The pelagic propagule's toolkit: An exploration of the morphology, swimming capacity and behaviour of marine invertebrate propagules

by

© Emaline M. Montgomery

A Dissertation submitted to the School of Graduate Studies in partial fulfillment of the requirements for the degree of

Doctor of Philosophy in Marine Biology,

Department of Ocean Sciences, Faculty of Science,

Memorial University of Newfoundland

June 2017

St. John's, Newfoundland and Labrador

Abstract

The pelagic propagules of benthic marine animals often exhibit behavioural responses to biotic and abiotic cues. These behaviours have implications for understanding the ecological trade-offs among complex developmental strategies in the marine environment, and have practical implications for population management and aquaculture. But the lack of life stage-specific data leaves critical questions unanswered, including: (1) Why are pelagic propagules so diverse in size, colour, and development mode; and (2) do certain combinations of traits yield propagules that are better adapted to survive in the plankton and under certain environments? My PhD research explores these questions by examining the variation in echinoderm propagule morphology, locomotion and behaviour during ontogeny, and in response to abiotic cues. Firstly, I examined how egg colour patterns of lecithotrophic echinoderms correlated with behavioural, morphological, geographic and phylogenetic variables. Overall, I found that eggs that developed externally (pelagic and externally-brooded eggs) had bright colours, compared to the typically pale colour intensity of internally-brooded eggs. Additionally, my analysis suggested geographic location as a potential driver of the evolution of colour diversity through the selection of better-adapted pigments in response to ecological pressure. I then undertook a critical assessment of swimming capacity and sensory ability in propagules from four co-occurring North Atlantic echinoderms with two different types of pelagic development: the sea stars *Asterias rubens* (planktotrophic) and *Crossaster papposus* (lecithottrophic), the sea urchin *Strongylocentrotus droebachiensis* (planktotrophic), and

the sea cucumber *Cucumaria frondosa* (lecithotrophic) at two different temperatures. Propagule swimming speed increased with ontogeny in two of the four species (the sea stars *A. rubens* and *C. papposus*) but did not uniformly increase with temperature. Contrary to initial assumptions, some lecithotrophic propagules emerged as the fastest swimmers (e.g., 1.2 mm s^{-1} in the brachiolaria of *C. papposus*). Lastly, in a study of phototaxis involving the same focal species, variation in swimming speed and trajectory were detected when propagules were exposed to three different light colours. Taken together, the data generated by my PhD work provide a framework to assess the adaptive value of pelagic propagules to benthic animals, to examine the trade-offs of complex life-history strategies, and to enhance modeling of larval dispersal in the marine environment.

Acknowledgements

Firstly, I would like to express my sincerest appreciation to my supervisor, Annie Mercier for the tireless dedication and mentorship she provided during this PhD. I have learned much from our work together, and her attention to detail and success in the field continues to be an inspiration.

My sincere thanks also goes to my collaborator, Jean-Francois Hamel, for his motivation and passion for natural history. I will always remember to tell a story through my publications, not just a list of numbers.

I would also like to thank my committee members: Kurt Gamperl, Garth Fletcher and Don Deibel, for their insightful comments and encouragement, but also for their questions that helped me tackle my research from different perspectives.

I would also like to acknowledge the support and encouragement of Chris Parrish, who provided guidance and a sounding board like I was one of his own students.

Many thanks to Don Stansbury (DFO) and the crew of the CCGS *Teleost* for the opportunity to study the deep sea on a research cruise, and to Memorial field services and the Ocean Sciences Centre's workshop for their invaluable assistance with animal collections and experimental logistics.

I thank my fellow labmates, past and present, for all the late nights in the lab and all the fun of the last four years. Special thanks to Katie Verkaik, Camilla Parzanini and Justine Ammendolia for their help with raising thousands of baby echinoderms and staring at dots in a dish.

Finally, I would like to thank my parents for their encouragement and advice (from one island to another), and my good friends Nathan Gentry and Tyler Brown for always being there when I needed it most.

Table of Contents

Lists of Tables

List of Figures

List of Appendices

Co-Authorship Statements

Thesis Chapter	Type of Work	Journal	Status
3	Meta-analysis	Advances in Marine Biology	Published January 2017
4	Experimental	Marine Biology	Published March 2017
5	Experimental	Journal of Experimental Marine Biology and Ecology	Submited April 2017
2+6	Review	TBD	Submission planned for Spring 2017

Expected order of authorship (all manuscripts)

Montgomery, Emaline M., Hamel, Jean-Francois, Mercier, Annie

Author responsibilities (all manuscripts)

E. M. and A. M. designed the projects. E. M. performed the data collection, analysis, wrote the manuscripts and made figures and tables. J.-F. H. contributed original data (Chapter 3 only). A.M. and J.-F. H. provided comments on the manuscripts and contributed ideas for interpretation and significance of the data.

Chapter 1. General Introduction

1.1. Life-History Strategies

The diversity and origins of animal life histories have fascinated biologists for centuries. Aptly described as a schedule of reproduction and survival (Brommer 2000), life-history traits and strategies are directly linked to the growth, fitness and ecological roles of animals (Kalinka and Tomancak 2012, Marshall and Morgan 2011, McEdward 2000). Most animal taxa have a so-called biphasic life history, including marine (Bishop and Brandhorst 2003, Pechenik 1999) and terrestrial invertebrates (Rieger 1994) and amphibians (Rieger 1994, Voss and Smith 2005). Following a period of embryonic development, an intermediate form is produced (the larva), which is morphologically and often physically isolated from the adult form. The larva continues to develop before undergoing a reorganization or metamorphosis to produce the juvenile, which will ultimately grow into a new adult. It is important to note that many species that utilize these strategies (especially benthic marine invertebrates) undergo a continuum of intermediate larval stages before reaching the final adult phase. Therefore, the term complex life history might be more accurate than biphasic to encompass the diversity of larval stages and pivotal time points in the ontogeny of marine invertebrates.

The development of diverse propagule forms and complex life-history strategies are thought to explain the wide variety of niches and ecological roles filled by animals in the marine environments (Kalinka and Tomancak 2012, McEdward 2000). I will use the term ‘propagule’ throughout the thesis as a convention to encompass both the embryos

and larvae of a species. Propagule forms range from simple, spheroid, shapes (e.g., planula larva; Fig. 1.1A) to elaborate structures with appendages and skeletal structures (e.g., pluteus larva; Fig. 1.1D). The predominance of marine invertebrate species that develop through a larval stage suggests that this strategy has been immensely successful for colonizing and surviving in oceanic habitats (Pechenik 1999, Poulin *et al.* 2001, Riginos *et al.* 2011). The evolution and maintenance of sensory abilities in invertebrate propagules is likely a major contributor to this success. Marine species that are sessile or sedentary as adults (demersal/benthic) are thought to experience stronger selective pressures to disperse their offspring than floating/swimming (pelagic) species (Poulin *et al.* 2001). Thus, most benthic invertebrates utilize a benthopelagic complex life cycle (Mileikovsky 1971, Pechenik 1999, Poulin *et al.* 2001), whereby free-swimming embryos and larvae develop in the water column and return to the benthos following metamorphosis. During the pelagic period, larvae can disperse widely to colonize new habitats and facilitate gene flow (Hart and Marko 2010, Marshall and Morgan 2011, Pechenik 1999). The selective pressures experienced by extant adults and larvae utilizing benthopelagic strategies have direct implications for understanding the evolution of marine animals and their continuing interactions with changing ocean environments.

1.2. Origins of Marine Propagules

The diversity of life-history variation present among marine invertebrate phyla is staggering. The fact that completely opposite life histories can be found even among closely related species generates many critical questions including: 1. Why are marine

larvae so diverse in form across similar environments? 2. How did such diversity emerge? The origin of biphasic life cycles in marine invertebrates is thought to overlap with the origin of the early animals (Degnan and Degnan 2006, Sly *et al.* 2003). However, there is extensive and rigorous debate about the morphology and reproductive characteristics of the earliest ancestor(s), and whether biphasic life cycles emerged at the evolution of bilateral body plans or of all animals (Degnan and Degnan 2006, Marlow *et al.* 2014, Nielsen 2013, Raff 2008).

Planktotrophic larvae (feeding on external food sources) are generally accepted to be the ancestral condition in animals (Nielsen 1998, 2009, Strathmann 1993). Early larvae may not have had a dedicated gut, but rather, absorbed nutrients from particles captured by cilia (Nielsen 1998). Nutrient absorption is still seen among modern larvae, although the maintenance of such an ability may supplement energy reserves rather than be a mandatory process (Jaeckle and Manahan 1989, Manahan 1990, Manahan and Crisp 1982). One hypothesis suggests that feeding in larvae developed as a secondary consequence of dispersal (Degnan and Degnan 2006). During the pelagic period, propagules are initially exposed to benthic predators such as filter feeders and sea anemones, and to floating/swimming predators such as jellies, zooplankton, shrimps and fishes later in their development (Johnson and Shanks 2003, Mercier *et al.* 2013a, Pennington *et al.* 1986). The pelagic period can be reduced by consuming nutrients to accelerate maturation (Miller 1993). Planktotrophy could therefore have evolved to mitigate the risks of longer exposure to predators.

Among modern phyla, lecithotrophic larval development (relying on maternal provision) is common among cold-water and deep-sea species (Marshall *et al.* 2012), and in seasonal environments with fluctuating phytoplankton blooms (Marshall and Burgess 2015). Species with non-feeding larvae generally produce large, yolky, eggs and exhibit lower fecundities than species with feeding larvae (Strathmann 1993). With maternal reserves, non-feeding larvae are often predicted to spend less time developing in the water column than planktotrophs (shorter pelagic propagule duration [PPD]; Strathmann 1977). Other putative benefits of lecithotrophy include reduced predation by benthic predators, and little risk associated with seasonally patchy food availability in the plankton. Initially, the shift to lecithotrophy was thought to incur a steep cost to maternal energetics, fecundity and dispersal abilities (noted by Pechenik 1999); but recent evidence has challenged the notion that non-feeding larvae have shorther PPDs than planktotrophs (Mercier *et al.* 2013b), and demonstrated that the survival of juveniles is enhanced in species with maternal investment via transgenerational effects (Krug *et al.* 2012). However, given that planktotrophs and lecithotrophs can successfully coexist in similar environments, and that not all species have shifted to an intermediate state of feeding between these two modes, lecithotrophy likely is not adaptive for all marine invertebrate species. In fact, hybrid nutritional modes exist between planktotrophy and lecithotrophy whereby propagules are provisioned with maternal lipid but can feed facultatively (e.g., Allen *et al.* 2006, Emlet 1986, McEdward 1997, Miller 1993, Miner *et al.* 2005). Propagule nutritional modes may therefore be described as a continuum rather than a

dichotomy of feeding vs. non-feeding (see Chapter 2 for further discussion of invertebrate life histories).

1.3. Importance of Marine Propagules: Ecology, Aquaculture, Conservation

In the plankton, small propagules can drift with the currents to reach new habitats for colonization, thereby minimizing inbreeding and preventing competition for limited resources (Table 1.1; Cowen and Sponaugle 2009, Paulay and Meyer 2006, Scheltema 1986). Pelagic larvae are thought to be able to escape benthic predators during their pelagic duration (Pechenik 1999, Pennington *et al.* 1986). For benthic adults with limited or no dispersal capabilities, such a strategy could greatly enhance fitness if the cost to produce such larvae was low. Larvae in most phyla are also equipped with sensory structures that enable them to explore and assess their immediate environment (e.g., sensory cilia, gravireceptors, eyes, and olfactory cells; Hadfield 2011, Nordstrom *et al.* 2003, Tamburri *et al.* 1996). Thus, larvae can, to some degree, select an optimum habitat for future juvenile and/or adult survival based on a hierarchy of chemical and biological cues. It is currently unclear whether sensory abilities developed secondarily to locomotory modifications such as ciliation. Given that many sensory structures across diverse animal phyla utilize cilia coupled to sensory cells, it can be difficult to disentangle the origins of locomotory and sensory function in larvae. However, there are also clear disadvantages to having a dispersive larval stage (Table 1.1). Pelagic larvae are often exposed to chaotic and ever changing environmental conditions in the plankton. Their small size constrains locomotion significantly and, thus, they may end up far away from a

suitable habitat due to currents or mixing patterns they cannot escape (Paulay and Meyer 2006, Scheltema 1986). Pelagic larvae also face exposure to bacterial and viral agents in addition to pelagic predators (Pechenik 1999), whereas parental protection (brooding/encapsulation) removes these threats (e.g. *Buccinum scalariforme,* see Appendix 1). Thus, the diversity of modifications to the general benthopelagic life history likely emerged to mitigate a suite of risks (Rieger 1994).

Understanding the modifications and adaptations of different propagule types is not only important from an ecological perspective, but also has implications for aquaculture and marine conservation. The reliable and efficient culture of fish and invertebrate species is economically important and has the potential to solve issues of world hunger (Frankic and Hershner 2003, Neori *et al.* 2004, Tacon 1997). Although it comes with drawbacks of its own, aquaculture can reduce destructive fishing practices, especially in the case of benthic species, such as scallops and ground fishes that are captured using trawl nets. One of the challenges facing aquaculture development is the successful reproduction and maintenance of animals in captive settings. Therefore, understanding the environmental preferences and reproductive biology of species is critical for the success and cost-effectiveness of the operations. Studies examining the adaptations and behaviours of marine larvae can provide valuable insights towards optimizing the transition from larvae to juvenile in aquaculture settings.

Species with benthopelagic life histories also require special attention in the context of marine conservation since propagule dispersal can be affected by numerous natural and anthropogenic phenomena. Identification of dispersal patterns and nursery

sites is therefore critical to the choice of marine protected areas (MPAs). Anthropogenic pressures, combined with ocean warming and associated climate-change scenarios, will likely have major impacts on the ability of propagules to survive and disperse (Byrne and Przeslawski 2013, Przeslawski *et al.* 2015). Hence, continued research on life histories and the biology of propagules will facilitate effective policy development in the years to come.

1.4. Current State of Knowledge in the Larval Ecology of Benthic Invertebrates

While the study of marine larval biology and ecology dates back more than a hundred years and has yielded seminal papers of major significance over the past 30 years (Eckman 1996, Jablonski and Lutz 1983, Marshall and Morgan 2011, Mercier *et al.* 2013b, Monro and Marshall 2015, Pechenik 1999, Poulin *et al.* 2001, Young 1990), knowledge of the fundamental role and significance of propagules in benthic animals is far from complete. Firstly, species of economic importance have been prioritized. For example, scallops (Lagos *et al.* 2016, Liu *et al.* 2016, Loor *et al.* 2016) and lobsters (Day *et al.* 2016, Small *et al.* 2016, Wakabayashi and Phillips 2016) dominated in a 2016 literature search of marine invertebrates. However, there is a relative shortage of studies focused on simpler, basal clades such as sponges and non-coral and cold-water cnidarians. Evidence is emerging to suggest homology of form, gene expression and sensory capabilities among marine larvae (Hadfield and Koehl 2004, Lacalli *et al.* 1990, Marlow *et al.* 2014, Pechenik 1999). Hence, modern primitive phyla can provide a useful window into the lives of early animals. There is also a shortage of studies on ciliated

propagules relative to propagules that swim using appendages (e.g. crustacean larvae) or via muscular contractions (e.g. late annelid larvae). This may be because ciliated propagules are typically weaker swimmers, often assumed to be passive particles, compared to appendage-bearing larvae, and because they are also harder to manipulate. Finally, there are a limited number of studies on lecithotrophic (non-feeding) species within phyla where multiple types of life histories have evolved (e.g., Mollusca, Annelida, Echinodermata). This is particularly surprising in phyla like Echinodermata, which are believed to be dominated by lecithotrophic species (~68% extant species; Uthicke *et al.* 2009). Comparing the fundamental differences between planktotrophs and lecithotrophs is important for understanding ecological and evolutionary processes, but also for optimizing aquaculture programs and conservation initiatives.

1.5. Echinodermata: A Focal Phylum for Comparative Study

The phylum Echinodermata (Fig. 1.2) is extensively studied (from community ecology to biomedical research), but provides an excellent example of the shortcomings that currently exist in larval ecological research. There are several key reasons why taxonomic and life-history based biases have emerged. Feeding larvae (such as those of the sea urchin genus *Strongylocentrotus*) are relatively easy to culture and can be stimulated to develop over a short period by manipulating culture temperature. In addition, most commercial echinoderms are planktotrophs (e.g., *Strongylocentrotus* and *Holothuria* spp.). This economic incentive has driven research of larval behaviour from an aquaculture and biogeographical perspective. Because lecithotrophic species have very

different life-history traits relative to planktotrophs, including lower fecundity, and since they may be less abundant in heavily studied environments (e.g. coral reefs), they tend to be misrepresented or overlooked. Such a lopsided approach to larval ecology is potentially problematic when predictions of larval dispersal for an area are required for the delimitation of ecological reserves and for optimizing aquaculture practices in commercial lecithotrophic echinoderms (e.g. the emerging *Cucumaria frondosa* industry in Newfoundland, Canada).

The Echinodermata are also an important group from an ecological and evolutionary point of view. There are representatives in nearly all marine habitats and echinoderm species with vastly different life-history strategies often co-exist in the same area. Echinoderms play critical roles in the marine environment as keystone species (e.g. *Pisaster ochraceus*; Paine 1969) and as preferred prey items (e.g. *Strongylocentrotus* spp.; Kvitek *et al.* 1998). In addition, echinoderms belong to the super phylum Deuterostomia (Grobben 1908), and share developmental features with organisms belonging to more complex phyla such as Chordata. Yet echinoderms still maintain many features seen in simpler organisms such as ellipsoid, mono-ciliated propagules. Echinoderm propagules display incredible variation in morphology and colour, even within genera, providing great opportunities to explore the environmental drivers of propagule phenotypes (see Chapter 3).

The co-existence of echinoderm species with different life histories can be seen prominently in temperate and cold-water environments. In the North Atlantic, four common species exist in similar habitats despite filling different ecological roles

(predators, prey) and possessing two different developmental modes (planktotrophy, lecithotrophy): the sea stars *Asterias rubens* and *Crossaster papposus*, the sea urchin *Strongylocentrotus droebachiensis* and the sea cucumber *Cucumaria frondosa* (Fig. 1.3). These features make these four species an ideal focus for comparative studies of propagule adaptions, locomotion and behaviours.

Asterias rubens (the common sea star; synonymous with *A. vulgaris*; Fig. 1.3A; Mah and Hansson 2011) is a widespread sea star species that colonizes temperate and subarctic regions on both sides of the Atlantic. The typical depth range for this species is from near surface to 200 m. Individuals generally reach 10-30 cm in diameter and range in colour from orange to purple or brown. *Asterias rubens* feeds actively on benthic molluscs such as mussels and snails, and more rarely on other echinoderms. It predominantly colonizes rocky habitats where it can often be found on boulders and vertical cliffs. This species is planktotrophic with a late spring spawning season (Table 1.2). Fecundity for a large female can be ~2.5 million oocytes (Fish and Fish 2011). The oocytes are small (0.1 mm) and cream in colour.

Strongylocentrotus droebachiensis (the green sea urchin; Fig. 1.3B; Kroh and Hansson 2012) is a ubiquitous member of circumpolar communities ranging in North America from the Artic to New Jersey, and from Alaska to Puget Sound. Depth range for this species is most commonly from intertidal to 200 m. Individuals of this species grow up to 15 cm in diameter. Urchin populations in the North Atlantic can destroy the habitat of other species through massive consumption of seaweeds, creating 'urchin barrens' (Gagnon *et al.* 2004). This species is also planktotrophic with a late spring spawning

season (Table 1.2). Fecundity is ~175,000 oocytes per female (Dupont *et al.* 2013). The oocytes are small (0.15 mm) and yellowish. There is a small but growing fishery associated with this species in both Pacific and Atlantic Canada.

