining how additional data and new interpretations support or challenge current hypotheses. For enhanced clarity, factors that influence offspring size variation are summarized based on two concepts: the mean optimal offspring size and the variability of offspring size. Studies on seasonal changes in offspring size (due to a combination of factors, e.g. food availability, temperature and salinity) were excluded, to avoid comparing influential factors of offspring size at different levels. Studies on latitudinal and geographical changes in offspring size (e.g. Dugan, Wenner & Hubbard, 1991) were also not used as they may involve genetic components. However, the stochastic developmental events (developmental variation, i.e. Vogt et al., 2008) are discussed as important influential factors on the variability of offspring size.

In summary, this review aims to synthesize what is known on the occurrence of offspring size variations within different marine invertebrate taxa at the various scales,

identify factors susceptible of mediating offspring size, and illustrate the main gaps and consequent weaknesses in current knowledge. By drawing from recent literature and providing a fresh outlook grounded in principles of evolutionary and reproductive biology, it is our hope that this work will highlight new avenues for the study of offspring size and orient future research in this field.

Offspring size in marine invertebrates

1. Assessment of definitions and methods

Marine invertebrates form a taxonomically rich assemblage with diversified reproductive modes (Fig. 2-1). Investigations of offspring size variation in marine taxa have taken different angles (parental effects, phenotype-fitness relationship) and covered various levels (within/among species, populations or clutches), generating a rich literature that includes a few reviews (Marshall & Keough, 2007; Marshall et al., 2008b). Over the years, methodologies have been developed and simplified assumptions made in an effort to define broad concepts. The strengths and weaknesses of those approaches have recently been highlighted (e.g. Jacobs & Podolsky, 2010). However, no critical assessment of definitions and measurement methods, central to proper data analysis and development of unified concepts, has ever been undertaken.

Marshall and Keough (2007) reported an average coefficient of variation (CV overall, within species) of 9%, based on data of egg diameter and larva length in 102 species of marine invertebrates across 7 phyla. The same authors proposed that variance in offspring size varied with development modes, i.e. direct developer (~15%) >

lecithotroph (~10%) > planktotroph (~5%). The main issue with this and later reviews is the definition of development patterns by a single term that intermixes morphology-based (direct *vs* indirect development) and nutritionally-based (feeding = planktotrophic *vs* non-feeding = lecithotrophic) factors. Marshall and Keough (2007) took the term 'direct developer' to mean "any development whereby the offspring are fully formed juveniles independent of maternal nutrition sources". This type of oversimplification has lingered in the literature since Thorson (1950) used it to refer to gastropods possessing a lecithotrophic veliger larva that developed into a benthic juvenile inside a protective capsule. Thorson (1950) used the term only once when mentioning that many prosobranchs "have a direct development without any pelagic life…". Chia (1974) later advocated that direct development should be restricted to the absence of a larval stage, and others concurred that direct development should only apply to species that produce a juvenile directly from the gastrula without any intermediate (larval) stage (Jablonski & Lutz, 1983; McEdward & Janies, 1993). Unfortunately these recommendations were not heeded in a number of later studies.

As a result, Marshall et al. (2008b) kept the simplified meaning when suggesting that offspring size variation should be determined by the ability of a female to predict the relationship between offspring size and performance, and proposed that "there is less potential for conflicting selection pressures on offspring size in direct developers because they have fewer life-history stages, making the relationship between offspring size and performance more likely to be predictable". They further analyzed data on offspring size variation among and within females in 25 species of marine invertebrates, and found that

the CV of offspring size within a clutch (=within brood) was lower in 'direct developers' than what they called 'indirect developers' (lecithotrophic and planktotrophic species). While not invalid, the terminology used by Marshall et al. (2008b) and others (e.g. Teske et al., 2007) is not explicit and thus may obscure or restrict the comparisons. For instance, the assumption regarding direct *vs* indirect developers is more adequately expressed in terms of the habitat in which development occurs, i.e. benthic mothers should be more apt to anticipate the conditions experienced by their offspring if the latter are benthic than pelagic (irrespective of whether they undergo direct or indirect development, they feed or not, or they are afforded protection or not).

McEdward and his collaborators (e.g. McEdward & Janies, 1997; McEdward & Miner, 2001) clearly established that the common use of few terms (lecithotrophy, planktotrophy, brooding) is ambiguous: "For example, lecithotrophy indicates that the offspring utilize endogenous nutritional reserves and do not need to feed. However, it is not specified whether development is pelagic or benthic, protected or free-living, or involves larval stages." They proposed to classify the developmental patterns of Echinodermata on the basis of three life-history characters, including morphogenesis (complex larval, simple larval, direct), nutritional mode (planktotrophic, lecithotrophic), and developmental habitat (pelagic, benthic) (McEdward & Janies, 1997). Furthermore, Poulin et al. (2001) used similar criteria for marine invertebrates as a whole to define eight possible developmental patterns based on three independent two-state characters (free/protected, pelagic/benthic and feeding/non-feeding). Only by using clear hierarchical terminology can we separately test whether habitat, nutrition, parental care

and morphogenesis have an influence on offspring size variation and interpret those results appropriately. Stricter multi-level definitions also make it easier to identify and suitably treat species with offspring that undergo mixed modes (e.g. benthic phase followed by pelagic phase) and to identify characters that are rarely combined (e.g. pelagic-protected).

Jacobs & Podolsky (2010) identified another potential flaw in the data analyzed by Marshall et al. (2008b), i.e. measurement of CV was based on diameter in 'direct developers', whereas it was a combination of diameter and volume in 'indirect developers'. Jacobs & Podolsky (2010) reiterated the findings of Schmalhausen (1935) which showed that CVs for measurements of length, surface and volume differ on the scale of 1:2:3. Jacobs & Podolsky (2010) further analyzed data on size variation based on diameter, and contrary to Marshall and colleagues, found no correlation between offspring size variation and development mode (although they were still using the same ambiguous definitions). We agree that measurement of offspring size is a primordial consideration. Common determinants of offspring size in the literature include oocyte/egg/settler diameter, surface area, volume and weight. However, the majority of analyses are based on egg diameter and larva length, which may not be the most appropriate or universal measurements, especially for some brooding species that release fully-formed juveniles. The sea anemone *Aulactinia stella* (Fig. 2-1a, b) provides a clear example of this. Fifty-seven juveniles of *A. stella* were measured for basal diameter, basal area, volume (basal area × height) and weight. Results showed that basal diameter, basal area and volume were all correlated with juvenile weight, but that volume was a better

indicator of weight than the two other measures (Fig. 2-2a). Volume may also be a more accurate size descriptor for life stages with a complex/plastic morphology (e.g. Fig. 2-1d, j, l).

The review of Jacobs & Podolsky (2010) also examined the strengths and weaknesses of the statistical methods used to measure offspring size variability, including Levene's test, the use of the coefficient of variation (CV) in F-tests, and analysis of covariance (ANCOVA). To remove the influence of mean size on offspring size variation (standard deviation), Jacobs & Podolsky (2010) recommended comparing the standard deviation of offspring size, with mean size as a covariate (ANCOVA). However, ANCOVAs do not remove the influence of mean size in comparisons of offspring size variation either (Taylor, 1961). A thorough discussion of the statistical methods will be presented later in this review.

A fourth major source of bias that can significantly affect comparisons and models is the use of data obtained from offspring that were not naturally-released, e.g. forcibly extracted or from induced release. In our study, five individuals of the sea anemone *A. stella* were monitored weekly for one year. Sizes of naturally-released juveniles were measured and the individuals were dissected at the end of the experimental period. Size variation of extracted juveniles was much greater than that of naturally-released juveniles from the same parent (Fig. 2-2b). Thus, the extraction (or forced release) of offspring at any stage could lead to a larger size variation, especially in species that rear offspring for a long period (i.e. more than one year in *A. stella*) or in species that produce large propagules or overlapping generations of oocytes. Again, the

CV of juvenile volume was a better indicator of CV of juvenile weight than was the CV of diameter or basal area (Fig. 2-2b). Previous studies were often based on induced release of larvae after temperature shock (Luttikhuizen, Honkoop & Drent, 2010), light shock (Allen, Buckley & Marshall, 2008; Dias & Marshall, 2010), vigorous shaking (Allen, Zakas & Podolsky, 2006) or with the use of chemicals such as KCl (Byrne et al., 2008). The absence of related effects has not been demonstrated for these techniques yet, thus information on natural size variation and size range should always be provided for comparison with extracted/induced results.

Bias in the measurement of offspring size variation due to temporal changes over the annual cycle or to the scale of the spawning period have been reported, especially in species that encapsulate offspring (Thompson, 1958; Ito, 1997). For example, the size of eggs in naturally-released egg capsules of the gastropod *Haloa japonica* decreased significantly from the beginning toward the end of the spawning period (~120 days, Ito, 1997). Furthermore, temporal changes in offspring size were observed at an even smaller scale within the reproductive period, i.e. diel variation. For example, in the bryozoan *Bugula neritina* that releases larvae daily at dawn, Kosman & Pernet (2009) measured larva size in hourly samples from adult colonies in field mesocosms between 06:00 and 18:00 and found that it decreased as the day progressed. Hence, the combination of temporal factors and natural *vs* extracted offspring could introduce large biases in assessments of offspring size variation. The asteroid echinoderm *Solaster endeca* (Fig. 2-1i), which releases pelagic lecithotrophic eggs, was used here to test the influence of temporal factors and natural *vs* extracted offspring. Size variation of eggs of *S. endeca*

naturally-released in April 2010 was compared with size variation of oocytes extracted in October-November 2010. The overall CV of naturally spawned propagule size was an order of magnitude lower than that of oocytes extracted in October and November (3% *vs* 30%). This is partly because, in most taxa, final meiotic maturation occurs only in the brief instants before oocyte release and complex changes are associated with spawning/fertilization, e.g. detachment of follicle cells, germinal vesicle breakdown, hydration of jelly coat and elevation of fertilization (vitelline) envelope (Giese, Pearse & Pearse, 1987). Thus, future studies on naturally released offspring should be preferred especially when investigating offspring size and its effects on performance. Studies should also consider the influence of temporal changes in offspring size, especially the time period of any extraction/inducement (i.e. before/during/after the spawning season).

Strip-spawning is another frequently used method, where the adult is induced to spawn and the oocytes are fertilized under laboratory conditions to obtain offspring (Marshall, Styan & Keough, 2000; Rius et al., 2009). Sperm concentration may alter fertilization success of different sizes of oocytes, especially for broadcasting species (Marshall, Styan & Keough, 2002). This is due to the fact that larger oocytes have higher chances of being fertilized under low sperm concentrations, whereas smaller oocytes experience lower chances of lethal polyspermy under high sperm concentrations. The strip-spawning technique is usually conducted using high sperm concentrations that will favour oocytes of certain sizes. Thus, the size distribution of the obtained embryos/larvae are likely shifted compared to those obtained under natural conditions in the field, and estimating size variation in later life stages is biased accordingly.

2. Proposed revisions to framework

a. Classification of offspring developmental modes and statistical approach

As mentioned above, the main issue in a number of previous studies (e.g. Marshall & Keough, 2007; Jacobs & Podolsky, 2010) is the definition of development patterns by a single term that intermixes morphology-based (direct or indirect development) and nutritionally-based (feeding or non-feeding) factors. Many species encapsulate or brood-protect their offspring until the release of fully-formed juveniles ('direct developers' according to those reviews), but they still undergo distinct larval stages (planktotrophic or lecithotrophic) in the capsules or brood site. For example, while the review of Marshall & Keough (2007) classified 20 species as 'direct developers', a closer exanimation showed that only one of those species (the gastropod *Crepidula adunca*) does not have any intermediate larval stage (the purest definition of a direct developer). While classifying offspring into only two (non-direct and direct developers) or three types (planktotrophic, lecithotrophic and direct developer) may have provided a simple and useful framework in early conceptualizations of offspring size, it becomes ambiguous when trying to integrate a wider range of species and make life-history-based comparisons. Consequently, a more circumspect study of the relationship between offspring size variation and development modes requires a more accurate classification of offspring types. It is our belief that unambiguous multi-factorial classification, based on clear hierarchical terminology, is the way forward. Thus we propose that morphogenesis (simplified, complex; the former involving a complex larval stage, and the later involving either a brief/simple or no larval stage), developmental habitat (benthic, pelagic, both),

care (free: no form of protection during development; protected: under parental care until the fully-developed juvenile stage, both: under parental care for a portion of the development) and nutrition (feeding: planktotrophic development, non-feeding: lecithotrophic development) should be used to classify developmental patterns. Table 2-1 illustrates the use of the proposed hierarchical criteria in defining the various developmental modes shown in Fig. 2-1.

Based on this classification, we reassessed the relationship between inter-specific size variation and the four factors (morphogenesis, habitat, care, nutrition) in 116 species of marine invertebrates (Appendix 2-1), using data from the review of Marshall & Keough (2007), combined with our own data and data from recent papers (e.g. Collin, 2010). Following Jacobs & Podolsky (2010), we have been cautious with the statistical analysis of variability. First, only data with the same dimensionality (diameter or length) were compared. Jacobs & Podolsky (2010) indicated that comparison of variability using CV could be problematic unless the relationship between the standard deviation and the mean was linear and had a y-intercept of zero. Reassessing the data from the review of Marshall & Keough (2007), they found that the relationship between standard deviation (SD, square root of variance) and the mean was logarithmic (instead of linear), and suggested that the use of a ratio (CV) does not effectively correct for a relationship between SD and the mean. In fact, variance (V_i) and mean (μ) are related in the form of a power function (Taylor's power law, Taylor, 1961):

$$V_i = \alpha\mu^{\beta}, \alpha \text{ and } \beta \text{ are constants} \quad (1)$$

$$V_i = SD^2 \quad (2)$$

Based on (1) and (2),

$$\mathbf{SD = \alpha^{1/2}\ \mu^{\beta/2}} \quad \mathbf{(3)}$$

In addition,

$$CV = SD/\mu \quad (4)$$

Based on (3) and (4),

$$\mathbf{CV = \alpha^{1/2}\ \mu^{\beta/2-1}}$$

Where α is a sampling parameter of less immediate ecological interest and β is an index of aggregation characteristics, and is species-specific (Taylor, 1961). Taylor (1961) reviewed β in several species, including viruses, invertebrates and fishes, and reported that it varied from 0.7 to 3. Thus, when $\beta \neq 2$, CV will be influenced by the mean (μ), and using CVs will be problematic in comparisons of size variation. In addition, using ANCOVAs on SD with mean as covariate, as suggested by Jacobs & Podolsky (2010), assumes a linear relation between SD and mean, making it problematic as well. To effectively correct the relationship between SD and mean, a logarithmic transformation of SD (lgSD) and mean (lgMean) is more appropriate. We re-examined the influence of developmental criteria on offspring size variation among the 116 species of marine invertebrates mentioned above, using one-way ANCOVAs on lgSD with lgMean as covariate. Results showed that offspring size variation was significantly smaller in species with feeding larvae than in species with non-feeding larvae ($F = 7.68$, $p = 0.007$), whereas habitat ($F = 0.84$, $p = 0.436$), care ($F = 1.86$, $p = 0.160$) and morphogenesis ($F =$

0.33, p = 0.566) did not have significant effects. Although nutrition has a significant influence on offspring size variation, it is worth mentioning that larval nutrition modes are strongly related to oocyte size. More precisely, planktotrophic eggs/larvae (feeding) are generally smaller than lecithotrophic eggs/larvae (non-feeding) (Strathmann, 1978).

Importantly, the three different statistical methods, including ANOVAs on CV, ANCOVAs on SD, both with mean as covariate, and ANCOVAs on lgSD with lgMean as covariate, gave different results (Table 2-2). This emphasizes the need for future studies to provide the mean and SD of offspring size, and to use a more appropriate analysis (we suggest ANCOVAs on lgSD with lgMean as covariate) for comparison of offspring size variation, especially at the inter-specific level. The implications are not as strong for intra-specific comparisons where the mean offspring size is similar among clutches.

b. Structural organization: unitary vs modular species

A number of fundamental biological concepts have been developed from the study of unitary organisms (mammals, birds, fishes) and later extended to marine invertebrates without considering the fact that many of the latter exhibit a modular organization (e.g. ascidians, bryozoans, sponges, corals). The fundamental difference between modular/colonial and unitary/solitary morphologies is often overlooked in ecological and biological studies that use marine benthic invertebrates as models. For example, datasets mixing unitary and modular taxa have been used to explore concepts and correlates involving dispersal abilities (Shanks, 2009), connectivity (Weersing & Toonen, 2009) and offspring size variability (Marshall & Keough, 2007; Marshall et al., 2008b). The importance of distinguishing unitary and modular organization in the context

of evolutionary biology has nevertheless been emphasized (Vuorisalo & Tuomi, 1986; Hughes, 2005). The distinction is particularly significant in the study of phenotypic plasticity: modular organisms do not exhibit a fixed morphology and may thus adjust their phenotype throughout their life in response to environmental fluctuations (i.e. number, size and arrangement of modules vary significantly among individuals and over time), whereas the adult phenotype of unitary forms is determined and varies minimally in a lifetime and among individuals (Pineda-Krch & Poore, 2004).

Furthermore, while the distinction of individual and group selection is clear in unitary organisms, phenotypic selection in most modular organisms has a hierarchical causal structure: groups function as interactive units that modify the fitness components at a lower level, consisting of the reproductive units which actually propagate genetic units (Tuomi & Vuorisalo, 1989). Hence, concepts of offspring size variations (and other life-history characters) in modular organisms can be investigated among groups of colonies (inter-population), among colonies (inter-individual), or among the smallest reproductive units (inter-module inside a colony). The latter is typically not considered. One aspect of phenotypic plasticity that has been investigated at various structural levels in modular organisms is the temperature-size rule (the inverse relationship between temperature during ontogeny and final body size in ectotherms). The rule was found to apply only to larval parenchymal cells and colony modules (autozooids), but not to the volume of whole mature colonies or any other structural level in bryozoans (Atkinson, Morley & Hughes, 2006). Also, comparative analyses among gorgonian corals found a decoupling of evolution at the polyp and branch levels indicating that evolutionary

change in polyp phenotype does not imply a change at the colony level, or *vice versa* (Sánchez & Lasker, 2003).

In the case of modular species, we may wonder what is considered the mother: the polyp, the colony or the genet? Also, whether offspring phenotype (= modular phenotype; polyp, zooid) really influences the final adult phenotype (= colonial phenotype)? As well, while growth is an important component of fitness, should the size and number of modules (e.g. Marshall & Keough, 2004b) or the whole colony size (mass, surface area, e.g. Marshall, Bolton & Keough, 2003; 2006) be examined as a more accurate indicator for the effects of offspring size on post-metamorphic performance in modular organisms? This ambiguity does not exist in unitary species.

This is extremely relevant in our efforts to conceptualize maternal effects on offspring phenotype in marine taxa and to explore eventual linkages in phenotypes between populations (e.g. Marshall et al., 2008b). It is therefore legitimate to wonder whether and how offspring size strategies developed by unitary and modular organisms might differ. One-way ANCOVA was used to test the influence of structural organization (colonial *vs* unitary) on offspring size variation (lgSD) with lgMean as covariate in the 116 species of marine invertebrates listed in the Appendix 2-1. Offspring size variation was significantly higher in unitary than in colonial species ($F = 4.32$, $p = 0.040$). We conclude that future studies should take the previously overlooked factor of structural organization into consideration when comparing and conceptualizing offspring size variations among different taxa.

c. Mediators of offspring size variation

Marshall et al. (2008a) suggested a few sources of offspring size variation, including seasonal variation, maternal age and spawning sequence, maternal size and maternal nutrition. However, this summary should be interpreted with caution, because the sources of offspring size variation proposed by Marshall et al. (2008a) operate at different levels. For example, the authors suggested that seasonal variations mediate offspring size due to changes in the combination of temperature, salinity, food availability and maternal size. However, the influence of each factor was not examined.

Another possible source of confusion is that offspring size variation can be viewed from two angles: (1) plasticity in mean offspring size (the production of constantly larger or smaller offspring), which achieves the maximum within-generation mean fitness; (2) the variability of offspring size (the simultaneous production of offspring of variable sizes), which achieves the minimum among-generation variation in reproductive success (Crean & Marshall, 2009). The two aspects are related, but not the same. For example, Marshall et al. (2008a) reviewed the literature on the influence of maternal nutrition on offspring size, and suggested that it could have mixed effects, either leading to an increase (anticipatory maternal effects, AME) or decrease (selfish maternal effects, SME) of offspring size. However, there is no evidence that AME or SME will affect offspring size *variability*. Another good example is a study on the lobster *Homarus americanus* in Îles-de-la-Madeleine, eastern Canada (Ouellet & Plante, 2004). The newly-hatched larvae of *H. americanus* from small females were significantly smaller

than those from larger females (Ouellet & Plante, 2004). However, the *variances* on mean larval size at hatching in the two size groups were similar.

In this respect, previous studies have identified a number of factors that influence mean offspring size, whereas factors affecting variability in offspring size at the intra-specific level have received far less attention. Thus, in the next sections, we will review and discuss factors that mediate offspring size variation from the two angles separately.

3. Factors that mediate mean offspring size

The phenotype of an organism is generally determined by three factors, including genes, the environment and developmental variations (Vogt et al., 2008). Offspring size, as a joint phenotype of two individuals (the offspring and its mother), is largely determined by the phenotype of the females (including maternal size, age and nutritional state), as well as the biotic and abiotic conditions they experienced (Marshall & Keough, 2007) . We will herein review the influences of maternal phenotypes as well as the biotic and abiotic conditions experienced by mother and offspring on mean offspring size (Table 2-2).

a. Maternal phenotype

Aspects of the maternal phenotype, i.e. size and age, have been reported to affect offspring size in a few marine invertebrates (Ito, 1997; Marshall et al., 2000, 2003). For example, mean egg size increased with maternal size in the gastropod *Haloa japonica* (Ito, 1997). Larger colonies of the colonial bryozoan *Bugula neritina* produced larger larvae, and colonies that derived from these larger larvae produced larger offspring

(Marshall et al., 2003). Although the mechanism is not clear, Marshall et al. (2003) suggested that larval size could be under genetic control. Furthermore, maternal size could influence offspring size by determining the female packing/packaging ability, especially for egg-encapsulating species. For example, the shell lengths of newly-hatched juveniles were greater in larger (shell length > 80 mm) than smaller females (< 80 mm) of the whelk *Buccinum undatum* (Nasution et al., 2010). Nasution et al. (2010) found that female size in *B. undatum* had a strong positive linear relationship with capsule size, and capsule size had a positive relationship with hatchling shell length following a Monod function. Thus, they proposed that the morphological (packing) constraint of capsules for the small females was limiting offspring size, because the whelk *B. undatum* secretes capsules in a pallial oviduct, and moulds and hardens them in a ventral pedal gland.

In contrast, studies on molluscs closely related to the above and other marine invertebrates have shown that maternal size exhibits no relationship or a negative relationship with offspring size (Bridges & Heppell, 1996; Chaparro et al., 1999; Ilano, Fujinaga & Nakao, 2004; Collin, 2010). For example, hatchling size of the whelk *Buccinum isaotakii* was not related to the shell length of the female (Ilano et al., 2004), due to the presence of different proportions of nurse eggs. Egg diameter of the gastropod *Crepidula dilatata* was independent of shell length of females; however, length of hatching juveniles increased with maternal size (Chaparro et al., 1999). The latter authors suggested that the increase in hatchling size with female size was due to the increased amount of nurse eggs available for embryos in larger females, instead of increased egg size.

Before drawing any general conclusion on the relationship between maternal and offspring size in marine invertebrates, it should be noted that this link was mainly studied in mollusc species that exhibit parental care (brooding or encapsulation) during a portion of the offspring development (Table 2-3). Thus, future studies should endeavour to provide more information on other phyla and on broadcasting species with pelagic eggs and/or larvae. In addition, measurement of offspring size mixed oocytes diameter/volume and shell length of hatchlings in various studies (Table 2-3), whereas adult size shifted from lorica length in rotifers, number of setae-bearing segments in annelids, shell length in gastropods, abdomen width and carapace length in crustaceans, and weights in bryozoans and chordates (Table 2-3). More uniform measures and correlations between maternal and offspring size should ideally be made to obtain a clearer idea of the relationship between the two.

Maternal age was shown to influence offspring size in marine fishes (Berkeley, Chapman & Sogard, 2004). However, due to the difficulty associated with accurate aging, this type of research is generally lacking in marine invertebrates, except for a few short-lived or ephemeral species that only reproduce once in their lifetime (for these species, maternal age is equivalent to spawning sequence). For example, the nudibranch *Adalaria proxima* produced larger eggs in the first laid masses than the subsequent ones (Jones, Todd & Lambert, 1996). Mean egg volume of the gastropod *Haloa japonica* decreased with spawning sequence (Ito, 1997). More precisely, egg size decreased significantly from the beginning toward the end of the spawning period (~120 days) following the model proposed by Begon and Parker (1986). Females of *H. japonica* can reproduce only

once in their lifetime, and they do not feed sufficiently during the reproductive season (Begon & Parker, 1986). Thus, to avoid increased chances of mortality in the later reproductive period, females lay more eggs and larger eggs at the beginning of the reproductive period (Begon & Parker, 1986). Larva length may vary following an even smaller time scale, i.e. during the process of fertilization (Marshall, Steinberg & Evans, 2004). For example, in the broadcasting sea urchins, *Holopneustes purpurescens* and *Heliocidaris erythrogramma,* under an "intermediate" sperm concentration (50% fertilization success) in the laboratory, oocytes that had not been previously exposed to sperm produced larger larvae, compared to oocytes that had been exposed to sperm before but had not been fertilized (Marshall et al., 2004). The authors suggested that changes in offspring size were due to size-dependant fertilization: larger oocytes were preferentially fertilized at a given sperm concentration. Changes in offspring size over time further highlight the need for studies across multiple life-history stages. Moreover, considering the influence of spawning sequence, future studies should repeatedly measure offspring size throughout the reproductive period, instead of taking measurements from a single reproductive event.

b. External conditions experienced by parents

Intra-specific competition experienced by mothers could interact with maternal genotype and influence offspring size, i.e. the density of conspecific juveniles/adults can mediate offspring size variation (Allen et al., 2008; Luttikhuizen et al., 2010). The effect of conspecific density on offspring size was suggested to be a combination of pre- and post-zygotic factors, because conspecific density could directly influence sperm

concentration as well as conspecific competition (Crean & Marshall, 2008). For example, egg size of the broadcasting bivalve *Macoma balthica* decreased with adult density, because adult density determined sperm concentration in the field (Luttikhuizen et al., 2010). Colonies of the bryozoan *Bugula neritina* from field locations with low densities of conspecifics produced larvae that were 13.8% smaller than those of colonies from high density areas (Allen et al., 2008). Allen et al. (2008) suggested that *B. neritina* adjusted larval size according to conspecific densities experienced, and proposed that the increased size under high conspecific density may benefit offspring by enhancing the chance of dispersal to escape a crowded environment.

Beside sperm concentration, polyandry could also provide some explanation for intra-clutch offspring size variation under high conspecific density (Sprenger, Anthes & Michiels, 2008). For example, oocytes are generally fertilized internally by copulation with multiple males in the hermaphroditic nudibranch *Chelidonura sandrana* (Sprenger et al., 2008). Focal "female" individuals of *C. sandrana* mated with different "males" produced significantly longer veliger larvae, compared to individuals that mated multiple times with the same partner (Sprenger et al., 2008). Two mechanisms were proposed to explain the effects of sperm diversity on offspring size: (1) "females" of *C. sandrana* cannot predict the environmental condition that their planktonic larvae will face, thus they fertilize with mixed sperm from different males to increases the possibility of producing some offspring with optimal fitness; (2) "females" of *C. sandrana* may use the number of different mating partners as an indication of high conspecific competition, thus, they produce larger offspring that may have a better survival and broader dispersal

(Sprenger et al., 2010). Both mechanisms were interpreted as a genetic bet-hedging strategy to decrease the variance of offspring fitness under unpredictable environmental conditions (Fox & Rauter, 2003).

Conflict between family members could also determine mean offspring size, especially competition over food and other resources among siblings and between parents and offspring (Kamel, Oyarzun & Grosberg, 2010). Family conflicts acting on offspring size is particularly relevant in poecilogonic species (Kamel et al., 2010). Poecilogony is the presence of more than one distinctive kind of nutritional development (planktotrophic, lecithotrophic) in the same sexually reproducing species. For example, in the polychaete *Boccardia proboscidea* one type of female produces capsules containing a mix of unfertilized nurse eggs, planktotrophic and adelphophagic progeny (Kamel et al., 2010). Adelphophagic progeny consume nurse eggs and also cannibalize planktotrophic siblings developing in the same capsule. Females of *B. proboscidea* can increase the number of nurse eggs inside each capsule, and also actively tears open each capsule, expelling the contents from the tube (Kamel et al., 2010). Early opening of capsules is believed to decrease cannibalism on planktonic larvae, and might be a response to locally unfavourable conditions (i.e. by promoting dispersal).

Inter-specific competition is another important factor determining optimal offspring size (or size variation) among populations or individuals, but very few studies have been conducted on this topic. In the brooding bryozoan *Watersipora subtorquata*, females that experienced inter-specific competition (from other species of bryozoans, ascidians, polychaetes, and barnacles) produced larger offspring than colonies free of

competition in the field (Marshall & Keough, 2009). The latter authors suggested that increased offspring size in *W. subtorquata* was an adaptive response to competition: females adaptively produce larger offspring which have a higher dispersal potential and thus a higher chance to escape the competitive environment.