Crossaster papposus (the common sun star; Fig. 1.3C; Mah and Hansson 2016) is an active predator of other echinoderms (e.g., sea cucumbers, sea urchins). It can be found in circumpolar regions of the North Atlantic and North Pacific. Individuals of this species can grow up to 34 cm in diameter. Habitat preferences include rocky and sandy bottoms. *Crossaster papposus* is rarely found intertidally and is more sensitive to temperature fluctuations than *A. rubens*. Depth range for this species is just below the surface to 300 m. *Crossaster papposus* is lecithotrophic with an early spring spawning season (Table 1.2). Fecundity is unknown, but likely in the order of thousands to tens of thousands of oocytes per female, values typical for lecithotrophic echinoderms (McClintock and Pearse 1986). The oocytes produced by the species are large (0.65 mm) and red.

Cucumaria frondosa (the orange-footed sea cucumber; Fig. 1.3D; Paulay and Hansson 2013) is the most common sea cucumber in the North Atlantic. It also occurs in the North Pacific and in the Arctic. The usual depth range for this species is 30-300 m. Individuals can reach up to 50 cm in length. They can often be found in rocky areas between boulders, extending their feeding tentacles out to capture plankton and suspended organic particles. *Cucumaria frondosa* is a lecithotrophic species with a spawning season in early spring (Table 1.2). Fecundity of *C. frondosa* is up to ~12,000 oocytes per mature female (Hamel and Mercier 1996). The oocytes produced by this

species are large (0.9 mm) and orange-red. A fishery for *C. frondosa* has recently emerged in Atlantic Canada, and there is a huge market for all sea cucumber products in Asia, making sustainable management of stocks of global concern (Anderson *et al.* 2011, Purcell *et al.* 2013).

1.6. Main Objectives and Thesis Structure

The overarching questions and goals covered by my PhD thesis can be summarized into four main points:

Objective 1: To examine the extent and ecological significance of egg colour diversity among lecithotrophic echinoderm propagules.

Objective 2: To quantify and compare the swimming capacity of planktotrophic and lecithotrophic echinoderm propagules under various conditions.

Objective 3: To quantify and compare the photosensitivity of planktotrophic and lecithotrophic propagules.

Objective 4: To review current knowledge of sensory capabilities in ciliated propagules, and to tease out relationships between life histories and behavioural and locomotory patterns in major marine phyla.

In Chapter 2, I present relevant definitions, outline the significance of propagule ecology from egg to juvenile, and discuss the applications of my thesis work. The following section (Chapter 3) presents a meta-analysis of the adaptive value of egg pigmentation in lecithotrophic echinoderms. Next, I measure the swimming capacity of two types of echinoderm propagules under ambient environmental conditions (Chapter 4)

and the corresponding sensory behaviour of the same species in response to varied light colour and intensity (Chapter 5). Finally, I present a critical assessment of propagule sensory behaviour in the context of swimming capacity and larval nutritional mode in the major marine phyla (Chapter 6). A final section (Chapter 7) summarizes the main findings of this thesis.

Chapter 3 was published in the January 2017 issue of *Advances in Marine Biology* and Chapter 4 was published in *Marine Biology* in March 2017. Chapter 5 is currently being prepared for journal submission. Chapters 2 and 6 will be combined and prepared for submission as a review/synthesis paper. Another recently published paper of relevance to this thesis is provided in Appendix 1.

1.7. References

Allen, J. D., C. Zakas, and R. D. Podolsky. 2006. Effects of egg size reduction and larval feeding on juvenile quality for a species with facultative-feeding development. *Journal of Experimental Marine Biology and Ecology* **331**:186-197.

Anderson, S. C., J. M. Flemming, R. Watson, and H. K. Lotze. 2011. Serial exploitation of global sea cucumber fisheries. *Fish and Fisheries* **12**:317-339.

Bishop, C. D., and B. P. Brandhorst. 2003. On nitric oxide signaling, metamorphosis, and the evolution of biphasic life cycles. *Evolution & Development* **5**:542-550.

Brommer, J. E. 2000. The evolution of fitness in life-history theory. *Biological Reviews of the Cambridge Philosophical Society* **75**:377-404.

Byrne, M., and R. Przeslawski. 2013. Multistressor impacts of warming and acidification of the ocean on marine invertebrates' life histories. *Integrative and Comparative Biology* **53**:582-596.

Cowen, R. K., and S. Sponaugle. 2009. Larval dispersal and marine population connectivity. *Marine Science* **1**.

Day, R. D., R. D. McCauley, Q. P. Fitzgibbon, and J. M. Semmens. 2016. Seismic air gun exposure during early-stage embryonic development does not negatively affect spiny lobster *Jasus edwardsii* larvae (Decapoda: Palinuridae). *Scientific Reports* **6**.

Degnan, S. M., and B. M. Degnan. 2006. The origin of the pelagobenthic metazoan life cycle: what's sex got to do with it? *Integrative and Comparative Biology* **46**:683-690.

Dupont, S., N. Dorey, M. Stumpp, F. Melzner, and M. Thorndyke. 2013. Long-term and trans-life-cycle effects of exposure to ocean acidification in the green sea urchin *Strongylocentrotus droebachiensis*. *Marine Biology* **160**:1835-1843.

Eckman, J. E. 1996. Closing the larval loop: linking larval ecology to the population dynamics of marine benthic invertebrates. *Journal of Experimental Marine Biology and Ecology* **200**:207-237.

Emlet, R. B. 1986. Facultative planktotrophy in the tropical echinoid *Clypeaster rosaceus* and a comparison with obligate planktotrophy in *Clypeaster dubdeoressus* (Clypeasteroida: Echinoidea). *Journal of Experimental Marine Biology and Ecology* **95**:183-202.

Fish, J. D., and S. Fish. 2011. *A Student's Guide to the Seashore*. Cambridge University Press, Cambridge, U.K.

Frankic, A., and C. Hershner. 2003. Sustainable aquaculture: developing the promise of aquaculture. *Aquaculture International* **11**:517-530.

Gagnon, P., J. Himmelman, and L. Johnson. 2004. Temporal variation in community interfaces: kelp-bed boundary dynamics adjacent to persistent urchin barrens. *Marine Biology* **144**:1191-1203.

Hadfield, M. G. 2011. Biofilms and marine invertebrate larvae: what bacteria produce that larvae use to choose settlement sites. *Annual Review of Marine Science* **3**:453-470.

Hadfield, M. G., and M. A. R. Koehl. 2004. Rapid behavioral responses of an invertebrate larva to dissolved settlement cue. *Biological Bulletin* **207**:28-43.

Hamel, J., and A. Mercier. 1996. Gonad morphology and gametogenesis of the sea cucumber *Cucumaria frondosa*. *Beche-de-mer Information Bulletin*:22-33.

Hart, M. W., and P. B. Marko. 2010. It's about time: divergence, demography, and the evolution of developmental modes in marine invertebrates. *Integrative and Comparative Biology*:icq068.

Jablonski, D., and R. A. Lutz. 1983. Larval ecology of marine benthic invertebrates: paleobiological implications. *Biological Reviews* **58**:21-89.

Jaeckle, W., and D. T. Manahan. 1989. Feeding by a "nonfeeding" larva: uptake of dissolved amino acids from seawater by lecithotrophic larvae of the gastropod *Haliotis rufescens*. *Marine Biology* **103**:87-94.

Johnson, K. B., and A. L. Shanks. 2003. Low rates of predation on planktonic marine invertebrate larvae. *Marine Ecology Progress Series* **248**:125-139.

Kalinka, A. T., and P. Tomancak. 2012. The evolution of early animal embryos: conservation or divergence? *Trends in Ecology & Evolution* **27**:385-393.

Kroh, A., and H. Hansson. 2012. *Strongylocentrotus droebachiensis* (O.F. Müller, 1776). in *World Echinoidea Database*, A. Kroh and R. Mooi, eds. World Register of Marine Species.

Krug, P. J., D. Gordon, and M. R. Romero. 2012. Seasonal polyphenism in larval type: rearing environment influences the development mode expressed by adults in the sea slug *Alderia willowi*. *Integrative and Comparative Biology* **52**:161-172.

Kvitek, R. G., P. Iampietro, and C. E. Bowlby. 1998. Sea otters and benthic prey communities: A direct test of the sea otter as keystone predator in Washington State. *Marine Mammal Science* **14**:895-902.

Lacalli, T., T. Gilmour, and J. West. 1990. Ciliary band innervation in the bipinnaria larva of *Piaster ochraceus*. *Philosophical Transactions of the Royal Society B: Biological Sciences* **330**:371-390.

Lagos, N. A., S. Benítez, C. Duarte, M. A. Lardies, B. R. Broitman, C. Tapia, P. Tapia, S. Widdicombe, and C. A. Vargas. 2016. Effects of temperature and ocean acidification on shell characteristics of *Argopecten purpuratus:* implications for scallop aquaculture in an upwelling-influenced area. *Aquaculture Environment Interactions* **8**:357-370.

Liu, W., C. Pearce, R. McKinley, and I. Forster. 2016. Nutritional value of selected species of microalgae for larvae and early post-set juveniles of the Pacific geoduck clam, *Panopea generosa*. *Aquaculture* **452**:326-341.

Loor, A., D. Ortega, C. Lodeiros, and S. Sonnenholzner. 2016. Early life cycle and effects of microalgal diets on larval development of the spiny rock-scallop, *Spondylus limbatus* (Sowerby II, 1847). *Aquaculture* **450**:328-334.

Mah, C., and H. Hansson. 2011. *Asterias rubens* Linnaeus, 1758. in *World Asteroidea Database*, C. Mah, ed. World Register of Marine Species

Mah, C., and H. Hansson. 2016. Crossaster *papposus* (Linnaeus, 1767). in *World Asteroidea Database*, C. Mah, ed. World Register of Marine Species.

Manahan, D. T. 1990. Adaptations by invertebrate larvae for nutrient acquisition from seawater. *American Zoologist* **30**:147-160.

Manahan, D. T., and D. J. Crisp. 1982. The role of dissolved organic material in the nutrition of pelagic larvae: Amino acid uptake by bivalve veligers. *American Zoologist* **22**:635-646.

Marlow, H., M. A. Tosches, R. Tomer, P. R. Steinmetz, A. Lauri, T. Larsson, and D. Arendt. 2014. Larval body patterning and apical organs are conserved in animal evolution. *BMC Biology* **12**:7.

Marshall, D. J., and S. C. Burgess. 2015. Deconstructing environmental predictability: seasonality, environmental colour and the biogeography of marine life histories. *Ecology Letters* **18**:174-181.

Marshall, D. J., P. J. Krug, E. K. Kupriyanova, M. Byrne, and R. B. Emlet. 2012. The biogeography of marine invertebrate life histories. *Annual Review of Ecology and Systematics* **43**:97-114.

Marshall, D. J., and S. G. Morgan. 2011. Ecological and evolutionary consequences of linked life-history stages in the sea. *Current Biology* **21**:R718-R725.

McClintock, J. B., and J. S. Pearse. 1986. Organic and energetic content of eggs and juveniles of antarctic echinoids and asterids with lecithotrophic development. *Comparative Biochemistry and Physiology Part A* **85**:341-345.

McEdward, L. R. 1997. Reproductive strategies of marine benthic invertebrates revisited: Facultative feeding by planktotrophic larvae. *American Naturalist* **150**:48-72.

McEdward, L. R. 2000. Adaptive evolution of larvae and life cycles. 403-409 in *Seminars in Cell & Developmental Biology* Elsevier.

Mercier, A., E. J. Doncaster, and J.-F. Hamel. 2013a. Contrasting predation rates on planktotrophic and lecithotrophic propagules by marine benthic invertebrates. *Journal of Experimental Marine Biology and Ecology* **449**:100-110.

Mercier, A., M. A. Sewell, and J.-F. Hamel. 2013b. Pelagic propagule duration and developmental mode: Reassessment of a fading link. *Global Ecology and Biogeography* **22**:517-530.

Mileikovsky, S. 1971. Types of larval development in marine bottom invertebrates, their distribution and ecological significance: a re-evaluation. *Marine Biology* **10**:193-213.

Miller, S. E. 1993. Larval period and its influence on post-larval life history: Comparison of lecithotrophy and facultative planktotrophy in the aoelid nudibranch *Phestilla sibogae*. *Marine Biology* **117**:635-645.

Miner, B. G., L. A. McEdward, and L. R. McEdward. 2005. The relationship between egg size and the duration of the facultative feeding period in marine invertebrate larvae. *Journal of Experimental Marine Biology and Ecology* **321**:135-144.

Monro, K., and D. J. Marshall. 2015. The biogeography of fertilization mode in the sea. *Global Ecology and Biogeography* **24**:1499-1509.

Neori, A., T. Chopin, M. Troell, A. H. Buschmann, G. P. Kraemer, C. Halling, M. Shpigel, and C. Yarish. 2004. Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. *Aquaculture* **231**:361-391.

Nielsen, C. 1998. Origin and evolution of animal life cycles. *Biological Reviews of the Cambridge Philosophical Society* **73**:125-155.

Nielsen, C. 2009. How did indirect development With planktotrophic larvae evolve? *Biological Bulletin* **216**:203-215.

Nielsen, C. 2013. Life cycle evolution: was the eumetazoan ancestor a holopelagic, planktotrophic gastraea. *BMC Evolutionary Biology* **13**:1-18.

Nordstrom, K., R. Wallen, J. Seymour, and D. Nilsson. 2003. A simple visual system without neurons in jellyfish larvae. *Philosophical Transactions of the Royal Society B: Biological Sciences* **270**:2349-2354.

Paine, R. T. 1969. A note on trophic complexity and community stability. *American Naturalist* **103**:91-93.

Paulay, G., and H. Hansson. 2013. *Cucumaria frondosa* (Gunnerus, 1767). World Register of Marine Species

Paulay, G., and C. Meyer. 2006. Dispersal and divergence across the greatest ocean region: do larvae matter? *Integrative and Comparative Biology* **46**:269-281.

Pechenik, J. A. 1999. On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. *Marine Ecology Progress Series* **177**:269-297.

Pennington, J. T., S. S. Rumrill, and F.-S. Chia. 1986. Stage-specific predation upon embryos and larvae of the Pacific sand dollar, *Dendraster excentricus*, by 11 species of common zooplanktonic predators. *Bulletin of Marine Science* **39**:234-240.

Poulin, E., S. V. Boletzky, and J.-P. Feral. 2001. Combined ecological factors permit classification of developmental patterns in benthic marine invertebrates: A discussion note. *Journal of Experimental Marine Biology and Ecology* **257**:109-115.

Przeslawski, R., M. Byrne, and C. Mellin. 2015. A review and meta‐analysis of the effects of multiple abiotic stressors on marine embryos and larvae. *Global Change Biology* **21**:2122-2140.

Purcell, S. W., A. Mercier, C. Conand, J. F. Hamel, M. V. Toral‐Granda, A. Lovatelli, and S. Uthicke. 2013. Sea cucumber fisheries: global analysis of stocks, management measures and drivers of overfishing. *Fish and Fisheries* **14**:34-59.

Raff, R. A. 2008. Origins of the other metazoan body plans: the evolution of larval forms. *Philosophical Transactions of the Royal Society B: Biological Sciences* **363**:1473-1479.

Rieger, R. M. 1994. The biphasic life cycle—a central theme of metazoan evolution. *American Zoologist* **34**:484-491.

Riginos, C., K. E. Douglas, Y. Jin, D. F. Shanahan, and E. A. Treml. 2011. Effects of geography and life history traits on genetic differentiation in benthic marine fishes. *Ecography* **34**:566-575.

Scheltema, R. S. 1986. On dispersal and planktonic larvae of benthic invertebrates: an eclectic overview and summary of problems. *Bulletin of Marine Science* **39**:290-322.

Sly, B. J., M. S. Snoke, and R. A. Raff. 2003. Who came first-larvae or adults? Origins of bilaterian metazoan larvae. *International Journal of Developmental Biology* **47**:623-632.

Small, D. P., P. Calosi, D. Boothroyd, S. Widdicombe, and J. I. Spicer. 2016. The sensitivity of the early benthic juvenile stage of the European lobster *Homarus gammarus* (L.) to elevated pCO2 and temperature. *Marine Biology* **163**:1-12.

Strathmann, R. R. 1977. Egg size, larval development, and juvenile size in benthic marine invertebrates. *American Naturalist* **111**:373-376.

Strathmann, R. R. 1993. Hypotheses on the origins of marine larvae. *Annual Review of Ecology and Systematics* **24**:89-117.

Tacon, A. 1997. Feeding tomorrow's fish: Keys for sustainability. *Feeding Tomorrow's Fish* **22**:11-33.

Tamburri, M. N., C. M. Finelli, D. S. Wethey, and R. K. Zimmer-Faust. 1996. Chemical induction of larval settlement behavior in flow. *Biological Bulletin* **191**:367-373.

Uthicke, S., B. Schaffelke, and M. Byrne. 2009. A boom-bust phylum? Ecological and evolutionary consequences of density variations in echinoderms. *Ecological Monographs* **79**:3-24.

Voss, S., and J. Smith. 2005. Evolution of salamander life cycles. *Genetics* **170**:275-281.

Wakabayashi, K., and B. F. Phillips. 2016. Morphological descriptions of laboratory reared larvae and post-larvae of the Australian shovel-nosed lobster *Thenus australiensis* Burton & Davie, 2007 (Decapoda, Scyllaridae). *Crustaceana* **89**:97-117.

Young, C. M. 1990. Larval ecology of marine invertebrates: a sesquicentennial history. *Ophelia* **32**:1-48.

1.8. Tables and Figures

Table 1.1. Weighing the risks of pelagic propagules.

Advantages	Disadvantages
Dispersal to colonize new habitats	Dispersal too far away from ideal habitat
Pelagic life prevents benthic predation	Pelagic life enables pelagic predation
Dispersal protects adult resources	Risky in low population densities
No parental care required	Greater exposure to environmental change
Dispersal prevents inbreeding	Risk exposure to disease or biological agents
Access to locations for juvenile survival	Small body size can impede locomotion

Table 1.2. Summary of biological traits associate with focal echinoderm species.

Species	Propagule Type	Spawning Season	Testable Stages	Fecundity ($\times 10^3$ female^{-1})	Egg diameter (mm)
S. droebachiensis	Planktotrophic	Late spring	6	175 (Dupont *et al.* 2013)	0.15
A. rubens			4	2500 (Fish and Fish 2011)	0.10
C. frondosa	Lecithotrophic	Spring	4	12 (Hamel and Mercier 1996)	0.90
C. papposus				Unknown	0.65

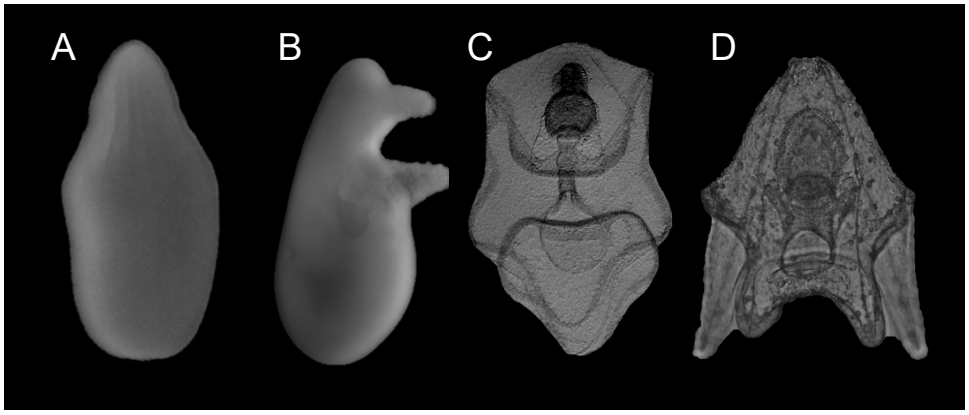


Figure 1.1. Examples of marine larvae. A) Planula larva (sea anemone, *Urticina felina*). B) Brachiolaria (sea star, *Crossaster papposus*). C) Late bipinnaria (sea star, *Asterias rubens*). D) Pluteus (sea urchin, *Strongylocentrotus droebachiensis*).

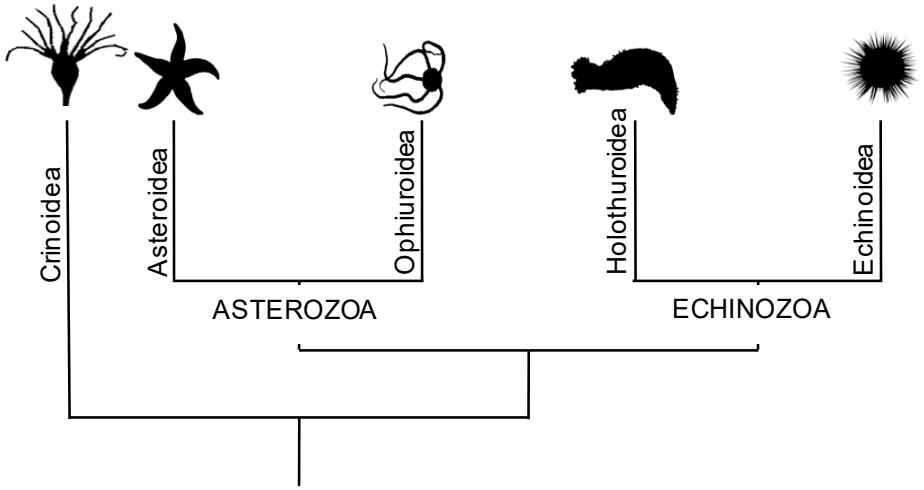


Figure 1.2. Currently accepted phylogeny of Echinodermata (based on Reich et al. 2015).

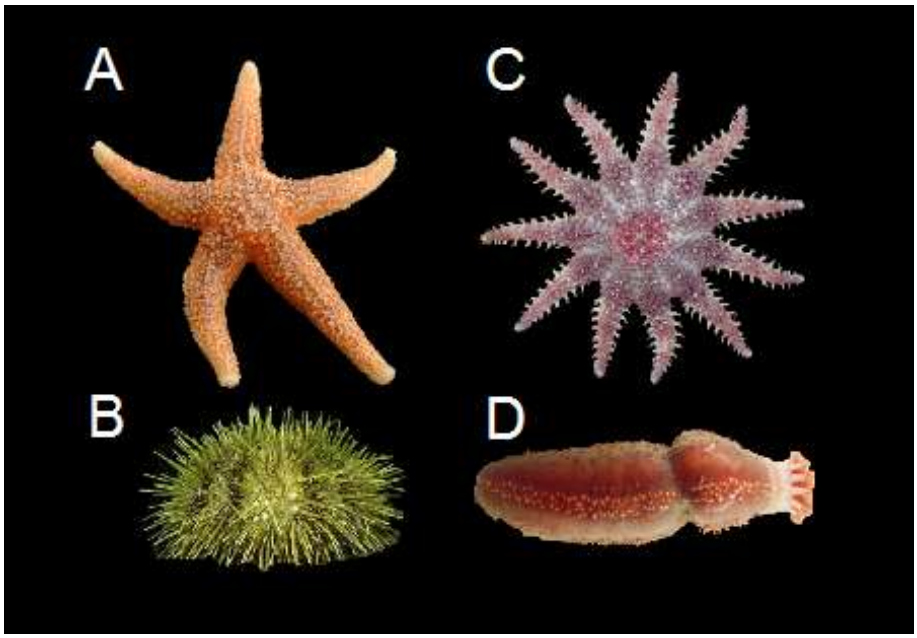


Figure 1.3. Adults of the focal echinoderm species. A) *Asterias rubens*. B) *Strongylocentrotus droebachiensis*. C) *Crossaster papposus*. D) *Cucumaria frondosa*.

Chapter 2. Propagule Ecology from Egg to Juvenile

2.1. Classification of Marine Propagules

Marine animals with complex life histories have evolved a diversity of forms, life styles and reproductive modes. Organizing the similarities and differences among propagules corresponding to different life-history strategies is therefore critical for scientific study and comparison. To this effect, the classification of marine propagules has undergone several revisions over the last few decades, each with their own take on what features need to be included in each category (e.g., early definitions - Thorson 1949; swimming capabilities - Jablonski and Lutz 1983; development type - McEdward and Janies 1993; ecologically-based categories - Poulin *et al.* 2001). However, the consensus among authors is that marine propagules can be broken into categories based on key life-history strategies such as nutritional mode or development location. For this review, I will utilize the categories proposed by Poulin *et al.* 2001 with the addition of metamorphosis type (see below).

Five main categories can be used to characterize marine propagules: (1) development type, (2) development location, (3) nutritional mode, (4) protection level, and (5) metamorphosis type (summarized in Table 2.1). Development type is determined by the presence of larval stages. *Direct* developing species progress from an embryo to a juvenile with no intermediate (larval) life stage (Poulin *et al.* 2001). In contrast, *indirect* development involves one or more intermediate larval forms between embryonic and

juvenile forms (Poulin *et al.* 2001). Species with egg capsules (protected) are often wrongly called direct developers (Ilano *et al.* 2004, Martel and Chia 1991) because juveniles emerge fully formed from the capsules. However, this overlooks the fact that many of them exhibit intra-capsular larval forms (see Appendix 1 for an example of intra-capsular development). Though direct developers can exhibit differences in development location and other features, any additional categories presented here will pertain only to indirect developers including: development location, nutritional mode, protection level and metamorphosis type. Propagule location during development, distinguishes *pelagic* propagules that develop away from the benthos and *benthic* propagules that develop close to, or on the benthos (either on the substrate or other organisms). Some species with encapsulated or brooded development first develop close to the benthos before being released as swimming larvae in the pelagic environment, highlighting the need for a *mixed* category. This is the case for many gastropods (Pechenik 1979).

Nutritional mode is probably the most common method of distinguishing among different types of propagules. Like development location, it is a continuum of nutritional types, from exclusively maternal to exclusively external sources (Poulin *et al.* 2001, Strathmann 1978, 2007). Propagules that utilize external sources of nutrition (e.g. food capture) are classified as *planktotrophic*, whereas propagules that utilize maternally-deposited energy reserves (yolk) and do not feed externally are classified as *lecithotrophic* (Poulin *et al.* 2001, Strathmann 1978). Some propagules can opportunistically feed, while also relying on maternal energy reserves; this intermediate condition is called *facultative planktotrophy* and has been identified in mollusc and

echinoderm species (Allen *et al.* 2006, Miller 1993, Miner *et al.* 2005). Planktotrophic propagules tend to be smaller than their lecithotrophic counterparts, which are more-yolky and often highly buoyant (Christiansen and Fenchel 1979, McEdward 1986, Moran and McAlister 2009).

Protection level during development ranges from *protected* [housed within protective capsules (for an example see Appendix 1: *Buccinum scalariforme*) or brooded on/inside the adult body] to *free living* (unprotected development), with some species termed *mixed* as their propagules undergo both protected and free-living periods. For instance, planula larvae of the sea anemone *Urticina felina* are protected in the adult body for the first part of development before being released to develop as free-living propagules (Mercier *et al.* 2011). Several polychaetes (e.g., *Heteromastus filiformis*) and gastropods (e.g., *Aeolidiella glauca*) also have a period of pre-hatch protected development (in gelatinous egg masses or capsules) before emerging in the plankton as larvae (Pechenik 1979). For species with complex life-history patterns, the use of 'mixed' and continuum-based categories for comparative studies are required to ensure that all life-history aspects can be included and understood (Caswell 1981, Pechenik 1979).

Metamorphosis is the transition between an intermediate larval form and the juvenile form (Burke 1983, Degnan and Degnan 2010). This process can be broken into two types: *simple*, in which the larval body becomes the juvenile body (commonly seen in basal organisms) or *complex*, in which the larval body is destroyed, consumed or manipulated in some way to generate the juvenile (commonly seen in more derived organisms).

Taken together, these five categories can be used to thoroughly describe and sort marine species to examine ecological and evolutionary patterns and trends. Examining the presence of sensory behaviour among different larval types can provide further information on how propagules have evolved to meet the needs of their environments and the trade-offs associated with different life histories. Since life-history strategies are a foundation of other biological processes, standardizing how I define propagules will be important for this review and other similar synthetic papers in the future. The purpose of this review is to determine the current state of knowledge of larval sensory abilities, behaviour and the intersection of these features with nutritional modes.

2.2. Importance of Cilia

Examples of ciliated propagules can be found in nearly all marine phyla. These propagules utilize bands or clusters of cilia for propulsion, which can be modified to play sensory functions. These cilia can also be used for feeding by planktotrophic propagules (e.g., pluteus and brachiolaria larvae of echinoderms, veliger larvae of molluscs). Although ciliated propagules are considered weak swimmers, relative to those that utilize appendages for propulsion (e.g. crustacean nauplii; Emlet 1994), slight modifications of their swimming speed (even two-fold increases) in response to environmental cues can affect behavioural processes like settlement (Abelson and Denny 1997, Pizarro and Thomason 2008).

There are compelling morphological similarities among ciliated larvae associated with different taxa and life-history strategies. Many species have at least one larval form

that is (1) ellipsoid, and (2) possesses a cluster of relatively long cilia in the apical region. In many groups, the emergence of this "apical tuft" along the developmental timeline is concurrent with the emergence of sensory behaviour (Byrne *et al.* 2007, Hadfield *et al.* 2000, Marlow *et al.* 2014). There has been debate as to the homology of this sensory apical tuft, with some authors suggesting it is convergent among invertebrate taxa (Dunn *et al.* 2007) and others demonstrating homology across a large evolutionary range of taxa (Marlow *et al.* 2014). Regardless of the evolutionary relatedness, the functional importance of apical cilia is clear to sensory behaviour in marine propagules (Byrne *et al.* 2007, Hadfield *et al.* 2000, Marlow *et al.* 2014).

2.3. Sensory Behaviour

Sensory processes can be broken into three chronological components: (1) detection, (2) translation and (3) response (Briffa and Greenaway 2011). Firstly, the individuals must possess specialized cellular machinery for the detection of stimuli. These features can include: sensory cells, specialized ion channels, receptor proteins, signal transduction molecules and signal transduction pathways (Jacobs *et al.* 2007, Jékely 2011). Though it might be assumed that complex assemblages of these specialized cells are coupled to the evolution of a true epidermis, adult sponges are still able to undergo coordinated contraction behaviour, even though they have a rudimentary epidermis with no discrete nervous elements to transmit the signal (Adams *et al.* 2010, Elliott and Leys 2010). Following detection, individuals must "assess" the signal and translate the information into a response. Such translation might involve chemical or electrical

signaling, depending on the complexity of the organism (Briffa and Greenaway 2011). Finally, there is a response (or no response) based on the detection and assessment of the original cue. Responses can be simple changes in morphology and body positioning or more complex patterns of locomotion and taxis.

Understanding how each of these steps function is important. Relative to vertebrates and terrestrial invertebrates, sensory structures in marine invertebrate propagules remain understudied (noted by Hadfield 2011, Pawlik 1992, Svane and Dolmer 1995, Tran and Hadfield 2013). Most studies on larval behaviour to date have only focused on the response phase of larvae to cues, rather than on the signal-detection or signal-translation steps (Pawlik 1992). Behaviour types (e.g. bold vs. shy) have been described in the adults of three cnidarian and one echinoderm species (Gherardi *et al.* 2012, Pruitt *et al.* 2012), but they have not been reported among early life stages.

2.4. Types and Mechanisms of Sensory Detection

Several main types of stimuli are perceivable by marine propagules including: photic, chemical (both from biotic and abiotic sources), thermal and positional (gravity) cues. Other stimuli could include water flow, presence of physical objects and magnetic fields, though much less focus has been placed on these. Sensory stimuli can act alone, but also can provide a hierarchy of signals along depth, horizontal and temporal gradients. For the purpose of this review, I will be focusing on sensory detection in ciliated propagules only, as these forms have been described as more basal (Nielsen 2008) or more passive (Emlet 1994) than propagules with appendages and are relatively

underrepresented in the literature. A baseline understanding of sensory detection mechanisms will be beneficial for future studies of these organisms but also in more complex systems (Arendt *et al.* 2009, Jacobs *et al.* 2007). For example, the sensory cell types and organization patterns seen in larval sea stars are thought to have provided a blueprint for evolution of complex larval forms in higher taxa (Lacalli *et al.* 1990). A summary of sensory detection mechanisms can be found in Table 2 for five major phyla with ciliated propagules: Porifera, Cnidaria, Annelida, Mollusca and Echinodermata.

2.4.1. Photosensitivity

Photosensitivity likely drove vertical migration, navigation and reproductive processes in early animals (Arendt *et al.* 2009). As light was crucial for animals evolving in the photic zone, photosensitive cellular structures are found in even the most basal of modern marine animals (Arendt *et al.* 2009, Nilsson 2009). Light-sensitive photoreceptor cells (PRC) and shading pigment cells (SPC) are the most basic components of photosensitivity in animals (Arendt *et al.* 2009). In simple systems, these sensory cell clusters are coupled to locomotory ciliated cells (LCC), that are responsible for directional swimming in response to signaling from the light sensitive cells nearby (Arendt *et al.* 2009). Opsin or opsin-like proteins are presumed to be some of the earliest light-sensitive pigments used by animal photoreceptors (Arendt *et al.* 2009, Raible *et al.* 2006). They can be found in the genome of many marine taxa ranging from cnidarians to echinoderms, and this suggests that a form of opsin may have been present in an early

animal ancestor, although taxa-specific forms of opsin have since emerged (Arendt *et al.* 2009, Raible *et al.* 2006).

Simple sensory cell clusters including PRC-SPC-LCC cells have been detected in demosponge larvae, which represent some of the simplest and most short-lived larval forms in the marine environment; despite their simplicity, they still exhibit predictable phototactic behaviours and morphological changes (Leys and Degnan 2001, Maldonado 2006, Maldonado *et al.* 2003). To build more complex photosystems, PRC clusters can be duplicated and compressed into discrete organs that are innervated by motor neurons or other such nervous elements (Arendt *et al.* 2009). An example of this duplication can be seen in the proto-eyes found in box jellyfish larvae (Nordstrom *et al.* 2003) without nervous system support. In comparison, so called "eyespots" innervated by larval nervous systems can be found in groups with more complex larval forms such as Annelida (Arendt *et al.* 2002), Mollusca (Chia and Koss 1983, Nielsen 2004) and Echinodermata (presumed by expression of opsin; Raible *et al.* 2006). Calcified larvae, like those present in Echinodermata, may also have precursors to more complex, adult structures, such as the ossicle-based micro-lenses in ophiuroids (Aizenberg *et al.* 2001). However, it remains unclear whether differences in photosensitivity exist among propagules with different life-history strategies such as planktotrophs versus lecithotrophs.

2.4.2. Chemosensitivity

Site-specific settlement has been well-established across diverse biphasic marine phyla ranging from sponges to echinoderms (Hadfield 1986, 2011, Pawlik 1992,

Tamburri *et al.* 1996, Webster *et al.* 2013). Certain types of chemical compounds released from bacteria (e.g. ligands), encrusting coralline algae, or conspecifics (e.g. amino acids, pheromones) can act as attractants or repellants to settling larvae (for a review see Hadfield 2011, Pawlik 1992). Detection of certain ions (e.g. cesium and potassium) is also thought to promote metamorphosis in some species, perhaps through depolarization from disrupted Na+/K+ ATPases (Hadfield *et al.* 2000, Leitz 1997). Propagules have also shown sensitivity to changes in ocean salinity (Metaxas and Young 1998) and pH, though it is difficult to disentangle whether behavioural responses to these stimuli are due to osmotic / physiological changes rather than detection via sensory machinery.

The cellular mechanisms behind the detection of chemical cues are less well-known. Specialized cellular receptors, transmembrane proteins and ion channels have all been proposed; though they can be difficult to detect because the pharmacological assays for many of these systems were designed for vertebrates, not invertebrates (Hadfield 2011, Holm *et al.* 1998). Chemical detection has also been proposed as a function of the apical cilia tufts seen in many ellipse-shaped larvae (see below for a description). Long cilia in this apical region in some annelid larvae make frequent contact with the substrate during settlement, which may facilitate olfactory sampling of benthic conditions (Hadfield 2011). Boundary-layer effects close to the substrate may enhance such sensory perception at small scales (Weissburg 2000). Bleaching of ciliated cells in the apical region also affects settlement and metamorphosis in mollusc larvae, reinforcing the hypothesis that this region could be important for detection and response to external chemical cues (Hadfield *et al.* 2000).

2.4.3. Gravisensitivity

Propagules can control their position in the water column through morphological features such as asymmetric density distribution and physical structures designed to interact with low Reynold's number conditions (Butman *et al.* 1988, Latz and Forward 1977, McCarthy *et al.* 2002, McDonald 2004, Mogami *et al.* 1988). To actively detect orientation, statocysts (small, hard structures within a fluid-filled space, Bender and Frye 2009) can be used. The statocyst settles at the lowest point in the fluid cavity when the organism changes position (Bender and Frye 2009). Such gravireceptors are present in nearly all adult metazoans and many larvae (Bender and Frye 2009).

The best described larval statocysts are found in mollusc veligers and pediveligers (O'Brien and Degnan 2003). They are similar in structure to those found in adults but are modified for the larval body form. Statocysts have also been reported in larval annelids (Smith 1989) but have not yet been detected in the larvae of echinoderms, cnidarians or poriferans, despite observations of sensitivity to orientation and depth in these groups (Brooke and Young 2005, Leys and Degnan 2001, Mogami *et al.* 1988). However, passive morphological mechanisms are not enough to explain the vertical distribution patterns and body orientation of larval echinoderms (Mogami *et al.* 1988). Adult echinoderms have statocysts and predictable righting behaviours when they are turned upside down (Ehlers 1997, Lawrence and Cowell 1996). Therefore, it is likely that echinoderm propagules possess such structures as well. Adult cnidarians possess relatively sophisticated statocysts but these have not yet been found in their larvae (Brooke and Young 2005). In contrast, orientation relative to gravity may be driven by

passive mechanisms in poriferan larvae since adults have no such structure and larvae have clear density-asymmetry in the vertical plane (Leys and Degnan 2001).

2.5. Measuring Behavioural Responses

The measurement of sensory behaviour depends on the type of stimulus and the type of information investigators are interested in. Two common ways to assess propagule behaviour are: (1) directional movement in response to stimulus (taxis; Kingsford *et al.* 2002) and (2) activity level (degree of response to stimulus; Marshall *et al.* 2003). Taxis can reveal information about preferred conditions, which has ecological implications for propagule location in the water column and settlement. Knowledge of preferred conditions can also be economically beneficial in aquaculture settings to optimize development time, cost-effectiveness and animal health (Butler IV *et al.* 2011, Gisbert and Williot 1997). Patterns of taxis in response to light (phototaxis), salinity (chemotaxis) and temperature (thermotaxis) have been well defined in some taxa, but few studies directly compare tactic behaviour among species with different life histories.

Activity level on one hand is useful for determining the magnitude of response to external cues. Measurements may include beating rate of appendages/cilia (Chan *et al.* 2013, Willows *et al.* 1997) and swimming speed (Chan *et al.* 2013, Emlet 1994, Hidu and Haskin 1978). Since sensory cells in ciliated propagules are often coupled to ciliated cells, locomotory output becomes a useful proxy for sensory response. To this effect, swimming speeds are relatively easy to quantify and are a useful starting point for intra- and inter-specific comparisons. However, the bulk of swimming capacity studies to date

have been conducted in phyla with mixed development modes (e.g. Mollusca, Annelida, Echinodermata) and have mainly been focused on propagules with planktotrophic development. Previously reported speed values for ciliated propagules range between 0.1-30.0 mm s^{-1} in Porifera (Maldonado 2006), Cnidaria (Harii *et al.* 2002, Mileikovsky 1973), Mollusca (Chia *et al.* 1984) and Echinodermata (Podolsky and Emlet 1993).