Other environmental conditions experienced by the mother, i.e. habitat, temperature and food availability, can influence offspring size. The initial size of juveniles has been related to adult habitat in marine invertebrates (Solé-Cava, Thorpe & Kaye, 1985; Moran, 1999). For example, lobster larvae captured in offshore waters were larger than those in inshore waters near Nova Scotia, Canada (Harding, Kenchington & Zheng, 1993). Moran (1999) reviewed the initial hatchling length of several species from three marine gastropod taxa, and found a trend with subtidal species having larger initial juvenile sizes than intertidal relatives, which was attributed in part to contrasting causes of juvenile mortality in the two environments. Moran (1999) suggested that abiotic stresses including desiccation, extreme temperatures, fluctuating salinity, as well as biotic stresses including predation in intertidal habitats, were the major causes of juvenile mortality. Thus, parental investment in smaller and more numerous offspring was likely to be favoured in highly variable and unpredictable intertidal habitats. On the other hand, biotic stresses (e.g. predation) are the primary causes of juvenile mortality in the subtidal habitats, thus, larger juvenile size are favoured to increase the chance of survival (Moran, 1999). However, the interpretation should be made with caution, considering that other selective factors besides predation and desiccation (i.e. substrate types, food availability, prey size) could also shape the optimal offspring size. For example, the interaction

between juvenile sea anemones and their specialized predator was driven both by the size of the prey and the size of the predator (Chapter 5). More precisely, *Urticina felina* juveniles (< 12 mg) were more vulnerable to subadults of the nudibranch *Aeolidia papillosa*, as no adult nudibranchs fed on them. In addition, subadult nudibranchs fed more frequently on the large juveniles of *U. felina* than on small ones. A completely different scenario was observed in interactions between nudibranchs and the much larger prey represented by *Aulactinia stella* juveniles (up to 200 mg). Larger juveniles of *A. stella* suffered higher predation rates when exposed to adult nudibranchs than subadult ones. Subadult nudibranchs were less inclined to feed on *A. stella* juveniles than adult nudibranchs, and the predation rates of subadult nudibranchs on large *A. stella* juveniles was lower than that on the small ones. Thus, the size-performance relationship is highly variable and determined by an interaction between offspring size and external factors (i.e. predator size) especially at the post-metamorphic stage (Chapter 5). Comprehensive research especially at the intra-specific level is needed to formulate a better explanation for offspring size selection in differing habitats.

Temperature has also been proposed to mediate offspring size in marine invertebrates (Simonini & Prevedelli, 2003; Collin & Salazar, 2010). For example, the polychaete *Dinophilus gyrociliatus* produced smaller eggs at 30°C, and larger eggs at lower temperatures between 12 to 24°C (Simonini & Prevedelli, 2003). Egg diameter and hatchling length produced at 23°C were larger than those produced at 28°C in two species of gastropods, *Crepidula atrasolea* and *C. ustulatulina* (Collin & Salazar, 2010). Collin & Salazar (2010) suggested that temperature-mediated size change may be due to

the relationship between size and oxygen supply and consumption. However, studies are needed to clarify the relationship between temperature and offspring size since contrasting results exist: e.g. Steer et al. (2004) studied the egg size of the squid *Euprymna tasmanica*, and found that egg size was not related to temperature, but rather to maternal nutrition.

Maternal nutrition, including food availability and diet type, has been reported to mediate offspring size in several marine invertebrates (Chester, 1996; Cheung & Lam, 1999; Steer et al., 2004). For example, starved females of the nudibranch *Tenellia adspersa* produced significantly smaller eggs than well-fed females (Chester, 1996). Similarly, females of the squid *Euprymna tasmanica* that were reared under low food availability produced smaller eggs compared to those reared under high food availability (Steer et al., 2004). The latter authors suggested that less-fed females cannot provide as much maternal nutrition as well-fed females. On the other hand, increased food availability was also shown to decrease or have no influence on offspring size in marine invertebrates (Cheung & Lam, 1999). It was suggested that the influence of food availability on offspring size was determined by "whether mothers have an opportunity to reproduce at some later stage and/or whether maternal nutrition is a good indicator of offspring nutrition" (Marshall et al., 2008a). Furthermore, the type/quality of the diet could also affect offspring size. For example, females of the greenlip abalone *Haliotis laevigata* that fed on red seaweed produced significantly smaller eggs than those that fed on a low level arachidonic acid diet (Graham et al., 2006).

Other factors, e.g. salinity, pollution, and manipulations of the females have been suggested to mediate offspring size. In the estuarine crab *Chasmagnathus granulata*, eggs from females maintained at a salinity of 15 had on average larger diameters than the eggs of females maintained at 20 and 32 (Gimenez & Anger, 2001). The average size of larvae produced by females exposed to copper was 12% larger than that of larvae from unexposed colonies of the bryozoan *Bugula neritina* (Marshall, 2008). In addition, manipulations on maternal size have been shown to mediate offspring size in *B. neritina*, i.e. halved colonies produced smaller larvae than unmanipulated colonies (Marshall & Keough, 2004b).

4. Factors that mediate variability in offspring size

Previous studies have identified a number of factors that influence the mean offspring size (see above; Table 2-3), but factors affecting variability in offspring size at the intra-specific level in marine invertebrates have received far less attention (Table 2-4).

One of the simplest explanations for offspring size variation posits that the production of uniformly larger offspring is constrained by physiological processes. For example, a few studies that tested within-clutch offspring size variations have suggested that they are due to physiological constraints preventing mothers from producing offspring of identical size, rather than to a diversified bet-hedging strategy (e.g. Einum & Fleming, 2004). Nevertheless, data have been published in support of an adaptive maternal strategy that would ensure the survival of some offspring under unpredictable conditions (Marshall et al., 2008b). On-going debates on the two theories will be discussed in the next Section.

A review of the literature shows that unpredictable environments may not always elicit parents to favour greater offspring size variability in marine invertebrates, although measures of "unpredictability" are drastically simplified. For example, the bryozoan *Bugula neritina* experiencing variable levels of conspecific competition (achieved by manipulating densities) were not shown to produce offspring with larger size variations (Allen et al., 2008). Two possible explanations were proposed: (1) the power of the analysis was not sufficient to detect subtle offspring size variation; or (2) environmental variation did not cause offspring size variation in *B. neritina*. On the other hand, constant environments causing larger variability in offspring size have been reported in the greenlip abalone *Haliotis laevigata* (Graham et al., 2006). Size variation in *H. laevigata* eggs increased over time when the adults were constantly fed a certain diet which was deemed "stressful" as it resulted in weight loss in the greenlip abalone (Graham et al., 2006). Similarly, marked offspring size variations were detected during and after parental care in species that brood offspring internally to mature demersal larvae or benthic juveniles (Chapters 3& 4), in spite of the fact that such strategies should, in theory, enable parents to predict the offspring environment.

Specific factors have also been investigated. For instance, temperature-related stress may influence the variability of offspring size (Jacobs & Podolsky, 2010). Egg masses of the intertidal gastropod *Melanochlamys diomedea* were reared under laboratory conditions at temperatures of 23, 26 and 29 °C (based on the temperature range in the field). Jacobs & Podolsky (2010) suggested that the increase of temperature from 23 to 29°C reflected an increasing level of stress. The size of hatchlings from highly stressed

adults was more variable than that of hatchlings from less stressed adults when embryos were exposed to low or medium stress, but it was less variable when embryos were exposed to high stress (Jacobs & Podolsky, 2010). The authors proposed that maternal effects reduced offspring size variation when embryos experienced conditions similar to the adults (i.e. more predictable environment).

Furthermore, Marshall & Keough (2004b) found that halved colonies of the bryozoan *B. neritina* produced larvae of a more variable size than unmanipulated colonies. Crean & Marshall (2009) suggested the large intra-clutch size variation after the manipulation of maternal size (simulating a predation event) was caused by physiological constraints due to the shift of resources from reproduction to growth (recovery).

Maternal size was also suggested to influence the variability of offspring size (Marshall et al., 2000). For example, smaller colonies of ascidian *Pyura stolonifera* produced smaller eggs but with larger intra-clutch size variation, compared to larger colonies (Marshall et al., 2000). On the other hand, a study on the gastropods *Crepidula ustulatulina* and *C. atrasolea* showed no relationship between intra-individual egg size variation and maternal size (Collin, 2010), and the authors suggested that factors responsible for the variation were not clear.

Another interesting source of variability in the size of offspring relates to developmental variation. Studies on developmental variation (or developmental noise) are rare, due to the lack of suitable model organisms (Vogt et al., 2008) especially in marine invertebrates. One well-studied model species is the freshwater marbled crayfish (parthenogenetic strain of *Procambarus alleni*). Vogt et al. (2008) studied the offspring

phenotypic variation from embryonic to adult stages among batch-mates from one *P. alleni*. They detected large size variations in isogenic batch-mates that were reared under the same environmental conditions with excess availability of food, and they found that size variation was enhanced remarkably after the juveniles reached the first feeding stage. Vogt et al. (2008) suggested "individual decision", i.e. "how much to feed and how often to feed and probably also slight differences in metabolism, which increase with time, are the main causes for this phenomenon". Furthermore, they proposed that developmental variation can be produced in all life stages and could change over the lifetime, suggesting that developmental variation is of great significance for clonal organisms to adapt to variable environments.

5. Bet-hedging hypotheses

Although there are numerous debates on the determination of optimal offspring size, three major hypotheses have been proposed: (1) the size-number trade-off (Smith & Fretwell, 1974), (2) the safe-harbour hypothesis (Shine, 1978), and (3) bet hedging, or variation in offspring size (Philippi & Seger, 1989). The first two theories are based on the assumption that large offspring result from greater parental investment and possibly benefit from higher individual fitness, and "predict a dichotomy in egg size in different species" (Levitan, 2000). However, the generality of this assumption is not clear, especially when being tested in the field (Monro, Sinclair Taylor & Marshall, 2010). These theories cannot adequately explain the widely observed within-clutch offspring size variations in some species of marine invertebrates, which could be better explained by the bet-hedging theory (reviewed by Marshall et al., 2008b), a concept that has

received much attention (mainly in Chordata and Arthropoda) but remains hard to assess (Simons, 2011).

Bet hedging is a strategy that decreases the temporal variance in fitness by sacrificing arithmetic mean fitness in unpredictable environments (Philippi & Seger, 1989). Bet hedging is applied to achieve maximum long-term fitness, which is measured as the geometric mean of the yearly/generational fitness contributions and is sensitive to large fitness variations (Olofsson, Ripa & Jonzén, 2009). As suggested by Olofsson *et al.* (2009), an individual could use two types of bet hedging to decrease the variance in fitness between years/generations: (1) conservative bet hedging, which involves producing fewer but larger offspring (conservative bet hedging is a low-risk strategy which produces offspring larger than the optimal size in a stable environment); and (2) diversified bet hedging, which involves producing offspring of various sizes. Conservative and diversified bet-hedging hypotheses work on the two aspects of offspring size variation, the mean and the variability, respectively.

The diversified bet-hedging hypothesis assumes that by producing offspring of different phenotypes at least some of them will survive to contribute to parental fitness. Studies on offspring size variation of marine invertebrates have so far mainly focused on this hypothesis, e.g. when females cannot predict the environment that offspring will experience, increasing variance in offspring size is proposed to be favoured (Crean & Marshall, 2009). On the other hand, studies that tested within-clutch offspring size variations have also suggested that this variation is due to physiological constraints preventing mothers from producing offspring of identical size, rather than to an adaptive

strategy (e.g. Einum & Fleming, 2004). Marshall et al. (2008b) argued that the use of optimality models (i.e. Smith-Fretwell fitness function) in these studies could partly account for their conclusion that diversified bet hedging is not adaptive. As suggested by Marshall et al. (2008b), the Smith-Fretwell fitness function (Smith & Fretwell, 1974), or the assumption that individual offspring fitness increases with the amount of energy invested in them by the parent (generally translating into offspring size), is problematic, because increased offspring size could cause lower fitness due to greater risks of polyspermy, predation, etc.

Marshall et al. (2008b) provided the first theoretical support to the diversified bet-hedging hypothesis as an adaptation to an unpredictable environment by comparing variation in egg size within and among clutches (= within-brood and between-brood) in marine invertebrates, and suggested that the two should be considered separately under unpredictable environment conditions. They found high offspring size variation among mothers and low variation within mother in 'direct developers'. As mentioned earlier, the definition of direct developer in the paper is not accurate because it indiscriminately refers to those species that produce non-pelagic benthic larvae, or brood to juveniles inside their bodies or in capsules, overlooking the fact that these species may still have a larval stage (i.e. indirect development). Marshall et al. (2008b) proposed that mothers of 'direct developers' (benthic or encapsulated offspring with low dispersal ability) were able to produce optimal sized offspring according to environmental conditions. On the other hand, mothers of 'indirect developers' (taken to mean free/unprotected lecithotrophic and planktotrophic eggs/larvae) produced offspring of various sizes to

adapt to the unpredictable environment (Marshall et al., 2008b). However, as mentioned earlier, the conclusions of Marshall et al. (2008b) are difficult to reconcile, because (1) they compared CVs on diameter and volume; (2) they used an oversimplified classification of developmental modes; and (3) the ambiguous relationship between development modes and environmental prediction. For instance, lecithotrophic larvae may experience unpredictable conditions due to their long competency periods, e.g. once released, non-feeding larvae of brooding cold-water soft corals were observed to remain free-swimming in the water column for more than 100 days before settlement (Sun, Hamel & Mercier, 2010). In addition, the conclusion of Marshall et al. (2008b) could be biased because they compared CVs, which are significantly influenced by the mean. Thus, to gain a better understanding of the relationship between offspring size variation and reproductive modes, further research should take more factors into consideration, including actual competency period, and should use a more appropriate statistical analysis (see Section "Classification of offspring developmental modes and statistical approach").

Adaptive coin flipping was the third proposed type of bet hedging, which is a strategy of diversifying the egg size at individual or population level (inter-clutch or inter-individual variation) (Cooper & Kaplan, 1982; Kaplan & Cooper, 1984). The adaptive coin flipping strategy could be achieved by one single female reproducing repeatedly and producing eggs of a different mean size each time, or by several females producing eggs of a different mean size at the same time (Kaplan & Cooper, 1984). For an individual organism, Olofsson et al. (2009) suggested that the optimal bet-hedging

strategy is a combination of the three hypotheses mentioned above, more precisely, females should produce relatively large propagules, and also vary the mean propagule size of a clutch between years and the sizes of the propagules within a clutch. Furthermore, Olofsson et al. (2009) proposed that phenotypic variation within a population that was assumed to be due to non-adaptive variation (e.g. Einum & Fleming, 2004), instead can be the result of females having this mixed strategy.

As suggested by Ripa et al. (2010), whether a particular strategy is a bet-hedging strategy depends on the environment. In addition, because bet-hedging traits are generally only over longer time scales (ideally across generations), testing bet-hedging responses to environmental change is rare and difficult (Simons, 2011). Thus, we suggest that more case studies are required before drawing any general hypothesis, and models should accommodate the ever-shifting selective environmental factors that affect offspring size, together with aspects of parental genotypes and life histories.

Conclusions

Clearly, offspring size variation is a very complex topic, and the unambiguous classification of reproductive modes and the choice of statistical methods are key to accurately identifying the variables that may act as selective pressures on offspring size and size variation. Future studies should take into consideration the appropriate classification of development modes and the impact of extraction of offspring or the inducement of spawning discussed earlier. Naturally-released offspring should be the focus of studies of size variation whenever possible. An optimal standardized

measurement of offspring size should also be developed (weight, volume, surface area or diameter), and this measurement used to make inter-species comparison. Weight and volume are more accurate measures than surface area and diameter, especially for species with contractile or polymorphic offspring.

In addition, research on offspring size variation and size-related performance in benthic marine invertebrates remains taxonomically-biased. Studies on factors that mediate offspring size variation, including mean offspring size and the variability of offspring size, have largely focused on two phyla, the Bryozoa and Mollusca (Tables 2-3, 4). Data on other phyla are comparatively scarce, and performance in offspring of different sizes has very rarely been studied experimentally. In addition, offspring size variation could be mediated by several influential factors (Table 2-4), and the respective influences of these factors may be species-dependent. More comprehensive studies testing different influential factors should thus be performed, as was done with the brooding bryozoan *Bugula neritina* (Table 2-5), to gain a thorough understanding of offspring size variation in a given species. It is important to study the occurrence of offspring size variations within different taxa at the intra-species level, and identify both factors and mechanisms responsible for mediating offspring size before drawing general theories on offspring size variation. Also, the comparisons of offspring size variation at more detailed levels, e.g. at intra-clutch level or at intra-specific level but at different ontogenetic stages, will contribute to our understanding of the function and evolution of offspring size variation.

By drawing from recent literature and providing a fresh outlook grounded in principles of evolutionary and reproductive biology, it is our hope that this work will highlight new avenues for the study of offspring size and orient future research in this field. We suggest that the following topics deserve more attention:

1. Offspring size variation across life-history stages

Size variation has most often been studied separately in eggs, larvae or juveniles after their release into the environment. However, there are very few integrative studies taking into account the significance of offspring size at successive life-history stages (eggs, embryos, larvae, juveniles) within a species (Ito, 1997). Furthermore, some of the studies used offspring which were experimentally manipulated to reduce their size, i.e. by isolating blastomeres from embryos (Sinervo, 1993). These investigations are interesting in that they partially reveal the influence of initial offspring size on their subsequent size and performance and may help distinguish maternal from genetic effects. However, studies on naturally-released offspring of different sizes bring more information relative to the influence of offspring size on their performance in nature. In addition, research on brooding species is limited and generally confined to post release stages. What happens before the offspring are released is largely overlooked, i.e. at which life stage is size variation initiated and does mean variance increase or decrease throughout protected development? Brooding species make ideal models since they offer a stable/predictable environment (e.g. capsules, internal cavity, and brooding chamber) to their offspring for a portion of their development.

For broadcast spawning species, variation in offspring size across life history could be due to (1) size-related growth rates across life history, i.e. when juveniles reach a certain size, the growth rates slow down; (2) size-related survival across life history, i.e. smaller/larger eggs have higher fertilization rates under different sperm concentrations in the environment, or smaller/larger offspring have lower survival rates under biotic or abiotic pressure, including food availability and predation. For brooding species the strategy might be more complex. For example, *Urticina felina* from the northwest Atlantic is an internally-brooding sea anemone which releases larvae between July and September. Oocytes of *U. felina* detach from gamete-bearing mesenteries and float freely in the gastrovascular cavity and tentacles in April, where they get fertilized and develop into embryos in June/July. The average surface area of oocytes and embryos was ~0.4 mm^2, much smaller than the average surface area of larvae, ~1.0 mm^2. However, size variation at the oocyte stage was much smaller than at the embryonic stage or larval and juvenile stage (Fig. 2-3, Chapter 3). The large size differences between eggs/embryos and larvae as well as the large size variation at the larval stage were found to be due to the ability of sibling embryos to fuse together and form 'mega-larvae' (Mercier, Sun & Hamel, 2011, Chapter 3).

2. Studying offspring size variation and the bet-hedging hypothesis in brooding species

As discussed previously, research on offspring size variation has largely focused on egg size in broadcast spawning and egg-encapsulating species; however, very little information has been obtained from brooding (viviparous or live-bearing) species,

whether they incubate to the larval or the juvenile stage. For example, among the 102 marine invertebrates reviewed by Marshall and Keough (2007), the most frequently studied species were broadcasting (37.3%) or encapsulating (39.2%) species, and only 23.5% were brooding species (combining species that brood offspring to larvae or to juveniles). Data from true live-bearing species, with life-history strategies analogous to placental or viviparous vertebrates, are rare (i.e. 3 Echinodermata). In addition, the few explicit studies of offspring phenotype plasticity have mainly focused on benthic *colonial* brooding invertebrates (ascidians and bryozoans) and a few planktonic *unitary* brooders (crustaceans), whereas data are generally lacking for benthic *solitary/unitary* (non-colonial) brooders. Thus, more studies on unitary brooding species are needed, with complementary comparative work on colonial brooding species and solitary broadcast spawning species.

While parental care did not significantly influence offspring size variation at the inter-specific level (ANCOVA, $F = 1.86$, $p = 0.160$), as mentioned in the Section "Classification of offspring developmental modes and statistical approach", offspring size variation in species with post-zygotic parental care, especially brooding species, may be influenced by a closer and prolonged relationship between mother and offspring. Internally brooding species are a great model to test the bet-hedging theory, because the mothers can presumably predict the environment experienced by their offspring. For example, will offspring size variation be lower at stages when the environment in which they are growing is predictable (inside the mother) than the variation at stages when the

environment in which they will be released is slightly less predictable (in the field, around the mother)?

Brooding species may have a species-specific strategy to increase offspring size significantly during the period of parental care. For example, the embryos of the internally-brooding sea anemone *Urticina felina* are able to fuse and form 'mega-larvae', causing a significant increment in size variation from the larval stage onward (Mercier et al., 2011, Chapter 3). Another brooding species with a strategy to increase offspring size is the sea anemone *Aulactinia stella* (Chapter 4). Adults of *A. stella* are live-bearing, brooding offspring inside the gastrovascular cavity for a long period of time (to >1 year), and are able to release juveniles at any time of the year, with a peak between July and October. The long non-fixed brooding period, the co-existence of different cohorts of juveniles, and intra-brood feeding and competition best explains offspring size variations in *A. stella* (Chapter 4). Adelphophagic species provide other extreme examples for the complex scheme of offspring size variation, considering that some of them can manipulate offspring size variation during the reproductive period (Kamel et al., 2010). For example, females can actively pull each capsule until it tears, expelling its contents to increase the survival of smaller planktonic larvae. Thus, for adelphophagic species, data on size variation of naturally-hatched offspring is needed. Clearly, it is important to investigate the mechanisms causing offspring size variation carefully, especially for species with parental care, although determining whether they are expressions of parental or offspring strategies to increase their respective fitness, or both, might prove challenging.

3. Size-performance relationship across multiple life-history stages and across generations

The offspring size-performance relationship is key in determining optimal offspring size; however, the first question is whether an optimal offspring size truly always evolves to maximize parental fitness. Optimal offspring size may change during ontogeny, due to different selective factors acting across life stages (Crean & Marshall, 2008). Considering the diversity of life histories in marine invertebrates, the fact that offspring with different sizes may be favoured at different life stages may mean that adults will favour producing offspring of various sizes, supporting the bet-hedging hypothesis (Toonen & Pawlik, 2001a). In addition, marine invertebrate species with a complex life cycle may undergo changes in offspring size throughout ontogeny. For example, Crean and Marshall (2008) found that the broadcasting ascidian *Styela plicata* produced larger eggs at low compared to high adult densities. However, the overall increase in egg size in individuals at low densities was due to the increased size of follicle cell, and the size of ovicells was smaller compared to those at high densities. Thus, although individuals at high densities produced smaller eggs, their embryos were in fact larger than those of individuals exposed to low densities. Crean and Marshall (2008) suggested that smaller egg size of individuals at high density could decrease the possibility of polyspermy, and their larger embryo size could favour greater dispersal and low conspecific competition. Consequently, Crean and Marshall (2008) proposed that broadcasting marine invertebrates could "adaptively adjust the properties of their gametes in response to the risk of a combination of both pre- and post-zygotic factors". For marine

invertebrate species with a complex life cycle, research has shown that the effects of offspring size on performance could change throughout ontogeny (Rius et al., 2009). Studies of size-related offspring performance have almost exclusively focused on a single life stage (especially the post-metamorphic stage), whereas very little empirical data exist on size-related fitness across multiple life-history stages (Rius et al., 2009). To gain a better understanding of the evolutionary advantages of offspring sizes, integrative experiments will need to explore size-performance relationships across multiple ontogenetic stages, including pre-metamorphic stages, juvenile stages and adulthood.

In addition, a few other shortfalls should be addressed and adjusted for the benefit of future research on size-performance relationship. First, information on size variation of naturally-released offspring is generally lacking or not clearly reported in most of the literature (Allen et al., 2008; Dias & Marshall, 2009). Without this background knowledge, it is difficult to discern if the offspring used for studying fitness are typically "large" or "small", and thus the influence of size on offspring performance might be underestimated. Second, research on post-metamorphic growth has revealed that larger offspring retained larger sizes; however, the rates of size increment (growth) were not compared. For instance, in the solitary ascidian *Microcosmus squamiger*, Rius et al. (2009) found that larger larvae stayed larger during juvenile stages over 11 weeks of observation in the field. However, the growth rates were higher in the smaller group than the larger one (Rius et al., 2009). Thus, it is possible that the size variation diminishes with time (smaller juveniles could reach the size of the larger ones in a given time period). More long-term studies on true growth rates are needed to understand how long the larger

offspring maintain their advantage in size and whether or not they grow faster. Third, in addition to thorough studies across life-history stages, research across generations is ideally needed to explore the influence of offspring size on reproduction. For instance, Dias & Marshall (2009) found that colonies from larger offspring have larger reproductive output (calculated as fecundity × 2nd generation offspring size) in the bryozoan *Celleporaria* sp. However, research across generations could be challenging, especially for species that take a long time (several years) to reach reproductive maturity.

4. Size-performance relationship under optimal or non-optimal environmental conditions

The offspring size-performance relation is not always positive, and depends on environmental conditions: theory predicts that larger offspring may have better fitness in stable conditions (K-selection), and smaller more abundant offspring may have more advantages under unstable conditions (r-selection). However, experimental evidence is generally lacking, and information needed to explore the effects of optimal or non-optimal conditions (i.e. predation pressure, thermal stress, food availability, pollution) on the performance of offspring of various sizes. For example, will the offspring size difference persist or dissipate under optimal conditions, especially high food availability? Will it be the same under low food availability? It will be interesting to assess to what extent the size of offspring will influence behaviour under uncongenial conditions.

In terms of biotic factors, the main environmental fluctuations include conspecific adult density, and predation pressure. While the former has been examined (Allen et al., 2008; Luttikhuizen et al., 2010), there is very limited research on the effects of offspring

size on predation rates. Intuitively, offspring size will not necessarily confer the same advantage depending on whether the settlers face opportunistic/omnivorous predators (e.g. non- or mildly- selective grazers) or a specialized predator. Furthermore, in spite of the general assumption that marine invertebrate offspring are widely palatable, very limited research has been done to support it. Lindquist and Hay (1996) proposed that larvae exhibited chemical defence toward fish predators, and a number of studies on lecithotrophic larvae of sponges, hydroids, bryozoans and corals have shown that these larvae were unpalatable to sympatric corals, sea anemones and fishes (Lindquist & Hay, 1996). Laboratory experiments will provide useful information in this area (Chapter 5), and experimental trials in the field would be very valuable for understanding the survival of offspring of various sizes.

The offspring size-performance relationship is likely context-dependent. For example, Marshall et al. (2003) found that larger larvae of the brooding bryozoan *Bugula neritina* survived better, compared to smaller ones. On the other hand, Marshall (2008) found that larvae produced by colonies exposed to copper were larger than those from unexposed colonies. The larger larvae from copper-exposed mothers survived better under conditions with copper pollution stress, compared to those from mothers not exposed to copper. However, they had a poorer performance in the field under stress, i.e. intra-specific competition. In addition, studies have shown that the offspring size-performance relationship is highly dependent on offspring experiences, i.e. artificially delayed metamorphosis (Dias & Marshall, 2009), and the local environment. Controlled laboratory conditions have been shown to underestimate the effect of offspring size on

their performance, compared to natural conditions in the field (Monro et al., 2010). Thus, it is important to conduct experimental studies under naturally varying environmental conditions, or use a field component to confirm data collected from the laboratory. Data from a small number of field studies have already shown that the effects of offspring size in native environments could be very different from the results obtained under laboratory conditions (i.e. intra-specific competition, Allen et al. 2008). In this context, laboratory conditions with environmental variations are preferable to static conditions.

5. Size-related biochemical, physiological and morphological features of offspring

It is often assumed that offspring size is a good indicator of fitness, taking for granted that larger offspring will perform better than smaller ones (but see above). However, this assumption has not been confirmed experimentally. In addition, even if it is true that larger offspring have more energy content, it is still not persuasive to conclude that larger offspring have higher fitness without considering their metabolic rates. Thus, combined studies on biochemical markers as well as metabolic rates in offspring of various sizes are needed. Besides metabolic rates and biochemical composition, other information on size-related offspring biology is also needed, i.e. ultrastructural and cellular differences, as well as genetic and molecular evidence (e.g. acquisition of allorecogntion).

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Tables

Table 2-1. Some of the various offspring developmental modes in marine invertebrates, with corresponding photos in Fig. 2-1.

Photos in Fig. 2-1	Species	Phylum	Structural Organization	Offspring Morphogenesis	Offspring Habitat	Offspring Care	Offspring Nutrition[a]
A,B	*Aulactinia stella*	Cnidaria	Unitary	Abbreviated	Benthic	Protected	Non-feeding
C,D	*Henricia lisa*	Echinodermata	Unitary	Abbreviated	Benthic	Both[b]	Non-feeding
E,F	*Urticina felina*	Cnidaria	Unitary	Abbreviated	Both	Both[c]	Non-feeding
G,H	*Drifa* sp.	Cnidaria	Colonial	Abbreviated	Both	Both[c]	Non-feeding
I,J	*Solaster endeca*	Echinodermata	Unitary	Abbreviated	Pelagic	Free	Non-feeding
K,L	*Isostichopus fuscus*	Echinodermata	Unitary	Complex	Pelagic	Free	Feeding
M	*Didemnum* sp.	Chordata	Colonial	Complex	Pelagic	Free	Feeding
N,O	*Lambis lambis*	Mollusca	Unitary	Complex	Both	Both[c]	Feeding
P	*Lebbeus groenlandicus*	Arthropoda	Unitary	Complex	Pelagic	Both[c]	Feeding

a. To metamorphosis.
b. Some offspring are brooded and other develop in the water column.
c. Offspring are brooded/encapsulated to a certain stage, then released.

Table 2-2. Comparison of different statistical methods for analyzing mediators of offspring size variability using data in the Appendix 2-1 ($p < 0.05$).

	Effects			
Statistics	**Nutrition**	**Habitat**	**Parental care**	**Morphogenesis**
ANOVAs on CV	Not significant	Significant	Significant	Significant
ANCOVAs on SD with mean as covariate	Not significant	Not significant	Not significant	Not significant
ANCOVAs on lgSD with lgMean as covariate	Significant	Not significant	Not significant	Not significant

Table 2-3. Factors proposed to influence mean optimal offspring size in marine invertebrates.