Though swimming speeds are a useful metric to assess behavioural responses, swimming trajectories (patterns) may be more informative and ecologically relevant. Swimming trajectories can be quantified via particle-tracking techniques for video recording (Chan 2012, Denoël *et al.* 2013, Faimali *et al.* 2006; see Chapter 4) or through more sophisticated methods such as particle image velocimetry, a technique that uses a "sheet" of laser light to precisely track flow patterns around moving objects (Koehl and Reidenbach 2007, Koehl and Hadfield 2010). Propagules tend to display predictable swimming patterns that can be modified in several ways (e.g. helical swimming in ciliated propagules; Cragg 1980, Jékely *et al.* 2008, McHenry 2001). The loop diameter and distance between the loops can be changed by helically-swimming larvae in response to environmental stimuli like light, salinity, and temperature (Pizarro and Thomason 2008). A study of settlement in coral planulae found that swimming trajectories were a better predictor of displacement than swimming speeds (Pizarro and Thomason 2008). Helical paths are thought to enable phototactic behaviour in propagules without complex light sensing machinery since rotations expose all sides of the body to directional stimulus (Jékely *et al.* 2008). Taken together, swimming trajectories may be more ecologically relevant than swimming speed, since modifying trajectory complexity can

alter displacement over time without the organism having to change speed (Pizarro and Thomason 2008).

2.6. Practical Applications of Sensory Behavioural Responses

2.6.1. Dispersal models

Marine propagules have commonly been considered passive particles at the mercy of ocean currents. The scale at which propagules are viewed and modelled, and the degree of focus placed on biological components, are inconsistent across the literature (Metaxas and Saunders 2009). In large-scale studies (over km), morphological and behavioural variables have sometimes been excluded in the past for the sake of model simplicity. However recent studies have shown that even at these large geographic scales, small-scale larval behaviours (over cm) have the greatest effect for ciliated, weakly swimming larvae (<1 cm s^{-1}), especially close to the benthos where benthic boundary layer effects are prominent (Robins *et al.* 2013, Wildish 2009). Propagules swimming with speeds in the range of 0.2–5 mm s^{-1} are predicted to swim autonomously in the viscous sublayer of the benthic boundary layer (Wildish 2009). In this region, even small modifications (e.g., a twofold increase) in swimming speed can significantly influence interactions with the benthos and settlement processes (Abelson and Denny 1997, Gross *et al.* 1992).

While the importance of propagule behaviour has been recognized with respect to displacement and settlement (Morgan 2014, Pringle *et al.* 2014, Robins *et al.* 2013), 40% of dispersal models from 2013 to present exclude behavioural parameters such as taxis or swimming patterns (Fig. 2.1, Appendix 2). Vertical migration behaviours were excluded

in 65% of studies; 75% did not use species-specific speed data, and 80% did not utilize settlement specificity (where applicable; Fig. 2.1). This may represent a disconnect between researchers interested in the ecological aspects of dispersal and those focused mainly on the oceanographic controls of dispersal. Efforts to incorporate propagule behaviour are also hindered by a shortage of empirical data, and by an incomplete understanding of ontogenetic changes in locomotory abilities (Robins *et al.* 2013) and of the fundamental differences among propagules with different life histories. Models are only as good as their parameters, and thus, working to merge ecological and oceanographic interests will only improve model resolution. This will be useful, especially close to the benthos, which is a focal region of interest for predictions of recruitment (Metaxas and Saunders 2009, Robins *et al.* 2013).

2.6.2. Ecotoxicology

Survival has been used as a reliable measure of exposure to environmental toxins. It is relatively easy to quantify and can be measured in a dose-dependent manner. However, the information provided by survival rates is limited at intermediate concentrations since it does not explain *why* some individuals survive while others do not (Hartmann *et al.* 2016, Parker 2016). Recently, measures of sensory responses (swimming speed and patterns) have effectively been used as an indication of stress following exposure to environmental toxins in amphibians (Bridges 1997, Chen *et al.* 2009), fishes(Chen *et al.* 2014, Floyd *et al.* 2008) and the larvae of corals (Reichelt-Brushett and Harrison 2000), mussels (Beiras and His 1995) and echinoderms (Morgana

et al. 2016). These studies indicate that the combined use of sensory behaviours and survival can provide a more holistic view of responses to unfavourable conditions. Embryos and larvae can be considered the most sensitive and susceptible life stages in the marine environment, as they often lack protective and regulatory mechanisms that exist in adults (Pechenik 1999, Strathmann 1993). Therefore, changes in propagule swimming behaviours during exposure to unfavourable conditions could be informative as to why certain individuals or populations are more resistant to toxicants than others. Since propagule behaviour is critical for dispersal and settlement patterns, disruption of these predictable patterns could have significant consequences not just in the lifetime of the organisms, but for future populations as well.

2.6.3. Climate change

Survival and morphological changes are often measured in response to climate change scenarios in the laboratory. Specific to marine environments, this can include modifications of temperature (Pörtner 2001), salinity (Richmond and Woodin 1996) and pH (Kurihara 2008), either independently or in combination. Calcifying propagules are often used for this type of research as carbonate deposition/dissolution is predicted to be affected by a changing ocean chemistry. Fewer studies have been performed on lecithotrophic and soft-bodied larval stages. One study on the lecithotrophic larvae of the sea star *Crossaster papposus* found that larvae were relatively resilient to the effects of reduced seawater pH when morphological and survival data were collected (Dupont *et al.* 2010). However, swimming patterns and other behavioural responses could have been

different than under ambient conditions. Another study on the sea cucumber *Cucumaria frondosa* found that low pH conditions compromised oogenesis and egg quality (Verkaik *et al.* 2016). The use of long-term exposure studies during egg production will be critical to understanding how lecithotrophic species may be impacted by a changing ocean, as larval energy supplies and survival in these species are driven by maternal investment.

Predictable and quantifiable behavioural responses provide a reliable baseline with which to compare responses under different climate change scenarios. Propagule interaction with altered seawater chemistry and temperature may affect normal processes like phototaxis and predator avoidance. Reduced salinity was shown to reverse the photopositivity of developing brachyuran crab larvae (Latz and Forward 1977), and reduced pH has been shown to decrease the sensitivity of larval fish to predatory olfactory cues. This makes them more susceptible to being captured during development (Dixson *et al.* 2010). The intersection of morphological and physiological stress associated with our changing oceans with shifts in behavioural patterns could have serious implications for recruitment and the long-term population ecology of benthic marine organisms, as reductions in fecundity or changes in other life-history traits can have long reaching concequences. To tackle this dilemma, studies that assess how "status quo" responses might be affected by changing conditions will need to be cross-referenced with the physiological (e.g. ion regulation, reduced ability to calcify) and biomechanical (e.g. ocean viscosity change, weaker shells) stress experienced by organisms. Testing the full spectrum of propagule types will also be important as morphological and buoyancy

differences among them may provide clues as to which species may be the most resilient to changing oceanic conditions.

2.7. References

Abelson, A., and M. Denny. 1997. Settlement of marine organisms in flow. *Annual Review of Ecology and Systematics* **28**:317-339.

Adams, E. D., G. G. Goss, and S. P. Leys. 2010. Freshwater sponges have functional, sealing epithelia with high transepithelial resistance and negative transepithelial potential. *PLoS One* **5**:e15040.

Aizenberg, J., A. Tkachenko, S. Weiner, L. Addadi, and G. Hendler. 2001. Calcitic microlenses as part of the photoreceptor system in brittlestars. *Nature* **412**:819-822.

Allen, J. D., C. Zakas, and R. D. Podolsky. 2006. Effects of egg size reduction and larval feeding on juvenile quality for a species with facultative-feeding development. *Journal of Experimental Marine Biology and Ecology* **331**:186-197.

Arendt, D., H. Hausen, and G. Purschke. 2009. The 'division of labour' model of eye evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364**:2809-2817.

Arendt, D., K. Tessmar, M.-I. M. de Campos-Baptista, A. Dorresteijn, and J. Wittbrodt. 2002. Development of pigment-cup eyes in the polychaete *Platynereis dumerilii* and evolutionary conservation of larval eyes in Bilateria. *Development* **129**:1143-1154.

Beiras, R., and E. His. 1995. Effects of dissolved mercury on embryogenesis, survival, growth of *Mytilus galloprovincialis* mussel larvae. *Marine Ecology Progress Series* **126**:185-189.

Bender, J. A., and M. A. Frye. 2009. Invertebrate solutions for sensing gravity. *Current Biology* **19**:R186-R190.

Bridges, C. M. 1997. Tadpole swimming performance and activity affected by acute exposure to sublethal levels of carbaryl. *Environmental Toxicology and Chemistry* **16**:1935-1939.

Briffa, M., and J. Greenaway. 2011. High in situ repeatability of behaviour indicates animal personality in the beadlet anemone *Actinia equina* (Cnidaria). *PLoS One* **6**:e21963.

Brooke, S., and C. Young. 2005. Embryogenesis and larval biology of the ahermatypic scleractinian *Oculina varicosa*. *Marine Biology* **146**:665-675.

Burke, R. D. 1983. The induction of metamorphosis of marine invertebrate larvae: Stimulus and response. *Canadian Journal of Zoology* **61**:1701-1719.

Butler IV, M. J., C. B. Paris, J. S. Goldstein, H. Matsuda, and R. K. Cowen. 2011. Behavior constrains the dispersal of long-lived spiny lobster larvae. *Marine Ecology Progress Series* **422**:223-237.

Butman, C. A., J. P. Grassle, and E. J. Buskey. 1988. Horizontal swimming and gravitational sinking of *Capitella* sp. I (Annelida: Polychaeta) larvae: implications for settlement. *Ophelia* **29**:43-57.

Byrne, M., Y. Nakajima, F. C. Chee, and R. D. Burke. 2007. Apical organs in echinoderm larvae: insights into larval evolution in the Ambulacraria. *Evolution & Development* **9**:432-445.

Caswell, H. 1981. The evolution of" mixed" life histories in marine invertebrates and elsewhere. *American Naturalist* **117**:529-536.

Chan, K. Y. 2012. Biomechanics of larval morphology affect swimming: Insights from the sand dollars *Dendraster excentricus*. *Integrative and Comparative Biology* **52**:458-469.

Chan, K. Y. K., H. Jiang, and D. K. Padilla. 2013. Swimming speed of larval snail does not correlate with size and ciliary beat frequency. *PLoS One* **8**:e82764.

Chen, T.-H., J. A. Gross, and W. H. Karasov. 2009. Chronic exposure to pentavalent arsenic of larval leopard frogs (*Rana pipiens*): bioaccumulation and reduced swimming performance. *Ecotoxicology* **18**:587-593.

Chen, T.-H., C.-C. Lin, and P.-J. Meng. 2014. Zinc oxide nanoparticles alter hatching and larval locomotor activity in zebrafish (*Danio rerio*). *Journal of Hazardous Materials* **277**:134-140.

Chia, F.-S., and R. Koss. 1983. Fine structure of the larval eyes of *Rostanga pulchra* (Mollusca, Opisthobranchia, Nudibranchia). *Zoomorphology* **102**:1-10.

Chia, F. S., J. Buckland, and C. M. Young. 1984. Locomotion of marine invertebrate larvae - A review. *Canadian Journal of Zoology* **62**:1205-1222.

Christiansen, F., and T. Fenchel. 1979. Evolution of marine invertebrate reproductive patterns. *Theoretical Population Biology* **16**:267-282.

Cragg, S. M. 1980. Swimming behaviour of the larvae of *Pecten maximus* (Bivalvia). *Journal Marine Biology Association of U.K.* **60**:551-564.

Degnan, S. M., and B. M. Degnan. 2010. The initiation of metamorphosis as an ancient polyphenic trait and its role in metazoan life-cycle evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**:641-651.

Denoël, M., S. Libon, P. Kestemont, C. Brasseur, J.-F. Focant, and E. De Pauw. 2013. Effects of a sublethal pesticide exposure on locomotor behavior: a video-tracking analysis in larval amphibians. *Chemosphere* **90**:945-951.

Dixson, D. L., P. L. Munday, and G. P. Jones. 2010. Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. *Ecology Letters* **13**:68-75.

Dunn, E. F., V. N. Moy, L. M. Angerer, R. C. Angerer, R. L. Morris, and K. J. Peterson. 2007. Molecular paleoecology: using gene regulatory analysis to address the origins of complex life cycles in the late Precambrian. *Evolution & Development* **9**:10-24.

Dupont, S., B. Lundve, and M. Thorndyke. 2010. Near future ocean acidification increases growth rate of the lecithotrphic larvae and juvenilles of the sea star *Crossaster papposus*. *Journal of Experimental Zoology* **314B**:382-389.

Ehlers, U. 1997. Ultrastructure of the statocysts in the apodous sea cucumber *Leptosynapta inhaerens* (Holothuroidea, Echinodermata). *Acta Zoologica* **78**:61-68.

Elliott, G. R. D., and S. P. Leys. 2010. Evidence for glutamate, GABA and NO in coordinating behaviour in the sponge, *Ephydatia muelleri* (Demospongiae, Spongillidae). *Journal of Experimental Biology* **213**:2310-2321.

Emlet, R. B. 1994. Body form and patterns of ciliation in nonfeeding larvae of echinoderms - Functional solutions to swimming in the plankton. *American Zoologist* **34**:570-585.

Faimali, M., F. Garaventa, V. Piazza, G. Greco, C. Corra, F. Magillo, M. Pittore, E. Giacco, L. Gallus, and C. Falugi. 2006. Swimming speed alteration of larvae of *Balanus amphitrite* as a behavioural end-point for laboratory toxicological bioassays. *Marine Biology* **149**:87-96.

Floyd, E. Y., J. P. Geist, and I. Werner. 2008. Acute, sublethal exposure to a pyrethroid insecticide alters behavior, growth, and predation risk in larvae of the fathead minnow (*Pimephales promelas*). *Environmental Toxicology and Chemistry* **27**:1780-1787.

Gherardi, F., L. Aquiloni, and E. Tricario. 2012. Behavioral plasticity, behavioral syndromes and animal personality in crustacean decapods: An imperfect map is better than no map. *Current Zoology* **58**:567-579.

Gisbert, E., and P. Williot. 1997. Larval behaviour and effect of the timing of initial feeding on growth and survival of Siberian sturgeon (*Acipenser baeri*) larvae under small scale hatchery production. *Aquaculture* **156**:63-76.

Gross, T. F., F. E. Werner, and J. E. Eckman. 1992. Numerical modeling of larval settlement in turbulent bottom boundary layers. *Journal of Marine Research* **50**:611-642.

Hadfield, M. G. 1986. Settlement and recruitment of marine invertebrates: A perspective and some proposals. *Bulletin of Marine Science* **39**:418-425.

Hadfield, M. G. 2011. Biofilms and marine invertebrate larvae: what bacteria produce that larvae use to choose settlement sites. *Annual Review of Marine Science* **3**:453-470.

Hadfield, M. G., E. A. Meleshkevitch, and D. Y. Boudko. 2000. The apical sensory organ of a gastropod veliger is a receptor for settlement cues. *Biological Bulletin* **198**:67-76.

Harii, S., H. Kayanne, H. Takigawa, T. Hayashibara, and M. Yamamoto. 2002. Larval survivorship, competency periods and settlement of two brooding corals, *Heliopora coerulea* and *Pocillopora damicornis*. *Marine Biology* **141**:39-46.

Hartmann, J. T., S. Beggel, K. Auerswald, B. C. Stoeckle, and J. Geist. 2016. Establishing mussel behavior as a biomarker in ecotoxicology. *Aquatic Toxicology* **170**:279-288.

Hidu, H., and H. H. Haskin. 1978. Swimming speeds of oyster larvae *Crassostrea virginica* in different salinities and temperatures. *Estuaries* **1**:252-255.

Holm, E. R., B. T. Nedved, E. Carpizo-Ituarte, and M. G. Hadfield. 1998. Metamorphic-signal transduction in *Hydroides elegans* (Polychaeta: Serpulidae) is not mediated by a G protein. *Biological Bulletin* **195**:21-29.

Ilano, A. S., K. Fujinaga, and S. Nakao. 2004. Mating, development and effects of female size on offspring number and size in the neogastropod *Buccinum isaotakii* (Kira, 1959). *Journal of Molluscan Studies* **70**:277-282.

Jablonski, D., and R. A. Lutz. 1983. Larval ecology of marine benthic invertebrates: paleobiological implications. *Biological Reviews* **58**:21-89.

Jacobs, D. K., N. Nakanishi, D. Yuan, A. Camara, S. A. Nichols, and V. Hartenstein. 2007. Evolution of sensory structures in basal metazoa. *Integrative and Comparative Biology* **47**:712-723.

Jékely, G. 2011. Origin and early evolution of neural circuits for the control of ciliary locomotion. *Philosophical Transactions of the Royal Society B: Biological Sciences* **278**:914-922.

Jékely, G., J. Colombelli, H. Hausen, K. Guy, E. Stelzer, F. Nédélec, and D. Arendt. 2008. Mechanism of phototaxis in marine zooplankton. *Nature* **456**:395-399.

Kingsford, M. J., J. M. Leis, A. Shanks, K. C. Lindeman, S. G. Morgan, and J. Pineda. 2002. Sensory environments, larval abilities and local self-recruitment. *Bulletin of Marine Science* **70**:309-340.

Koehl, M., and M. A. Reidenbach. 2007. Swimming by microscopic organisms in ambient water flow. *Experiments in Fluids* **43**:755-768.

Koehl, M. A. R., and M. G. Hadfield. 2010. Hydrodynamics of larval settlement from a larva's point of view. *Integrative and Comparative Biology* **50**:539-551.

Kurihara, H. 2008. Effects of CO2-driven ocean acidification on the early developmental stages of invertebrates. *Marine Ecology Progress Series* **373**:275-284.

Lacalli, T., T. Gilmour, and J. West. 1990. Ciliary band innervation in the bipinnaria larva of *Piaster ochraceus*. *Philosophical Transactions of the Royal Society B: Biological Sciences* **330**:371-390.

Latz, M. I., and R. B. Forward. 1977. The effect of salinity upon phototaxis and geotaxis in a larval crustacean. *Biological Bulletin* **153**:163-179.

Lawrence, J. M., and B. C. Cowell. 1996. The righting response as an indication of stress in *Stichaster striatus* (Echinodermata, Asteroidea). *Marine and Freshwater Behaviour and Physiology* **27**:239-248.

Leitz, T. 1997. Induction of settlement and metamorphosis of cnidarian larvae: signals and signal transduction. *Invertebrate Reproduction and Development* **31**:109-122.

Leys, S. P., and B. M. Degnan. 2001. Cytological basis of photoresponsive behavior in a sponge larva. *Biological Bulletin* **201**:323-338.

Maldonado, M. 2006. The ecology of the sponge larva. *Canadian Journal of Zoology* **84**:175-194.

Maldonado, M., M. Durfort, D. A. McCarthy, and C. M. Young. 2003. The cellular basis of photobehavior in the tufted parenchymella larva of demosponges. *Marine Biology* **143**:427-441.

Marlow, H., M. A. Tosches, R. Tomer, P. R. Steinmetz, A. Lauri, T. Larsson, and D. Arendt. 2014. Larval body patterning and apical organs are conserved in animal evolution. *BMC Biology* **12**:7.

Marshall, D. J., J. A. Pechenik, and M. J. Keough. 2003. Larval activity levels and delayed metamorphosis affect post-larval performance in the colonial ascidian *Diplosoma listerianum*. *Marine Ecology Progress Series* **246**:153-162.

Martel, A., and F.-S. Chia. 1991. Drifting and dispersal of small bivalves and gastropods with direct development. *Journal of Experimental Marine Biology and Ecology* **150**:131-147.