Factor tested	Conclusion	Phylum	Species	Type[a]	Measure[b]	Organisation[c]	Reference
1. Maternal phenotype							
Maternal size	Stage I larva sizes from females with smaller carapace length were significantly smaller than mean larva sizes from females with larger carapace length	Arthropoda	*Homarus americanus*	LB	LL	U	(Ouellet & Plante, 2004)
Maternal size	Egg size not related to maternal carapace length	Arthropoda	*Pagurus longicarpus*	LB	OD	U	(Damiani, 2003)
Maternal size	Egg size not related to maternal size	Arthropoda	*Scyllarides squammosus*	LB	OD	U	(DeMartini & Williams, 2001)
Maternal size	Embryo size not related to number of setae-bearing segments of female	Annelida	*Streblospio benedicti*	LB	EV	U	(Bridges & Heppell, 1996)
Maternal size	Colonies with larger weight produced larger larvae	Bryozoa	*Bugula neritina*	LB	LS	C	(Marshall et al., 2003)
Maternal size	Egg size increased with maternal weight	Chordata	*Pyura stolonifera*	P	OD	C	(Marshall et al., 2000)
Maternal size	Hatchling size not related to maternal shell length	Mollusca	*Buccinum cyaneum*	EN	JL	U	(Miloslavich & Dufresne, 1994)
Maternal size	Hatchling size not related to maternal shell length	Mollusca	*Buccinum isaotakii*	EN	JL	U	(Ilano et al., 2004)
Maternal size	Maternal shell length had strong positive linear relationship with capsule size, and capsule size had positive relationship with hatchling size	Mollusca	*Buccinum undatum*	EN	JL	U	(Nasution et al., 2010)

Maternal size	Egg size not related to maternal shell length	Mollusca	*Crepidula atrasolea*	EN	OD	U	(Collin, 2010)
Maternal size	Egg size not related to maternal shell length	Mollusca	*Crepidula dilatata*	EN	OD	U	(Chaparro et al., 1999)
Maternal size	Mean size juveniles at hatching increased with maternal shell length	Mollusca	*Crepidula dilatata*	EN	JL	U	(Chaparro et al., 1999)
Maternal size	Egg size not related to maternal shell length	Mollusca	*Crepidula ustulatulina*	EN	OD	U	(Collin, 2010)
Maternal size	Egg size increased with maternal shell length	Mollusca	*Haloa japonica*	EN	OV	U	(Ito, 1997)
Maternal size	Egg size increased with maternal lorica length	Rotifera	*Keratella cochlearis*	LB	OV	U	(Green, 1998)
Maternal age (spawning sequence)	Females produced larger eggs in the first laid masses than subsequent egg masses	Chordata	*Adalaria proxima*	EN	OD	U	(Jones et al., 1996)
Maternal age (spawning sequence)	Egg size decreased with spawning sequence	Mollusca	*Haloa japonica*	EN	OV	U	(Ito, 1997)
Diel variation	Larval size decreased as day progressed	Bryozoa	*Bugula neritina*	LB	LV	C	(Kosman & Pernet, 2009)
Mating order	Larvae generated from oocytes exposed to sperm the first time were larger, compared to those from oocytes previously exposed to sperm but unfertilized	Echinodermata	*Heliocidaris erythrogramma*	P	LL	U	(Marshall et al., 2004)
Mating order	Larvae generated from oocytes exposed to sperm the first time were larger, compared to those from oocytes previously exposed to sperm but unfertilized	Echinodermata	*Holopneustes purpurescens*	P	LL	U	(Marshall et al., 2004)

2. Maternal experience
(including biotic and abiotic environmental factors)

Adult density (as proxy of intra-specific competition)	Colonies produced larger larvae at high adult densities and smaller larvae at low densities	Bryozoa	*Bugula neritina*	LB	LS	C	(Allen et al., 2008)
Adult density (as proxy of intra-specific competition)	Colonies produced smaller eggs at high densities. However, eggs had larger ovicells, so yielded larger embryos	Chordata	*Styela plicata*	P	OS	C	(Crean & Marshall, 2008)
Adult density (as proxy of sperm concentration)	Egg size decreased with adult density	Mollusca	*Macoma balthica*	P	OD	U	(Luttikhuizen et al., 2010)
Copulation with multiple males	Polyandrous individuals produced significantly larger veliger larvae	Mollusca	*Chelidonura sandrana*	EN	LL	U	(Sprenger et al., 2008)
Interspecific competition	Colonies experiencing higher inter-specific competition produced larger larvae	Bryozoa	*Watersipora subroquata*	LB	LS	C	(Marshall & Keough, 2009)
Manipulation on maternal size	Halved colonies produced smaller larvae than unmanipulated colonies	Bryozoa	*Bugula neritina*	LB	LS	C	(Marshall & Keough, 2004b)
Temperature	Smallest eggs generally produced at higher temperatures	Annelida	*Dinophilus gyrociliatus*	EN	OD	U	(Simonini & Prevedelli, 2003)
Temperature	Egg and hatchling larger at 23°C than 28°C	Mollusca	*Crepidula atrasolea*	EN	OD, JL	U	(Collin & Salazar, 2010)
Temperature	Egg and hatchling larger at 23°C than 28°C	Mollusca	*Crepidula ustulatulina*	EN	OD, JL	U	(Collin & Salazar, 2010)
Temperature	Temperature did not influence egg size	Mollusca	*Euprymna tasmanica*	EN	OV	U	(Steer et al., 2004)

Maternal nutrition	Eggs produced by starved females significantly smaller than those produced by fed individuals	Chordata	*Tenellia adspersa*	EN	OD	U	(Chester, 1996)
Maternal nutrition	Females fed high food concentrations produced larger eggs	Mollusca	*Euprymna tasmanica*	EN	OV	U	(Steer et al., 2004)
Maternal nutrition	Size of eggs not affected by food availability to females	Mollusca	*Nassarius festivus*	EN	OD	U	(Cheung & Lam, 1999)
Maternal nutrition	Maternal diet influenced egg size: females fed on seaweed produced significantly smaller eggs than females fed on a low level arachidonic acid diet	Mollusca	*Haliotis laevigata*	P	OD	U	(Graham et al., 2006)
Maternal nutrition	Eggs produced by starved females significantly smaller than those produced by fed individuals	Chordata	*Tenellia adspersa*	EN	OD	U	(Chester, 1996)
Salinity	Larger eggs diameters in females maintained at 15 than 20 and 32	Arthropoda	*Chasmagnathus granulata*	EN	OD	U	(Gimenez & Anger, 2001)
Exposure to pollution stress	Colonies exposed to pollution stress (copper) produced larger larvae	Bryozoa	*Bugula neritina*	LB	LS	C	(Marshall, 2008)

a. Pelagic P, Encapsulated EN, Brooding to juvenile JB, Brooding to larva LB
b. Oocyte/egg diameter OD, Oocyte/egg surface area OS, Oocyte/egg volume OV, Embryo volume EV, Larval surface area LS, Larval length LL, Larval volume LV, Juvenile length JL
c. Unitary U, Colonial C

Table 2-4. Factors proposed to mediate the variability of offspring size in marine invertebrates.

Factors tested	Conclusion	Phylum	Species	Type[a]	Measure[b]	Parental care[c]	Organisation[d]	Reference
Nutritional stress	Egg size variation increased over time when females constantly fed on certain diet	Mollusca	*Haliotis laevigata*	P	OD	F	U	(Graham et al., 2006)
Temperature	Hatchling size from highly stressed adult (29°C) more variable than those from less stressed adults (23, 26°C) when embryos exposed to low or medium stress, but less variable when embryos exposed to high stress	Mollusca	*Melanochlamys diomedea*	EN	JL	Both	U	(Jacobs & Podolsky, 2010)
Manipulation on maternal size	Halved colonies produced more variable larvae than unmanipulated colonies	Bryozoa	*Bugula neritina*	LB	LS	Both	C	(Marshall & Keough, 2004b)
Maternal size	Smaller females produced eggs with larger intra-clutch size variation, compared to larger females	Chordata	*Pyura stolonifera*	P	OD	F	U	(Marshall et al., 2000)
Maternal size	Egg size variation not related to maternal size	Mollusca	*Crepidula atrasolea*	EN	OD, JL	P	U	(Collin, 2010)
Maternal size	Egg size variation not related to maternal size	Mollusca	*Crepidula ustulatulina*	EN	OD, JL	P	U	(Collin, 2010)

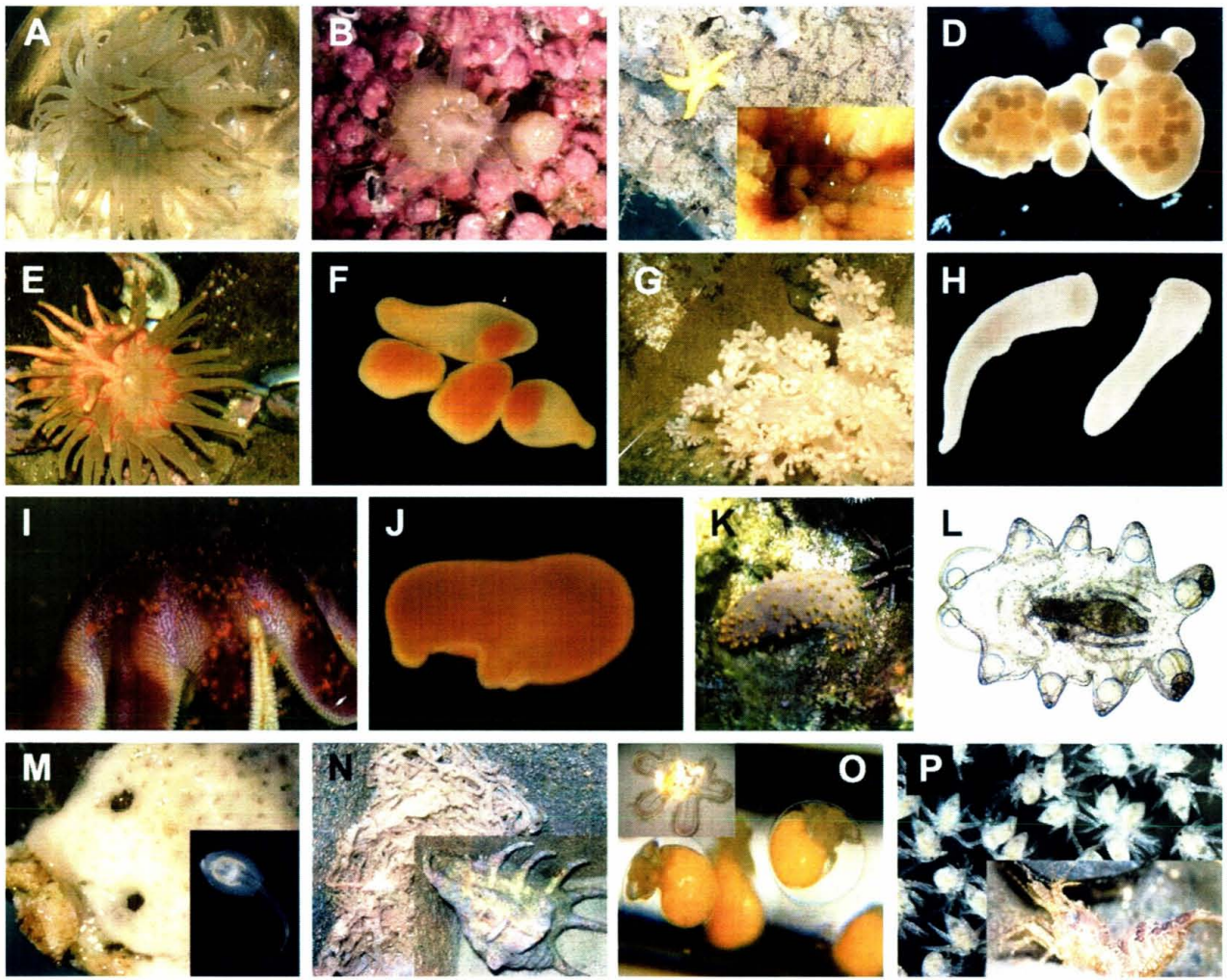
a. Pelagic P, Encapsulated EN, Brooding to juvenile JB, Brooding to larva LB
b. Oocyte/egg diameter OD, Juvenile length JL, Larval surface area LS
c. Parental care F, P, Both
d. Unitary U, Colonial C

Table 2-5. Offspring size and size variability in the brooding bryozoan *Bugula neritina.*

Source of variation	Conclusion	Reference
1. Mean offspring size		
Maternal size	Larger colonies produced larger larvae, and colonies that derived from these larger larvae produced larger offspring	(Marshall et al., 2003)
Manipulation on maternal size	Halved colonies produced smaller larvae than unmanipulated colonies	(Marshall & Keough, 2004b)
Adult density (intraspecific competition)	Colonies produced larger larvae at high densities and smaller larvae at low densities	(Allen et al., 2008)
Exposure to pollution stress	Colonies exposed to pollution stress (copper) produced larger offspring	(Marshall, 2008)
Diel variation	Size of larvae decreased as the day progressed	(Kosman & Pernet, 2009)
2. Offspring size variability		
Manipulation on maternal size	Halved colonies produced more variably-sized larvae than unmanipulated colonies	(Marshall & Keough, 2004b)
Adult density (intraspecific competition)	Colonies in high-density and low-density environments produced offspring with similar size variations	(Allen et al., 2008)

Figures

Fig. 2-1 (next page). Sample of the diversity of offspring developmental modes and phenotypes in marine invertebrate taxa. Details provided in Table 2-1. a) The live-bearing sea anemone *Aulactinia stella* (~6 cm in diameter). b) Juveniles of *A. stella* (0.5-1 cm in diameter). c) The sea star *Henricia lisa* (~7 cm in diameter). In this species some offspring are brooded as shown in insert (embryos ~1.2 mm), while others develop freely. d) Late brachiolaria larvae of *H. lisa* (~2.1 mm) undergoing metamorphosis. e). The brooding sea anemone *Urticina felina* (~10 cm in diameter). h) Planula larvae of *U. felina* (1~2 mm long). i) Spawning female sea star *Solaster endeca* (~25 cm in diameter). j) Brachiolaria larva of *S. endeca* (1.2 mm). k) The sea cucumber *Isostichopus fuscus* (~28 cm long). l) Auricularia larva of *I. fuscus* (1.1 mm long). m) The ascidian *Didemnum* sp. (~3 cm span) with insert showing its tadpole larva (8 mm long). n) Egg mass of the gastropod *Lambis lambis* (10 cm span) with insert showing the adult (~15 cm long). o) Veliger larvae of *L. lambis* developing inside the egg mass before hatching (0.7 mm), with insert showing the hatched free-swimming veliger larva (0.9-1.1 mm). p) Nauplius larvae (0.3 mm) of the shrimp *Lebbeus groenlandicus*, with insert showing the adult (9 cm long).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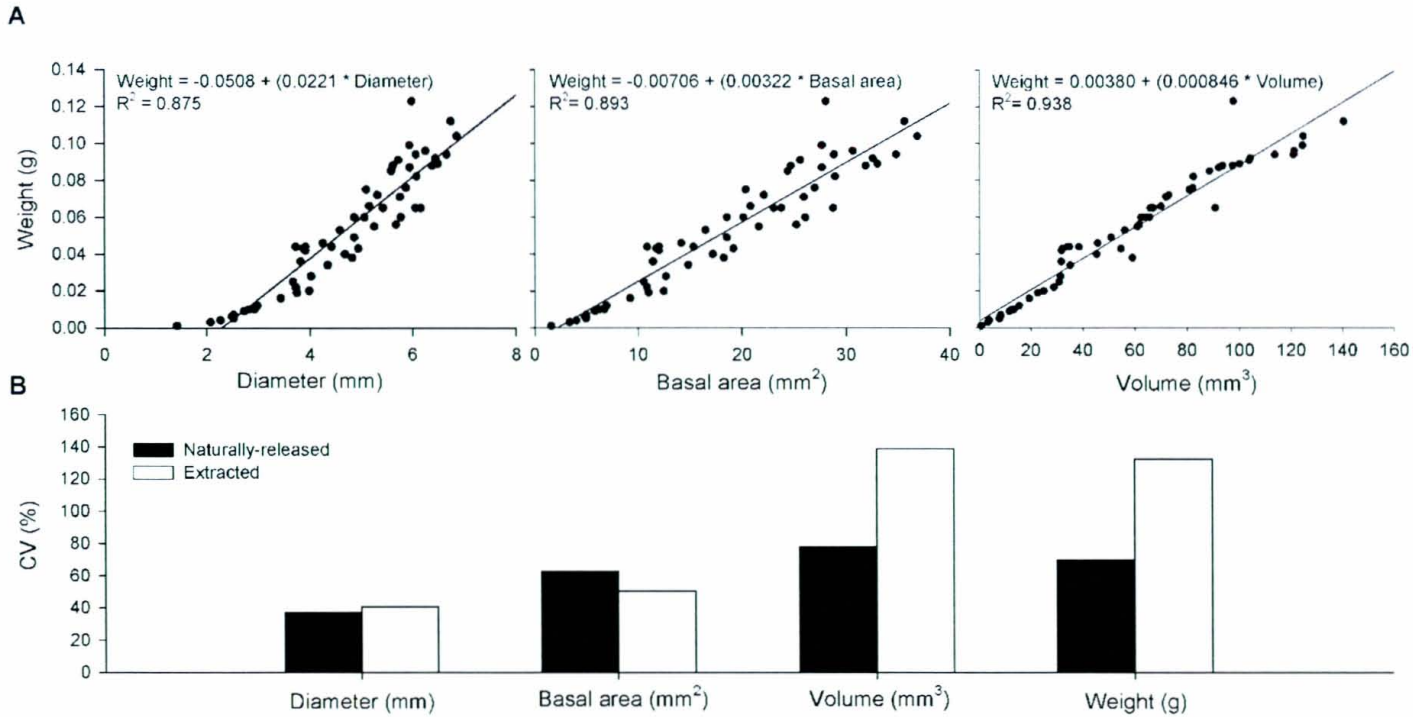

Fig. 2-2. a) Relationships between basal diameter (mm), basal area (mm^2) and volume (mm^3) and juvenile weight (g) in *Aulactinia stella*; b) Coefficient of variation (CV) of basal diameter, basal area, volume, and weight (g) in naturally-released *vs* extracted *Aulactinia stella* juveniles from the same parent

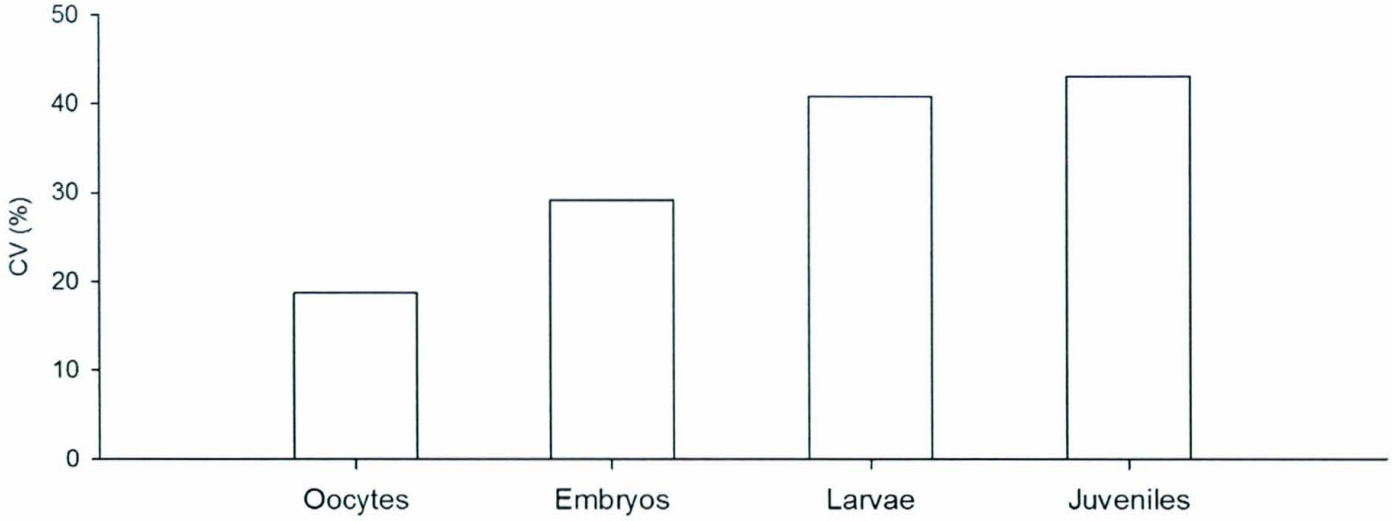

Fig. 2-3 Overall size variation expressed as CV (%) of mean surface area (mm^2) in oocytes, embryos, planula larvae and juveniles of *Urticina felina* (n = 4 - 6 broods)

Appendix

Appendix 2-A. Offspring size variation in marine invertebrates, including data from the literature and new data from the present study (bold).

Phylum	Species	Morpho-genesis[a]	Offspring habitat[b]	Offspring care[c]	Offspring nutrition[d]	Organi-sation[e]	Offspring size (µm)	CV (%)	SD[f]	Source
Annelida	*Bushiella abnormis*	COM	Both	Both	NF	U	185	21.62	40.00	(Hess, 1993)
	Circeis armoricana	COM	Both	Both	NF	U	167	10.17	16.98	(Hess, 1993)
	Hydroides dianthus	COM	PL	F	FF	U	60.7	8.23	5.00	(Toonen & Pawlik, 2001b)
	Paradexiospira vitrea	COM	Both	Both	NF	U	196	9.18	17.99	(Hess, 1993)
	Phragmatopoma lapidosa	COM	PL	F	FF	U	90.4	4.09	3.70	(McCarthy, Young & Emson, 2003)
	Pileolaria berkeleyana	COM	Both	Both	NF	U	169	7.69	13.00	(Hess, 1993)
	Protolaeospira eximia	COM	Both	Both	NF	U	199	10.55	20.99	(Hess, 1993)
Bryozoa	*Bugula neritina*	COM	Both	Both	NF	C	271	6.9	18.70	(Marshall et al., 2003)
	Bugula simplex	COM	Both	Both	NF	C	207	19.43	40.22	(Wendt, 2000)

	Bugula stolonifera	COM	Both	Both	NF	C	160	7.9	12.64	(Wendt, 2000)
	Bugula turrita	COM	Both	Both	NF	C	202	6.64	13.41	(Wendt, 2000)
	Watersipora subtorquata	COM	Both	Both	NF	C	323.18	11	35.55	(Marshall & Keough, 2004a)
Chordata	*Ciona intestinalis*	COM	PL	F	NF	U	145	5.17	7.50	(Marshall & Keough, 2007)
	Diplosoma listerianum	COM	PL	F	NF	C	976	9.32	90.96	(Marshall & Keough, 2007)
	Pyura fissa	COM	PL	F	NF	U	175.78	5.21	9.16	(Marshall & Keough, 2007)
	Pyura stolonifera	COM	PL	F	NF	C	269	9.18	24.69	(Marshall & Keough, 2007)
	Styela plicata	COM	PL	F	NF	U	163	7.9	12.88	(Marshall & Keough, 2007)
Cnidaria	*Acropora hyacinthus*	SIM	PL	F	NF	C	553	4.7	25.99	(Baird et al., 2001)
	Acropora millepora	SIM	PL	F	NF	C	541	5.91	31.97	(Baird et al., 2001)
	Acropora spathulata	SIM	PL	F	NF	C	557	9.33	51.97	(Baird et al., 2001)
	Astreopora myriophthalma	SIM	PL	F	NF	C	538	3.38	18.18	(Baird et al., 2001)

Aulactinia stella	**SIM**	**B**	**P**	**NF**	**U**	**4692.3**	**29.4**	**1379.54**	**Present study**
Cyanea capillata	**SIM**	**PL**	**Both**	**NF**	**U**	**157.6**	**17.1**	**26.95**	**Present study**
Favites halicora	SIM	PL	F	NF	C	401	8.7	34.89	(Baird et al., 2001)
Gersemia rubiformis	**SIM**	**Both**	**Both**	**NF**	**C**	**455.3**	**14.9**	**67.84**	**Present study**
Goniastrea retiformis	SIM	PL	F	NF	C	371	5.66	21.00	(Baird et al., 2001)
Heliopora coerulea	SIM	B	Both	NF	C	3700	10.81	399.97	(Harii et al., 2002)
Pachyseris speciosa	SIM	PL	F	NF	C	368	5.16	18.99	(Baird et al., 2001)
Pocillopora damicornis	SIM	Both	Both	NF	C	1000	20	200.00	(Harii et al., 2002)
Montipora digitata	SIM	PL	F	NF	C	337	12.16	40.98	(Baird et al., 2001)
Stomphia coccinea	**SIM**	**B**	**P**	**NF**	**U**	**618**	**18.4**	**113.71**	**Present study**
Tubularia mesenbryanthemum	SIM	B	Both	NF	C	305	10.88	33.18	(Yamashita et al., 2003)
Urticina felina	**SIM**	**Both**	**Both**	**NF**	**U**	**676.4**	**9.3**	**62.91**	**Present study**

Crustacea	*Balanus balanoides*	COM	Both	Both	FF	U	283	4.94	13.98	(Barnes & Barnes, 1965)
	Chthamalus dentatus	COM	Both	Both	FF	C	190.9	4.6	8.78	(Achituv & Wortzlavski, 1983)
	Geryon fenneri	COM	Both	Both	FF	U	567	2.64	14.97	(Hines, 1988)
	Euterpina acutifrons	COM	PL	Both	FF	U	62.4	0.7	0.44	(Guisande, Sanchez & Manciro, 1996)
	Geryon quinquedens	COM	Both	Both	FF	U	731	3.83	28.00	(Hines, 1988)
	Octomeris angulosa	COM	Both	Both	FF	U	211.7	3.77	7.98	(Achituv & Wortzlavski, 1983)
	Pagurus longicarpus	COM	Both	P	FF	U	410	6.09	24.97	(Damiani, 2003)
	Verruca stroemia	COM	Both	Both	FF	--	565	5.3	29.95	(Barnes, 1953)
Echinodermata	*Arbacia lixula*	COM	PL	F	FF	U	76.6	4.3	3.29	(George, Cellario & Fenaux, 1990)
	Asterina minor	COM	PL	F	NF	U	437	6.86	29.98	(Komatsu et al., 1979)
	Astrobrachion constrictum	COM	PL	F	NF	U	415	13.49	55.98	(Stewart & Mladenov, 1994)

Astropecten gisselbrechti	COM	PL	F	NF	U	353	5.09	17.97	(Komatsu & Nojima, 1985)
Clypeaster rosaceus	COM	PL	F	FF	U	280.3	2.74	7.68	(Emlet, 1986)
Clypeaster subdepressus	COM	PL	F	FF	U	152.6	2.29	3.49	(Emlet, 1986)
Crossaster papposus	**COM**	**PL**	**F**	**NF**	**U**	**833.7**	**2.98**	**24.84**	**Present study**
Cucumaria frondosa	**COM**	**PL**	**F**	**NF**	**U**	**622.7**	**3.93**	**24.47**	**Present study**
Dendraster excentricus	COM	PL	F	FF	U	129	3.5	4.52	(Podolsky, 2002)
Diplasterias brucei	--	B	P	--	U	3000	20	600.00	(Bosch & Pearse, 1990)
Echinaster morph 1	COM	PL	F	NF	U	840	4.76	39.98	(Scheibling & Lawrence, 1982)
Echinaster morph 2	COM	B	F	NF	U	960	5.2	49.92	(Scheibling & Lawrence, 1982)
Leptosynapta clarki	COM	B	P	FF	U	2000	52	1040.00	(Sewell, 1994)
Holothuria scabra	COM	PL	F	FF	U	157	2.27	3.56	(Ramofafia, Battaglene & Byrne, 2000)
Luidia foliolata	COM	PL	F	FF	U	144.3	4.78	6.90	(George, 1994)

Luidia quinaria	COM	PL	F	FF	U	124	4.91	6.09	(Komatsu, Oguro & Kano, 1982)
Luidia maculata	COM	PL	F	FF	U	173	3.46	5.99	(Komatsu et al., 1994)
Patiriella regularis	COM	PL	F	FF	U	197	1.92	3.78	(Byrne & Barker, 1991)
Phyllacanthus imperialis	COM	PL	F	NF	U	507	6.29	31.89	(Olson, Cameron & Young, 1993)
Pisaster brevispinus	COM	PL	F	FF	U	165	3.33	5.49	(Fraser et al., 1981)
Pisaster ochraceus	COM	PL	F	FF	U	163	3.68	6.00	(Fraser et al., 1981)
Porania antarctica	COM	PL	F	FF	U	548	9.23	50.58	(Bosch, 1989)
Porania sp	COM	B	F	NF	U	554	17.08	94.62	(Bosch, 1989)
Psolidium bullatum	COM	PL	F	NF	U	330	5.15	17.00	(McEuen & Chia, 1991)
Psolus chitinoides	COM	PL	F	NF	U	627	5.58	34.99	(McEuen & Chia, 1991)
Pteraster militaris	COM	B	P	NF	U	2171	25.1	544.92	(McClary & Mladenov, 1990)
Solaster endeca	**COM**	**PL**	**F**	**NF**	**U**	**951.2**	**3.61**	**34.34**	**Present study**

	Strongylocentrotus droebachiensis	**COM**	**PL**	**F**	**FF**	**U**	**173.1**	**5.85**	**10.13**	**Present study**
Mollusca	*Acanthina spirata*	COM	B	P	FF	U	671	8.64	57.97	(Spight, 1976)
	Adalaria proxima	COM	Both	Both	FF	U	168	4.57	7.68	(Jones et al., 1996)
	Aeolidia papillosa	**COM**	**Both**	**Both**	**FF**	**U**	**47.4**	**12.2**	**5.78**	**Present study**
	Babylonia areolata	COM	Both	Both	FF	U	425.7	5.4	22.99	(Chaitanawisuti & Kritsanapuntu, 1997)
	Brachidontes virgiliae	COM	PL	F	FF	U	383	13.31	50.98	(Bernard, Davies & Hodgson, 1988)
	Buccinum cyaneum	COM	B	P	FF	U	1520	19.078	289.99	(Miloslavich & Dufresne, 1994)
	Bulla gouldiana	COM	Both	Both	FF	U	84.5	4.52	3.82	(Farfan & Ramirez, 1988)
	Calliostoma zizyphinum	COM	Both	P	NF	U	300	3.12	9.36	(Holmes, 1997)
	Cantharidus callichroa	COM	B	P	FF	U	446	10.76	47.99	(Son & Hong, 1994)
	Chlamys asperrima	COM	PL	F	FF	U	71.2	5.67	4.04	(Styan & Butler, 2000)

Chlamys bifrons	COM	PL	F	FF	U	116.5	2.66	3.10	(Styan & Butler, 2000)
Crepidula adunca	SIM	B	P	--	U	2200	25.71	565.62	(Collin, 2000)
Crepidula atrasolea	COM	B	P	NF	U	328.1	3.87	12.70	(Collin, 2010)
Crepidula dilatata	COM	Both	Both	FF	U	218	3.66	7.98	(Gallardo, 1977)
Crepidula dilatata	COM	B	P	FF	U	234	7.86	18.39	(Gallardo, 1977)
Crepidula ustulatulin	COM	B	P	NF	U	285.7	4.87	13.91	(Collin, 2010)
Crucibulum quiriquina	COM	Both	Both	FF	U	325.8	6.59	21.47	(Veliz, Guisado & Winkler, 2001)
Crucibulum quiriquina	COM	B	P	FF	U	720	17.12	123.26	(Veliz et al., 2001)
Cymatium cutaceum	COM	Both	Both	FF	U	151	5.03	7.60	(Ramon, 1991)
Cymatium corrugatum	COM	Both	Both	FF	U	216	3.425	7.40	(Ramon, 1991)
Cypraea caputdraconis	COM	Both	Both	FF	U	112	5.1	5.71	(Osorio, Gallardo & Atan, 1992)
Cypraecassis testiculus	COM	Both	Both	FF	U	149	10.06	14.99	(Hugues & Hughes, 1987)

Dendropoma corrodens	--	B	P	FF	U	512	11.52	58.98	(Miloslavich & Penchaszadeh, 1992)
Dendropoma petraeum	COM	B	P	FF	C	756	10.73	81.12	(Calvo, Templado & Penchaszadeh, 1998)
Drupella cornus	COM	Both	Both	FF	U	170	1.47	2.50	(Turner, 1992)
Engoniophos unicinctus	COM	B	P	FF	U	1007.5	23.69	238.68	(Miloslavich & Penchaszadeh, 1994)
Haminoea vesicula	COM	Both	Both	FF	U	90	3.33	3.00	(Gibson & Fu-Shiang, 1989)
Littorina obtusata	**COM**	**Both**	**Both**	**FF**	**U**	**201.2**	**8.32**	**16.74**	**Present study**
Macoma mitchelli	COM	PL	F	FF	U	59	3.89	2.30	(Kennedy, Lutz & Fuller, 1989)
Odostomia columbiana	COM	Both	Both	FF	U	74	2.17	1.61	(Collin & Wise, 1997)
Ostrea edulis	COM	Both	Both	NF	U	202	5.94	12.00	(Foighil, 1989; Jonsson et al., 1999)
Petaloconchus montereyensis	COM	B	P	FF	C	1450	5.51	79.90	(Hadfield, 1989)
Polinices lewisii	COM	Both	Both	FF	U	235.4	2.33	5.48	(Pedersen & Page, 2000)

Searlesia dira	COM	B	P	FF	U	1490	18.12	269.99	(Rivest, 1983)
Sepioteuthis australis	--	Both	P	--	U	4800	13.12	629.76	(Steer, Moltschaniwskyj & Jordan, 2003)
Strombina francesae	SIM	B	P	--	U	571	6.12	34.95	(Cipriani & Penchaszadeh, 1993)
Strombina pumilio	SIM	B	P	--	U	947	10.24	96.97	(Cipriani & Penchaszadeh, 1993)
Strombus costatus	COM	Both	Both	FF	U	262	2.29	6.00	(Davis, Bolton & Stoner, 1993)
Strombus gigas	COM	Both	Both	FF	U	225	7.56	17.01	(Davis et al., 1993)
Strombus raninus	COM	Both	Both	FF	U	140	2.85	3.99	(Davis et al., 1993)
Thais emarginata	COM	B	P	FF	U	1330	13.23	175.96	(Spight, 1976)
Tridacna squamosa	COM	PL	F	FF	U	158	4.43	7.00	(Fitt & Trench, 1981)
Nucella crassilabrum	COM	B	P	FF	U	1131	8.71	98.51	(Gallardo, 1979)
Nucella lapillus	COM	B	P	FF	U	1270	14.4	182.88	(Etter, 1989)

	Velutina velutina	**COM**	**Both**	**Both**	**FF**	**U**	**269.7**	**6.74**	**18.18**	**Present study**
	Vermetus sp	COM	B	P	NF	U	240	5.83	13.99	(Miloslavich & Penchaszadeh, 1992)
Platyhelminthes	*Stylochus ellipticus*	COM	Both	Both	FF	U	68.5	4.52	3.10	(Chintala & Kennedy, 1993)

a. Complex COM, Abbreviated ABB
b. Benthic B, Pelagic PL
c. Free F, Protected P. Protected can be further subdivided into IB (internally brooded); EB (externally brooded); EN (encapsulated).
d. Feeding FF, Non-feeding NF
e. Colonial C, Unitary U
f. For published results, SD was back calculated using Mean × CV.
Notes: Mixed strategies are harder to define; for example, encapsulated development followed by release of a pelagic larval stage or internal brooding followed by release of benthic larvae. ND = Not determined.

CHAPTER 3 : **Marked shifts in offspring size elicited by frequent fusion among siblings in an internally brooding marine invertebrate**

A version of this chapter has been submitted to The American Naturalist

Abstract

While offspring size is a widely studied concept in evolutionary ecology, mechanisms that may affect offspring phenotype in species with post-zygotic parental care are incompletely understood. Here we examined the impact of fusion among siblings (chimerism) on ontogenetic shifts in offspring size in the brooding sea anemone *Urticina felina*. Fusion occurred only among brood-protected embryos in *U. felina,* whereas it occurred post release among settling larvae of soft corals studied here and previously. Two products of fusion were evidenced in *U. felina*: morphologically-aberrant (multi-headed) offspring and large homogeneous offspring coined 'mega-larvae'. The frequent occurrence (~77%) of mega-larvae indicates that they are the primary product of fusion, which drove an increase in offspring size and within-clutch size variation prior to release. In addition, lipid signatures suggest that bi-headed juveniles represent by-products that do not reach adulthood. Not only were occurrences of mega-larvae common in the populations studied, they increased with maternal fecundity, suggesting that fusion among maternal siblings may be a form of kin cooperation integral to the reproductive success of *U. felina*, which warrants investigation in other live-bearing invertebrate taxa.

Introduction

Offspring size is among the most widely studied forms of phenotypic variability and is central to fundamental concepts in evolutionary ecology (Smith and Fretwell 1974; Bernardo 1996; Uller 2008). A well-recognized tenet is that while offspring size influences the fitness of both mothers and offspring, selection acts to maximize maternal fitness with respect to offspring provisioning. This gave rise to the size-number trade-off hypothesis, whereby a finite amount of resources allows mothers to either produce a small number of well-provisioned offspring or more numerous poorly-provisioned ones (Smith and Fretwell 1974; Bernardo 1996). To date, studies have largely focused on establishing whether variation in offspring size is an adaptive response to local conditions, on the importance of this variation, and on the factors that may drive it. Much less studied are the mechanisms that underlie variations in offspring size. In species that exhibit post-zygotic (post-oviposition) parental care, interactions and conflicts with the parent or among siblings may also act on offspring size.

Offspring size plasticity has been studied in mammals (Charnov and Ernest 2006), birds (Krist 2011), reptiles (Sinervo 1990), fish (Hendry et al. 2001; Einum and Fleming 2004) and marine invertebrates (Marshall and Keough 2007; Allen et al. 2008). While studies on vertebrates have included species with and without parental care, in marine invertebrates the focus has largely been on the propagules of broadcast-spawning species or post-release stages of a few brooding species. Overall, hypotheses of adaptive bet-hedging (e.g. Marshall et al. 2008) and physiological constraints (e.g. Einum and Fleming

2004) that tried to explain offspring variation within clutches have both found support in the literature. Marked size variations within clutches were recently suggested to illustrate the adaptive bet-hedging concept. However, more complex schemes have also been evidenced in live-bearing (viviparous) organisms (Jorgensen et al. 2011), questioning the universality of a simplified theoretical approach. Because parent-offspring conflicts are expected to increase during parental care (Trivers 1974), live-bearing species provide great opportunities for the study of offspring size variations driven by various forms of parental and sibling interactions.

Post-zygotic interactions known to influence offspring size arise with viviparity (Crespi and Semeniuk 2004), including adelphophagy (cannibalism among siblings, e.g. Kamel et al. 2010) and matrotrophy (offspring feeding on mother's tissues, e.g. Pollux and Reznick 2011). We propose that heterogeneic fusion (chimerism) during early ontogeny is another key determinant of offspring phenotypic plasticity. The natural occurrence of chimerism reported in protists, fungi, plants and animals (Pineda-Krch and Lehtilä 2004) challenges the concept of an individual on which many principles of ecology and evolution rely (Santelices 1999; Rinkevich 2000; Folse and Roughgarden 2010). Compared to clonal species, direct evidence of this phenomenon in unitary aclonal species remains quite limited; it has only recently been documented in such an organism, the sea anemone *Urticina felina* (Mercier et al. 2011).

In an effort to shed new light on the ecological significance of this unique form of phenotypic plasticity, the present work investigated the impact of natural fusion on offspring size in brood-protecting cnidarians, focusing on the cosmopolitan boreal

species *Urticina felina*. Internal brooding (a form of viviparity or live-bearing) is a common type of parental care believed to elicit parent-offspring and sibling rivalries in marine invertebrates (McClary and Mladenov 1990) and fish (Jorgensen et al. 2011). To date, studies on brooding species of benthic marine invertebrates (sponges, ascidians and soft corals) have only reported fusion among post-release larvae, i.e. following the period of parental care. Our specific aims were to (1) elucidate the size structure and plasticity of pre-metamorphic offspring in *U. felina,* (2) conduct a first investigation of fusibility at various ontogenetic stages in this species, (3) characterize the two types of fusion products using lipid markers and (4) contrast our fusion results with findings in colonial species of cnidarians. For the latter we used data from the literature and we conducted a complementary study on the soft corals *Drifa* sp. and *Duva florida*.

Materials and Methods

Main study on sea anemones *Urticina felina*

Collection and maintenance. *Urticina felina* is a gonochoric aclonal sea anemone with a cosmopolitan circumboreal distribution (Hayward and Ryland 1990; Van Ofwegen et al. 2001). It is common in the North Atlantic from the lower intertidal zone down to 400 m (Chia 1976; Solé-Cava et al. 1985). Evidence of chimerism in *U. felina* was initially noted after the natural release of larvae by laboratory-maintained adults in August 2008, when the presence of several fused settlers was observed (Mercier et al. 2011). Following this, adults of *U. felina* were collected at a depth of 10 m off the Avalon Peninsula (Newfoundland, Canada) between March and July 2009 (n = 22) and in June

2010 (n = 46). The collection site (Island Cove) is a relatively protected and calm area that harbours a diversified community of suspension feeding organisms. Several brooding females were detected upon collection: 3 in 2009 and 13 in 2010. Each of them was placed in a tank together with 3-4 males. Another group of 5 brooding females was identified in 2011 among females that had been collected in the previous year. Holding tanks (20-40 L) were supplied with unfiltered running seawater (including planktic food), at temperatures that followed the ambient cycle (0-10 °C), under natural photoperiod. The size of brooding mothers in this study varied from 45.7 to 212.9 g drained weight.

Study of pre-metamorphic stages. Females of *U. felina* brood their offspring to mature larvae freely inside the gastrovascular cavity (coelenteron) and the tentacles; propagules are easy to detect through the thin transparent epithelium. The earliest propagules (oocytes) were collected from five mothers through a small clip in the tentacles in April and May 2011. Embryos were obtained by clipping the tentacles of six brooding mothers in June 2010. During the larval release period (July-September of 2009 and 2010), larvae emitted through the mouth of the females in several major planulation events were collected at the surface of the water column within 24 h post release. Propagules were photographed under a Nikon SMZ1500 stereomicroscope attached to a Nikon DXM1200F digital camera, and processed using Simple PCI (v. 6.0) to measure surface area for analysis of offspring size structure from oocytes to larvae. Moreover, 6 samples (12-15 larvae per sample) of small (0.54-0.76 mm^2) and large (0.83-1.42 mm^2) larvae were collected from each brooding females (n = 3) in July 2009 and placed in 2 ml

chloroform under nitrogen at -20 °C for comparative analysis of major lipid classes (see method below).

Fusibility trials. Evidence of fused embryos and larvae within broods was obtained previously (Mercier et al. 2011). To determine whether post-release larvae could still fuse, a total of 30 low-density trials were conducted on 93 larvae released from three mothers, including 15 trials on kin larvae, and 15 trials on mixed larvae. A further 27 high-density trials were conducted, including 18 trials on 874 kin larvae collected from four mothers, and nine trials on 420 larvae released from nine mothers. Low-density trials consisted of 2-4 larvae placed in a 1-ml pipette tip (mimicking pre-release intimacy of propagules within the tentacles) kept in 50-ml beakers. The beakers were maintained in a thermostatic bath of running ambient seawater (6-10°C), and half of the seawater inside the beakers was renewed every other day (using seawater surrounding the brooding adults to account for the possible influence of chemical cues). High-density trials consisted of groups of 20-30 larvae placed in 3-ml vessels inside a 250-ml beaker with unfiltered seawater under slow flow-through conditions (again using seawater present around the brooding adults). The occurrence of fusion was monitored for five weeks until metamorphosis and settlement of most propagules (> 50%). Similar trials were also conducted on naturally expelled and extracted embryos. However, results were inconclusive because embryos could not survive outside the mother, despite several attempts under rearing conditions that proved successful for larvae.

Study of post-metamorphic stages. This study compared settlers developed from the two fusion products, including singleton juveniles originating from mega-larvae and

morphologically-aberrant juveniles (bi-headed sectorial chimeras). All were obtained from larvae that were naturally released in August 2008 and reared in a flow-through system (as described in the Maintenance section) with the presence of ***coralline algae*** as substratum. Six 20-month-old juveniles, including three singletons (5.0-7.8 mg wet weight) and three sectorial bi-headed chimeras (1.0-2.9 mg) were preserved in 2 ml chloroform and kept under nitrogen at -20 °C for lipid composition analysis.

Lipids analysis. Extraction and analysis of lipids were based on standard methods for aquatic samples (Parrish 1999). Total lipids were extracted with a mixture of chloroform and methanol 2:1 (v:v). Lipid classes were determined using thin layer chromatography with flame ionization detection (TLC/FID) with a MARK V Iatroscan (Iatron Laboratories, Tokyo, Japan). Lipids were separated in a three stage development system. The first separation consisted of 25-min and 20-min developments in 99: 1: 0.05 hexane: diethyl ether: formic acid. The second separation consisted of a 40-min development in 79: 20: 1 hexane: diethyl ether: formic acid. The last separation consisted of 15-min developments in 100% acetone followed by 10-min developments in 5:4:1 chloroform: methanol: chloroform-extracted-water. After each separation, the rods were scanned and the data were processed using the PeakSimple Chromatography software (V3.88, SRI Instruments, USA).

Complementary study of soft corals *Drifa* sp. and *Duva florida*

Drifa sp. and *Duva florida* are two common internally-brooding soft corals in the northwest Atlantic. Specimens were collected at 500-1240 m depth off Newfoundland as detailed in previous work (Sun 2009). Larvae of *Drifa* sp. were released naturally from

August 2007 to June 2008 under laboratory conditions (Sun et al. 2009; Sun et al. 2010), whereas larvae of *D. florida* were extracted from adult colonies (Sun 2009; Sun et al. 2011). Fusion was detected during studies of life history; larvae released/extracted from the same date were maintained together without consideration of kinship.

Data analysis

Shapiro-Wilk normality tests were performed to examine the distribution of offspring size at different stages (oocytes, embryos and larvae) in the broods. Relationships with maternal fecundity and weight were examined using Spearman rank order correlations and linear regressions. Within-clutch size variation of embryos and larvae were examined using Mann-Whitney rank sum test and *t*-test, respectively. Mann-Whitney rank sum tests were used to examine the total lipid content ($\mu g\ ind^{-1}$) and lipid concentration ($\mu g\ mm^{-3}$) in large (mega-larvae) and small larvae. In addition, *t*-tests were used to examine the proportions of all major lipid classes (> 1% of total lipids) in both large and small larvae, as well as the total lipid concentration ($\mu g\ mg^{-3}$) and the proportions of all major lipid classes in the singleton juveniles originating from mega-larvae *vs* morphologically-aberrant juveniles.

Results

Analysis of pre-metamorphic stages in *Urticina felina*

The size of oocytes in *U. felina* typically ranged from 0.2 to 0.5 mm^2 (mean ± SD of 0.36 ± 0.07 mm^2 and maximum of 0.60 mm^2, Fig. 3-1). Early embryos were 0.31 ± 0.09 mm^2 with a maximum size of 0.73 mm^2 (Fig. 3-1). Most larvae were much larger than oocytes

and embryos (Fig. 3-2A, B), measuring up to 4.5 mm^2 (Fig. 3-1), in contrast to previous data reported in sea anemones, where size of embryos and fully developed larvae is similar to size of oocytes/eggs (app. 3-A). Moreover, normal size distribution of propagules of *U. felina* became less frequent as development progressed. Three out of five females (60.0%) had a statistically normal egg size distribution, one out of six females (13.2%) had a normal embryo size distribution, and only one out of twelve females (8.3%) had a normal larva size distribution.

The large larvae (> 0.60 mm^2) comprised a minority of incompletely fused (morphologically-aberrant) larvae (Fig. 3-2C), the number of which was not related to maternal fecundity, measured as the total number of offspring released ($r_s = 0.51$, $p = 0.089$, $n = 12$), or to the weight of brooding mothers ($r_s = 0.45$, $p = 0.136$, $n = 12$). Details on the types and relative abundance of visibly chimeric entities are available in Mercier et al. (2011). Most large larvae were morphologically normal (Fig. 3-2B) yet in the same size range as visibly fused larvae (Fig. 3-1), indicating the existence of homogeneous chimeras formed by full fusion (coined mega-larvae). Two thirds (66.7%) of those mega-larvae measured 0.6-1.2 mm^2, a size estimated to correspond to 2-6 fused siblings; only 0.1% of them were >3.0 mm^2, combining 24-43 fused siblings. The proportion of all fusion products (combining morphologically-aberrant larvae and mega-larvae) varied from 43.2% to 98.8% in the 12 broods examined, with a mean of 76.9 ± 21.3% (± SE). The proportion of mega-larvae varied from 43.2% to 97.9% in those broods (76.5 ± 21.2%). While the number of mega-larvae followed a linear relationship with maternal

fecundity ($F = 370.04$, $r = 0.98$, $p < 0.001$, Fig. 3-3), it was not significantly related to maternal weight ($r_s = 0.41$, $p = 0.137$).

Within-clutch offspring size variations (CV of surface area) increased throughout early development, i.e. within-clutch size variability of embryos ($U = 2.00$, $p = 0.017$) and larvae ($t = -2.44$, $p = 0.027$) was significantly greater than that of oocytes (Fig. 3-4). The overall offspring size variations at population level also increased throughout development (Fig. 3-4).

Larvae were composed of hydrocarbons (HC), wax and steryl esters (WE/SE), triacylglycerols (TG), free fatty acids (FFA), sterols (ST), acetone mobile polar lipids (AMPL) and phospholipids (PL). Total lipid content (μg ind^{-1}) was significantly greater in mega-larvae than in small larvae (Mann-Whitney, $U = 0.00$, $p < 0.001$, app. 3-B), whereas lipid concentration (μg mm^{-3}) was not ($U = 20567.50$, $p = 0.974$). In addition, the proportions of all major lipid classes (> 1% of total lipids) were similar in both large and small larvae (app. 3-B).

Analysis of juveniles in *Urticina felina*

Based on wet weight, 20-month-old bi-headed juveniles (1.90 ± 0.95 mg; mean ± SD) were significantly smaller ($t = 4.63$, $p = 0.010$) than singletons originating from mega-larvae (6.43 ± 1.40 mg), despite their comparable size range at larval release (Fig. 3-1). Both types of juveniles were mainly composed of hydrocarbons (HC), free fatty acids (FFA), sterols (ST), acetone mobile polar lipids (AMPL) and phospholipids (PL). However, total lipids accounted for 9.1-18.5% of wet weight in bi-headed juveniles, and only 2.7-5.7% in singletons. Furthermore, total lipid concentration was 134.9 ± 27.2 μg

mg^{-1} (± SE) in bi-headed juveniles, which was significantly higher (t = -3.12, p = 0.035) than in singletons (45.1 ± 9.0 μg mg^{-1}). Among the major lipid classes, only AMPL were significantly more concentrated in bi-headed juveniles (t = -3.25, p = 0.031). Polar lipids (AMPL and PL), were the major lipid classes in both types of juveniles, comprising 54.7 % of lipids in singletons and 69.0 % in bi-headed juveniles. The proportion of HC was significantly higher in singletons than in bi-headed juveniles (t = 4.21, p = 0.014), whereas the proportion of PL was significantly higher in chimeras than in singletons (t = -3.03, p = 0.039).

Fusion in sea anemones and soft corals

Larvae of *U. felina*, whether they were siblings from the same brood or not, did not fuse together post release at the time of metamorphosis or settlement. In all low-density trials, larvae settled either without any contact or slightly touching each other, without fusing. Similar results were obtained in high-density trials: although a few larvae (<10) stuck briefly together, one of the two partners always died and no viable chimeras were ever observed.

In contrast, newly-released larvae of *Drifa* sp. typically stuck together when they came into contact (Fig. 3-2D). Approximately 5% of post-release larvae fused naturally (~10 out of 200 larvae) and grew into two-polyp colonies (Fig. 3-2E). Fusion between two larvae generally occurred in the water column during the process of settlement, 1-2 d post release. It is worth mentioning that while no morphologically-aberrant chimeras were detected among newly-released larvae (such as in *U. felina*), the length of larvae varied markedly, from ~0.5 mm to 5 mm (Sun et al. 2010). The smallest larvae were

roughly the size of vitellogenic oocytes (0.49 ± 0.02 mm; from histology) (Sun et al. 2010) but the largest larvae were up to ten times larger. Post-release larvae of *Duva florida* also stuck together when they came into contact, and had the capacity to settle and fuse with one another to form two-polyp colonies (Fig. 3-2F).

Discussion

Offspring size variation caused by frequent fusion in *Urticina felina*

The unitary cnidarian *U. felina* illustrates a set of conditions that favour fusion among siblings at an earlier stage than previously reported in colonial invertebrates (i.e. among brooded embryos rather than post release during gregarious settlement). The initial results, which were based solely on morphologically-aberrant chimeras, led us to believe that fusion in this species was relatively infrequent (< 4%) (Mercier et al. 2011) and consistent with the hypothesis of the "imperfect system" (Feldgarden and Yund 1992). A closer look at the developmental biology of *U. felina* highlighted a different scenario: fused embryos can also develop into larger yet morphologically-homogeneous mega-larvae (resulting in large settlers), which are quite abundant. The present analysis of propagule size frequencies fully supports this assumption. While the early embryos of *U. felina* were typically the same size as the eggs, on average ~77% of the larvae were much larger. Because embryos and larvae do not feed (the mouth only opens upon metamorphosis), active feeding cannot explain the size increment, and trans-membranous feeding is unlikely to drive such marked growth. The number of mega-larvae was significantly related to maternal fecundity, whereas the number of sectorial chimeras was

not, indicating that (1) incomplete fusion is an infrequent by-product and (2) the occurrence of fusion, indicated by the number of mega-larvae, depends on size of the brood (i.e. higher fecundity increasing chances of fusion and/or competition among kin). In contrast, maternal size did not directly influence rates of fusion.

Natural fusion was determined to occur only among maternal siblings (embryos) of a clutch, indicating that the allorecognition system matures before the fully-developed larval stage in *U. felina*. Alternatively, it may illustrate the conspecific acceptance threshold theory (Reeve 1989) which predicts that thresholds for fusion become more restrictive as the frequency of interactions with more distantly related individuals increases (e.g. upon release from the brood in *U. felina*).

Fusion: a more complex strategy in unitary sea anemone than in colonial soft corals

Apart from microchimerism (cell movement between mother and foetus or between twins) and rare cases of dispermic chimeras indirectly detected via tissue analysis in mammals, chimerism is predominantly studied in colonial marine invertebrates that exhibit asexual reproduction (Pineda-Krch and Lehtilä 2004). The case of *U. felina* is the first direct observation of natural fusion in a unitary aclonal invertebrate. Several benefits of chimerism have been suggested, including increased genetic variability and body size, and improved survival, growth and reproductive output (Buss 1982; Amar et al. 2008). The only two-polyp colonies were formed by fusion of larvae or polyps in the cold-water soft corals studied here. Thus, fusion may be a strategy to compensate for the slow

growth rates of cold-water corals, as suggested for the tropical scleractinian *Siderastrea stellata* (Neves and da Silveira 2003).

In *U. felina*, two products of fusion occur: morphologically-aberrant offspring (e.g. bi-headed sectorial chimeras) and morphologically-homogeneous mega-larvae. The present work showed that total lipid concentration ($\mu g\ mm^{-3}$) was similar and lipid content greater in mega-larvae when compared to small siblings, supporting that they are formed by fusion and consequently possess greater lipid reserves. In a separate study, we found that mega-larvae had better pre-metamorphic performance than the smaller non-chimeric larvae (Chapter 5). In the present work, the morphologically-aberrant chimeric state did not show any fitness advantage (possibly even the inverse) over the mega-larvae originating from the full fusion of sibling embryos.

In addition, morphologically-aberrant chimeras were smaller than singletons at a corresponding age despite originating from similar-sized larvae at release and contained more lipids than the sum of two juveniles would predict, as well as higher lipid concentrations, largely due to more abundant acetone mobile polar lipids (AMPL). The latter, which include gylcolipids, pigments and monoacylglycerols, were proposed to constitute an indicator of stress in a study of scallops (*Placopecten magellanicus*) where a sharper decrease of AMPL occurred in animals having the greatest increase in growth (Parrish et al. 1998). In the present study, the smaller size, greater levels of AMPL and higher lipid concentrations are all consistent with a slower growth in visibly chimeric juveniles, possibly indicative of greater stress and/or inability to metabolize lipids.

Furthermore, morphologically-aberrant adults or juveniles *U. felina* have not been reported in the field, suggesting they do no exhibit long-term survival.

Conclusions and future directions

A striking shift in offspring size occurs during the brood-protecting phase in *U. felina*, and fusion among siblings was shown to play a key role in this phenomenon. Fusion in *U. felina* occurs only during the brooded embryonic phase and not among post-release larvae, in stark contrast with soft coral relatives studied here and elsewhere. This suggests earlier maturation of the allorecognition system in unitary than colonial cnidarians, consistent with the belief that coloniality in most marine organisms has evolved from solitary ancestors (Beklemishev 1969). In the present study, fusing/fused offspring (fused embryos, mega-larvae and morphologically aberrant larvae) of *U. felina* were observed in brooding mothers freshly collected from the field on several occasions, indicating that fusion occurs readily in the natural environment. Whether fusion is only resulting from the failure of the allorecognition system (as currently advocated) or whether it is enhanced by a mother's condition (temperature, wave action, conspecific density, etc.) would be an interesting topic for future studies.

Fusion among brooded siblings is a previously overlooked mechanism that can generate important offspring size variations. We propose that the development of mega-larvae through fusion in *U. felina* represents a form of kin cooperation conferring size-related fitness advantage. This mechanism might be selected for in situations where settlement of the progeny occurs gregariously shortly after release (philopatry), which is the case in brooding species that release fully-formed larvae. Results from the present and

on-going studies support the adaptive role of mega-larvae that possess more lipid reserves and exhibit better survival and greater dispersive abilities. For example, larger larvae of the sea anemone *Urticina felina* outperformed small siblings, i.e. a higher proportion of the larger larvae were buoyant and had a greater survival than their smaller siblings under suboptimal conditions (Chapter 5). Whether fusion of embryos also occurs during the brooding phase in corals (as the marked difference in larval sizes suggests), or in other viviparous taxa, should be explicitly investigated, starting with those in which post-release fusion has already been reported. Determining whether the duration of brooding favours the production of mega-larvae and whether the latter exhibit increased post-metamorphic performance represent the logical next steps. Furthermore, molecular studies are needed to clarify the benefits of chimerism, i.e., whether genetic variability translates into more versatile physiological qualities enabling chimeras to better cope with environmental changes. Finally, the impact of fusion at later stages (among larvae and settlers) in colonial organisms also deserves more attention in the context of offspring size variation theories.

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Figures

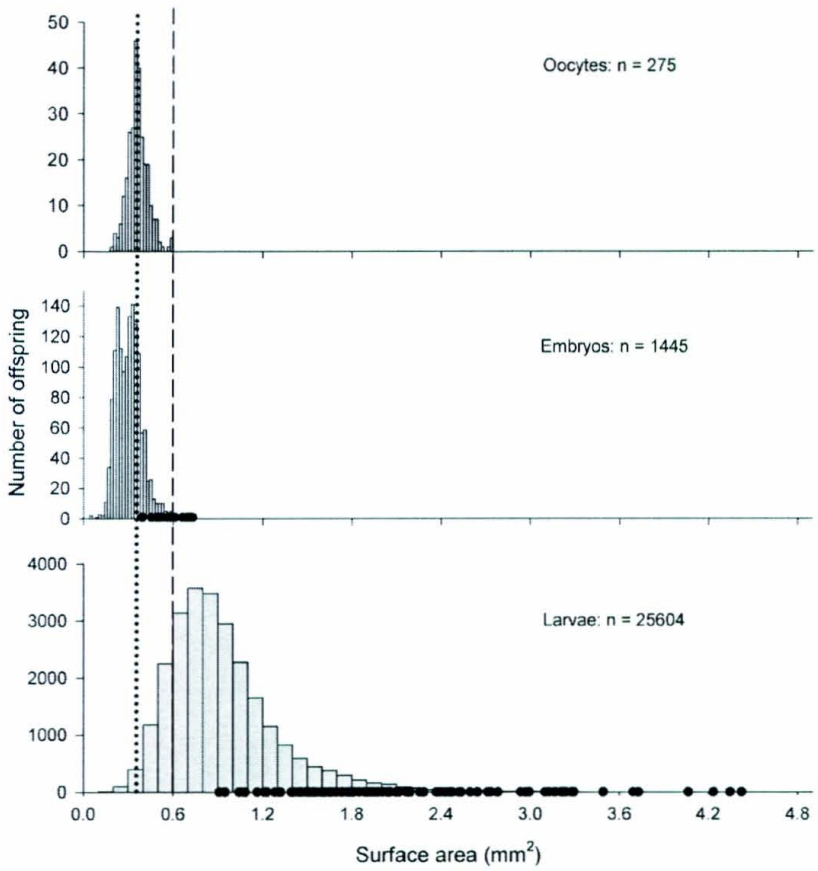


Fig. 3-1. Size frequency distributions of oocytes (from 5 females), embryos (6 females) and larvae (12 females) of *Urticina felina*. Each filled circles represents one morphologically-aberrant chimeric offspring. Value on each graph indicates number of offspring. Dotted line shows the average size of oocytes (0.36 mm^2) and dashed line indicates the maximum expected size based on maximum size of oocytes (0.60 mm^2). Note the variable y-axis scales; size distributions of oocytes and embryos established from subsamples.