McCarthy, D. A., R. B. Forward Jr, and C. M. Young. 2002. Ontogeny of phototaxis and geotaxis during larval development of the sabellariid polychaete *Phragmatopoma lapidosa*. *Marine Ecology Progress Series* **241**:215-220.

McDonald, K. 2004. Patterns in early embryonic motility: Effects of size and environmental temperature on vertical velocities of sinking and swimming echinoid blastulae. *Biological Bulletin* **207**:93-102.

McEdward, L. R. 1986. Comparative morphometrics of echinoderm larvae I. Some relationships between egg size and initial larval form in echinoids. *Journal of Experimental Marine Biology and Ecology* **96**:251-265.

McEdward, L. R., and D. A. Janies. 1993. Life cycle evolution in Asteroids: What is a larva. *Biological Bulletin* **184**:255-268.

McHenry, M. J. 2001. Mechanisms of helical swimming: asymmetries in the morphology, movement and mechanics of larvae of the ascidian *Distaplia occidentalis*. *Journal of Experimental Biology* **204**:2959-2973.

Mercier, A., Z. Sun, and J.-F. Hamel. 2011. Internal brooding favours pre-metamorphic chimerism in a non-colonial cnidarian, the sea anemone *Urticina felina*. *Proceedings of the Royal Society of London B: Biological Sciences*:rspb20110605.

Metaxas, A., and M. Saunders. 2009. Quantifying the "bio-" components in biophysical models of larval transport in marine benthic invertebrates: Advances and pitfalls. *Biological Bulletin* **216**:257-272.

Metaxas, A., and C. M. Young. 1998. Behaviour of echinoid larvae around sharp haloclines: effects of the salinity gradient and dietary conditioning. *Marine Biology* **131**:443-459.

Mileikovsky, S. A. 1973. Speed of active movement of pelagic larvae of marine bottom invertebrates and their ability to regulate their vertical position. *Marine Biology* **23**:11-17.

Miller, S. E. 1993. Larval period and its influence on post-larval life history: Comparison of lecithotrophy and facultative planktotrophy in the aoelid nudibranch *Phestilla sibogae*. *Marine Biology* **117**:635-645.

Miner, B. G., L. A. McEdward, and L. R. McEdward. 2005. The relationship between egg size and the duration of the facultative feeding period in marine invertebrate larvae. *Journal of Experimental Marine Biology and Ecology* **321**:135-144.

Mogami, Y., C. Oobayashi, T. Yamaguchi, Y. Ogiso, and S. A. Baba. 1988. Negative geotaxis in sea urchin larvae: A possible role of mechanoreception in the late stages of development. *Journal of Experimental Biology* **137**:141-156.

Moran, A. L., and J. S. McAlister. 2009. Egg size as a life history character of marine invertebrates: Is it all it's cracked up to be? *Biological Bulletin* **216**:226-242.

Morgan, S. G. 2014. Behaviorally mediated larval transport in upwelling systems. *Advances in Oceanography* **2014**:1-18.

Morgana, S., C. Gambardella, C. Falugi, R. Pronzato, F. Garaventa, and M. Faimali. 2016. Swimming speed alteration in the early developmental stages of *Paracentrotus lividus* sea urchin as ecotoxicological endpoint. *Marine Environmental Research* **115**:11-19.

Nielsen, C. 2004. Trochophora larvae: Cell‐lineages, ciliary bands, and body regions. 1. Annelida and Mollusca. *Journal of Experimental Zoology* **302**:35-68.

Nielsen, C. 2008. Six major steps in animal evolution: are we derived sponge larvae? *Evolution & Development* **10**:241-257.

Nilsson, D.-E. 2009. The evolution of eyes and visually guided behaviour. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364**:2833-2847.

Nordstrom, K., R. Wallen, J. Seymour, and D. Nilsson. 2003. A simple visual system without neurons in jellyfish larvae. *Philosophical Transactions of the Royal Society B: Biological Sciences* **270**:2349-2354.

O'Brien, E. K., and B. M. Degnan. 2003. Expression of Pax258 in the gastropod statocyst: insights into the antiquity of metazoan geosensory organs. *Evolution & Development* **5**:572-578.

Parker, M. O. 2016. Adult vertebrate behavioural aquatic toxicology: reliability and validity. *Aquatic Toxicology* **170**:323-329.

Pawlik, J. R. 1992. Chemical ecology of the settlement of benthic marine invertebrates. *Oceanography and Marine Biology* **30**:273-335.

Pechenik, J. A. 1979. Role of encapsulation in invertebrate life histories. *American Naturalist* **114**:859-870.

Pechenik, J. A. 1999. On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. *Marine Ecology Progress Series* **177**:269-297.

Pizarro, V., and J. Thomason. 2008. How do swimming ability and behaviour affect the dispersal of coral larvae. 464-467 in *Proceedings of the 11th International Coral Reef Symposium, Fort Lauderdale, Florida, 7*.

Podolsky, R. D., and R. B. Emlet. 1993. Separating the effects of temperature and viscosity on swimming and water movement by sand dollar larvae (*Dendraster excentricus*). *Journal of Experimental Biology* **176**:207-222.

Pörtner, H. 2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* **88**:137-146.

Poulin, E., S. V. Boletzky, and J.-P. Feral. 2001. Combined ecological factors permit classification of developmental patterns in benthic marine invertebrates: A discussion note. *Journal of Experimental Marine Biology and Ecology* **257**:109-115.

Pringle, J. M., J. E. Byers, P. Pappalardo, J. P. Wares, and D. Marshall. 2014. Circulation constrains the evolution of larval development modes and life histories in the coastal ocean. *Ecology* **95**:1022-1032.

Pruitt, J. N., J. J. Stachowicz, and A. Sih. 2012. Behavioral types of predator and prey jointly determine prey survival: potential implications for the maintenance of within-species behavioral variation. *American Naturalist* **179**:217-227.

Raible, F., K. Tessmar-Raible, E. Arboleda, T. Kaller, P. Bork, D. Arendt, and M. I. Arnone. 2006. Opsins and clusters of sensory G-protein-coupled receptors in the sea urchin genome. *Developmental Biology* **300**:461-475.

Reichelt-Brushett, A. J., and P. L. Harrison. 2000. The effect of copper on the settlement success of larvae from the scleractinian coral *Acropora tenuis*. *Marine Pollution Bulletin* **41**:385-391.

Richmond, C. E., and S. A. Woodin. 1996. Short-term fluctuations in salinity: Effects on planktonic invertebrate larvae. *Marine Ecology Progress Series* **133**:167-177.

Robins, P. E., S. P. Neill, L. Giménez, S. R. Jenkins, and S. K. Malham. 2013. Physical and biological controls on larval dispersal and connectivity in a highly energetic shelf sea. *Limnology and Oceanography* **58**:505-524.

Smith, R. I. 1989. Observations on spawning behavior of *Eupolymnia nebulosa*, and comparisons with *Lanice conchilega* (Annelida, Polychaeta, Terebellidae). *Bulletin of Marine Science* **45**:406-414.

Strathmann, R. R. 1978. The evolution and loss of feeding larval stages of marine invertebrates. *Evolution* **32**:894-906.

Strathmann, R. R. 1993. Hypotheses on the origins of marine larvae. *Annual Review of Ecology and Systematics* **24**:89-117.

Strathmann, R. R. 2007. Three functionally distinct kinds of pelagic development. *Bulletin of Marine Science* **81**:167-179.

Svane, I., and P. Dolmer. 1995. Perception of light at settlement: A comparative study of two invertebrate larvae, a scyphozoan planula and a simple ascidian tadpole. *Journal of Experimental Marine Biology and Ecology* **187**:51-61.

Tamburri, M. N., C. M. Finelli, D. S. Wethey, and R. K. Zimmer-Faust. 1996. Chemical induction of larval settlement behavior in flow. *Biological Bulletin* **191**:367-373.

Thorson, G. 1949. Reproductive and larval ecology of marine bottom invertebrates. *Biological Reviews* **25**:1-45.

Tran, C., and M. G. Hadfield. 2013. Localization of sensory mechanisms utilized by coral planulae to detect settlement cues. *Invertebrate Biology* **132**:195-206.

Verkaik, K., J.-F. Hamel, and A. Mercier. 2016. Carry-over effects of ocean acidification in a cold-water lecithotrophic holothuroid. *Marine Ecology Progress Series* **557**:189-206.

Webster, N. S., S. Uthicke, E. S. Botte, F. Flores, and A. P. Negri. 2013. Ocean acidification reduces induction of coral settlement by crustose coralline algae. *Global Change Biology* **19**:303-315.

Weissburg, M. J. 2000. The fluid dynamical context of chemosensory behavior. *Biological Bulletin* **198**:188-202.

Wildish, D. 2009. Benthic boundary layer effects. *Elements of Physical Oceanography: A Derivative of the Encyclopedia of Ocean Sciences*:199.

Willows, A., G. A. Pavlova, and N. E. Phillips. 1997. Modulation of ciliary beat frequency by neuropeptides from identified molluscan neurons. *Journal of Experimental Biology* **200**:1433-1439.

2.9. Tables and figures

Table 2.1. Classification of marine propagules

Development Type	Development Location	Nutritional Mode	Protection Level	Metamorphosis Type
Direct				
Indirect	Pelagic	Planktotrophic	Free-living	Simple
	Mixed	Facultative planktotrophic	Mixed	
	Benthic	Lecithotrophic	Protected	Complex

Table 2.2. Summary of sensory detection machinery and sensory responses to external environmental cues in five phyla of marine invertebrates as described in the text

Phylum	Porifera	Cnidaria	Annelida	Mollusca	Echinodermata
Light					
Cellular mechanism[1]	Yes	Yes	Yes	Yes	Likely[3]
Behavioural response[2]	Yes	Yes	Yes	Yes	Yes
Chemical					
Cellular mechanism	Likely	Yes	Yes	Yes	Yes
Behavioural response	Yes	Yes	Yes	Yes	Yes
Positional					
Cellular mechanism	No	Likely	Yes	Yes	Likely
Behavioural response	Yes	Yes	Yes	Yes	Yes

[1] Cellular mechanism includes: specialized cellular structures and/or signal transduction pathways. 'Yes' indicates studies have confirmed the mechanism used, 'No' indicates studies have confirmed no detection structures are present for that specific cue. 'Likely' indicates cellular mechanisms likely present because structures can be found in adults and the larval behavioural responses exist

[2] Behavioural responses include changes in movement, direction and body orientation in response to sensory cues

[3] Orientation is probably controlled by passive morphological features such as asymmetric density

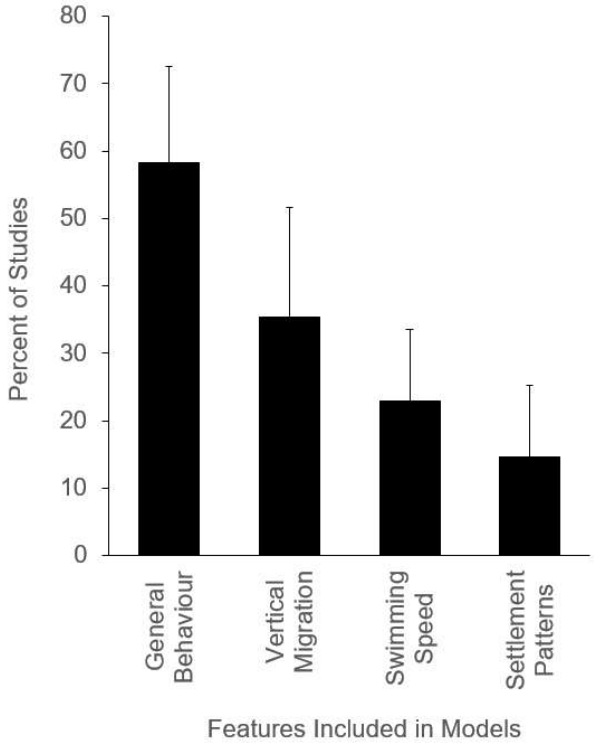


Figure 2.1. Survey of dispersal papers utilizing biophysical models from 2013-2015. Bars indicate percent of papers that included behaviour in their biophysical models ±SD (N = 9-11 papers per year). 'General behaviour' included any type of behaviour that is actively controlled by the propagule during development such as: 'vertical migration', 'swimming speed' and 'settlement patterns'.

Chapter 3. Patterns and Drivers of Egg Pigment Intensity and Colour Diversity in the Ocean: A Meta-Analysis of Phylum Echinodermata

A version of this chapter has been published in Vol 76 of the journal Advances in Marine Biology, in January 2017 (and it is featured on the cover).

3.1. Abstract

Egg pigmentation is proposed to serve numerous ecological, physiological and adaptive functions in egg-laying animals. Despite the predominance and taxonomic diversity of egg-laying animals, syntheses of the putative functions and drivers of egg pigmentation are relatively narrow in scope, centering almost exclusively on birds. Non-vertebrate and aquatic species are essentially overlooked, yet many of them produce maternally-provisioned eggs in strikingly varied colours, from pale yellow to bright red or green. Here we explore how these colour patterns correlate with behavioural, morphological, geographic and phylogenetic variables in extant classes of Echinodermata, a phylum that has close phylogenetic ties with chordates and representatives in nearly all marine environments. Results of multivariate analyses show that intensely pigmented eggs are characteristic of pelagic or external development whereas, pale eggs are commonly brooded internally. Of the five egg colours catalogued, orange and yellow are the most common. Yellow eggs are a primitive character, associated with all types of development (predominant in internal brooders), whereas green eggs are always pelagic, occur in the most derived orders of each class and are restricted to the Indo-Pacific. Orange eggs are

geographically ubiquitous and may represent a “universal” egg pigment that functions well under a diversity of environmental conditions. Finally, green occurs chiefly in class Holothuroidea and Ophiuroidea, orange in Asteroidea, yellow in Echinoidea and brown in Holothuroidea. By examining an unprecedented combination of egg colours/intensities and reproductive strategies, this phylum-wide study sheds new light on the role and drivers of egg pigmentation, drawing parallels with theories developed from the study of more derived vertebrate taxa. The primary use of pigments (of any colour) to protect externally developing eggs from oxidative damage and predation is supported by the comparatively pale colour of equally large, internally-brooded eggs. Secondarily, geographic location drives the evolution of egg colour diversity, presumably through the selection of better-adapted, more-costly pigments in response to ecological pressure.

3.2. Introduction

The most primitive and widely used reproductive strategy in the animal kingdom involves the laying of eggs (Blackburn 1999). It is exhibited by an overwhelming majority of taxa, including members of Arthropoda (insects, spiders, crustaceans), Mollusca (bivalves, gastropods), Annelida (segmented worms), Platyhelminthes (flat worms), Cnidaria (corals, sea anemones), Echinodermata (sea stars, sea urchins) and Chordata (birds, reptiles, fishes) [for a review see Blackburn 1999]. Egg-laying can follow internal fertilization (i.e. oviparity) with or without the synthesis of a protective shell (e.g. birds); or it may involve the release of unfertilized eggs (i.e. oocytes) that are fertilized externally (i.e. ovuliparity; Blackburn 1999, Lodé 2012, Ostrovsky *et al.* 2015, Wourms 1994), as seen in frogs, fishes, arthropods, and most aquatic invertebrates. A small number of terrestrial and aquatic animals incubate fertilized eggs for a period before release (i.e. ovo-viviparity; Blackburn 1999, Lodé 2012, Wourms 1994). Parental investment in progeny via these various reproductive strategies, leads to a broad range of egg phenotypes (Blount 2004, McEdward and Morgan 2001, Monaghan *et al.* 1998, Sargent *et al.* 1987). Such differences in developmental nutrition are critical from an evolutionary point of view (Ostrovsky *et al.* 2015). While egg-laying modes are particularly diverse and taxonomically widespread in the ocean, where they first evolved, our understanding of egg phenotypes in marine animals lags behind that of terrestrial animals, especially with respect to the distribution and purpose of egg colour. Interspecific variation in egg colour is particularly widely studied in avian ecology, where trade-offs may involve crypsis, mimicry, UV protection, structural integrity, and sexual

selection (Cassey *et al.* 2012, Hanley *et al.* 2015, Kilner 2006, Maurer *et al.* 2014, Svensson and Wong 2011). While the marine realm offers equally striking examples of brightly coloured eggs, the reason for this has been comparatively understudied, despite the fact it may offer valuable insights into evolutionary patterns.

Cnidarians, molluscs, annelids, teleost fishes, and echinoderms are among the most notable marine taxa to possess large oocytes/eggs ranging in colour from neon pink to dark green (e.g. Cheesman *et al.* 1967, Hamel and Mercier 1996, Lindquist and Hay 1996, McEuen 1988). Lecithotrophic (maternally provisioned, non-feeding, yolky) propagules are particularly colourful and often retain their colour, opacity and intensity until settlement (Wray 1996). In contrast, planktotrophic (feeding) propagules tend to be smaller and either transparent or faintly coloured (generally, coloured eggs in this group develop into transparent embryos and larvae). The relatively large size and bright colour of lecithotrophic oocytes could increase the risk of predation by visual predators in the pelagic environment due to enhanced visibility relative to planktotrophic propagules (Iyengar and Harvell 2001, Vaughn and Allen 2010). Despite these potential consequences, species with pelagic lecithotrophic development are common and ecologically important in temperate and polar waters, where they often co-occur with planktotrophs (Marshall *et al.* 2012, Monro and Marshall 2015, Pearse and Bosch 1994). Parental provisioning among lecithotrophs has been well studied from the perspective of energetics and nutrition, whereas other features such as egg pigmentation remain poorly understood. The early origin of pigments and maternal provisioning in the animal tree of life, and the link between bright eggs colours and lecithotrophy in many clades (e.g.

Hamel and Mercier 1996, Lindquist and Hay 1996, McEuen 1988, Ostrovsky *et al.* 2015), suggest as yet unresolved evolutionary patterns that warrant further investigation.

Pigments are known to play a variety of roles in biological systems, including plants (Alkema and Seager 1982), fishes (Losey *et al.* 1999), and bacteria (Soliev *et al.* 2011); for a review see Svensson and Wong 2011. Carotenoids are one of the most widespread and diverse pigment classes (Cheesman *et al.* 1967, Svensson and Wong 2011); they are fundamental for internal functions such as physiology, electron transport, cell signalling, and enzymatic activity (Pereira *et al.* 2014, Svensson and Wong 2011). But they also provide colouration for camouflage, sexual signals, and warning signals in animals ranging from simple invertebrates to higher vertebrates (Grether *et al.* 2001, Olson and Owens 1998, Stoehr 2006, Svensson and Wong 2011). Animals obtain carotenoids and other pigments from their diet (Grether *et al.* 2001, Svensson and Wong 2011) and modify them subsequently to generate new colours through the addition of proteins (e.g. carotenoid-protein complexes) or the overlay of multiple pigment classes, such as the stacking of carotenoids and melanin in the feathers of birds (Grether *et al.* 2001, McGraw *et al.* 2004). Yet in many species, these changes in pigmentation are extremely costly and reserved only for the most critical of processes, e.g. red pigments used for external body ornamentation and sexual selection in many species (Grether *et al.* 2001, Olson and Owens 1998).

In oocytes/eggs, pigmentation is a product of maternal investment that imparts external colouration, to prevent oxidation (from UV damage) and regulates cellular functions, and is associated with toxicity to predators in various taxa (McGraw *et al.*

2005, Nicola and Monroy-Oddo 1952, Winters *et al.* 2014). Diet composition has been shown to affect both lipid deposition and egg yolk colour in vertebrates (e.g. chickens, *Gallus gallus*; Ferrante *et al.* 2011). Egg and offspring colour can be directly influenced by maternal investment in locusts, relative to specific environmental variables (Tanaka and Maeno 2006). Brightly coloured eggs are an indicator of good maternal and offspring health in salmonid fishes (Craik 1985), and influence male mate choice in gobiids (Amundsen and Forsgren 2001). The yellow, red, and green eggs of lecithotrophic echinoderms exhibit toxicity and unpalatability in some Antarctic, North Atlantic, and North Pacific species (Iyengar and Harvell 2001, Mercier *et al.* 2013a, Sewell and Levitan 1992). These conspicuous colours have been proposed to act as aposematic (warning) colouration for visual predators like shrimps and fishes (Iyengar and Harvell 2001).

While the physiological and biochemical roles of major pigments have been well studied in most animal taxa (Svensson and Wong 2011), the ecological significance of egg colour diversity remains relatively unexplored, especially in aquatic systems and among non-vertebrate taxa. Echinodermata are well suited to phylum-wide comparisons of egg colour for several reasons. Representatives of this phylum thrive in nearly all marine habitats, and across broad latitudinal and bathymetric ranges. Furthermore, echinoderms are deuterostomes (a developmental feature shared with vertebrates) and many species produce maternally provisioned (yolky) eggs that may be free living (pelagic or benthic) or internally/externally brooded. They also display a staggering assortment of egg colours, including yellow, red, orange, green, and black. Despite the

large body of literature dedicated to reproductive strategies in echinoderms, to our knowledge the prevalence or purpose of colour diversity and intensity among their propagules is not explored beyond proposed relationships with lipid deposition and aposematic colouration (Iyengar and Harvell 2001).

Brooding and broadcast-spawning echinoderms exist in similar habitats but possess dramatically different life-history characteristics. This raises critical questions including: (1) Why are lecithotrophic propagules so brightly pigmented; and (2) is egg pigmentation in the ocean randomly distributed across phylogenies, life histories and regions? While the provenance and potential role of pigmentation has been examined in various marine species, no study has analyzed interspecific patterns to explain the exceptional diversity of their egg colours. The present study explores these questions by reviewing egg colour (including both pigment intensity and pigment colour) among lecithotrophic echinoderms and conducting a suite of multivariate analyses to test possible relationships with key biotic and abiotic variables; these include development site (parental care), egg size, egg buoyancy, adult size, phylogeny, and geographic location.

3.3. Study of Egg Metrics, and Biotic and Abiotic Factors

3.3.1. Dataset collection

A comprehensive dataset of egg colours in lecithotrophic echinoderms from all over the world was gathered from the primary literature, with complementary data obtained from Google Image Searches and academic blogs (Figure 3.1, Appendix 3A, $N =$

126 records). Because egg colour in marine taxa is not currently considered to have clear biological or ecological value, this variable is not reported consistently. Searches were therefore conducted in a hierarchical fashion, starting with broad scale ecological papers down to reports of egg colour in developmental and species-specific papers. Keywords used included egg, oocyte, colour, pigment, spawning, and the names of known lecithotrophic species. Though comprehensive, this dataset may not include all anecdotal accounts of egg colour within larger studies. Egg diameter in the full dataset ranged from 150-3400 μm and adult body size from 1-60 cm in length (or diameter in the case of radially symmetrical animals). Geographic location and phylogeny were obtained from the World Registry of Marine Species (WoRMS, last access November 2015, http://www.marinespecies.org) and the Ocean Biogeographic Information System (OBIS, last access November 2015 http://iobis.org/). As ranges of occurrence can be very broad and/or not well defined for most species, the geographic analysis centered on ocean basins instead of more precise coordinates or latitudes.

3.3.2. Standardization of variables for colour assessment

Locally accessible echinoderm species from the North Atlantic were examined to ground truth egg colour metrics in the dataset. Coastal species included the sea stars *Solaster endeca* (8-10 cm radius), *Henricia sanguinolenta* (2-5 cm) and *Crossaster papposus* (5-10 cm), and the sea cucumbers *Cucumaria frondosa* (10-15 cm contracted length) and *Psolus fabricii* (10-15 cm). They were collected by SCUBA between 10-20 m depth in southeast Newfoundland (eastern Canada). Deep-sea species included the sea

stars *Henricia lisa* (2-5 cm radius) and *Hippasteria phrygiana* (8-15 cm); they were collected aboard the CCGS *Teleost* along the continental slope (northeast Newfoundland) between 700-1450 m depth. All species and individuals were housed in 375-L tanks provided with flow-through seawater at temperatures ranging from 0-5 °C (see Mercier and Hamel 2010 for a description). Images of eggs and embryos from natural spawning were taken with an Olympus TG-2 digital camera for in-depth colour analysis (see methods below).

Where possible, egg colours listed in publications were verified with images provided in supplemental documents or through Google Image Search. Egg colours of local North Atlantic species were confirmed from natural spawning events in the laboratory. To minimize ambiguity and inaccurate descriptions in the literature, egg colours obtained from the primary literature were grouped into six main families (red, orange, brown, yellow, green, and grey; Table 3.1), and colour intensities were grouped into 3 categories (pale, standard, and bright; Table 3.1). Corresponding quantitative definitions were devised, based on an analysis of egg colour images captured during the present study, using Adobe Photoshop. Colour families were attributed a range of red ratios on the red, green, blue (RGB) additive primaries scale, whereas percent saturation was used to quantify colour intensity from pale to intense (Table 3.1). The defined ranges should be broad enough to account for any device-specific colour variations. Figure 3.2 outlines the distributions of colour families and intensities in the primary dataset.

3.3.3. Subset generation and data analysis

To tease out the drivers of egg colour intensity and diversity, subsets of the main dataset were examined. To be included in a subset, records had to be complete for all factors of interest and each factor combination had to be represented by a minimum of 3 records. Factor analysis of mixed data (FAMD) and hierarchical clustering of principal components (HCPC) were conducted using the FactoMineR package for R Statistical Software (Lê *et al.* 2008). FAMD analysis is similar to multivariate principle component analysis (PCA), but unlike PCA, FAMD combines both qualitative and quantitative variables. This makes FAMD ideal for meta-analyses of mixed variable data (Ch *et al.* 2010, Panneton *et al.* 2013).

We first tested all species in the dataset with complete records to determine general groupings based on all variables: egg colour family, egg colour intensity, egg size, development mode, ocean basin, adult size, and taxonomic class ($N = 78$, Appendix 3B). To tease out more detailed associations, we tested the hypothesis that egg colour was not randomly distributed among geographic locations, using the same subset as above without phylogeny as a factor (egg colour family, egg colour intensity, egg size, development mode, ocean basin, and adult size; $N = 78$, Appendix 3B). Thereafter, we tested whether egg buoyancy correlated with egg colour and development mode, independent of geographic location ($N = 56$, Appendix 3C). We also tested whether certain egg colours were phylogenetically linked in the four main extant classes, Echinoidea (sea urchins), Asteroidea (sea stars), Holothuroidea (sea cucumbers), and Ophiuroidea (brittle stars), independent of geographic location and development mode (*N*

= 103, Appendix 3D). HCPC trees were cut at the relative highest change in inertia, or statistical difference between the number of available groupings (Lê *et al.* 2008). Clusters were analysed using the proportion of individuals in each cluster that possessed a non-random grouping of qualitative variables and/or a non-random mean difference from the global population among tested quantitative variables; see Lê *et al.* 2008 for details. All statistical analyses were conducted at $\alpha = 0.05$.

3.4. Drivers of Egg Pigmentation Intensity and Diversity

Five egg colours were catalogued in the whole dataset (Figures 3.2-3.4) with orange and yellow being the most common (comprising 25% and 20% of species, respectively), followed by roughly equal occurrences of red (17%), brown (16%), and green (16%). Only 6% of species had grey or black eggs.

3.4.1. Overall patterns of egg colour relative to development site

Egg colour was not randomly distributed in the main dataset (summarized in Appendix 3E). Three main clusters emerged, corresponding to the three egg development sites tested here: pelagic, externally-brooded, and internally-brooded (Figure 3.5, HCPC clusters $P < 0.001$). Species with green eggs of average intensity were associated with pelagic development ($P < 0.001$). Orange egg colour was associated with externally-brooded development and pelagic development, and was characterized by bright intensity ($P < 0.001$). In contrast, internally brooding species tended to have yellow and brown eggs of pale intensity ($P < 0.001$). The following sections detail the results of the

multivariate analyses that further tease out the main patterns and drivers of egg pigmentation in lecithotrophic echinoderms.

3.4.2. Ocean basin, development mode, egg and adult size

Species with red eggs generally exhibit pelagic development (Fig. 3.6, HCPC $P < 0.001$) but showed no trend in geographic distribution or adult body size. Orange egg colour clustered with both pelagic and external-brooding development site ($P < 0.001$). Pelagic orange eggs were of average diameter and produced by species with average-sized adults whereas externally-brooded orange eggs were typically larger than average ($P = 0.005$). Orange eggs were also common in species with ubiquitous geographic distributions, independent of development mode ($P < 0.001$). In contrast, green eggs were only present in the Pacific and Indian oceans ($P < 0.001$); they were typically small in diameter ($P = 0.009$), pelagic ($P < 0.001$).

Brown and yellow egg colour was closely linked to pale pigment intensity ($P < 0.001$); they were most common in internally brooding species ($P < 0.001$) and in the Atlantic Ocean. Brown and yellow eggs were average in size but were typically produced by species with smaller than average adults ($P = 0.004$). Grey egg colour could not be tested formally as it is relatively rare among lecithotrophic echinoderms.

3.4.3. Buoyancy

There was a significant relationship among egg buoyancy, egg colour, and egg development site (Figure 3.7, HCPC $P < 0.001$, summarized in Appendix 3G). Positive buoyancy was linked with pelagic development (HCPC $P = 0.016$) and red/orange egg

colour families ($P < 0.001$). Negative buoyancy was associated with externally brooded eggs ($P < 0.001$) and with orange egg colour ($P < 0.001$). Orange egg colour was present in both buoyancy clusters consistent with the presence of two subsets of orange eggs identified above with different development sites (pelagic and externally brooded). Green and yellow egg colours did not cluster with either positive or negative buoyancy.

3.4.4. Taxonomic class

Overall, the full spectrum of egg colours (red, orange, yellow, brown, green, grey) was found in four of the five extant classes of echinoderms (Figure 3.8). The fifth class (Crinoidea) had only three representatives, and thus, could not be comprehensively analyzed. Clear patterns emerged from this phylogenetic analysis. Red and yellow egg colours appear early in the phylogeny, whereas the ability to produce green pigments appears among the most derived orders of Asteroidea, Echinoidea, Holothuroidea, and Ophiuroidea. The increase in available egg colour pigments with increasing distance from ancestral orders is also conserved within Holothuroidea, Echinoidea, and Ophiuroidea.

In addition, egg colour families were not randomly distributed across classes when analyzed independently of development mode and geographic location (Figure 3.9, HCPC $P < 0.001$, summarized in Appendix 3H). Green eggs were most common in Holothuroidea and Ophiuroidea ($P < 0.001$), orange eggs in Asteroidea ($P < 0.001$), brown eggs in Holothuroidea ($P < 0.001$) and yellow eggs in Echinoidea ($P < 0.001$).

3.5. Discussion

The pelago-benthic life cycles that exist in the aquatic realm offer a unique framework for the study of egg phenotype evolution, one that has no parallel in terrestrial systems where the mainly studied group, class Aves (birds), relies exclusively on external brooding. The study of Echinodermata is particularly valuable in this context, given the full spectrum of reproductive strategies displayed by members of this phylum and their evolutionary closeness to higher taxa (Cameron *et al.* 2000, Strathmann 2007). The present work showed that egg pigment intensities and colours are not distributed randomly across development types (e.g. pelagic, externally brooded, internally brooded), geographic locations, and phylogenies in lecithotrophic echinoderms. In interspecific comparisons, egg colour also appears to be intrinsically linked with egg size and adult size, depicting contrasting life-history strategies. These findings have major implications for our understanding of the selective pressures and constraints that may act on egg phenotypes across evolutionary and geological time. They could also find a practical use in developing identification keys for planktonic eggs in the ocean (Appendix 3I).

3.5.1. Development site explains pigment intensity but not colour diversity

Species with external fertilization and pelagic development must overcome different challenges than those faced by brooding species, including exposure of eggs to sunlight and other environmental pressures (Burgess *et al.* 2013, Gillespie and McClintock 2007). Whether free-spawned or brooded, lecithotrophic eggs/embryos obtain lipid reserves from maternal sources, and thus, do not require external nutrition

during development (Falkner *et al.* 2013, Prowse *et al.* 2008). These storage lipids (i.e. wax esters in echinoderm larvae) are susceptible to oxidative stress from oxygen free radicals, metabolites, and UV radiation (Blount 2004, Falkner *et al.* 2006, Villinski *et al.* 2002). Echinoderm adults and embryos have been previously shown to be sensitive to UV radiation, indicating that antioxidant pigments that can offset UV damages could play a role in this taxoniomic group (Häder *et al.* 2007). Pelagic lecithotrophic embryos and larvae are commonly buoyant (in ~75% of species analyzed here) and spend a portion of their development at or close to the ocean surface. Hence, floating propagules near the ocean surface presumably require more antioxidants, which could explain why nearly all pelagic, non-feeding propagules in the dataset possess intense pigments. This pigment is potentially a form of carotenoid, a pigment class found to have antioxidant function in other animals (Blount 2004, Vershinin 1999). Externally-brooded propagules also possessed intense pigmentation (~65% of species), probably because they are still exposed to some level of UV radiation and environmental fluctuation in shallow benthic environments. Apart from antioxidant activity, the brighter pigmentation of externally brooded propagules may also afford cryptic colouration (matching the adult body colour) to minimize predation during their development. This pattern is well illustrated in sea stars *Trophodiscus* sp. and among brooding cidaroid urchins, which brood larvae of a colour that matches that of the mother (Mah 2009). In contrast, internally-brooded propagules had pale brown or cream egg colours (~90% of species in dataset). This is not surprising given that internally brooded propagules are commonly not released until the feeding juvenile stage is reached, so their exposure to UV rays and free radicals is

minimal during early development. Once they begin to disperse, these juveniles could employ behavioural strategies to avoid exposure to light until the adult pigments start to develop. Taken together, the lack of intense egg pigments among internal brooders strongly suggests that bright pigments provide an adaptive value for pelagic or external development.

This dichotomy between benthic/brooded and pelagic development can be exemplified within echinoderm species that possess multiple types of eggs and larvae. The deep-sea asteroid *Henricia lisa* broods a few eggs under its body and free-spawns the remainder. The eggs and larvae produced for brooding are pale in colour relative to the propagules produced for pelagic development in *H. lisa* (Mercier and Hamel 2008a) (see insert Table 3.2). This colour difference suggests that maternal provisioning of pigments can vary across propagules of the same clutch. Evidence of deliberate alteration of larval energy reserves depending on reproductive strategy is relatively rare in echinoderms, but is common among annelids (Knott and McHugh 2012) and opisthobranchs (Krug 2009) with mixed modes of development (poecilogony). By increasing the concentration or type of pigments present in their free-spawned progeny, *Henricia* females could be enhancing offspring survival if such pigments are critical for lipid protection in the pelagic environment. The ability to deposit certain pigments may also be genetically controlled as seen in polychaete worms, where colour intensity variation is the result of selective pigment uptake from food (Sella and Marzoná 1983).

In addition to differences in pigment intensity, pelagic and brooded/benthic propagules also varied in buoyancy in the present study. Eggs that developed pelagically

could be positively, neutrally, or negatively buoyant, whereas brooded eggs were always negatively buoyant. This is not surprising, as it would be difficult, particularly for the external brooders, to keep floating eggs on or under the parent's body. In species with mixed types of development (like *Henricia* spp.), differing egg buoyancies could allow females to sort propagules at spawning. Positive egg buoyancy in echinoderms has historically been associated with lipid deposition by the mother (Emlet 1994). But the very large eggs seen in external brooders are not positively buoyant, despite major maternal yolk deposition (Emlet 1994). Such variation in buoyancy may be facilitated though manipulation of lipid to protein ratios. Some sea stars (e.g. *Meridiastra* spp.) with similar egg volumes produce eggs with different buoyancies by altering the total amount of lipid present while leaving the protein levels unchanged (Prowse *et al.* 2008). Thus, there may be an increased cost associated with production of positively buoyant eggs that explains egg size differences in species with pelagic versus brooded eggs. Overall, inter- and intraspecific variation in the intensity of egg pigmentation and egg buoyancy is clearly linked to development site in lecithotrophic echinoderms. However, these factors do not explain the broad variation in colouration seen among echinoderm eggs, suggesting that additional variables drove the evolution of egg pigment diversity in the ocean.

3.5.2. Why green? - The link between ocean basin and phylogenetic patterns of egg colour

Part of this puzzle is solved when geographic location is considered. The present study showed that echinoderm egg colours are non-randomly distributed among ocean basins. Green-coloured eggs, in particular, are exclusively associated with the Indo-Pacific, independent of latitude and clime (from temperate cold to tropical; see Mercier *et al.* 2013b for clime definitions). Presumably, species in the Pacific and Indian oceans must have been exposed to unique abiotic and/or biotic features that acted to select green egg pigments. This hypothesis is supported by evidence that green eggs may be convergent among four of the five extant echinoderm classes (the fifth being understudied). Green egg pigments are also restricted to the most derived orders of each class, inferring a more recent emergence relative to other pigments. This suggests that green pigments could require more time to emerge, consistent with the older age of the Pacific ocean basin (Larson and Chase 1972). The phylogenetic and spatial distribution of green eggs raises two main questions. Why did green evolve last in a limited number of species (what functional or ecological advantages did they gain)? What unique factor(s) in the Indo-Pacific basin drove this evolution?

The nature of phytoplankton communities might be at play since they occupy the base of the food web and animals mainly obtain pigments from vegetal and microbial sources through their diet. For instance, the relative proportions of green/brown photopigments like fucoxanthin and the distribution of diatom species that harbour them (Wright and Jeffrey 1987) differ across ocean basins, hemispheres, and geological times

(Cermeño and Falkowski 2009, Hasle 1976). A different functional source of pigment could thus contribute to the presence of green eggs in Indo-Pacific echinoderms. However, green eggs are clearly not a passive outcome of dietary pigment availability since (1) other egg colours occur in the Indo-Pacific and (2) species with red/orange eggs may co-occur and share a similar diet with green-egg producing species. Case in point, the sea star *Solaster endeca* produces red eggs in both the North Pacific and North Atlantic oceans, whereas species in this genus that are confined to the Pacific (*S. dawsoni* and *S. stimpsoni*) produce bright green eggs, even though all three species occupy similar habitats and commonly feed on the same echinoderm prey (Lambert 2000, Van Veldhuizen and Oakes 1981).

The recent evolution of green egg pigments and their predominance in the Indo-Pacific might have been driven by a physiological or functional superiority to red and yellow pigments. Blue-green eggs are common among passerine birds, even though they are potentially conspicuous to predators while in the nest (Hanley *et al.* 2008, Maurer *et al.* 2011); the more brightly coloured eggs are suggested to reflect maternal health and are involved in communication between parents (Hanley *et al.* 2008, Navarro *et al.* 2011). Increased UV protection offered by blue-green pigments is hypothesized to explain the choice of this eggshell colour (Cassey *et al.* 2012, Hanley *et al.* 2008, Maurer *et al.* 2011). If green pigments also provide enhanced UV protection to pelagic lecithotrophic eggs, over time there may be a similar shift in the colour spectrum of echinoderm eggs towards the blue-green region. In our dataset, species producing green-pigmented eggs are commonly distributed in the intertidal and shallow-water coastal regions of the Pacific

(e.g. *Cucumaria miniata*, *Cucumaria piperata* and *Meridiastra calcar*) while subtidal and deep-water species maintain red, yellow, and brown egg colours. The intertidal environment is presumably associated with increased exposure to solar radiation and predators, and this may require more efficient antioxidants but also more potent chemical deterrents. While the greater toxicity of green pigments has not been tested, green eggs were shown to be unpalatable (Sewell and Levitan 1992). Overall, while the green superiority hypothesis is attractive, it is still difficult to reconcile with the apparent absence of green eggs in the Atlantic basin, unless it reflects the lack of intertidally spawning lecithotrophs in this region (pers. obs.). Green eggs have only been reported in one lecithotrophic Atlantic species, the brittle star *Ophioderma brevispina.* This species can be found from the outer reefs of Central America to the temperate waters of Massachusetts, USA. However, *O. brevispina* was not listed as green-egged in our dataset because colour listings for this species are ambiguous, including "lemon yellow", "dark green", and "brown" that may vary among clutches and locations (Grave 1916, Hendler and Tyler 1986). If such a range in egg colours is indeed present, and not an artefact of inaccurate reporting, *O. brevispina* could represent the first parallel evolution of green egg pigments in an Atlantic species.