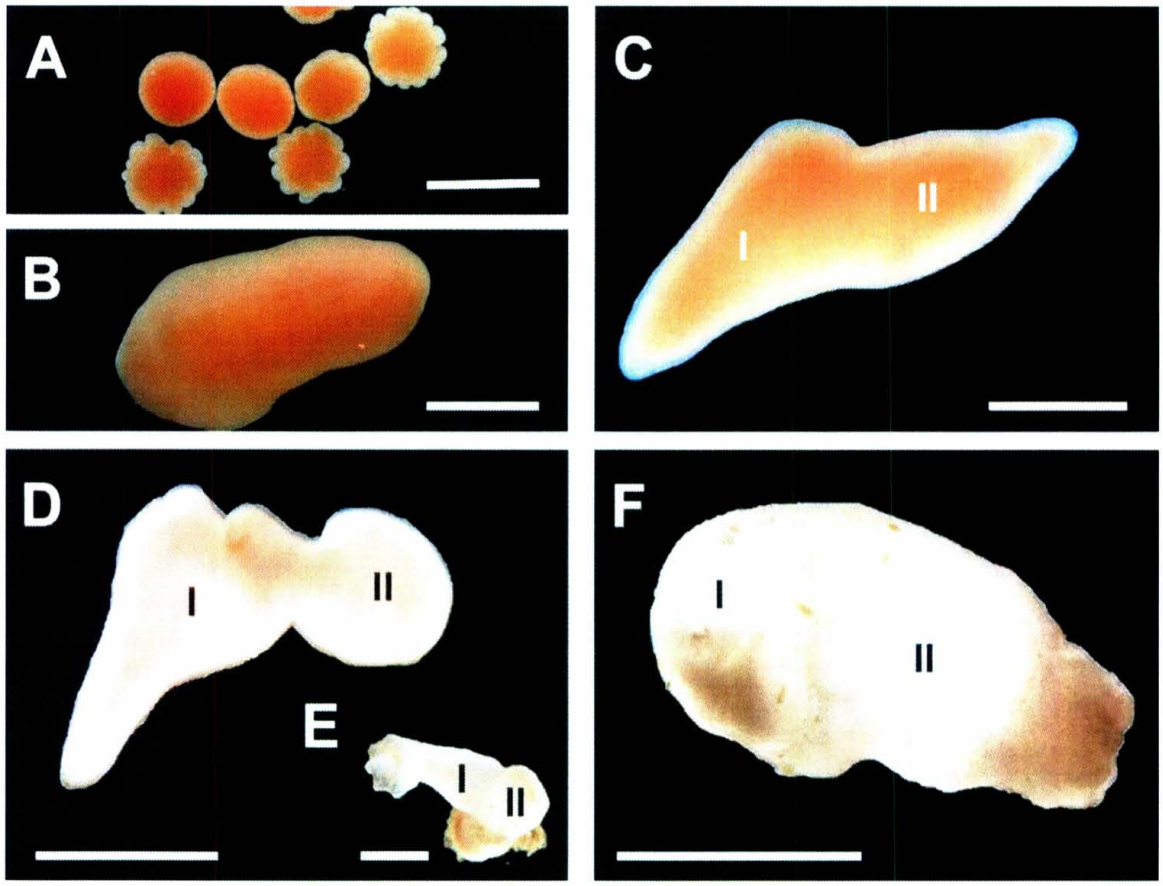


Fig. 3-2. Propagules and chimeras at different life stages in *Urticina felina* (A-C) and fusion among post-release larvae in two species of colonial soft corals (D-F). *Urticina felina:* Marked size difference between A) early embryos and B) mega-larva (same scale); C) Example of morphologically-aberrant larva composed of two distinguishable fused entities. Soft corals: D) Newly fused larvae of *Drifa* sp.; E) the same chimeric entity (two-polyp colony) after 50 d of growth; F) chimeric two-polyp colony developed from fused larvae in *Duva florida.* Roman numerals (I-II) identify different individuals. Scale bar represents 0.5 mm in C, and 1 mm in all other panels.

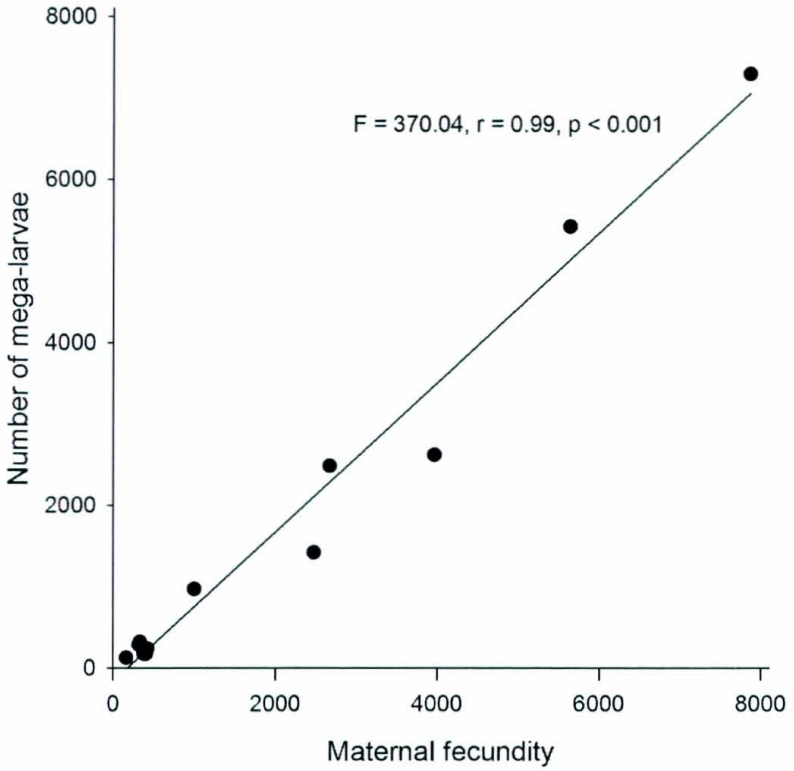


Fig. 3-3. Linear relationship between the number of mega-larvae (> 0.60 mm^2) and maternal fecundity (number of offspring released) in *Urticina felina*

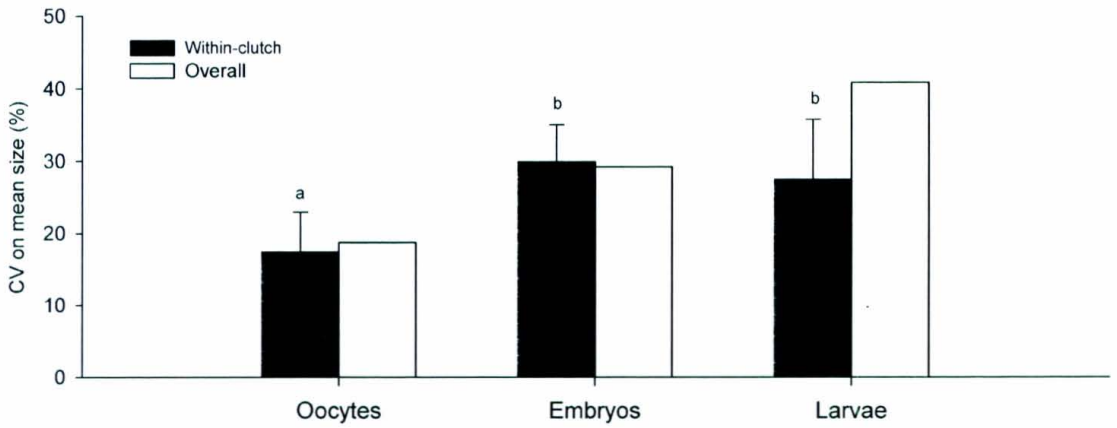


Fig. 3-4. Within-clutch and overall size variation (CV of surface area) in oocytes (n = 5 brooding females), embryos (n = 6) and larvae (n = 12) of *Urticina felina*. Values (± SD) with different superscript letters are significantly different (*t*-tests, $p < 0.05$).

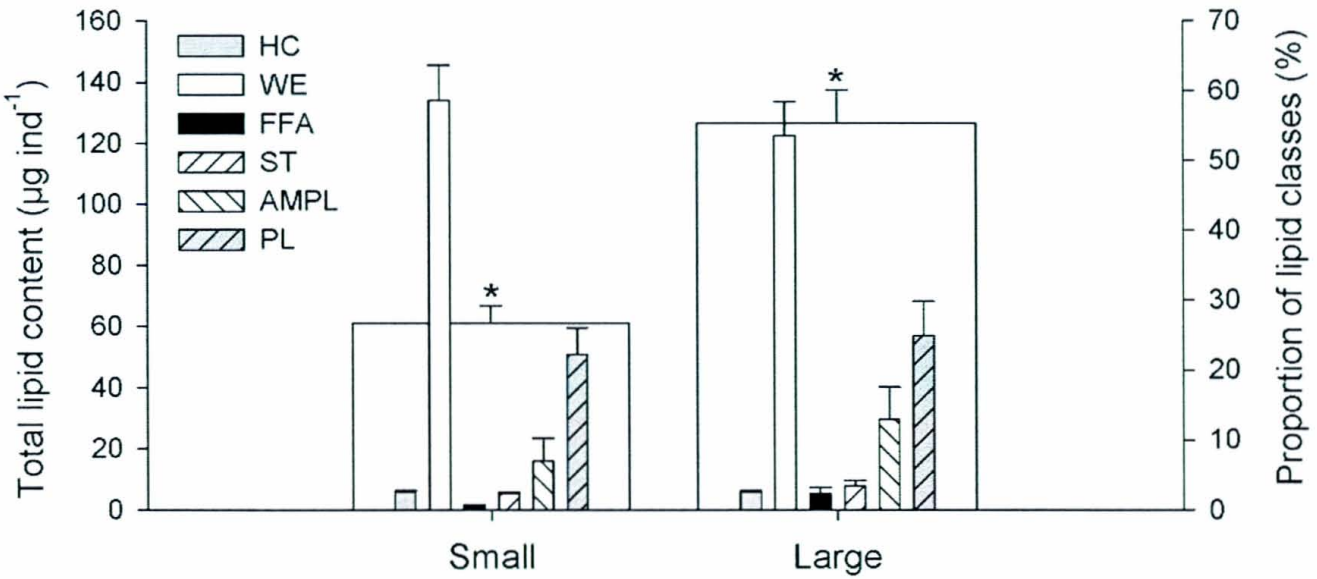
Appendices

Appendix 3-A. Size of eggs and larvae in *Urticina felina* and four other species of sea anemones

Species	Egg/embryo size (mm^2)	Larva size (mm^2)	Reference
Anthopleura ballii	0.1	0.1	(Davy and Turner 2003)
Entacmaea quadricolor	0.5	0.6	(Scott and Harrison 2007)
Heteractis crispa	0.3	0.3	(Scott and Harrison 2007)
Tealia (=*Urticina*) *crassicornis**	0.2-0.4	0.3	(Chia and Spaulding 1972)
Urticina felina	0.2-0.6	0.2- 4.5	This study

*A close relative of *U. felina*.

Appendix 3-B. Comparison of the lipid content and proportion of various lipid classes between small larvae and mega-larvae of *Urticina felina.*



Comparison between small larvae and mega-larvae (n = 9), on the basis of total lipid content (large bar, right axis) and proportion of various lipid classes (small histograms, left axis) at population level. HC: hydrocarbons; WE/SE: wax esters; FFA: free fatty acids; ST: sterols; AMPL: acetone mobile polar lipids; PL: phospholipids. Asterisks indicate significant differences between small and large larvae (t-tests, $p < 0.05$).

CHAPTER 4 : **Offspring size variations during and after parental care in a live-bearing cnidarian**

A version of this chapter has been submitted to Oecologia

Summary

Variations in offspring size are suggested to result from maternal effects or to reflect an adaptive strategy that ensures the survival of certain offspring in unpredictable environments (bet hedging). These assumptions have largely been examined in two of >30 animal phyla and studies on aquatic invertebrates have focused on egg-layers. Here we examined how currently proposed hypotheses held in a live-bearing marine species belonging to a neglected phylum. *Aulactinia stella* is a sessile internally-brooding cnidarian that releases fully-developed benthic juveniles, presumably enabling it to predict the environment experienced by offspring. Contrary to the general prediction of the bet-hedging theory, marked variations in juvenile size (>40%) were observed, both pre and post release. Within-brood variance of juvenile weight was not significantly related to parental weight, sampling month or environmental conditions, minimizing the influence of alternate parental effects. Total lipid concentration was significantly higher in small juveniles than in large ones and in adult tissues. Similarity analysis of major fatty acids revealed that large juveniles were more similar to adult tissues than small juveniles to adult tissues, suggesting an ontogenetic dietary shift upon acquisition of feeding organs. We propose that offspring size variations in *A. stella* are primarily mediated by: (1) The long, non-fixed brooding period and the co-existence of different cohorts. (2) Active feeding of offspring during parental care which presumably elicits competition with the parent and among siblings. These findings highlight

previously overlooked conflict-driven mechanisms acting on offspring phenotype in a viviparous species with extended parental care.

Key-words: bet hedging, conflict, marine invertebrate, phenotype plasticity, viviparity

Introduction

While a rich literature on animal ecology and evolution is dedicated to the study of offspring size variation, the current conceptual frameworks derive from a seemingly broad yet surprisingly low diversity of taxa. Models and hypotheses surrounding offspring size variations largely center on phylum Chordata, i.e. some of the most charismatic terrestrial (mammals, birds, reptiles, amphibians, e.g., Dziminski and Alford 2005; Charnov and Ernest 2006; Uller and Olsson 2010; Krist 2011) and aquatic models (fish, urochordates, e.g., Marshall et al. 2000; Schrader and Travis 2012). The other ~30 invertebrate phyla are comparatively understudied, with the exception of Arthropoda (insects, Fox and Czesak 2000; Gilboa and Nonacs 2006). In addition, within the eight major non-vertebrate marine phyla, studies focus on species that lay or broadcast eggs (Marshall and Keough 2007, Chapter 2); essentially leaving out the many live-bearing invertebrates with life-history strategies analogous to well-known vertebrate models (e.g. placental fishes, viviparous reptiles).

In this context, offspring size and size variations in marine invertebrates are commonly proposed to be mediated by environmental factors (Crean and Marshall 2009) and maternal phenotype, especially maternal size (Marshall et al. 2003). Much less consideration is given to sibling competition and parent-offspring conflicts, even though they are expected to increase during periods of parental care (Trivers 1974; Kamel et al. 2010a; Kamel et al. 2010b) and were shown to drive fecundity and clutch size in birds, insects and peociliid fish (Schrader and Travis 2012). Internal brooding of offspring is

reported from most major marine phyla, e.g., cnidarians (Dunn et al. 1980), molluscs (Beauchamp 1986), crustaceans (Baeza and Fernández 2002), echinoderms (McClary and Mladenov 1990), and chordates (Jorgensen et al. 2011). While mating systems of invertebrate brooders and pregnant vertebrates are strikingly similar, with clear evolutionary implications (Avise et al. 2011), the former receive much less attention. The closer relationship between mother and offspring and the more or less prolonged brood-protecting period suggest that offspring size variation likely follows a more complex scheme in brooding species (especially live-bearers that release juveniles) than in free spawning species. Thus, studies of viviparous invertebrate systems may provide significant insight in developing concepts of offspring size variations.

Lipids, as an energy source, play an important role in the reproduction and embryonic development of marine invertebrates (Wehrtmann and Graeve 1998; Pernet et al. 2002; Rosa et al. 2003). Fatty acids are major components of most lipid classes and some are essential for optimal health. They have commonly been used as trophic markers to provide information on dietary intake (Dalsgaard et al. 2003). For species that brood offspring until the juvenile stage, such markers can be used to distinguish nutrition sources available for juveniles: maternal nutrients stored as egg yolk and/or provided during development, and nutrients directly obtained from the diet of offspring while feeding inside the brooding mother.

The purpose of the present study was to explore how currently proposed hypotheses on offspring size variations would hold in a live-bearing marine species belonging to a previously neglected phylum, with key representatives (e.g. corals, sea

anemones) in nearly all aquatic ecosystems. *Aulactinia stella* (Verrill) (Cnidaria: Actiniaria) is an internally-brooding sea anemone (Dunn et al. 1980) that releases fully-formed benthic juveniles. Our objectives were to (1) characterize the brooding process in *A. stella* by long-term monitoring of adults under laboratory conditions, (2) assess size structure of juveniles, both during brooding and post release, relative to maternal phenotype, (3) compare lipid and fatty acid composition in adult tissues and juveniles of different sizes, and (4) use lipid signatures to elucidate size plasticity in *A. stella* juveniles and detect any shift from maternally-derived to dietary nutritional resources during early ontogeny. We believe this is the first explicit study of offspring phenotype and composition to be conducted both during and after a period of parental care in a marine invertebrate.

Materials and Methods

Adults of *A. stella* were collected at a depth of ~10 m off the Avalon Peninsula (Newfoundland, Canada) from March-July 2009, March-June 2010, and in January 2011. Individuals were distributed in flow-through holding tanks (20 L) for short-term storage before being transferred into experimental units (see below). Each holding tank held 6-10 individuals, and was supplied with unfiltered running seawater (~8 L min^{-1}), at ambient temperature 0-10°C, under natural photoperiod and planktic food supply.

Size of brooded juveniles in freshly collected adults

Forty adults were examined within 3 days of collection in March-June 2010 and January 2011 to estimate reproductive activity and natural size variation of offspring inside

brooding adults. Adult wet weight (after incision at the basal disk to drain excess water), basal disk diameter and contracted height were measured. Each specimen was dissected by removing the basal disk and cutting vertically along the septa. The presence of gamete-bearing mesenteries, i.e. oogenic mesenteries, was noted and numbers of juveniles were recorded on removal. Juvenile wet weight and volume (basal area × contracted height) were measured immediately after extraction. In addition, subsamples from 4 adults were collected and preserved for lipid and fatty acid analysis (see below).

Comparison of offspring size variation at release and during brooding

Adult *A. stella* were reared individually in 2-L flow-through containers for long-term monitoring of the release of juveniles from June 2009-March 2010 (n = 8) and April 2010-April 2011 (n = 8). All containers were supplied with unfiltered running seawater (~1.5 L min^{-1}), at ambient temperature under natural photoperiod and planktic food supply. Urchin gonads or shrimp (~0.5 g) were fed into the mouth of the sea anemones every other week. The natural release of juveniles by each brooding adult of *A. stella* was monitored weekly and wet weight (an accurate measurement of *A. stella* juvenile size; see results) measured as described for surgically-extracted juveniles. At the end of both experimental periods (March 2010 and April 2011), all adults (n = 16) were dissected as described above to assess brooding status. Wet weight of adults as well as number and wet weight of any brooded juveniles were also measured as described above.

Feeding experiment

During a preliminary study, some *A. stella* juveniles were observed with their tentacles extended while being extracted from brooding adults. Thus, feeding experiments were

conducted to test whether juveniles were capable of feeding on food obtained by the brooding adult (while nestling inside the gastrovascular cavity or along the mesenteries). Before the experiment, six adults (10.2-56.0 g) were transferred into separate 2-L containers under low flow (~0.5 L min^{-1}) and acclimatized overnight. Shrimp was used in the feeding experiment because individuals of *A. stella* had shown active feeding on shrimp fragments and the shrimp brightness made it easy to distinguish visually whether juveniles (translucent beige or greenish) were feeding on food ingested by the brooding adult. Shrimp paste (2 ml) was dropped on tentacles close to mouths of adults hourly for 6 consecutive hours. Adults were left overnight to provide enough time for full ingestion. They were examined 24 h after first feeding, as described above. All juveniles inside the brooding adult were collected and transferred to a Petri dish and the number of positively-feeding juveniles, i.e. those with traces of food in their gastrovascular cavity (Fig. 4-1a), was recorded.

Lipid composition

To compare lipid composition of adults and offspring, samples were collected of adult body wall (n = 11 from 4 adults, 2-3 samples per adult, from the basal disk) and oogenic mesenteries (n = 9 from 3 adults) and of whole juveniles of various sizes (n = 12 from 4 adults) in May-June 2010. Oogenic mesenteries were collected from the only three individuals with such tissue. Twelve juveniles were divided into 2 size classes to compare lipid composition, with small juveniles (n = 6) weighing 7-77 mg and large juveniles (n = 6) 122-308 mg. Samples were preserved in 2 ml chloroform under N_2 at -20°C for lipid and fatty acid analyses. Fatty acids were determined in the 3 individuals that possessed

gametes. For juvenile samples, only the smallest and largest juvenile from each adult were analysed. The small juvenile class (n = 3) weighed 8-77 mg, and the large juvenile class (n = 3) weighed 186-308 mg.

Extraction and analysis of lipids were based on standard methods (Parrish 1999). Lipid classes were determined using thin layer chromatography with flame ionization detection with a MARK V Iatroscan (Iatron Laboratories, Tokyo, Japan). Data were processed using the PeakSimple Chromatography software (V3.88, SRI Instruments, US). Fatty acid methyl esters (FAME) were analysed on a HP 6890 GC FID equipped with a HP 7683 autosampler. Peaks were identified using retention times from standards purchased from Supelco: 37 component FAME mix, Bacterial acid methyl ester mix, PUFA 1 and PUFA 3. Chromatograms were integrated using the Varian Galaxie Chromatography Data System, version 1.9.3.2. The Iatroscan determined derivatization efficiency for the samples was 76%. Lipid data are reported as % weight.

Data analysis

Parametric tests were used when assumptions of normality and equal variance were met; otherwise non-parametric counterparts were used. The relationship between juvenile weight and volume, as well as relationships between different variables and parent weight were determined using Spearman's rank order correlation. Within-brood coefficients of variation of mean weight (CVs) for surgically-extracted and naturally-released juveniles were compared using *t*-tests. Kruskal-Wallis one-way ANOVA on ranks were used to test the influence of sampling month on mean weight of juveniles. One-way ANOVA were used to test the influence of sampling month on within-brood CV of mean juvenile

weight. Weights of juveniles with and without traces of feeding were compared with *t*-tests.

Lipid and fatty acid proportions were analysed by ANOVA. Where assumptions of equal variance failed, ANOVA on ranks were used. Major fatty acids (> 1%) in adult body wall, oogenic mesentery, and large and small juveniles were compared using the Bray-Curtis similarity measurement and non-metric multi-dimensional scaling (MDS) analyses (Clarke and Warwick 2001). Variation in fatty acid composition among types of samples was subsequently tested for significance with ANOSIM (Analysis of similarities, Clarke and Warwick 2001). The R_{ANOSIM} statistic values varied from 0 (no difference among groups) to 1 (samples within the same group are more similar than samples from different groups). SIMPER (similarity percentage analysis, Clarke & Warwick 2001) was used to explore the relative contribution of individual fatty acid to dissimilarity among different types of samples.

Results

Like other sea anemones, *A. stella* lacks discrete ovaries, and oocytes grow within reproductive mesenteries between the retractor muscle and mesenterial filaments. Although *A. stella* is presumed to be a protandric hermaphrodite (Van Guelpen et al. 2005), no spermatozoa were detected in sea anemones studied here (n = 56). Juveniles of *A. stella* were brooded freely inside the gastrovascular cavity, and typically emitted individually through the mouth from August to October. Fully developed juveniles (Fig. 4-1a) up to 312 mg were released. Small juveniles (~5 mg) were also observed in the

tentacles of 3 adults in August and October 2010 (Fig. 4-1b, c). Furthermore, 2 adults were seen to release ~25 tiny propagules (< 5 mg) in mucus bundles through the mouth (Fig. 4-1d) or individually through tentacle tip pores (approximately 60% of these were < 1 mg). Unlike typical juveniles, these propagules, especially those < 1 mg, were covered with cilia, and were able to move rapidly in seawater (Fig. 4-1e). They had septa but their mouth and tentacles were not well-developed.

Offspring size variation during parental care in freshly collected adults

Among the 40 adults (1.1-56.0 g) dissected immediately after collection in April-May-June 2010, and January 2011, a total of 25 (62.5%) were brooding juveniles (Appendix 4-A). The proportion of brooding adults fluctuated from 50.0-88.9% in the 4 sampling months. Wet weights of 179 juveniles extracted from the brooding adults varied from 0.5 to 312 mg (Figs. 4-2a), with a mean of 59.3 mg and their volume varied from 0.4 to 395.2 mm^3, with a mean of 58.8 mm^3. The weight of juveniles was significantly correlated with their volume ($r_s = 0.94$, $n = 179$, $p < 0.005$) and thus was considered an accurate measurement of size.

Brood size (number of juveniles per brood) varied from 1 to 57 (Appendix 4-A), and was not significantly correlated with parent weight (Fig. 4-3a, $n = 25$, $p = 0.581$). In some cases, small adults brooded a large number of juveniles (> 10 juveniles) and large adults brooded few juveniles (down to one juvenile). However, brood weight (combined weight of all juveniles) was significantly related to parent weight (Fig. 4-3b). The mean weight of juveniles in a given brood varied from 5 to 275 mg, and was also significantly related to parent weight (Fig. 4-3c). However, it was not significantly different among

sampling months (January, April, May, June; H = 4.63, n = 25, p = 0.201). The overall coefficient of variation (CV) of mean weight of all juveniles (n = 179) was 111.8%. Within-brood CV was 3.7-143.1% in 19 adults that brooded > 1 juvenile (mean of 75.0%, Fig. 4-3d); it was not significantly correlated to parent weight (Fig. 4-3d, n = 19, p = 0.432) or to brood size (p = 0.819) and was not significantly affected by sampling month (F = 0.37, p = 0.699). The among-mother CV of brooded juveniles (calculated as SD/Mean of juvenile weight per female) was 45.7%.

Offspring size variation after natural release (post parental care)

Among 16 adult sea anemones (2.7-24.1 g) reared under laboratory conditions for long-term monitoring in 2 experimental periods (June 2009-March 2010, and April 2010-April 2011), 10 individuals (62.5%) were observed to release juveniles naturally (premature propagules mentioned earlier were excluded from this analysis). Three parents released a total of 15 juveniles in August and September 2009 and 7 released a total of 43 juveniles from August-October 2010. Weights of these naturally-released juveniles were 2-311 mg, with a mean of 76.2 mg (Fig. 4-2b). For parents releasing > 1 juveniles, within-brood CV of juvenile weight was 7.2-87.9% (mean of 40.5%), and it was not significantly related to parent weight (n = 9, p = 0.462) or brood size (p = 0.462). The among-mother CV of naturally-released juveniles was 97.3%.

Pre and post release comparisons of offspring size variation

At the end of the monitoring periods, in March 2010 and April 2011, 12 out of 16 adults (75%) were still brooding juveniles (> 6 mo after the last natural release). There were 1 to

16 juveniles per brood, for an overall total of 98 (Appendix 4-A). Their weight was 1-296 mg, with a mean of 33.9 mg. Only 1 adult did not release juveniles during the monitoring period and was not brooding at the end of the study. For parents that brooded > 1 juveniles, the within-brood CV of juvenile weight was 21.2-144.8% (mean of 83.7%) and not significantly related to parent weight ($n = 10$, $p = 0.275$) or to brood size ($p = 0.097$). In addition, within-brood CV on mean weight in brooded juveniles from parents maintained under captive conditions for about one year was not significantly different from that of brooded juveniles from parents examined immediately after collection from the field ($t = -0.61$, $n = 29$, $p = 0.546$). The among-mother CV of brooded juveniles (weight) was 84.6%.

Table 4-1 summarizes the variance in juvenile size measured across and within the various broods examined in this study. The overall CV of mean weight was higher in naturally-released than brooded juveniles across pooled broods. The mean CV was lower within-brood than among-mother at release but the inverse was seen in pre-release broods from field-collected adults.

Intra-brood feeding

Four adults (out of 6) were brooding 2 or 3 juveniles (total of 9) at the end of this study. The proportion of juveniles that fed on food ingested by the adult (ratio of juveniles with traces of feeding to the total number of brooded juveniles) was 50 -100%. Furthermore, mean weight of juveniles with traces of feeding (133.8 ± 58.8 mg, ± SD, $n = 6$) was greater than that of juveniles without any trace of feeding (57.8 ± 39.0 mg; $n = 3$), but the

difference was not significant ($p = 0.086$) due to the large variance in weight within the two groups.

Lipid composition and fatty acids

Adult tissues (body wall and oogenic mesenteries) and juveniles (large and small) were composed mainly of phospholipids (PL), sterols (ST), acetone mobile polar lipids (AMPL), triacylglycerols (TG), free fatty acids (FFA), hydrocarbons (HC), ethyl ketones (EK) and methyl esters (ME) (Appendix 4-B). Total lipid content (mean ± SE) accounted for 2.0 ± 0.2% of wet weight in adult body wall, 4.0 ± 0.2% in oogenic mesenteries, 3.3 ± 0.4% in large juveniles, and 5.0 ± 0.6% in small juveniles. Because lipids and fatty acids have not previously been studied in the genus *Aulactinia,* we provide a more complete outline and discussion in the Supporting Information (supplementary text). Here we focus on differences across sample types.

The polar lipid classes, AMPL and PL, were the most common lipids in the four types of samples, comprising 75.2 ± 2.6% in adult body wall, 60.1 ± 1.7% in oogenic mesenteries, 66.8 ± 4.0% in large juveniles and 62.7 ± 2.8% in small juveniles. The concentration of AMPL in large juveniles was not significantly different from that in the two types of adult tissue, but the concentration in small juveniles was significantly higher than that in adult body wall (Appendix 4-B). Proportions of AMPL did not vary significantly among the 4 types of samples. The concentrations of PL in large juveniles and small juveniles were not significantly different from those in oogenic mesenteries, but were significantly higher than in adult body wall. PL proportion in large juveniles was not significantly different from that in the 2 types of adult tissues; whereas PL

proportion in small juveniles was significantly higher than in adult body wall (Appendix 4-B).

Among some 50 fatty acids (FA) identified in the samples, there were 24 major ones (> 1% in at least one type of sample: Appendix 4-C), that accounted for > 90% of total FA in adult body wall, oogenic mesenteries, and juveniles. The proportion of polyunsaturated fatty acids (ΣPUFA), the most common FA group, was similar in all sample types (Appendix 4-C). Proportions of most major PUFAs were similar in large and small juveniles, except 20:2a and 20:5n-3 (EPA). EPA was the major PUFA in all samples, and its level in large juveniles was similar to that in the 2 types of adult tissue, but was significantly higher than in small juveniles. Besides EPA, the PUFAs that represented > 5% were 22:4n-6, 22:5n-3, and the essential fatty acids 20:4n-6 (ARA) and 22:6n-3 (DHA).

MDS showed FAs in large and small juveniles were more close to oogenic mesenteries than adult body wall (Fig. 4-4a). ANOSIM revealed fatty acid proportions were significantly different among juveniles and adult tissue, except between large and small juveniles ($p = 0.10$). Although fatty acids were not significantly different in large and small juveniles, R_{ANOSIM} revealed that large juveniles were more similar to adult tissue (*vs* oogenic mesenteries, $R = 0.679$; *vs* adult body wall, $R = 0.635$) than small juveniles (*vs* oogenic mesenteries, $R = 0.744$; *vs* adult body wall, $R = 0.726$). In addition, SIMPER analysis showed that similarity between large juveniles and adult tissue was greater than similarity between small juveniles and adult tissue (Fig. 4-4b), and that

essential EPA and DHA contributed to > 5% of the dissimilarity among different types of samples (Table 4-2).