3.5.3. Why red and yellow? - A North Atlantic study of crypsis

Egg colours in lecithotrophic echinoderms from the North Atlantic typically range from light yellow/brown to red. Interestingly, red/orange oocytes from this region appear to match the background when viewed under blue light (Fig. 3.10); i.e. the dominant

wavelength below ~5 m in the North Atlantic. As many visual predators are highly sensitive to blue-coloured objects, the absence of blue pigments might provide crypsis against predation for red and yellow eggs while at depth in the water column (Johnsen 2005, Umbers 2013). The fact that red colouration is primarily associated with crypsis in the deep sea among diverse species is likely not a coincidence (Johnsen 2005). In the North Atlantic, there are several deep-sea species with brightly pigmented eggs including the sea star *Hippasteria phrygiana* (red-orange), the sea anemones *Hormatia* spp. (red), *Allantactis parasitica* (red) and *Urticina* sp. (orange), and the deep-sea coral *Drifa* spp. (pink) [Mercier and Hamel 2008b, Sun *et al.* 2010, Sun *et al.* 2009]. Relative colour matching in the deep sea is reportedly enough for crypsis against visual invertebrate and vertebrate predators (Johnsen 2005). Background matching may also explain the predominance of red and orange eggs among echinoderm species that release buoyant eggs in North Atlantic waters.

Similar to many biological systems, red in echinoderms eggs has historically been attributed to aposematic colouration, i.e. to signal unpalatability or chemical defenses (Iyengar and Harvell 2001, McClintock and Baker 1997). While some North Atlantic species with red-coloured eggs are known to deter predators with chemically-defended larvae (Mercier *et al.* 2013a), there are several key problems associated with the aposematic colouration hypothesis that suggest it may not be the only explanation for the ubiquity of red/orange egg pigments. Firstly, chemical defense is not restricted to red or brightly coloured eggs/larvae. Case in point, *Acanthaster planci* is a tropical sea star with transparent, planktotrophic larvae that are chemically defended against fish predation

(Lucas *et al.* 1979). Secondly, in the North Atlantic, red-coloured eggs appeared to be nearly invisible under simulated subsurface conditions in the present study (Fig. 3.10). Thus, aposematic colouration may only be useful against certain types of visual predators. This hypothesis is further confounded when predation rates by non-visual benthic suspension feeders are considered, since the unpalatable compounds in red/orange coloured eggs also deter these types of predators (Mercier *et al.* 2013a). Although this mismatch raises the critical question of why unpalatable eggs would need to be visually undetectable, the specific colours that evolved among lecithotrophic echinoderm eggs are clearly region-specific and non-random. Therefore, it is possible that the use of pigments in eggs first evolved to preserve essential lipids from oxidation, and that region-specific benefits were later actively selected for, leading to prioritization of certain pigment types (and ultimately colours) over others.

3.5.4. Egg colours in the ocean and beyond

The fact that egg colours in echinoderms are not randomly distributed relative to development site (position in the water column) and geographic location (ocean basin) echoes findings reported in other taxa; although, the scope of the present study is unparalleled (phylum-wide and worldwide). Mollusc eggs that are laid in shallow waters can be bright red, orange, or green. While this intriguing diversity was deemed worthy of further study nearly 50 years ago (Cheesman *et al.* 1967), apparently the question was not pursued. The range of pigments reported in mollusc eggs exposed to solar radiation is strikingly similar to the colour palette seen here among pelagic lecithotrophic eggs in

echinoderms. The presence of these specific colours in the marine environment supports the hypothesis that the use of certain pigments may be convergent among marine animals facing strong selective pressures to protect exposed eggs.

The association between egg colour and life history in the phylum Echinodermata is also surprisingly similar to that reported in more derived taxa such as birds (Aves, Chordata), but with a key difference. Eggs like those seen in Echinodermata consist of yolk surrounded by a thin membrane. Maternal investment, is therefore, consolidated into these yolk packages, inherently constraining physiological and defensive functions of pigments. The evolution of external shells in terrestrial animals like birds resulted in a two-part protection system that may be independently manipulated relative to maternal condition, sexual selection, and environmental conditions (Cassey *et al.* 2012, Maurer *et al.* 2011, Maurer *et al.* 2014). Like oocytes of basal echinoderms, the yolk of bird eggs is coloured yellow from maternal deposition of antioxidants such as carotenoids. In birds, it is the shell that saw an evolution of colour diversity, from pure white to blue-green, through the use of other pigments such as biliverdin (Navarro *et al.* 2011). White characterizes the eggshells of ancestral birds and of species that brood in cavities (i.e. not exposed to intense UV radiations), illustrating their weaker need for protective pigments (Kilner 2006, Lack 1958, Maldowie 1886). This parallels the predominance of weakly pigmented (pale) eggs among internally brooding echinoderms in the present study. As for extant egg colour diversity, whether broad geographic or phylogenetic trends echoing the ones evidenced here also occur in birds is still not fully understood. This is because the literature typically focuses on the nesting ecology of discrete species/populations and

syntheses are uncommon. In one of the rare class-wide reviews, Kilner 2006 hypothesized that the diversity of eggshell colour and patterning in birds was largely driven by a co-evolutionary arms race around brood parasitism (i.e. to make eggs more or less conspicuous, depending on the perspective).

3.6. Future Directions

Taken together with results reported previously in more derived taxa, the findings of the present study suggest that increasing complexity in egg colour patterns may represent an evolutionary trend in reproductive traits that emerged as animals shifted from a predominantly r-selected type of egg production (planktotrophy), involving millions or thousands of eggs, to a K-type model, where fewer offspring are produced (lecithotrophy). A quantitative assessment of egg colour patterns that further transcends the major boundaries in animal evolution would be a valuable step forward in deciphering the origins and adaptive value of egg colour in aquatic and terrestrial systems. For instance, primitive animals like sea anemones and corals (phylum Cnidaria) also produce brilliantly coloured, non-feeding larvae but the ecological value of their pigments has not yet been explicitly explored. Key hypotheses to test across systems and taxa include: (1) egg pigmentation increases and diversifies as fecundity decreases, (2) pigment intensity is a function of exposure to UV and/or other sources of lipid oxidation, (3) selection of certain pigments over others is a function of ecological or energetic benefits, and (4) pigment intensity and colour can be directly manipulated by the level of maternal investment. Such findings would be invaluable to our understanding of how parental

investment can be tailored to fit the needs of the offspring of egg-laying taxa. If nothing else, future studies on reproductive biology and ecological trade-offs should pay closer attention to egg colour measurements and definitions, as egg pigments clearly are more than aesthetic.

3.7. Summary and Conclusions

While egg-laying is widespread in the animal kingdom and marine species with maternally-provisioned development produce some of the most strikingly coloured eggs, knowledge on the roles and putative drivers of egg pigmentation largely focuses on a small number of brooding avian taxa. Analyses and syntheses over broad taxonomic and geographic scales are wanting. The present phylum-wide study of Echinodermata exhibiting diverse life histories reveals that the colour, buoyancy, egg size, adult size and development site of eggs are generally linked within reproductive and life-history strategies. Yellow emerges as the primitive egg colour and is still the most common in internal brooders, while green eggs occur only in the most derived orders of each class and are restricted to the Indo-Pacific basin. The more intense pigmentation of pelagic and externally-brooded eggs compared to internally-brooded eggs of similar size strongly supports the hypothesis that pigmentation is actively selected for to protect propagules against UV radiation and, possibly, visual predators. Egg colours diversified from the ancestral yellow in response to local environmental and/or ecological pressures, through a selection for better-adapted yet more costly pigments (red/orange and green), yielding defined geographic and phylogenetic colour patterns. Further quantitative assessments of

egg colours and pigment types over broad scales could be used to determine whether and how fecundity and external pressures can mediate the nature and amount of maternal investment into egg pigmentation. The selective advantages of red and green pigments also need to be explored.

3.8. Acknowledgements

This work was supported by a doctoral scholarship from the Natural Sciences and Engineering Research Council of Canada (NSERC CGS-D) to E. Montgomery and by grants from NSERC and the Canadian Foundation for Innovation (CFI) to A. Mercier. The authors wish to thank M. Byrne (U. Sydney) for providing egg colour records from Australasia. The authors also wish to thank the anonymous reviewers for their constructive comments and suggestions. The crew of the CCGS *Teleost* and Memorial University field services are also acknowledged for their assistance and animal collections.

3.9. References

Alkema, J., and S. L. Seager. 1982. The chemical pigments of plants. *Journal of Chemical Education* **59**:183-186.

Amundsen, T., and E. Forsgren. 2001. Male mate choice selects for female coloration in a fish. *Proceedings of the National Academy of Sciences of the United States of America* **98**:13155-13160.

Blackburn, D. G. 1999. Viviparity and oviparity: evolution and reproductive strategies. Pp. 994-1003 in *Encyclopedia of Reproduction* Academic Press, New York, New York, USA.

Blount, J. D. 2004. Carotenoids and life-history evolution in animals. *Archives of Biochemistry and Biophysics* **430**:10-15.

Burgess, S. C., M. Bode, and D. J. Marshall. 2013. Costs of dispersal alter optimal offspring size in patchy habitats: combining theory and data for a marine invertebrate. *Functional Ecology* **27**:757-765.

Cameron, C. B., J. R. Garey, and B. J. Swalla. 2000. Evolution of the chordate body plan: New insights from phylogenetic analyses of deuterostome phyla. *Proceedings of the National Academy of Sciences of the United States of America* **97**:4469-4474.

Cassey, P., G. H. Thomas, S. J. Portugal, G. Maurer, M. E. Hauber, T. Grim, P. G. Lovell, and I. MikŠÍK. 2012. Why are birds' eggs colourful? Eggshell pigments co ‐ vary with life ‐ history and nesting ecology among British breeding non ‐ passerine birds. *Biological Journal of the Linnean Society* **106**:657-672.

Cermeño, P., and P. G. Falkowski. 2009. Controls on diatom biogeography in the ocean. *Science* **325**:1539-1541.

Ch, P., C. Andrés, M. C. Vergara Holguín, and G. A. Ligarreto Moreno. 2010. Clasificación de 42 líneas mejoradas de arveja (*Pisum sativum* L.) por caracteres morfológicos y comportamiento agronómico. *Revista Facultad Nacional de Agronomía* **63**:5543-5553.

Cheesman, D. F., W. L. Lee, and P. F. Zagalsky. 1967. Carotenoproteins in invertebrates. *Biological Reviews* **42**:131-160.

Craik, J. C. A. 1985. Egg quality and egg pigment content in salmonid fishes. *Aquaculture* **47**:61-88.

Emlet, R. B. 1994. Body form and patterns of ciliation in nonfeeding larvae of echinoderms - Functional solutions to swimming in the plankton. *American Zoologist* **34**:570-585.

Falkner, I., S. Barbosa, and M. Byrne. 2013. Reproductive biology of four ophiocomid ophiuroids in tropical and temperate Australia - reproductive cycle and oogenic strategies in species with different modes of development. *Invertebrate Reproduction and Development* **57**:189-199.

Falkner, I., M. Byrne, and M. A. Sewell. 2006. Maternal provisioning in *Ophionereis fasciata* and *O. schayeri*: brittle stars with contrasting modes of development. *Biological Bulletin* **211**:204-207.

Ferrante, V., D. Baroli, S. Marelli, M. G. Mangiagalli, and L. G. Cavalchini. 2011. Effect of tomato by-product diet supplementation on egg yolk colour. *Italian Journal of Animal Science* **2**:459-461.

Gillespie, J. M., and J. B. McClintock. 2007. Brooding in echinoderms: How can modern experimental techniques add to our historical perspective? *Journal of Experimental Marine Biology and Ecology* **342**:191-201.

Grave, C. 1916. *Ophiura brevispina* II. An embryological contribution and a study of the effect of yolk substance upon development and developmental processes. *Journal of Morphology* **27**:413-451.

Grether, G. F., J. Hudon, and J. A. Endler. 2001. Carotenoid scarcity, synthetic pteridine pigments and the evolution of sexual coloration in guppies (*Poecilia reticulata*). *Proceedings of the Royal Society of London. Series B: Biological Sciences* **268**:1245-1253.

Häder, D.-P., H. Kumar, R. Smith, and R. Worrest. 2007. Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. *Photochemical & Photobiological Sciences* **6**:267-285.

Hamel, J. F., and A. Mercier. 1996. Early development, settlement, growth, and spatial distribution of the sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea). *Canadian Journal of Fisheries and Aquatic Sciences* **53**:253-271.

Hanley, D., T. Grim, P. Cassey, and M. E. Hauber. 2015. Not so colourful after all: eggshell pigments constrain avian eggshell colour space. *Biology Letters* **11**.

Hanley, D., G. Heiber, and D. C. Dearborn. 2008. Testing an assumption of the sexual-signaling hypothesis: does blue-green egg color reflect maternal antioxidant capacity. *The Condor* **110**:767-771.

Hasle, G. R. 1976. The biogeography of some marine planktonic diatoms. 319-IN316 in *Deep Sea Research and Oceanographic Abstracts* Elsevier.

Hendler, G., and P. A. Tyler. 1986. The reproductive cycle of *Ophioderma brevispinum* (Echinodermata: Ophiuroidea). *Marine Ecology* **7**:115-122.

Iyengar, E. V., and C. D. Harvell. 2001. Predator deterrence of early developmental stages of temperate lecithotrophic asteroids and holoturoids. *Journal of Experimental Marine Biology and Ecology* **264**:171-188.

Johnsen, S. 2005. The red and the black: bioluminescence and the color of animals in the deep sea. *Integrative and Comparative Biology* **45**:234-246.

Kilner, R. M. 2006. The evolution of egg colour and patterning in birds. *Biological Reviews* **81**:383-406.

Knott, K. E., and D. McHugh. 2012. Introduction to symposium: poecilogony-A window on larval evolutionary transitions in marine invertebrates. *Integrative and Comparative Biology* **52**:120-127.

Krug, P. J. 2009. Not my "type": Larval dispersal dimorphisms and bet-hedging in opisthobranch life histories. *Biological Bulletin* **216**:355-372.

Lack, D. 1958. The significance of the colour of turdine eggs. *Ibis* **100**:145-166.

Lambert, P. 2000. *Sea stars of British Columbia, Southeast Alaska, and Puget Sound*. UBC Press, Vancouver.

Larson, R. L., and C. G. Chase. 1972. Late Mesozoic evolution of the western Pacific Ocean. *Geological Society of America Bulletin* **83**:3627-3644.

Lê, S., J. Josse, and F. Husson. 2008. FactoMineR: an R package for multivariate analysis. *Journal of Statistical Software* **25**:1-18.

Lindquist, N., and M. E. Hay. 1996. Palatability and chemical defense of marine invertebrate larvae. *Ecological Monographs*:431-450.

Lodé, T. 2012. Oviparity or viviparity? That is the question…. *Reproductive Biology* **12**:259-264.

Losey, G. S., T. W. Cronin, T. H. Goldsmith, D. Hyde, N. J. Marshall, and W. N. McFarland. 1999. The UV visual world of fishes: A review. *Journal of Fish Biology* **54**:921-943.

Lucas, J. S., R. J. Hart, M. E. Howden, and R. Salathe. 1979. Saponins in eggs and larvae of *Acanthaster planci* (L.)(Asteroidea) as chemical defences against planktivorous fish. *Journal of Experimental Marine Biology and Ecology* **40**:155-165.

Mah, C. L. 2009. The hidden treasure of *Trophodiscus*. in *The Echinoblog*.

Maldowie, A. M. 1886. Development and decay of the pigment layer in birds' eggs. *Journal of Anatomy and Physiology* **20**:225.

Marshall, D. J., P. J. Krug, E. K. Kupriyanova, M. Byrne, and R. B. Emlet. 2012. The biogeography of marine invertebrate life histories. *Annual Review of Ecology and Systematics* **43**:97-114.

Maurer, G., S. J. Portugal, and P. Cassey. 2011. Review: An embryo's eye view of avian eggshell pigmentation. *Journal of Avian Biology* **42**:494-504.

Maurer, G., S. J. Portugal, M. E. Hauber, I. Mikšík, D. G. D. Russell, and P. Cassey. 2014. First light for avian embryos: Eggshell thickness and pigmentation mediate variation in development and UV exposure in wild bird eggs. *Functional Ecology* **29**:209-218.

McClintock, J. B., and B. J. Baker. 1997. Palatability and chemical defense of eggs, embryos and larvae of shallow-water antarctic marine invertebrates. *Marine Ecology Progress Series* **154**:121-131.

McEdward, L. R., and K. H. Morgan. 2001. Interspecific relationships between egg size and the level of parental investment per offspring in echinoderms. *Biological Bulletin* **200**:33-50.

McEuen, F. S. 1988. Spawning behaviors of northeast Pacific sea cucumbers (Holothuroidea: Echinodermata). *Marine Biology* **98**:565-585.

McGraw, K. J., E. Adkins-Regan, and R. S. Parker. 2005. Maternally derived carotenoid pigments affect offspring survival, sex ratio, and sexual attractiveness in a colorful songbird. *Naturwissenschaften* **92**:375-380.

McGraw, K. J., K. Wakamatsu, S. Ito, P. M. Nolan, P. Jouventin, F. S. Dobson, R. E. Austic, R. J. Safran, L. M. Siefferman, and G. E. Hill. 2004. You can't judge a pigment by its color: Carotenoid and melanin content of yellow and brown feathers in swallows, bluebirds, penguins, and domestic chickens. *The Condor* **106**:390-395.

Mercier, A., E. J. Doncaster, and J.-F. Hamel. 2013a. Contrasting predation rates on planktotrophic and lecithotrophic propagules by marine benthic invertebrates. *Journal of Experimental Marine Biology and Ecology* **449**:100-110.

Mercier, A., and J.-F. Hamel. 2008a. Depth-related shift in life history strategies of a brooding and broadcasting deep-sea asteroid. *Marine Biology* **156**:205-223.

Mercier, A., and J.-F. Hamel. 2008b. Nature and role of newly described symbiotic associations between a sea anemone and gastropods at bathyal depths in the NW Atlantic. *Journal of Experimental Marine Biology and Ecology* **358**:57-69.

Mercier, A., and J.-F. Hamel. 2010. Synchronized breeding events in sympatric marine invertebrates: Role of behavior and fine temporal windows in maintaining reproductive isolation. *Behavioral Ecology and Sociobiology* **64**:1749-1765.

Mercier, A., M. A. Sewell, and J.-F. Hamel. 2013b. Pelagic propagule duration and developmental mode: Reassessment of a fading link. *Global Ecology and Biogeography* **22**:517-530.

Monaghan, P., R. G. Nager, and D. C. Houston. 1998. The price of eggs: increased investment in egg production reduces the offspring rearing capacity of parents. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **265**:1731-1735.

Monro, K., and D. J. Marshall. 2015. The biogeography of fertilization mode in the sea. *Global Ecology and Biogeography* **24**:1499-1509.

Navarro, C., T. Pérez-Contreras, J. M. Avilés, K. J. McGraw, and J. J. Soler. 2011. Blue-green eggshell coloration reflects yolk antioxidant content in spotless starlings *Sturnus unicolor*. *Journal of Avian Biology* **42**:538-543.