Discussion

This study provides new empirical data on offspring size variation in a live-bearing cnidarian. The size of *A. stella* juveniles varied markedly throughout brooding and at release, irrespective of parent size. Given the typically small clutches, prolonged brooding may be a strategy to increase survival of juveniles; however, extended care also tends to increase potential for conflicts. Results from feeding trials and lipid/fatty acid analysis suggest that early juveniles initially depend on pre-zygotic (egg) provisioning and dissolved nutrients, and that large juveniles, having developed functional feeding organs, start to actively ingest food captured by their parent. This strongly suggests that offspring size and size variation in *A. stella* is not adaptive but rather tributary of parent-offspring and sibling conflicts during parental care, a situation typified in oviparous vertebrates with postnatal care (birds) and invertebrates that encapsulate eggs (gastropods), but hardly ever discussed in viviparous taxa (Kamel et al. 2010a, b). The novel arena presented here will be useful in exploring evolutionary concepts (e.g. viviparity-driven conflict) through comparisons with analogous vertebrate systems (e.g. placental fish).

Benefits and costs of brooding

Parental care has been suggested to benefit juveniles in various ways, e.g. enhanced survival through parental food provision and protection against predators (Trumbo 1996).

In *A. stella*, soft-bodied offspring may be protected against opportunistic grazers (e.g. sea urchins, Simoncini and Miller 2007) and/or specialized predators (e.g. nudibranchs, Greenwood et al. 2004) in two ways. (1) Survival of juveniles may be enhanced by increment in size during parental care, as suggested by size-dependent survival of juveniles against specialized predators (i.e. nudibranchs *Aeolidia papillosa*: Chapter 5). (2) Brooding adults may time release to decrease predation pressure by avoiding peak abundance of specialized predators, which are typically ephemeral. The life span of *A. papillosa* in the NW Atlantic extends from October/November to the following July, similar to accounts in the NE Atlantic (Hall and Todd 1986). Brooding adults of *A. stella* release offspring chiefly in the fall, at a time when specialized predators are scarce or absent (i.e. the older generation died off in July after the reproductive season, and the new generation is still composed of small subadults: Chapter 5).

Size and number of tentacles and nematocyst types have been suggested to influence prey capture ability in corals and sea anemones (Madin 1988). Thus, brooding adults of *A. stella* likely are more efficient at capturing food than juveniles, and they could 'nurse' brooded juveniles until they become more efficient predators. For extremely small juveniles ($\leq$ 5 mg), which possess only tentacle buds and thus have limited prey capture ability, nutrition provided by brooding adults in the form of pre-zygotic reserves or dissolved nutrients would be crucial. Postvitellogenic transfer of nutrients from parent to juveniles (matrotrophy) has also been reported in internally brooding sea stars (McClary and Mladenov 1990) and live-bearing fish (Pollux and Reznick 2011).

Important costs to the mother have been observed in brooding marine invertebrates (Fernández et al. 2000), which affect investment in gametes and determine the trade-off between the cost of brooding and capacity to produce eggs (Brante et al. 2003). In *A. stella*, the cost of brooding could be more dramatic considering that juveniles are able to consume part of the food that brooding adults obtain (i.e. parent-offspring competition), which could partly explain why the number of offspring in a brood was generally small (1 to 57). Experimental studies on clutch size variations are needed to confirm this quantitatively.

Meanwhile, lipid and fatty acid analyses support the assumption that juveniles of *A. stella* undergo a dietary shift during parental care. EPA and DHA, which are important for reproduction and early development of marine invertebrates (Heras et al. 2000; Pernet et al. 2002), were the most important discriminating fatty acids among samples. The proportion of EPA was significantly higher in large juveniles and oogenic mesenteries than in small juveniles, which may reflect metabolizing EPA during early development or early growth and conservation of EPA during later growth. Conservation of EPA during metabolism, indicated by high EPA content, has also been suggested in the sympatric bivalve *Yoldia hyperborea* (Parrish et al. 2009). Furthermore, similarity analyses on the major fatty acids revealed that large juveniles clustered closer to the adult tissues than to smaller juveniles. In species that brood offspring to the juvenile stage, nutrition of juveniles can be obtained from two sources: (1) pre-zygotic (egg) provisioning by adults, and/or (2) later dietary uptake (usually nutrients obtained from the adult in dissolved form, here autonomous feeding). Results suggest that large juveniles feed more readily on

the diet of brooding adults inside the gastrovascular cavity than smaller siblings. This is supported by the mean weight of feeding juveniles being higher than that of non-feeding juveniles. In addition, small juveniles <10 mg were not well developed (i.e. had less functional tentacles and digestive system) consistent with a dependence on pre-zygotic provisioning and dissolved material that would generate a fatty acid signature different from that of the adult. In support of this, the MDS plot showed that the largest of the 'small' juveniles (77 mg, able to actively feed) was more similar to large juveniles and adult tissue than to its smaller siblings weighing 8 and 10 mg.

Offspring size variation

Offspring size variations in *A. stella* were typically > 40% and up to 129% in the overall population. Using Jacobs & Podolsky's (2010) conversion rate (x3) for CVs measured in length *vs* volume (= weight, Chapter 2), we find that overall CV of mean juvenile size in *A. stella* is generally higher than in 101 of the 102 species of marine invertebrates reviewed by Marshall & Keough (2007). Interestingly, the species with a comparably high CV is a live-bearing holothuroid echinoderm (the review included only three viviparous species, all in phylum Echinodermata). However, inter-specific comparison of offspring size variation should be made with caution, given issues with dimensionality highlighted by Jacobs & Podolsky (2010), and because CV is influenced by mean size, and thus best compared through analyses of covariance (Chapter 2).

Recent attempts have been made to relate offspring size plasticity to bet hedging, a concept that has received much attention (mainly in Chordata and Arthropoda) but remains hard to assess (Simons 2011). The simplified assumption of dynamic or

diversified bet hedging is that when females can predict the environment to which offspring will be exposed, producing offspring close to the mean optimal size will be favoured; otherwise, increasing variance in offspring size will be favoured to ensure survival under unpredictable environmental conditions (Marshall and Keough 2007; Marshall et al. 2008; Crean and Marshall 2009). In marine invertebrates with complex life histories, the ability of mothers to predict offspring performance has been proposed to depend on developmental mode, i.e. greater ability in mothers that produce benthic juveniles than in mothers that produce dispersive pelagic propagules (Marshall et al. 2008). Hence, the former should exhibit greater within-clutch and lower among-mother size variation than the latter (Marshall et al. 2008). Notwithstanding limitations in the categories used (no distinction between viviparous and encapsulated development of benthic juveniles) we have attempted to reconcile this general prediction with our data. *A. stella* is a long-lived sessile species that broods to fully-developed philopatric juveniles. Adults should thus be able to accurately predict the environment experienced by offspring both while inside the gastrovascular cavity (egg to juvenile), and upon their release. The among-mother variance in *A. stella* was much higher than the mean within-brood variance for newly-released juveniles (as predicted), but an inverse trend was observed in brooded juveniles extracted from freshly collected adults ($CV_{within} > CV_{among}$). The contrast between pre and post-release juveniles is intriguing. It highlights the need to conduct empirical tests of size variations across life stages within species and consider this in subsequent inter-specific comparisons, which is currently not the case.

Other maternal effects, more commonly identified as determinants of offspring size plasticity include maternal size and experience (reviewed by Marshall & Keough 2007). For example, smaller colonies of the urochordate *Pyura stolonifera* produced eggs with larger intra-clutch size variation, compared to larger colonies (Marshall et al. 2000). Here, the within-brood CV of juvenile size was not significantly related to parental size, indicating that adult phenotype is not the primary driver of offspring size variation in *A. stella*. Furthermore, CVs were similar whether measured in the broods of adults that were freshly collected from the field in different months or in the broods of adults maintained for ~1 year in (comparatively benign) laboratory conditions. Thus, parental environmental effects do not appear to be playing a major role either.

Offspring size variation appears to derive mainly from the brooding strategy itself and may thus be under the control of brooding adults to some degree. Brooded juveniles and oogenic mesenteries were observed at all sampling dates, including 6 mo after the main release event, indicating (1) a prolonged brooding period, (2) overlap between brooding and oogenesis, and (3) brooding of more than one cohort of juveniles per year, with possible generation overlap. Furthermore, brooding adults were observed to release offspring at any time when experiencing physical stress (e.g. after being teased or when their body wall was damaged), suggesting that the length of the brooding period is not fixed, despite the occurrence of an identified preferential release season (fall). This minimizes the risk of instantaneous brood mortality through parent mortality (predation) usually associated with viviparity (Jorgensen *et al.* 2011). As discussed earlier, extended brooding presumably contributes to fitness (greater chances that juveniles will survive to

reproduction) by protecting them until they reach a refuge size. But as juveniles grow, they may also compete with each other and with the brooding adult for potentially limited resources (e.g. food). Thus, in addition to initial parental investment, parent-offspring conflicts and interactions among siblings emerge as key mediators of offspring size and size variations in *A. stella*.

A different form of offspring size variation mediated by sibling interactions has been reported in the sympatric sea anemone *Urticina felina*. Internally-brooded embryos of *U. felina* are capable of fusing with their siblings to form large mega-larvae which exhibit better survival to settlement (Mercier et al. 2011, and Chapter 5). This mechanism is unlikely to occur in *A. stella* considering its much lower fecundity and the fact that the largest juveniles were more developed than the smallest ones (< 5 mg). Taken together these findings suggest that various forms of plasticity in offspring phenotype can be expected to arise in brooding (including live-bearing) taxa. As recently stated by Jorgensen *et al.* (2011) from a study of vivparous fish, optimality models based on a trade-off between egg size and fecundity "fall short of capturing the true complexity of the interactions that shape the evolution of offspring size." Future work on *A. stella* and similar understudied models could be instrumental in broadening our understanding of key concepts, including the effects of density and age-dependent factors on family conflicts, clutch size and offspring size plasticity.

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Tables

Table 4-1. Offspring size variation in *Aulactinia stella*, measured as coefficient of variation (CV) of mean weight at various scales and on various occasions.

Time	Cohort	CV of mean juvenile weight (%)					
		Overall population		Among-mother		Within-brood	
Pre-release (extracted, still brooded)	Field-Jan	80.8	111.8	63.3	45.7	83.6	75.0
	Field-Apr	87.9		--		--	
	Field-May	119.2		63.3		71.9	
	Field-Jun	72.5		64.8		66.1	
	Lab-year 1	85.2	79.8	58.7	84.6	60.1	83.7
	Lab-year 2	138.5		42.3		107.2	
Post-release (naturally-released)	Lab-year 1	131.4	128.8	126.9	97.3	42.5	40.5
	Lab-year 2	76.4		74.6		39.5	

Table 4-2. Discriminating fatty acids of the dissimilarity in samples of *Aulactinia stella* (with contribution to average dissimilarity > 5 %).

Type of samples	Fatty acids	Proportion (% weight)	Proportion (% weight)	Contribution (%)
Small juveniles *vs* Large juveniles	20:5n3 EPA	22.37	28.01	22.97
	16:0	6.29	5.5	8.36
	18:0	6.43	4.97	7.53
	22:6n3 DHA	4.79	6.2	5.77
	18:1n5?	6.03	4.77	5.15
Small juveniles *vs* Oogenic mesenteries	20:5n3 EPA	22.37	27.77	22.89
	16:0	6.29	6.82	10.44
	18:0	6.43	5.48	7.63
	22:5n3	7.19	8.87	7.16
	22:4n6?	5.45	6.73	6.49
	22:6n3 DHA	4.79	4.15	5.31
Large juveniles *vs* Oogenic mesenteries	22:6n3 DHA	6.2	4.15	13.11
	16:0	5.5	6.82	8.11
	22:4n6?	5.86	6.73	8.02
	16:3n4?	2.09	1.72	6.95
	22:1n9	4.53	3.49	6.46
	22:5n3	7.86	8.87	6.27
	20:5n3 EPA	28.01	27.77	5.64
	22:1n7	1.31	0.45	5.29
Small juveniles *vs* Adult body wall	20:5n3 EPA	22.37	24.32	10.54
	22:4n6?	5.45	8.56	8.31
	22:6n3 DHA	4.79	1.75	7.81
	22:1n9	4.08	6.94	7.36
	16:0	6.29	5.06	6.33
	18:1n9	3.25	1.29	5.67
	20:1n9	2.71	0.62	5.38
Large juveniles *vs* Adult body wall	22:6n3 DHA	6.2	1.75	13.78
	20:5n3 EPA	28.01	24.32	11.47
	22:4n6?	5.86	8.56	8.95
	22:1n9	4.53	6.94	7.58
	20:4n6 AA	3.27	5	5.74
	16:3n4?	2.09	3.68	5.68
Oogenic mesenteries *vs* Adult body wall	20:5n3 EPA	27.77	24.32	10.09
	22:1n9	3.49	6.94	9.74
	22:6n3 DHA	4.15	1.75	6.78
	16:0	6.82	5.06	6.51
	22:5n3	8.87	6.6	6.4
	22:4n6?	6.73	8.56	6.27
	16:3n4?	1.72	3.68	5.54

Figures

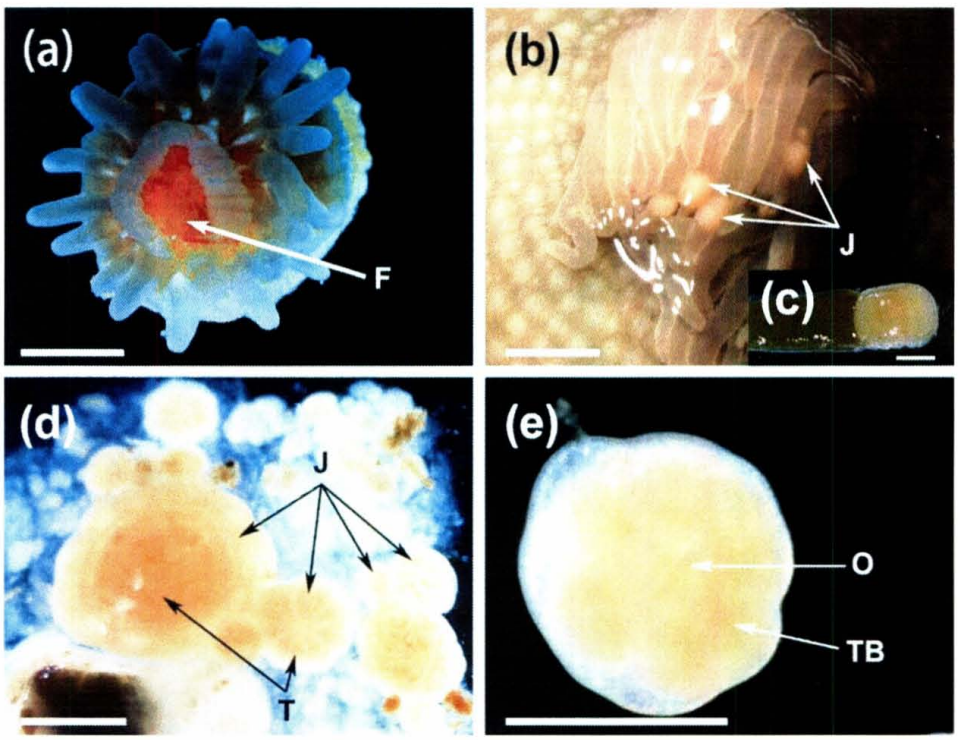

Fig. 4-1. *Aulactinia stella.* (a) Brooded juvenile; this one was scored as positive for intra-brood feeding based on presence of food (F) in the gastrovascular cavity. (b) Small juveniles (J) moving freely in the tentacles of a brooding adult. (c) Close-up of a small juvenile in (b). d) Size variation of offspring released in a mucus bundle, including tiny propagules and metamorphosing juveniles (J), with primary tentacles (T). (e) Close-up of a small metamorphosing juvenile in (d), showing oral pore (O) and tentacle buds (TB). Scale bar represents 2 mm in (a), 4 mm in (b), 1 mm in (c) and (d), and 0.5 mm in E.

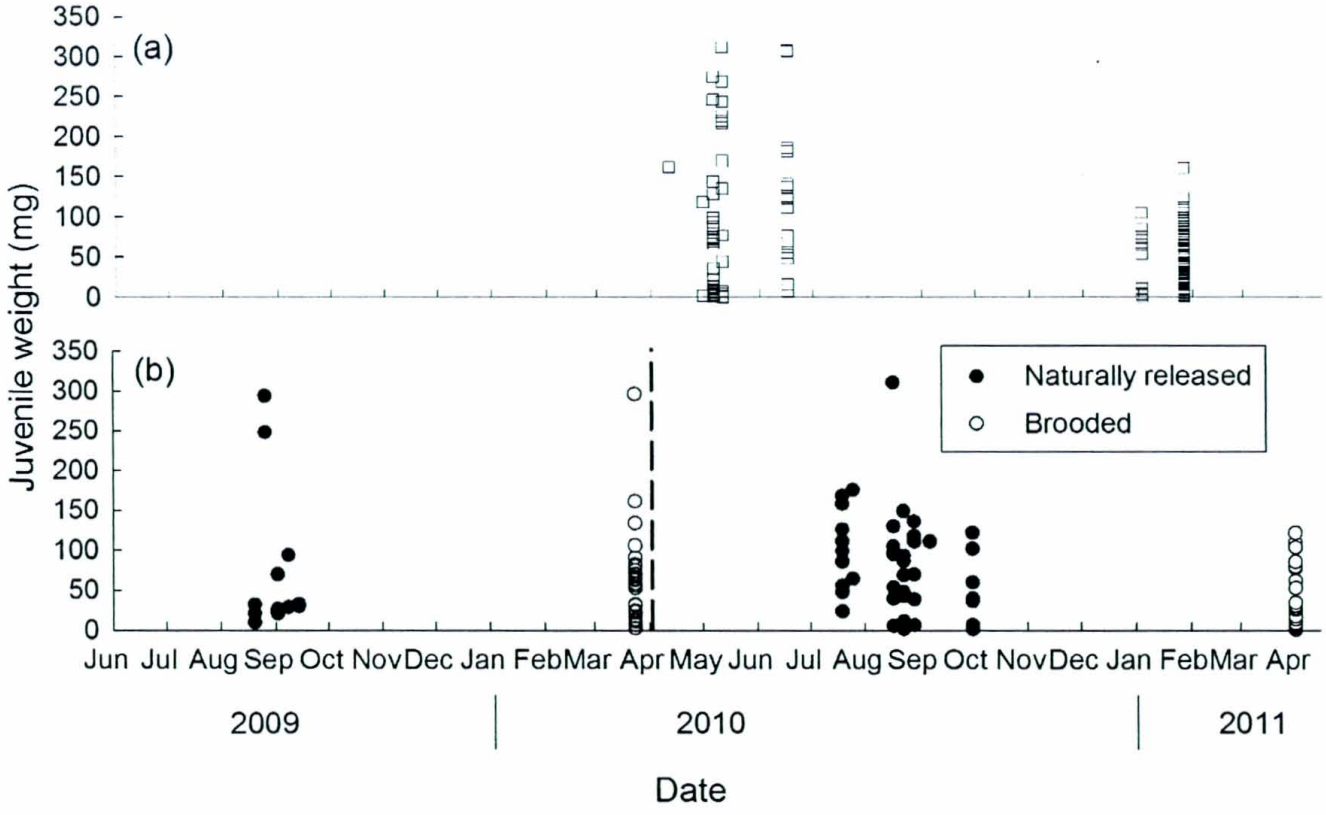

Fig. 4-2. *Aulactinia stella.* Distribution of juvenile sizes (wet weight) over time. (a) Juveniles surgically extracted immediately after collection of brooding adult. (b) Naturally-released juveniles and brooded juveniles in two experimental periods from 2009 to 2011. Dashed line separates the two experimental periods.

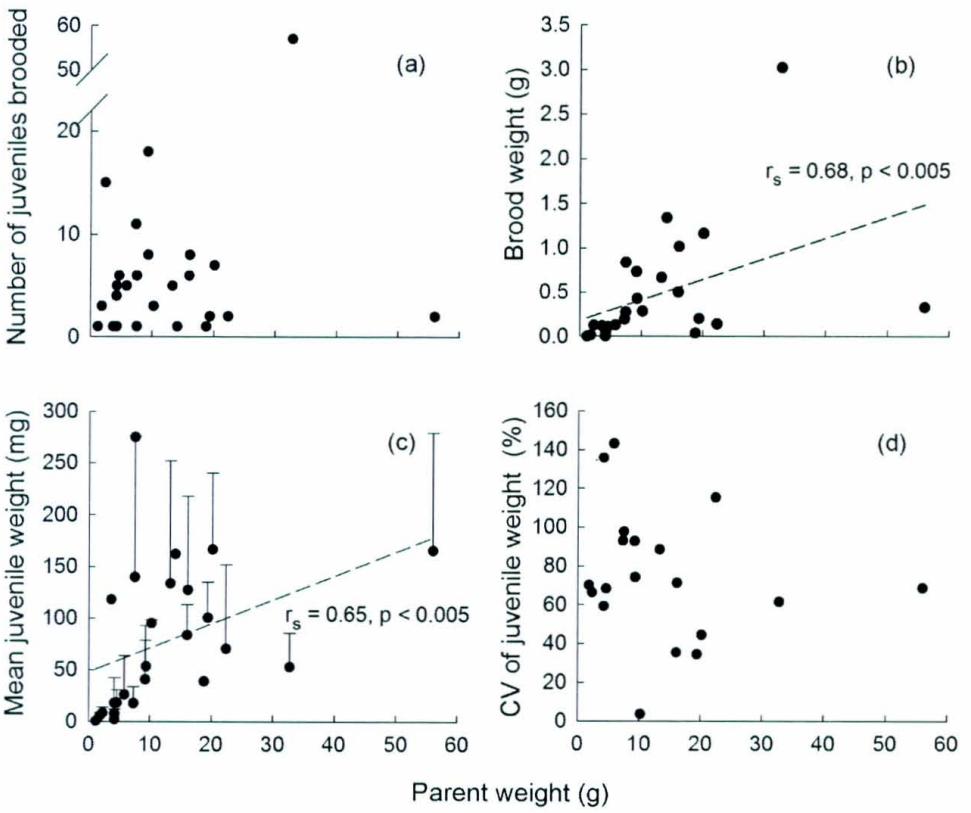

Fig. 4-3. *Aulactinia stella.* Influence of adult wet weight on: (a) the number of juveniles being brooded; (b) wet weight of entire brood (g); (c) mean (+SD) juvenile wet weight (mg); and (d) Within-brood CV of mean juvenile weight.

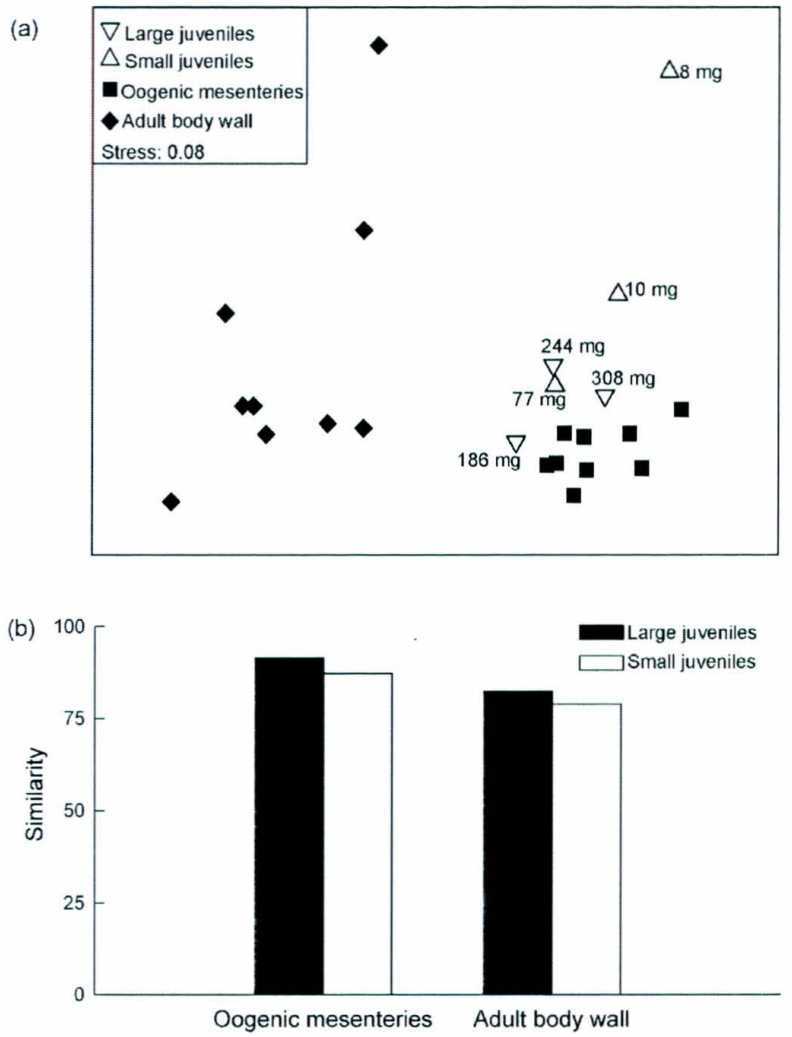

Fig. 4-4. *Aulactinia stella.* (a) Multidimensional scaling (MDS) 2-D plot of Bray-Curtis similarity index of major fatty acids from large and small juveniles (wet weight indicated), oogenic mesenteries and adult body wall. (b) Bray-Curtis similarity index between large and small juveniles with adult tissue (oogenic mesenteries and adult body wall).

Appendices

Appendix 4-A. Number and wet weight (Ww; mean ± SD) of naturally-released and brooded juveniles in adult *Aulactinia stella* of various sizes.

		Naturally-released juveniles		Brooded juveniles	
Ind[a]	Adult Ww (g)	Number	Ww (mg)	Number	Ww (mg)
I - 1	13.7	3	21.0 ± 11.0	2	118.1 ± 61.0
I - 2	8.1	2	271.0 ± 32.5	0	-
I - 3	9.5	10	38.1 ± 24.1	1	82.0
I - 4	13.3	0	-	3	20.2 ± 5.9
I - 5	15.4	0	-	7	97.8 ± 98.6
I - 6	12.2	0	-	10	65.3 ± 13.8
I - 7	9.9	0	-	1	31.0
I - 8	8.5	0	-	4	40.5 ± 39.5
II - 2	24.1	2	52.0 ± 5.6	14	32.6 ± 42.3
II - 3	2.7	9[b]	40.1 ± 35.3	11	11.3 ± 8.6
II - 4	7.4	4[c]	163.0 ± 11.8	15	20.7 ± 21.0
II - 5	15.2	5	116 ± 9	14	22.2 ± 32.2
II - 8	12.8	3	7.0 ± 5.0	16	13.2 ± 11.1
II - 6	13.0	1	311	0	-
II - 1	10.4	19	74.1 ± 37.9	0	-
II - 7	5.8	0	-	0	-
III-1	3.7	-	-	1	118.0
III-2	4.2	-	-	1	2.0
III-3	15.4	-	-	0	-
III-4	14.1	-	-	1	162.0
III-5	7.5	-	-	1	275.0
III-6	7.2	-	-	0	-
III-7	6.7	-	-	0	-
III-8	1.4	-	-	0	-
III-9	5.8	-	-	5	26.2 ± 37.5
III-10	3.2	-	-	0	-
III-11	2.1	-	-	0	-
III-12	2.3	-	-	15	8.3 ± 5.5
III-13	56.0	-	-	2	165.5 ± 113.8
III-14	10.5	-	-	0	-
III-15	22.3	-	-	2	70.5 ± 81.3
III-16	10.2	-	-	3	95.0 ± 3.5
III-17	23.4	-	-	0	-
III-18	16.0	-	-	6	83.7 ± 29.7

III-19	13.5	-	-	0	-
III-20	9.0	-	-	0	-
III-21	19.4	-	-	2	100.5 ± 34.6
III-22	13.3	-	-	5	133.6 ± 118.3
III-23	16.1	-	-	8	127.3 ± 90.7
III-24	1.8	-	-	3	5.2 ± 3.7
III-25	1.2	-	-	1	0.5
III-26	7.5	-	-	6	139.7 ± 136.3
III-27	20.1	-	-	7	166.4 ± 74.1
III-28	3.9	-	-	0	-
III-29	1.1	-	-	0	-
III-30	24.2	-	-	0	-
III-31	20.7	-	-	0	-
III-32	9.3	-	-	8	53.4 ± 39.6
III-33	4.2	-	-	4	18.0 ± 24.4
III-34	3.1	-	-	0	-
III-35	32.7	-	-	57	53.0 ± 32.7
III-36	7.3	-	-	11	17.6 ± 16.4
III-37	4.6	-	-	6	18.2 ± 12.4
III-38	4.2	-	-	5	7.6 ± 4.5
III-39	18.8	-	-	1	39.0
III-40	9.2	-	-	18	40.8 ± 37.8

a: Prefix I- identifies individuals that were monitored from June 2009 to March 2010, II- individuals that were monitored from April 2010 to April 2011, and III- individuals that were freshly collected from the field in March-June 2010 and January 2011.
b: Not including 14 tiny propagules released in mucus bundles in July and October 2010.
c: Not including 11 tiny propagules released in August 2010.

Appendix 4-B. Mean concentration and proportion of lipids in adult body wall, oogenic mesenteries and large and small brooded juveniles of the sea anemone *Aulactinia stella*. Values (mean ± SE) in the same row with different superscript letters are significantly different (one-way ANOVA, $p < 0.05$).

Lipids	Adult body wall (n = 11)		Oogenic mesenteries (n = 9)		Large juveniles (n = 6)		Small juveniles (n = 6)	
	Concentration ($\mu g\ mg^{-1}$)	Proportion (%)	Concentration ($\mu g\ mg^{-1}$)	Proportion (%)	Concentration ($\mu g\ mg^{-1}$)	Proportion (%)	Concentration ($\mu g\ mg^{-1}$)	Proportion (%)
Hydrocarbons	0.28 ± 0.07^{a}	1.47 ± 0.39^{AB}	0.30 ± 0.07^{a}	0.73 ± 0.13^{B}	0.49 ± 0.08^{ab}	1.52 ± 0.25^{AC}	6.30 ± 3.22^{b}	9.98 ± 4.28^{C}
Methyl Esters	0.21 ± 0.04^{a}	1.03 ± 0.16^{A}	0.96 ± 0.11^{b}	2.41 ± 0.24^{AB}	1.49 ± 0.63^{b}	4.24 ± 1.25^{B}	1.12 ± 0.42^{b}	2.44 ± 0.90^{AB}
Ethyl Ketones	0.21 ± 0.09^{a}	1.10 ± 0.47^{A}	0.86 ± 0.17^{ab}	2.10 ± 0.32^{A}	1.26 ± 0.72^{b}	3.43 ± 1.45^{A}	1.64 ± 0.32^{b}	3.17 ± 0.49^{A}
Triacylglycerols	0.38 ± 0.07^{a}	1.98 ± 0.47^{A}	8.11 ± 0.72^{b}	20.20 ± 1.34^{B}	3.39 ± 0.89^{c}	9.85 ± 1.61^{C}	3.20 ± 0.97^{c}	6.92 ± 2.00^{C}
Free Fatty Acids	0.38 ± 0.10^{a}	1.89 ± 0.43^{A}	0.64 ± 0.31^{a}	1.56 ± 0.58^{A}	0.16 ± 0.11^{a}	0.44 ± 0.28^{A}	2.40 ± 1.08^{a}	4.50 ± 2.07^{A}
Sterols	3.20 ± 0.31^{a}	16.25 ± 1.49^{A}	4.67 ± 0.73^{a}	11.38 ± 1.17^{B}	3.55 ± 0.27^{a}	11.36 ± 0.94^{B}	3.25 ± 0.61^{a}	7.94 ± 2.64^{B}
Acetone Mobile Polar Lipids	0.87 ± 0.17^{a}	4.93 ± 1.20^{A}	1.41 ± 0.21^{ab}	3.49 ± 0.40^{A}	1.32 ± 0.33^{ab}	4.44 ± 1.23^{A}	3.47 ± 0.70^{b}	6.73 ± 0.82^{A}
Phospholipids	14.36 ± 1.38^{a}	70.25 ± 2.86^{A}	22.54 ± 0.95^{bc}	56.58 ± 1.75^{B}	20.35 ± 2.85^{c}	62.4 ± 3.29^{AB}	27.15 ± 2.91^{b}	55.95 ± 2.98^{B}
Total	20.19 ± 1.48^{a}	--	39.57 ± 1.93^{b}	--	32.58 ± 4.33^{b}	--	49.75 ± 6.79^{c}	--

Appendix 4-C. Major fatty acids (> 1% of total fatty acids) in adult body wall, oogenic mesenteries, large and small brooded juveniles of the sea anemone *Aulactinia stella*. Values (mean ± SE) in the same row with different superscript letters are significantly different (one-way ANOVA, $p < 0.05$).

% Fatty acids	Adult body wall (n = 9)	Oogenic mesenteries (n = 9)	Large juveniles (n = 3)	Small juveniles (n = 3)
16:0	5.06 ± 0.61 [a]	6.82 ± 0.14 [b]	5.50 ± 0.19 [ab]	6.29 ± 1.76 [ab]
ι17:0	1.59 ± 0.12 [a]	0.77 ± 0.10 [b]	1.05 ± 0.07 [b]	1.04 ± 0.08 [b]
18:0	5.6 ± 0.39 [a]	5.48 ± 0.12 [a]	1.57 ± 0.20 [a]	6.43 ± 0.78 [a]
ΣSFA	15.26 ± 1.20 [a]	15.38 ± 0.27 [a]	14.03 ± 0.10 [a]	16.07 ± 3.39 [a]
15:1	2.71 ± 0.15 [a]	1.05 ± 0.14 [b]	1.43 ± 0.45 [b]	1.49 ± 0.08 [b]
16:1n-7	1.62 ± 0.17 [a]	2.39 ± 0.06 [b]	1.87 ± 0.13 [a]	2.69± 0.10 [b]
18:1n-9	1.29 ± 0.40 [a]	2.38 ± 0.29 [b]	2.06 ± 0.18 [ab]	3.25 ± 0.25 [b]
18:1n-7	1.46 ± 0.24 [a]	2.89 ± 0.05 [b]	2.68 ± 0.39 [b]	2.50 ± 0.14 [b]
18:1n-5? *	6.15 ± 0.28 [a]	5.36 ± 0.11 [a]	5.81 ± 0.88 [a]	5.00 ± 0.11 [a]
20:1n-11?	0.66 ± 0.13 [a]	1.06 ± 0.06 [b]	1.00 ± 0.20 [ab]	1.76 ± 0.06 [c]
20:1n-9	0.62 ± 0.09 [a]	1.73 ± 0.06 [b]	1.69 ± 0.27 [ab]	2.72 ± 0.44 [b]
20:1n-7?	1.74 ± 0.13 [a]	2.59 ± 0.06 [b]	2.58 ± 0.24 [b]	2.29 ± 0.10 [b]
22:1n-9	6.94 ± 0.33 [a]	3.49 ± 0.10 [b]	4.07 ± 0.15 [bc]	4.53 ± 0.44 [c]
22:1n-7	1.93 ± 0.16 [a]	0.45 ± 0.12 [b]	0.71 ± 0.35 [bc]	1.28 ± 0.14 [c]
ΣMUFA	26.42 ± 0.92 [a]	24.92 ± 0.3 [a]	25.50 ± 0.63 [a]	30.17 ± 1.39 [b]
16:2n-4	1.59 ± 0.12 [a]	0.45 ± 0.02 [b]	0.73 ± 0.07 [b]	0.66 ± 0.04 [b]
16:3n-4?	3.68 ± 0.26 [a]	1.72 ± 0.21 [b]	2.44 ± 0.69 [b]	1.70 ± 0.48 [b]
16:4n-3?	1.13 ± 0.15 [a]	0.44 ± 0.06 [b]	0.62 ± 0.15 [b]	0.47 ± 0.05 [b]
16:4n-1	1.28 ± 0.12 [a]	0.44 ± 0.06 [b]	0.46 ± 0.12 [b]	0.75 ± 0.19 [b]
20:2a?	0.74 ± 0.10 [a]	0.91 ± 0.09 [a]	0.75 ± 0.12 [a]	1.45 ± 0.38 [b]
20:2n-6	0.47 ± 0.06 [a]	1.08 ± 0.05 [b]	0.98 ± 0.18 [b]	0.93 ± 0.07 [b]
20:4n-6 ARA	5.00 ± 0.42 [a]	3.57 ± 0.18 [b]	3.37 ± 0.34 [b]	3.52 ± 0.23 [b]
20:5n-3 EPA	24.32 ± 1.11 [ab]	27.77 ± 0.35 [b]	28.01 ± 0.41 [b]	22.37 ± 2.46 [a]
22:4n-6?	8.56 ± 0.59 [a]	6.73 ± 0.35 [b]	5.28 ± 0.59 [b]	6.04 ± 0.49 [b]
22:5n-3	6.60 ± 0.34 [a]	8.87 ± 0.19 [b]	7.27 ± 0.61 [a]	7.77 ± 0.31 [ab]
22:6n-3 DHA	1.75 ± 0.18 [a]	4.15 ± 0.40 [b]	5.25 ± 0.66 [bc]	5.74 ± 0.63 [c]
ΣPUFA	58.32 ± 2.08 [a]	59.7 ± 0.42 [a]	60.48± 0.64 [a]	53.76 ± 4.51 [a]
Bacterial	6.80 ± 0.31 [a]	3.66 ± 0.15 [b]	4.37 ± 0.54 [b]	4.29 ± 0.05 [b]
P/S	4.04 ± 0.35 [a]	3.89 ± 0.09 [a]	4.31 ± 0.06 [a]	3.74 ± 0.96 [a]
Σn-3	35.12 ± 1.48 [a]	43.05 ± 0.63 [b]	38.12 ± 4.19 [ab]	43.21 ± 1.47 [b]
DHA/EPA ratio	0.07 ± 0.01 [a]	0.15 ± 0.02 [b]	0.23 ± 0.01 [c]	0.21 ± 0.02 [c]

* ? Identity FA not confirmed by comparison with a standard or by mass spectrometry, but by comparison with Ackman (1986) .

CHAPTER 5 : **The complexity of offspring size effects: variations across life stages and between species**

The manuscript in this chapter is in preparation for Oikos

Abstract

Optimality models of offspring size and number assume positive functions between parental investment and offspring size, and between offspring size and performance. In marine organisms with complex life cycles, the size-performance function is hard to grasp because measures of performance are varied and their relationships with size may not be consistent throughout early ontogeny. Here we examine size effects in pre-metamorphic (larval) and post-metamorphic (juvenile) stages of brooding marine invertebrates and show that they vary both intra-specifically (across life stages) and inter-specifically for the post-metamorphic stages. Larger offspring of the sea anemone *Urticina felina* outperformed small siblings, to some extent, at the larval stage (i.e. greater settlement and survival rates under suboptimal conditions), whereas smaller offspring were favoured by size-selective predation on 15-mo old juveniles. Post-metamorphic size-dependant mortality followed an inverse trend in a sympatric species with a different life-history strategy (*Aulactinia stella*) in which smaller juveniles suffered overall greater predation rates. Size differences in pre-metamorphic performance of *U. felina* were linked to total lipid contents of larvae and size-related mortality of post-metamorphic stages followed the predictions of a trade-off associated with prey size selection. These findings emphasize the challenge in gathering empirical support for a positive size-performance function in taxa that exhibit complex life cycles.

Introduction

A central tenet of life-history theory is the occurrence of a trade-off between the size and number of offspring produced (Smith and Fretwell 1974, Stearns 1992). This trade-off is driven by the balance between energy spent on individual offspring and parental fitness (Smith and Fretwell 1974), with two important underlying assumptions: (1) a negative relationship between offspring number and energy invested per offspring, and (2) a positive relationship between parental investment per offspring and offspring performance. Studies have suggested that offspring size, especially egg size, reflects parental investment (Jaeckle 1995) and the amount of energetic reserves available for metamorphosis and early growth (Marshall and Keough 2003). However, this notion has not been extensively tested, and offspring size apparently does not always relate to organic content (McEdward and Carson 1987).

Recent studies have proposed that size of offspring influences their pre-metamorphic performance, e.g. fertilization (Marshall et al. 2000) and time before settlement (Marshall and Keough 2003). For instance, large eggs of the broadcasting ascidian *Pyura stolonifera* achieved maximum fertilization at a lower sperm concentration than smaller eggs (Marshall et al. 2000). In addition, larger larvae were shown to have a greater ability to delay settlement in the absence of proper settlement cues in three species of colonial marine invertebrates (Marshall and Keough 2003). Offspring size may also influence post-metamorphic performance, including survival, growth, competition among conspecifics and even reproduction of the next generation

(Emlet and Sadro 2006, Marshall et al. 2006). For example, larger hatchling juveniles of the gastropod *Nucella ostrina* had higher survival rates and remained larger in size after 36-54 days in the field than the smaller hatchlings (Moran and Emlet 2001). Current studies of size-related offspring performance in marine organisms have almost exclusively focused on a single life stage (especially the post-metamorphic stage), whereas very little empirical data exist on size-related fitness across multiple life-history stages (Rius et al. 2009). To gain a better understanding of the evolutionary advantages of offspring size, empirical tests of the size-performance relationship should be carried out across multiple life-history stages, including pre-metamorphic stages, juvenile stages and adulthood.

Studies of offspring size effects in benthic marine organisms are largely centered on colonial bryozoans (Marshall and Keough 2008) and ascidians (Marshall and Keough 2005, Jacobs and Sherrard 2010), with fewer studies on solitary species, including sea urchins (Emlet and Hoegh-Guldberg 1997), gastropods (Moran and Emlet 2001) and barnacles (Emlet and Sadro 2006). While it is commonly assumed that size confers advantages, contrasting results have been reported (e.g. Marshall and Keough 2005 vs. Jacobs and Sherrard 2010). The influence of offspring size on their performance appears to be strongly mediated by external conditions, including predation (Rivest 1983, Barbeau and Scheibling 1994), competition (Marshall et al. 2006, Allen et al. 2008), temperature and habitat (Moran 1999, Collin and Salazar 2010). Predation is often identified as the most influential factor on offspring survival in sessile benthic organisms (Spight 1976). Although offspring size has been suggested to have a strong influence on

the resistance of juveniles to predation (Rivest 1983, Barbeau and Scheibling 1994), evidence to the contrary has also been obtained (Gosselin and Rehak 2007). It remains that the relationship between size and performance of juveniles under different types of predation pressure has rarely been studied in benthic marine species (Rivest 1983, Barbeau and Scheibling 1994).

In the present study, experimental trials were conducted to gain a better understanding of the effects of size on the performance of pre-metamorphic (larva) and post-metamorphic (juvenile) stages in the brooding sea anemone *Urticina felina*, which releases lecithotrophic larvae of various sizes (Mercier et al. 2011). Our specific aims were to: (1) verify the effects of size on behaviour, time to settlement and survival of larvae, (2) compare lipid composition in larvae of different sizes, and (3) test size-related survival of juveniles in the presence of different sizes of their specialized predator. To test whether the size-related survival of juveniles varies between species, predation trials were also conducted on the juveniles of the sympatric live-bearing sea anemone *Aulactinia stella.*

Materials and Methods

Time to settlement and survival of small and large larvae of *Urticina felina*

Adults of *Urticina felina* were collected at a depth of ~10 m off the Avalon Peninsula (Newfoundland, Canada) in June 2010, and were distributed into several holding tanks (20-40 L) supplied with unfiltered running seawater, at temperatures that followed the ambient annual cycle (0-10°C), under natural photoperiod. To compare the

behaviour of various sized larvae from the same brood, four brooding females (41.2 to 212.9 g drained weight, with visible embryos/larvae) were maintained individually during the larval release period (July to September 2010). Larvae were emitted through the mouth of the females, and were collected at the surface of the water column within 24 h post release.

Between 191 and 277 larvae were collected from each of the four brooding females and used to test the influence of larval size on their performance (i.e. buoyancy, survival and time to settlement). Larvae from the same brood were examined under a Nikon SMZ1500 stereomicroscope, and then classified into two classes (small and large) based on their surface area. The mean size of small larvae were between 48.8 and 67.0% of the size of large sibling larvae, yielding significant size differences in each of the broods (Mann-Whitney or *t*-tests, $p < 0.001$) as illustrated in Fig. 5-1.

Preliminary trials consistently showed that, regardless of size, the proportion of buoyant larvae dropped < 50% at 10 days post release when a rock (~4 cm^2) covered with coralline algae (*Clathromorphum* sp.) was offered (= optimal substratum for settlement), whereas it dropped to 50% at 18 days post release in bare containers (mimicking sub-optimal settlement conditions). Thus the experiment was divided into two segments to test the influence of larval size (1) on the behaviour under sub-optimal settlement conditions (without preferred substratum), and (2) on the behaviour of larvae when the optimal substratum was made available (by exposing the same larvae to this new condition). Day 18 was chosen as the midpoint for the settlement experiment as per results described above.

Groups of small and large sibling larvae (n = 29-48 per group; 3 groups for each size class in each female) were randomly distributed into six separate flow-through plastic containers (2-L). The containers were supplied with unfiltered running seawater (~1.5 L min^{-1}) and subjected to naturally fluctuating temperature and photoperiod (as described for adults). During the first experimental segment (days 1 to 18), containers were monitored every 2-4 days and larvae scored as: (1) buoyant (floating at the surface); (2) demersal, when larvae were on the bottom, but did not settle firmly; (3) settled, when they were firmly attached to the bottom or the sides of the container and could not be removed using a gentle jet of water. Survival rates, defined as the percent number of offspring remaining (in all categories) at a given time on the initial number of larvae were also recorded.

The second experimental segment (days 19 to 36) was performed to test the influence of larva size on behaviour upon encounter with an appropriate settlement substrate (coralline algae added on day 19). The proportion of larvae in different categories and survival rates were still recorded every 2-4 days. Categories "buoyant" and "demersal" remained the same as in the first experimental segment, but the category "settled" then included larvae settled on bare and natural substrata. The experiment was terminated on day 36 when almost no buoyant larvae were left.

Lipids in small and large *Urticina felina* larvae

Brooding adults (n = 3) of *Urticina felina* were collected at a depth of ~10 m off the Avalon Peninsula (Newfoundland, Canada) in July 2009, and maintained individually as described above to obtain sibling larvae for lipid analysis. Larvae were collected at the

surface of the water column within 24 h post release. Six samples of small and large larvae (12-15 larvae per sample) were collected from each brood (n = 3), measured and placed in 2 ml chloroform under nitrogen at -20°C for lipid analysis. In determination of lipid concentration (μg mm^{-3}), the mean volume of small larvae from the three brooding females varied from 0.23 to 0.38 mm^3, and that of large larvae varied from 0.44 to 0.98 mm^3.

Extraction and analysis of lipids were based on standard methods for aquatic samples (Parrish 1999). Total lipids were extracted with a mixture of chloroform and methanol 2:1 (v:v). Lipid classes were determined using thin layer chromatography with flame ionization detection (TLC/FID) with a MARK V Iatroscan (Iatron Laboratories, Tokyo, Japan). Lipids were separated in a three stage development system. The first separation consisted of 25-min and 20-min developments in 99:1:0.05 hexane: diethyl ether: formic acid. The second separation consisted of a 40-min development in 79:20:1 hexane: diethyl ether: formic acid. The last separation consisted of 15-min developments in 100% acetone followed by 10-min developments in 5:4:1 chloroform: methanol: chloroform-extracted-water. After each separation, the rods were scanned and the data were processed using the PeakSimple Chromatography software (V3.88, SRI Instruments, USA).

Size-related survival of juveniles in the presence of predators

The nudibranch *Aeolidia papillosa* is a specialized predator of a number of sea anemones (Hall and Todd 1986), including *Urticina felina* and *Aulactinia stella* (Greenwood et al. 2004). Preliminary experiments showed that *A. papillosa* could quickly

feed on small individuals of *U. felina* and *A. stella* (within 30 min of contact) and that small specimens of nudibranchs (subadults) that ingested juveniles of both sea anemone species were ready to feed again after ~24 h.

Large adult specimens of *A. papillosa* (n = 10, 3.8-19.3 g wet weight) were collected at a depth of ~10 m in December 2010 and January 2011 in Admirals Cove, Newfoundland, eastern Canada. Subadults of *A. papillosa* (n = 15, 0.02-0.6 g) were collected in May-August and in December 2010. Specimens of *A. papillosa* from the two categories were used to determine how efficient and selective they were in the presence of small and large juveniles of *U. felina* (15-mo old, Table 5-1).

The experimental trial consisted of one *A. papillosa* offered simultaneously one small and one large juvenile sea anemone as potential prey. The trials were performed in round containers (21 cm in diameter) kept individually in 20-L flow-through tanks, supplied with a gentle flow (~0.8 L min^{-1}) ensuring uniform exchange and current of water through four equally spaced 3-cm meshed holes (500 µm). Juveniles of *U. felina* were sorted and wet weighed (Table 5-1), then allowed to recuperate for 24 h before the experiment. Sixty-four trials (39 and 25 replicates for subadult and adult *A. papillosa*, respectively) were performed between December 2010 and January 2011. Three to 5 trials were run simultaneously, and new *U. felina* juveniles were used as prey in each trial. To make sure that the predators were hungry, the interval between each replicate run was a minimum of 3 days (as per preliminary results). At the onset of the trial, the predator was haphazardly introduced into the experimental container and left to acclimate for 1 h. Then, one small and one large juvenile sea anemone (sizes described above) were

introduced simultaneously and placed at equal distance and angle from the predator. Predation was monitored every 30 min until a positive response (i.e. predator feeding on a prey or prey totally eaten by the predator) was scored, or up to 7 h, after which time the experiment was considered null.

We also tested another species of sea anemone, *Aulactinia stella*, which is sympatric to *U. felina*. Adults of *A. stella* were collected at a depth of ~10 m off the Avalon Peninsula (Newfoundland, Canada) from March-June 2010, and in January 2011, and maintained under the laboratory conditions mentioned previously for *U. felina*. Juveniles of *A. stella* were collected after natural release events or extraction (Chapter 4), and divided into two size classes (Table 5-1). Forty-seven trials (28 and 19 replicates for subadult and adult *A. papillosa*, respectively) were performed between May and August 2010, and between December 2010 and January 2011, as the different life stages of *A. papillosa* were available solely in specific months of the year. Experimental procedures were identical to the ones outlined above for *U. felina*.

Data analysis

Nested analyses of variance (nested ANOVAs, parent as nested factor) were used to compare different variables in the performance of small and large sibling larvae of *Urticina felina* from different brooding females in two successive experimental segments. Relationships between mean larva size in a group and survival rates at the end of the two experimental segments were determined using Spearman's rank order correlation. Comparisons of different variables between small and large *U. felina* larvae at the population level (irrespective of parentage) in the settlement trials were made with *t*-tests.

Where assumptions of normality and equal variance failed, Mann-Whitney rank sum tests were used.

Pearson's correlation was used to test the relationship between mean larva size and lipid content per larva ($\mu g\ ind^{-1}$). Nested ANOVAs (parent as nested factor) were used to compare the proportions and the amount ($\mu g\ ind^{-1}$) and concentration ($\mu g\ mm^{-3}$) of major lipid classes in small and large larvae of *Urticina felina* from the same brood. The significance level for all tests was set at $p < 0.05$. Data are expressed as mean ± SE.

Results

Behaviour, time to settlement and survival of *Urticina felina* larvae

While the size range of larvae differed among the four brooding females (i.e. the smaller larvae of some females were similar in size to the larger larvae of other females), comparable behavioural distinctions between large and small siblings occurred in all of the broods in the two experimental segments (Fig. 5-1).

The mean survival rates among smaller larvae of a brood were significantly lower than among larger siblings at day 18 (73.0 ± 3.1% vs 90.6 ± 1.5%; $F_{4, 23} = 6.91$, $p = 0.002$) and at day 36 (57.3 ± 4.9% vs 80.7 ± 2.3%; $F_{4, 23} = 24.00$, $p < 0.001$, Fig. 5-1). The time required for the proportion of buoyant larvae to drop < 50% was 11.5 ± 1.7 days in small larvae of a brood, and 18.5 ± 3.5 days in large ones. The proportion of buoyant larvae was significantly lower in the smaller larvae of a brood than in their larger siblings ($F_{4, 23} = 22.89$, $p < 0.001$, Fig. 5-1) at day 18. However, at day 36 (18 days following the addition of the natural substratum), no significant differences occurred in the proportions

of buoyant larvae between small and large siblings ($F_{4, 23} = 2.20$, $p = 0.115$, Fig. 5-1). The inverse trend occurred in the proportion of settlers: no significant differences occurred at day 18 ($F_{4, 23} = 2.87$, $p = 0.057$, Fig. 5-1), whereas at day 36 the mean proportion of settlers (on all substrata) was lower among smaller larvae of a brood than larger siblings ($F_{4, 23} = 14.01$, $p < 0.001$). No significant differences were detected in the mean proportion of demersal larvae between small and large siblings at day 18 ($F_{4, 23} = 2.33$, $p = 0.101$) or day 36 ($F_{4, 23} = 0.81$, $p = 0.535$).

To examine the influence of larval size on settlement at the population level (irrespective of parentage), all trials of larvae measuring 0.59-1.14 mm^2 were pooled (small size class), and trials with larvae between 1.42 and 2.61 mm^2 were pooled (large size class). Following this procedure, the mean size of small larvae was 0.84 ± 0.01 mm^2 which represented 44.9% of the mean size of large larvae (1.87 ± 0.02 mm^2). The mean survival rates did not vary significantly between the two size classes at day 18 ($78.5 \pm 3.9\%$ vs $85.0 \pm 2.9\%$; $t = -1.32$, $df = 22$, $p = 0.201$) or day 36 ($63.7 \pm 6.1\%$ vs $74.3 \pm 3.6\%$; $t = 1.49$, $df = 22$, $p = 0.150$; Fig. 5-2). In addition, mean survival rate at day 18 was not correlated with mean larval size (Fig. 5-3, $r_s = 0.38$, $n = 24$, $p = 0.070$), however, it was at day 36 (Fig. 5-3, $r_s = 0.41$, $n = 24$, $p = 0.044$). It is worth mentioning that survival rate after 36 days was $33.2 \pm 2.0\%$ when mean larval size in a group was < 0.7 mm^2, compared to $74.1 \pm 2.6\%$ when mean size was 1.48 ± 0.1 mm^2 (Fig. 5-3). The proportion of buoyant larvae was significantly lower in the small size class than in the large size class both at day 18 ($21.1 \pm 2.5\%$ vs $55.1 \pm 6.0\%$; $U = 9.00$, n (small) = 12, n (large) = 12, $p < 0.001$) and day 36 ($0.9 \pm 0.5\%$ vs $5.0 \pm 1.0\%$; $U = 23.00$, n (small) = 12,

n (large) = 12, $p < 0.003$, Fig. 5-2). More larvae had settled at day 18 in the small than in the large size class ($t = 3.01$, df = 22, $p = 0.006$). However, the overall proportion of settled larvae was not significantly different between small (52.9 ± 5.5%) and large (62.9 ± 3.2%) larvae at day 36 ($t = -1.63$, df = 22, $p = 0.117$) at the population level. The proportion of demersal larvae in the small size class was higher than in the large size class at day 18 ($t = 4.10$, df = 22, $p < 0.001$), but was not significantly different at the end of the second experimental period on day 36 (U = 45.00, *n* (small) = 12, *n* (large) = 12, $p = 0.125$).

Lipid composition of *Urticina felina* larvae

Small and large larvae of *Urticina felina* were both composed of hydrocarbons (HC), wax and steryl esters (WE/SE), triacylglycerols (TG), free fatty acids (FFA), sterols (ST), acetone mobile polar lipids (AMPL) and phospholipids (PL). At the population level, irrespective of parentage, total lipid content (µg ind^{-1}) was positively related to average larval size (n = 6, r = 0.84, $p = 0.035$, Fig. 5-4A). In contrast, lipid concentration (µg mm^{-3}) was not related to average larval size (n = 6, r = -0.57, $p = 0.237$, Fig. 5-4B).

Similarly, at the population level, total lipid content (µg ind^{-1}) was significantly lower in small than in large larvae (U = 0.00, *n* (small) = 9, *n* (large) = 9, $p < 0.001$), whereas lipid concentration (µg mm^{-3}) was not (U = 40.00, *n* (small) = 9, *n* (large) = 9, $p = 1.000$). The amounts of most major lipid classes (µg ind^{-1}) were significantly lower in small than large larvae (Table 5-2), except HC ($t = 0.62$, df = 16, $p = 0.546$). The proportions of all major lipid classes (> 1% of total lipids) were similar in both small and

large larvae (Chapter 3). WE/SE was the most common lipid in both size classes, which comprised 53.5 ± 4.9% of total lipids in small and 58.6 ± 5.1% in large larvae.

A closer within-brood examination showed that total lipid content was significantly lower in small than in large sibling larvae of a brood ($F_{3, 17} = 15.99$, $p < 0.001$), due to the significantly lower amounts of WE/SE ($F_{3, 17} = 7.10$, $p = 0.005$) and PL ($F_{3, 17} = 3.78$, $p = 0.041$) in small siblings. The amounts of the remaining major lipid classes, including HC, FFA, ST and AMPL, were similar in all larvae inside a brood. The proportions of major lipid classes were similar in both small and large siblings, except for the proportion of HC, which was significantly higher in large larvae of a brood ($F_{3, 17} = 4.08$, $p = 0.033$).

Predation on juvenile sea anemones of different sizes

Juvenile *U. felina* of all sizes were more susceptible to predation by subadults than by adults of *Aeolidia papillosa* (Table 5-1). None of the adult nudibranchs fed on juvenile *U. felina* within the experimental period, whereas 73.8% of subadult nudibranchs did (Table 5-1). Among the latter, more fed on the larger prey offered. Specifically, 25.6% of subadult nudibranchs consumed the smaller *U. felina* juvenile, whereas 48.2% consumed the larger juvenile. The average time before feeding by subadult nudibranchs was 3.2 ± 0.5 h on small *U. felina* juveniles, and 4.2 ± 0.5 h on large juveniles, with no significant difference ($U = 62.50$, n (small) = 10, n (large) = 19, $p = 0.139$).

In contrast to *U. felina*, *A. stella* juveniles were more severely preyed upon by adults than by subadults of *Aeolidia papillosa* (Table 5-1). All adult nudibranchs tested (100%) fed within the experimental period, compared to only 64.3% of subadult

nudibranchs (Table 5-1). Small *A. stella* juveniles were more susceptible than large ones when facing the predation of subadult nudibranchs. More precisely, 39.3% of subadult nudibranchs fed on small juveniles *A. stella* with a mean time before feeding of 3.1 ± 0.8 h, whereas only 25.0% fed on larger juveniles with a similar mean time before feeding of 3.5 ± 0.4 h (t = 0.51, df= 16, p = 0.615).

On the other hand, larger *A. stella* juveniles were more susceptible than small ones to predation by adult nudibranchs. Specifically, 84.2% of adult nudibranchs fed on large *A. stella* juveniles with a mean time before feeding of 2.0 ± 0.2 h, whereas only 15.8% fed on small *A. stella* juveniles with a similar time before feeding of 1.8 ± 0.6 h (t = 0.27, df= 17, p = 0.792).

Discussion

The present work provides new experimental results (Table 5-3) in support of the assumption that offspring size influences pre-metamorphic as well as post-metamorphic performance, but following slightly different schemes than previously shown in benthic marine organisms (Marshall and Keough 2003, Allen et al. 2008, Jacobs and Sherrard 2010). In the sea anemone *Urticina felina*, smaller larvae of a brood had lower survival than larger siblings and exhibited an inverse trend in the proportion of buoyant larvae and settlers, suggesting that smaller larvae settled more rapidly under sub-optimal conditions, as per the desperate larva hypothesis (Elkin and Marshall 2007). In contrast, the settlement of larger siblings was apparently driven by the presence of optimal substratum. A lipid analysis indicated that differences in survival and time before settlement in small

and large sibling larvae may be due to the greater lipid content of the latter. The most abundant lipid class in all larvae was wax/steryl ester, which presumably provides larger larvae with more energy, enabling them to stay buoyant longer in the water column and to delay settlement until optimal conditions are encountered. The differences in survival and time before settlement at the intra-brood and population levels indicate that the relationship between larval size and performance is mediated by parentage. Inverse trends were evidenced when examining post-metamorphic competence in the form of susceptibility to predation by nudibranchs in juveniles of *U. felina* (< 12 mg) and those of a co-occurring sea anemone, *Aulactinia stella* (to 200 mg). Large juveniles of *U. felina* were more susceptible than small ones and were mostly preyed upon by subadult predators. On the other hand, in *A. stella* smaller juveniles were more vulnerable to subadult nudibranchs, whereas larger juveniles were more vulnerable to adult nudibranchs. Thus, the present study shows that the relationship between offspring size and performance can vary ontogenetically and among species.

Offspring size and performance in pre-metamorphic stages

Survival enhanced by larger offspring size has been reported in colonial invertebrates, e.g. bryozoans and ascidians (Marshall and Keough 2003, 2005) and corals (Isomura and Nishihira 2001). However, the relationship between offspring size and survival was suggested to vary with time, i.e. the effects only persisting for a short period of time (Marshall and Keough 2005). For example, colonies of the ascidian *Diplosoma listerianum* that developed from larger larvae had larger feeding structures and higher survival than those developed from smaller larvae after 2 weeks, but not after 3 weeks in

the field (Marshall and Keough 2005). Here, larger larvae of *U. felina* exhibited better survival than their smaller siblings, contrary to results in colonial ascidians (Marshall and Keough 2005). When mean larval size in a group was < 0.7 mm^2, survival rates were always lower than 50%. *Urticina felina* larvae > 0.6 mm^2 (coined mega-larvae) were shown to be formed by fusion of sibling embryos (Chapter 3). Greater survival rates in larger mega-larvae supports the adaptive role of fusion in creating longer-lived and more dispersive larvae in this species. However, it is worth mentioning that survival rates were similar in large and small size classes at the population level (irrespective of parentage), which suggests that parental effects are acting on the offspring size-performance relationship and stresses the importance of conducting future studies at the within-brood level.

Behavioural differences during settlement have been reported in many benthic marine organisms (reviewed by Raimondi and Keough 1990). The latter authors suggested that larval behaviour variability may be caused by "genetic variation among larvae, ontogenetic changes in behaviours, parental environmental effects, modification of response by other environmental cues, or the overriding of behavioural responses by physical process". However, the relative contribution of genetic and environmental factors to larval behaviour variability and the detailed mechanisms underlying this variability are still largely unknown. In the present study, larval size in *U. felina* not only significantly influenced the final results but also the dynamics of settlement. For example, proportions of buoyant larvae were lower in smaller than in larger siblings of a brood under sub-optimal settlement conditions before the addition of the natural substratum.

However, those proportions were not significantly different between the two size classes at the end of the experimental period (36 days). Similarly, the proportion of settled larvae at the population level was significantly higher in the smaller size class under sub-optimal settlement conditions, whereas the overall proportion of settled larvae was not significantly different at the end of the experimental period. These changes suggest that smaller individuals need to settle more rapidly, but that the ultimate settlement rates remain similar in both size classes.

The influence of offspring size on settlement behaviour (desperate larva theory) has been reported in colonial marine invertebrates (Marshall and Keough 2003, Elkin and Marshall 2007). For example, larger larvae of the bryozoan *Bugula neritina* had a more variable swimming period before settlement compared to smaller ones (Marshall and Keough 2003). Although small and large larvae were capable of settling, smaller larvae of *B. neritina* settled sooner than larger larvae, regardless of settlement surface (Marshall and Keough 2003). Similarly, a field study showed that the size of settlers in the bryozoan *Watersipora subtorquata* was larger on rough surfaces, compared to smooth plates, which suggested that smaller larvae were less selective for habitat (Marshall and Keough 2003). Based on our study, it is likely that the effects of larva size on swimming time could be levelled in the presence of a strong settlement inducer (optimal conditions) from the onset. However, the size-related variability in settlement behaviours among sibling larvae of *U. felina* may serve as a dispersal strategy, i.e. to maintain recruitment of some offspring (smaller in size) closer to the parental habitat (philopatry), while allowing the larger ones to disperse more widely, particularly when incentives for

settlement are weaker (e.g. sub-optimal environment, competition, predation). In brooding species that release fully formed larvae within a short time, such as *U. felina*, this strategy may have evolved to decrease the intrinsic effects of competition among sibling settlers. Offspring size variation as a strategy to decrease intra-species competition has been reported in other marine invertebrates. For instance, Marshall and Bolton (2007) found that larger egg size corresponded to longer planktonic period in three lecithotrophic species, the ascidians *Phallusia obesa* and *Ciona intestinalis* and the echinoid *Heliocidaris erythrogramma*, and suggested that offspring from large eggs would disperse further than those from small eggs, and that spreading of offspring may decrease intra-specific competition.

Larval size and lipid composition in *Urticina felina*

Offspring size, especially egg size, has been suggested to reflect parental investment per offspring and to be an indication of organic content in marine invertebrates (Jaeckle 1995). It has been shown that larval settlement behaviour and dispersal patterns might be determined via lipid content, composition and allocation (Harii et al. 2007), and that marine invertebrates with non-feeding larvae may mediate dispersal potential of their offspring by manipulating larval size, because small larvae tend to become less discriminating in their choice of settlement substrata as their energetic reserves run out (Marshall and Keough 2003).

Although larval size in *U. felina* does not reflect initial egg provisioning due to fusion among siblings (Mercier et al. 2011, Sun et al. pending revision), the total lipid content per larva ($\mu g\ ind^{-1}$) followed the predicted increase with size. Further

examination showed that the significantly lower lipid content in small than in large larvae within a brood was due to lower amounts of wax esters (WE) and phospholipids (PL). WE/SE was the most abundant lipid class in both small and large larvae of *U. felina*. WE are the major lipids considered to govern buoyancy and act as energy reserves in marine organisms (Lewis 1970, Nevenzel 1970), hence changes in the proportion of WE could influence the position of larvae in the water column, and control their dispersal. For example, Harii et al. (2007) found that the WE content changed significantly over time in the larvae of the hermatypic coral *Acropora tenuis*, and suggested that WE might be an energy source for metamorphosis and settlement. Thus, we propose that the lower amount of total lipids and especially WE/SE in small larvae of *U. felina* explains why they stay buoyant for a shorter period than larger siblings under non-optimal settlement conditions. It is worth mentioning that the total lipid content (μg ind^{-1}) in large larvae was solely due to scaling, since lipid concentration (μg mm^{-3}) was similar in all larvae. Studies on size-specific energy consumption are needed to confirm whether larger larvae have proportionally more energy reserves than smaller ones.

Offspring size and performance (as susceptibility to predation)

Offspring size has been suggested to influence resistance to predation (Rivest 1983, Barbeau and Scheibling 1994). Smaller hatchlings of the neogastropd *Searlesia dira* were preferentially selected by smaller hermit crab predators with left cheliped length < 6.0 mm; whereas larger crabs did not show any feeding preferences related to prey size (Rivest 1983). The role of body size in predator-prey interactions has been shown in marine invertebrates, fishes and insects (Juanes 1992, Lundvall et al. 1999,

Berger et al. 2006). For invertebrate predators, prey vulnerability was predicted to initially increase with size to a maximum and decrease thereafter. This dome-shaped function has been suggested to be a combined effect of the predator's ability to detect small prey and its ability to capture large prey (Christensen 1996, Lundvall et al. 1999). Feeding preferences of a predator of a given size is possibly decided by the combination of the energy intake efficiency (Stephens and Krebs 1986) and the cost of predation (Stephens and Krebs 1986, Juanes 1992). Smaller predators preferentially feeding on smaller prey have been reported in many marine invertebrates (Juanes 1992, Barbeau and Scheibling 1994).

In the present study, *U. felina* juveniles, irrespective of their size, were more vulnerable to subadults of the nudibranch *Aeolidia papillosa*, as no adult nudibranchs fed on them. This is likely because large adult nudibranchs are less inclined to spend energy preying on such small prey as *U. felina* juveniles (< 12 mg). Further support for this assumption is provided by the fact that large juveniles of *U. felina* were more frequently consumed by subadult nudibranchs than small ones. A completely different scenario was observed in interactions between nudibranchs and much larger prey, i.e. juveniles of the sea anemone *Aulactinia stella* (to 200 mg). Larger juveniles of *A. stella* suffered higher predation rates when exposed to adult nudibranchs than small ones. Subadult nudibranchs were less inclined to feed on *A. stella* juveniles than adult nudibranchs. Under the predation of subadult nudibranchs, small *A. stella* juveniles were consumed more frequently than large ones. The nudibranch *A. papillosa* uses mucus to counteract its prey's nematocysts (Greenwood et al. 2004), although it may still risk injury or death

when the prey is large enough (Conklin and Mariscal 1977). Thus, the different feeding preference of nudibranchs on the *A. stella* juveniles of various sizes is possibly related to the higher risk of injury from the prey's nematocysts for small subadult nudibranchs than for the adults. In summary, the interaction between juvenile sea anemones and their specialized predator seems driven both by the size of the prey and the size of the predator. To date more studies have focused on the influences of competition (conspecific densities; Allen et al. 2008) than predation as a biotic influence on post-metamorphic performance.

Taken together, our results indicate that the relationship between offspring size and performance is a difficult one to assess, being dependent on a complex suite of environmental and biotic factors encountered at different life stages, e.g. the availability of optimal substratum during settlement and the level and type of predation at the juvenile stage. Thus, the general assumption that larger offspring perform better does not hold true in the present study. Challenges to this common assumption have also be reported in vertebrates (Dibattista et al. 2007, Warner and Shine 2007, Maddox and Weatherhead 2008). Thus, the importance of offspring size may be overestimated relative to other traits in defining life-history strategies, and future studies on the effects of offspring size on their performance should give more consideration to ontogeny and the different influential factors.

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Tables

Table 5-1. Predation rates (%) and time (h) before feeding (mean ± SE) on juveniles of various sizes by the nudibranch *Aeolidia papillosa* within a 7-h experimental period. Data are shown for juveniles of two brooding sea anemones, *Urticina felina* and *Aulactinia stella*

Predator *Aeolidia papillosa*	*Urticina felina* juveniles					*Aulactinia stella* juveniles				
	Small (2.2 ± 0.1 mg)		Large (12.0 ± 1.0 mg)		No predation	Small (6.4 ± 0.3 mg)		Large (78.5 ± 3.0 mg)		No predation
	Proportion (%)	Time (h)	Proportion (%)	Time (h)	Proportion (%)	Proportion (%)	Time (h)	Proportion (%)	Time (h)	Proportion (%)
Subadults	25.6	3.2 ± 0.5	48.2	4.2 ± 0.5	26.2	39.3	3.1 ± 0.8	25.0	3.5 ± 0.4	35.7
Adults	0	--	0	--	100	15.8	1.8 ± 0.6	84.2	2.0 ± 0.2	0

Table 5-2. Mean lipid content (µg ind^{-1}) of major lipid classes (> 1% of total lipids) in small and large larvae of the sea anemone *Urticina felina.* Data are expressed as mean ± SE ($n = 9$). Values with different superscript letters are significantly different (t-tests, $p < 0.05$)

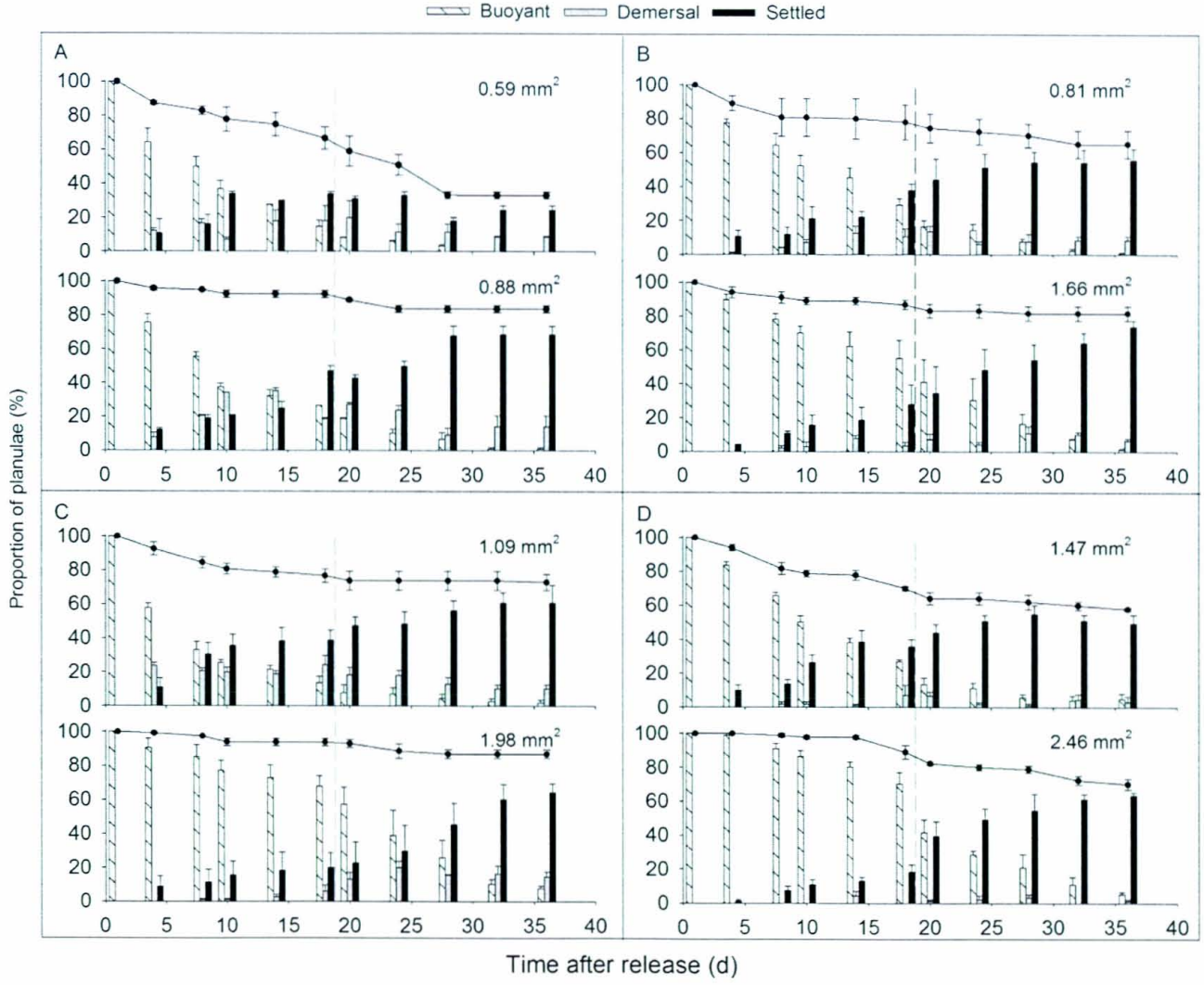
Lipids	Small larvae (127 larvae)	Large larvae (128 larvae)
Hydrocarbons (HC)	2.65 ± 0.56^{a}	3.03 ± 0.28^{a}
Wax and Steryl Esters (WE/SE)	37.32 ± 3.95^{a}	67.59 ± 7.85^{b}
Free Fatty Acids (FFA)	0.42 ± 0.12^{a}	2.82 ± 0.91^{b}
Sterols (ST)	1.53 ± 0.18^{a}	3.99 ± 0.87^{b}
Acetone Mobile Polar Lipids (AMPL)	4.22 ± 1.75^{a}	14.66 ± 4.36^{b}
Phospholipids (PL)	14.86 ± 3.45^{a}	34.07 ± 8.62^{b}

Table 5-3. Summary of size-based performance of offspring at different ontogenetic stages in brooding sea anemones

Species and life stage	Performance indicator	Small offspring	Large offspring
Urticina felina			
Larvae and new settlers, 18 d post release	Survival rate		
	within brood	Lower	Higher
	across broods	Similar	Similar
Larvae and settlers, 36 d post release	Survival rate		
	within brood	Lower	Higher
	across broods	Similar	Similar
Larvae, 18 d post release	Proportion of buoyant larvae		
	within brood	Lower	Higher
	across broods	Lower	Higher
Larvae, 36 d post release	Proportion of buoyant larvae		
	within brood	Similar	Similar
	across broods	Lower	Higher
Settlers, 18 d post release	Settlement rate (sub-optimal conditions)		
	within brood	Similar	Similar
	across broods	Higher	Lower
Settlers, 36 d post release	Settlement rate (optimal substratum on day 19)		
	within brood	Lower	Higher
	across broods	Similar	Similar
Juveniles, 15-16 months	Predation by *A. papillosa*		
	adults	Lower	Higher
	subadults	Similar	Similar
Aulactinia stella			
Juveniles, 0-8 months	Predation by *A. papillosa*		
	adults	Higher	Lower
	subadults	Lower	Higher

Figures

Fig. 5-1 (next page). *Urticina felina.* Behaviour (bars) and survival (line and associated data points) of small (upper panel) and large (lower panel) larvae collected from four brooding females (a-d). Mean larval size for each group is indicated in the top right corner of the graphs. Dashed lines indicate the introduction of the natural substratum on day 19. Data are expressed as mean ± SE (n = 3).


Buoyant
Demersal
Settled
A
0.59 mm²
0.88 mm²
B
0.81 mm²
1.66 mm²
C
1.09 mm²
1.98 mm²
D
1.47 mm²
2.46 mm²
Proportion of planulae (%)
Time after release (d)

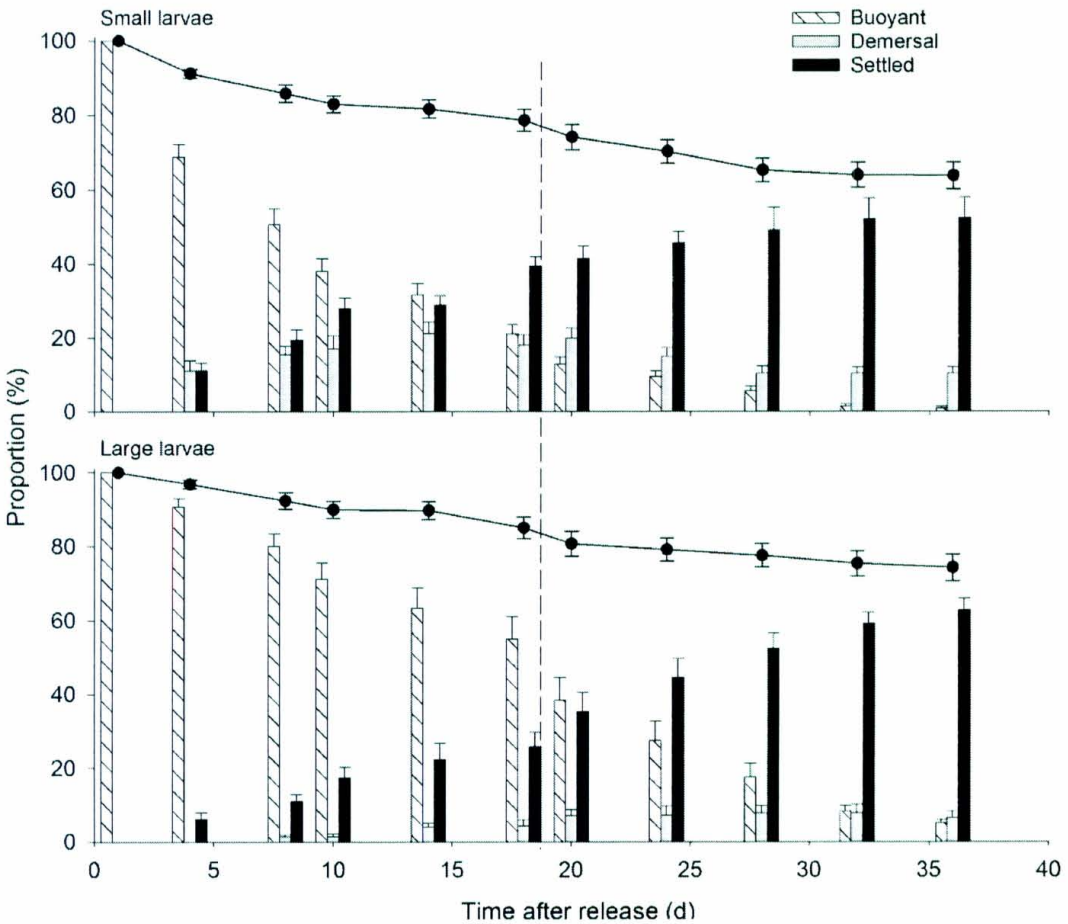


Fig. 5-2. *Urticina felina.* Proportions of buoyant, demersal and settled larvae (bars) over time and corresponding survival rates (line) in small (upper panel) and large (lower panel) larvae at the population level. Data were pooled across broods on the basis of mean size (0.84 *vs* 1.87 mm^2), and expressed as mean ± SE (n = 12, three replicates in each of four mothers). Dashed lines indicate the introduction of the natural substratum on day 19.

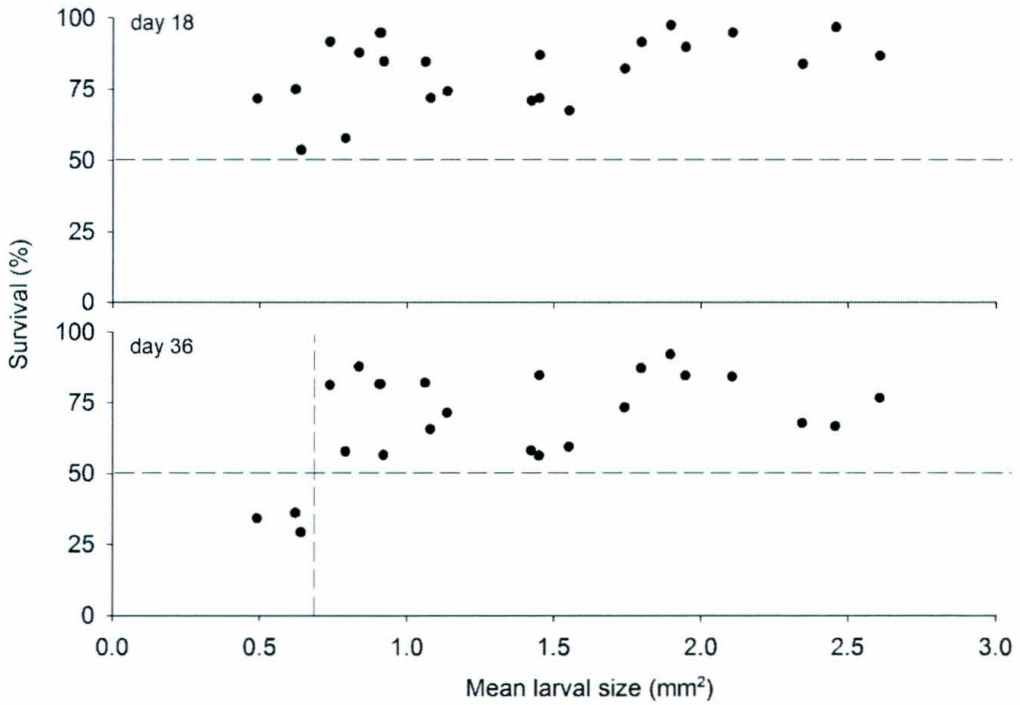


Fig. 5-3. *Urticina felina.* Relationship between mean larval size (n = 24 from four mothers) and survival at the end of the two experimental segments (upper panel at day 18, and lower panel at day 36). Horizontal dashed line indicates 50% survival rate, and vertical dash line indicates 0.7 mm^2 larval size.

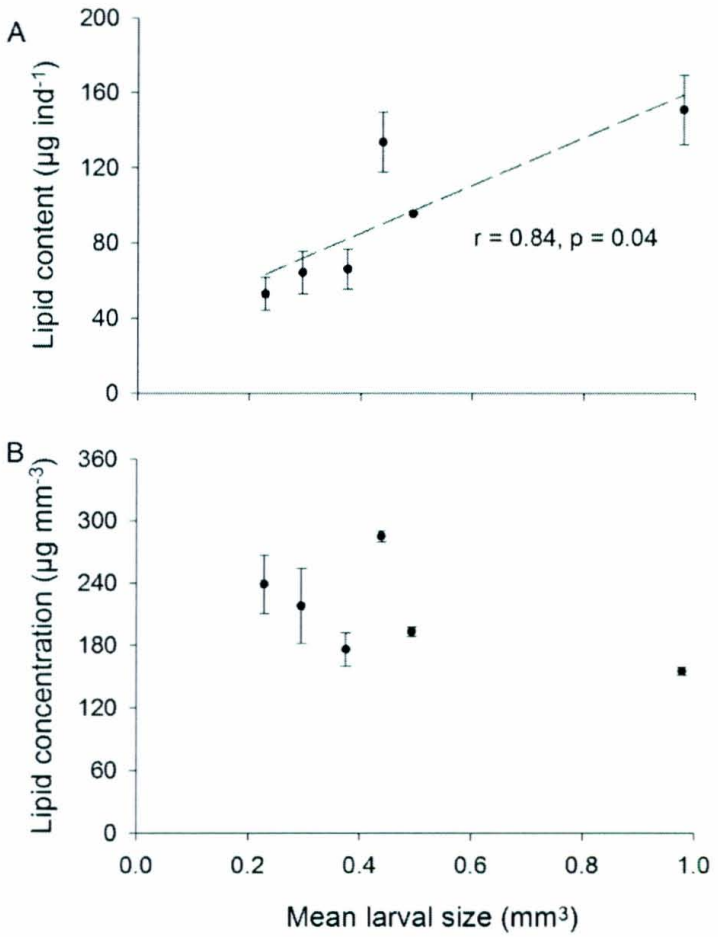


Fig. 5-4. *Urticina felina.* (a) Significant linear relationship between total lipid content and mean larval size. (b) Absence of relationship between lipid concentration (µg mm^{-3}) and mean larval size. Data are expressed as mean ± SE (n = 3).

CHAPTER 6 : **General conclusions**

Offspring size variation is of fundamental ecological and evolutionary importance (Smith & Fretwell 1974b, Bernardo 1996). It has been shown to be a dynamic and adaptive characteristic in marine invertebrates (Allen et al. 2008). Inter-specific size variation is interesting especially when comparing species with different development modes, and when comparing closely related species in which the mechanisms underpinning offspring size variation differ.

In reviewing the literature on this topic, I found that studies of the relationship between offspring size variation and development modes lacked a standardized and accurate classification of offspring types and have endeavoured to propose one (Chapter 2). Only by using clear hierarchical terminology can we separately test whether developmental habitat (benthic, pelagic, both), nutrition (feeding, non-feeding), parental care (free, protected, both) and morphogenesis (simplified, complex) have an influence on offspring size variation and interpret those results appropriately. In addition, I discovered that because the coefficient of variation of offspring size (CV) is influenced by mean offspring size, it is important to use proper statistical analysis to compare variability. ANCOVA on lgSD with lgMean as covariate was identified as the most suitable for comparison of offspring size variation, especially at the inter-specific level (Chapter 2). My review further emphasized that the few existing studies have mainly focus on benthic *colonial* brooding marine invertebrates (ascidians and bryozoans) and a few planktonic *unitary* (non-colonial) brooders (crustaceans), but data were generally lacking for benthic *unitary* brooders. Thus, more studies on unitary species that brood to

larvae or juveniles are needed, with complementary comparative work on colonial brooding species and unitary broadcast-spawning species (Chapter 2).

While inter-specific offspring size variation is impressive, intra-specific size variation is equally important for understanding the mechanisms that cause the variation as well as their influence on performance in every life-history stage. Size variation has primarily been studied separately in eggs, larvae or juveniles after their release into the environment. However, there are very few integrative studies taking into account the significance of offspring size at the successive life history stages (eggs, embryos, larvae, juveniles) within a species (Ito 1997). What happens before the offspring are released is generally overlooked, i.e. at which life stage is size variation initiated (i.e. oocytes, fertilized eggs, embryos, larvae or juveniles) and whether mean variance increases or decreases throughout development.

Offspring size variation in species with post-zygotic parental care, especially internally brooding species, display a more complex scheme than broadcast-spawning species, due to a close prolonged relationship between parent and offspring conducive to the development of co-operation and conflicts. Internally-brooding species exhibit strategies that may increase offspring size significantly during the period of parental care, therefore occurrences of offspring size variation should be investigated more thoroughly in viviparous taxa before formulating general theories. For example, the embryos of the internally-brooding sea anemone *Urticina felina* are able to fuse and form mega-larvae, causing a significant increment in size variation from the larval stage onward (Chapter 3). Occurrences of mega-larvae increased with maternal fecundity and were high in the

populations studied, suggesting that fusion among siblings can be viewed as an extreme case of kin cooperation integral to the reproductive strategy of *U. felina*. Another internally-brooding species with a strategy to increase offspring size is the sea anemone *Aulactinia stella* (Chapter 4). Adults of *A. stella* brood juveniles freely inside the gastrovascular cavity for a long period (to > 1 year), and are able to release juveniles at any time of the year, with a peak between July and October. The long non-fixed brooding period, the co-existence of different cohorts of juveniles and intra-brood competition likely mediate offspring size variations in *A. stella* (Chapter 4). There appears to be a trade-off to balance the conflict between juveniles and brooding adults. For example, the long brooding process increases adult fitness through increased offspring survival (by providing food and protection), however, it can also decrease adult fitness due to the intensified competition for food that develops among brooded siblings and with the adult. Clearly, it is important to investigate the mechanisms underlying offspring size variation carefully, especially for species with post-zygotic parental care, before formulating general theories. Differences among the various reproductive strategies should be examined more explicitly.

Offspring size plays an important role in performance at pre-metamorphic and post-metamorphic stages (Marshall et al. 2006, Phillips 2006, Allen et al. 2008, Chapter 5). Current studies of size-related offspring performance in marine invertebrates have almost exclusively focused on a single life stage (especially the post-metamorphic stage), whereas very little empirical data exist on size-related fitness across multiple life-history stages (Rius et al. 2009). For marine invertebrates species with a complex life cycle,

research has shown that the effects of offspring size on performance could change throughout ontogeny (Rius et al. 2009). The study outlined in Chapter 5 evidenced increased performance of larger larvae of the sea anemones *U. felina* at pre-metamorphic stages. Larger larvae displayed better dispersive abilities (i.e. were able to remain longer in the water column and were primarily driven to settle by the presence of an optimal substratum) and had higher survival rates at day 36 post release. On the other hand, the offspring size-performance relation at the post-metamorphic stages appears to be context-depend and strongly affected by external factors, i.e. predation pressure (Chapter 5), food availability (Sman et al. 2009) and competition (Marshall et al. 2006). For instance, the size-related post-metamorphic performance of sea anemones *U. felina* facing the specialized predator nudibranch *Aeolidia papillosa* depended on the sizes of both prey and predator (Chapter 5). Hence, the relationship between offspring size and performance depends on the complex suite of environmental and biotic factors encountered at different life stages, with the size advantage chiefly operating at the pre-metamorphic stage, and more complex interactions between offspring size and external factors occurring at the post-metamorphic stage. To date more studies have focused on the influences of competition (conspecific densities; Allen et al. 2008) than predation as a biotic factor on the post-metamorphic performance. Thus, to gain a better understanding of the size-related offspring fitness, integrative experiments under various environmental and biotic conditions are needed to study the size-performance relationship across multiple life-history stages, including pre-metamorphic stages, juvenile stages and adulthood.

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