Nicola, M., and A. Monroy-Oddo. 1952. The distribution of carotenoids in the eggs, ovaries and testicles of *Paracentrotus lividus*. *Cellular and Molecular Life Sciences* **8**:187-189.

Olson, V. A., and I. P. F. Owens. 1998. Costly sexual signals: are carotenoids rare, risky or required? *Trends in Ecology & Evolution* **13**:510-514.

Ostrovsky, A. N., S. Lidgard, D. P. Gordon, T. Schwaha, G. Genikhovich, and A. V. Ereskovsky. 2015. Matrotrophy and placentation in invertebrates: a new paradigm. *Biological Reviews*:n/a-n/a.

Panneton, B., A. Clément, and L. Lagacé. 2013. Potential of fluorescence spectroscopy for the characterisation of maple syrup flavours. *Journal of the Science of Food and Agriculture* **93**:3279-3285.

Pearse, J. S., and I. Bosch. 1994. Brooding in the Antarctic: Östergren had it nearly right. Pp. 111-120 in *Echinoderms Through Time*, A. Balkema, ed. Rotterdam.

Pereira, D. M., P. Valentão, and P. B. Andrade. 2014. Marine natural pigments: Chemistry, distribution and analysis. *Dyes and Pigments* **111**:124-134.

Prowse, T. A. A., M. A. Sewell, and M. Byrne. 2008. Fuels for development: evolution of maternal provisioning in asterinid sea stars. *Marine Biology* **153**:337-349.

Sargent, R. C., P. D. Taylor, and M. R. Gross. 1987. Parental care and the evolution of egg size in fishes. *American Naturalist* **129**:32-46.

Sella, G., and M. Marzoná. 1983. Inheritance, maternal influence and biochemical analysis of an egg color polymorphism in *Ophryotrocha diadema*. *Experientia* **39**:97-98.

Sewell, M. A., and D. R. Levitan. 1992. Fertilization success during a natural spawning of the dendrochirote sea cucumber *Cucumaria miniata*. *Bulletin of Marine Science* **51**:161-166.

Soliev, A. B., K. Hosokawa, and K. Enomoto. 2011. Bioactive pigments from marine bacteria: applications and physiological roles. *Evidence-Based Complementary and Alternative Medicine* **2011**.

Stoehr, A. M. 2006. Costly melanin ornaments: the importance of taxon? *Functional Ecology* **20**:276-281.

Strathmann, R. R. 2007. Three functionally distinct kinds of pelagic development. *Bulletin of Marine Science* **81**:167-179.

Sun, Z., J.-F. Hamel, and A. Mercier. 2010. Planulation periodicity, settlement preferences and growth of two deep-sea octocorals from the northwest Atlantic. *Marine Ecology Progress Series* **410**:71-87.

Sun, Z., J. F. Hamel, and A. Mercier. 2009. Planulation of deep-sea octocorals in the NW Atlantic. *Coral Reefs* **28**:781-781.

Svensson, P. A., and B. B. M. Wong. 2011. Carotenoid-based signals in behavioural ecology: A review. *Behaviour* **148**:131-189.

Tanaka, S., and K. Maeno. 2006. Phase-related body-color polyphenism in hatchlings of the desert locust, *Schistocerca gregaria*: Re-examination of the maternal and crowding effects. *Journal of Insect Physiology* **52**:1054-1061.

Umbers, K. D. L. 2013. On the perception, production and function of blue colouration in animals. *Journal of Zoology* **289**:229-242.

Van Veldhuizen, H. D., and V. J. Oakes. 1981. Behavioral-responses of 7 species of asteroids to the asteroid predator, *Solaster dawsoni*. *Oecologia* **48**:214-220.

Vaughn, D., and J. D. Allen. 2010. The peril of the plankton. *Integrative and Comparative Biology* **50**:552-570.

Vershinin, A. 1999. Biological functions of carotenoids‐diversity and evolution. *Biofactors* **10**:99-104.

Villinski, J. T., J. C. Villinski, M. Byrne, and R. A. Raff. 2002. Convergent maternal provisioning and life‐history evolution in echinoderms. *Evolution* **56**:1764-1775.

Winters, A. E., M. Stevens, C. Mitchell, S. P. Blomberg, and J. D. Blount. 2014. Maternal effects and warning signal honesty in eggs and offspring of an aposematic ladybird beetle. *Functional Ecology*.

Wourms, J. P. 1994. The challenges of piscine viviparity. *Israel Journal of Zoology* **40**:551-568.

Wray, G. A. 1996. Parallel evolution of nonfeeding larvae in echinoids. *Systematic Biology* **45**:308-322.

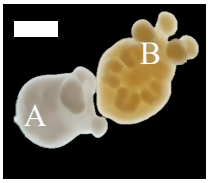
Wright, S. W., and S. W. Jeffrey. 1987. Fucoxanthin pigment markers of marine phytoplankton analysed by HPLC and HPTLC. *Marine Ecology Progress Series* **38**:259-266.

3.10. Tables

Table 3.1. Colour groupings and intensity categories with their corresponding qualitative descriptors in the literature. Each colour family is defined by a range of red content, based on the percent ratio of red to total red and green (R/R+G) on the RGB scale. Intensity is defined as percent colour saturation.

Colour Group	**Qualitative Descriptors**	**R / (R+G) (%)**	**Colour Intensity**	**Qualitative Descriptors**	**Saturation (%)**
Red	Red Pink Purple	100-75	Pale	Pale Dull Light	<50
Orange	Orange	74-55	Standard	No modifier listed	50-70
Brown	Brown Cream	74-40	Bright	Bright Intense Dark	>70
Yellow	Yellow	54-40	--	--	--
Green	Green	0-39	--	--	--
Grey	Grey White Black	--	--	--	--

Table 3.2. Within-species colour variation is a function of life history and buoyancy [positive = (+), negative = (-)] in two species of sea star. The insert photo shows the brachiolaria larvae of the sea star *Henricia lisa*. These larvae were spawned during the same event; the pale grey one (A) was brooded and the bright yellow one (B) broadcasted in the water column. Scale bar represents 1 mm.

Species	**Development Site**	**Egg Colour**	
Henricia lisa	Brooded (-)	Pale Grey (A)	
	Pelagic	Bright Yellow (B)	
Echinaster echinophorus	Pelagic (-)	Orange	
	Pelagic (+)	Black	

3.11. Figures

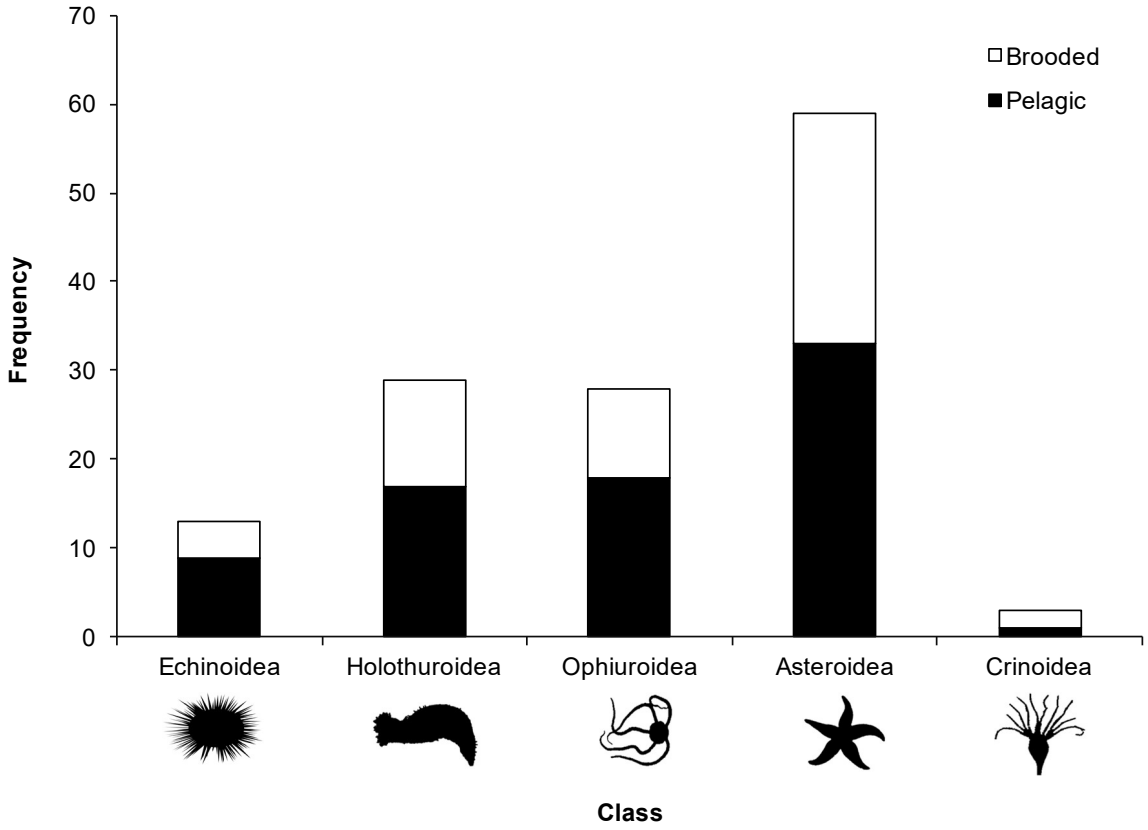


Figure 3.1. Distribution of echinoderm classes and development sites (brooded/benthic vs. pelagic) in the dataset (Appendix A). Frequency indicates number of species (total N = 126 records).

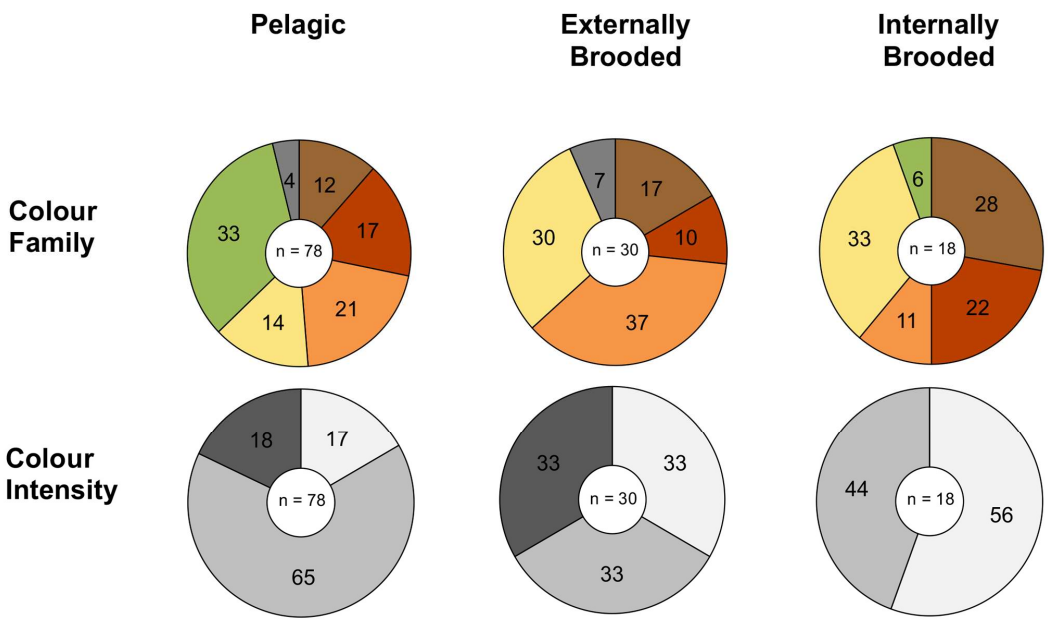


Figure 3.2. Percent distribution of egg colour family and intensity in the full dataset (Appendix A) sorted by development site: pelagic, externally brooded, and internally brooded. Shades of egg colour families (red, orange, brown, yellow, green, grey) are represented as closely as possible in the upper panels. Colour intensities from dark to pale are shown on a grey scale in the lower panels. Sample size is provided in the center of each pie. (total $N = 126$ records).

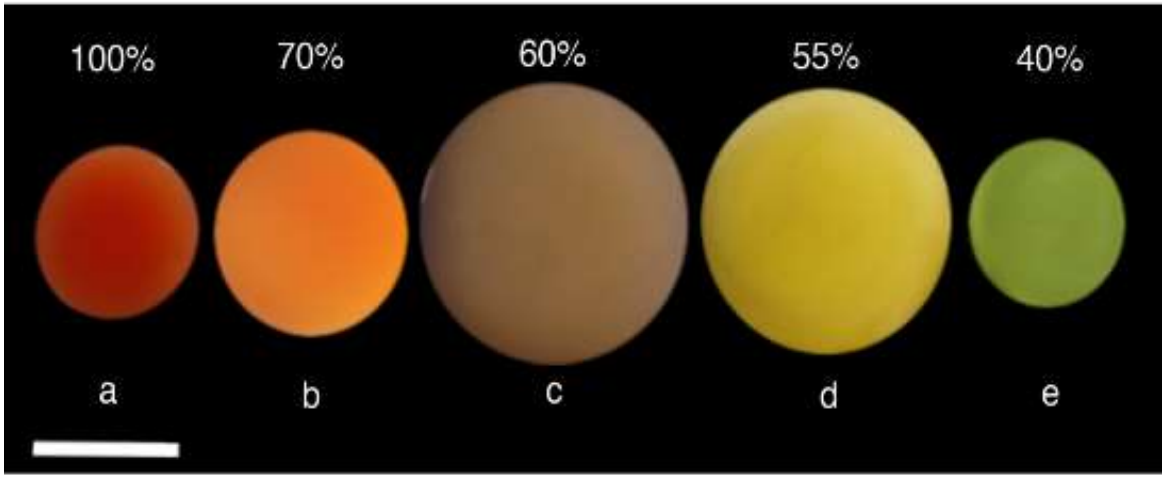


Figure 3.3. Egg colour and size diversity in lecithotrophic echinoderms. a) *Crossaster papposus* (freshly spawned egg). b) *Cucumaria frondosa* (freshly spawned egg). c) *Henricia lisa* (freshly spawned egg collected from under mother). d) *Pteraster abyssorum* (from live 60-celled embryo of size/colour consistent with egg). e) *Cucumaria miniata* (composite image from several photos, scaled to size). Percent values represent percent red ratio [R/(R+G) ratio; see Table 1 for method]. Scale bar represents 500 μm. Note that egg sizes shown here do not illustrate the general relationship between egg colour and egg size in the full dataset.

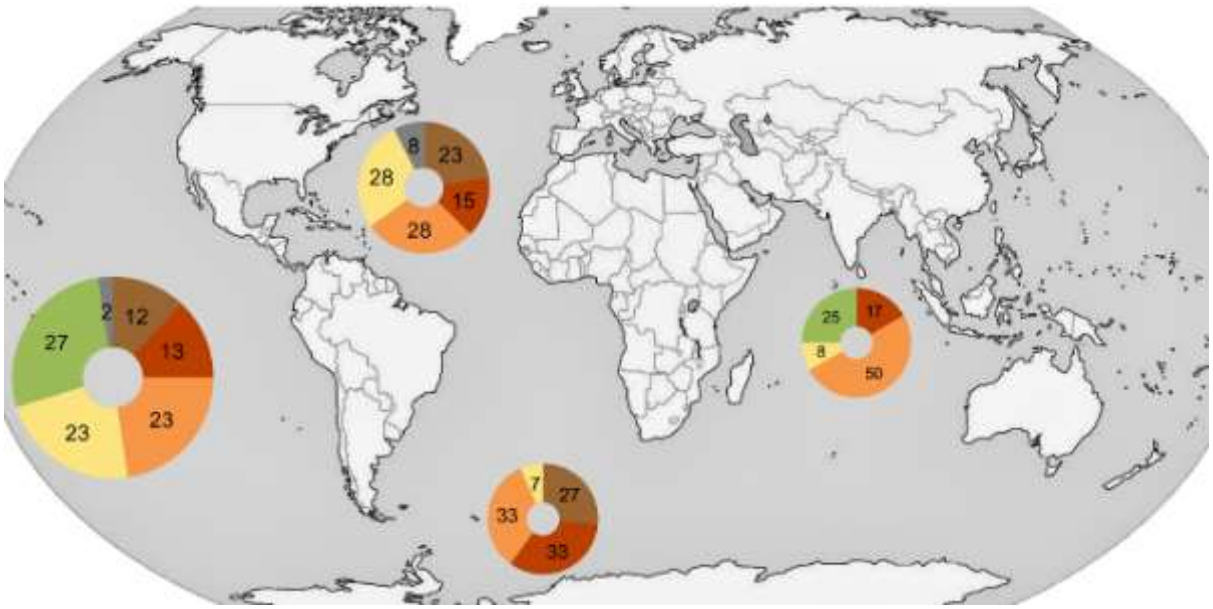


Figure 3.4. Global distribution of egg colour families in lecithotrophic echinoderms. Numbers indicate percent (%) of species with eggs of each colour (red, orange, brown, yellow, green, grey) found in the corresponding ocean basin. Distribution data obtained from OBIS. Species with cosmopolitan distributions are included in all relevant ocean basins. Size of pies reflects relative number of records (Appendix A, $N = 126$).

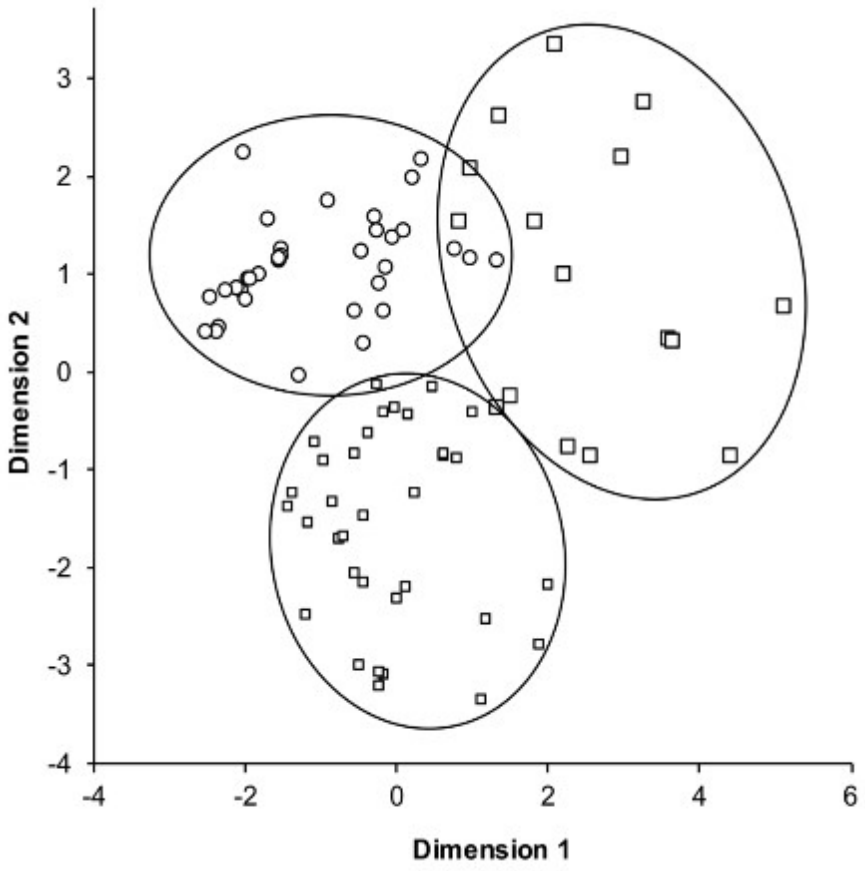


Figure 3.5. Relationship among egg colour, development mode, phylogeny, and ocean basin in lecithotrophic echinoderms. Dimension 1 = FAMD component with greatest variance. Dimension 2 = FAMD component with 2nd greatest variance. Symbol shape indicates developmental mode: circle = pelagic, large square = externally brooded, small square = internally brooded (N = 87, Appendix E).

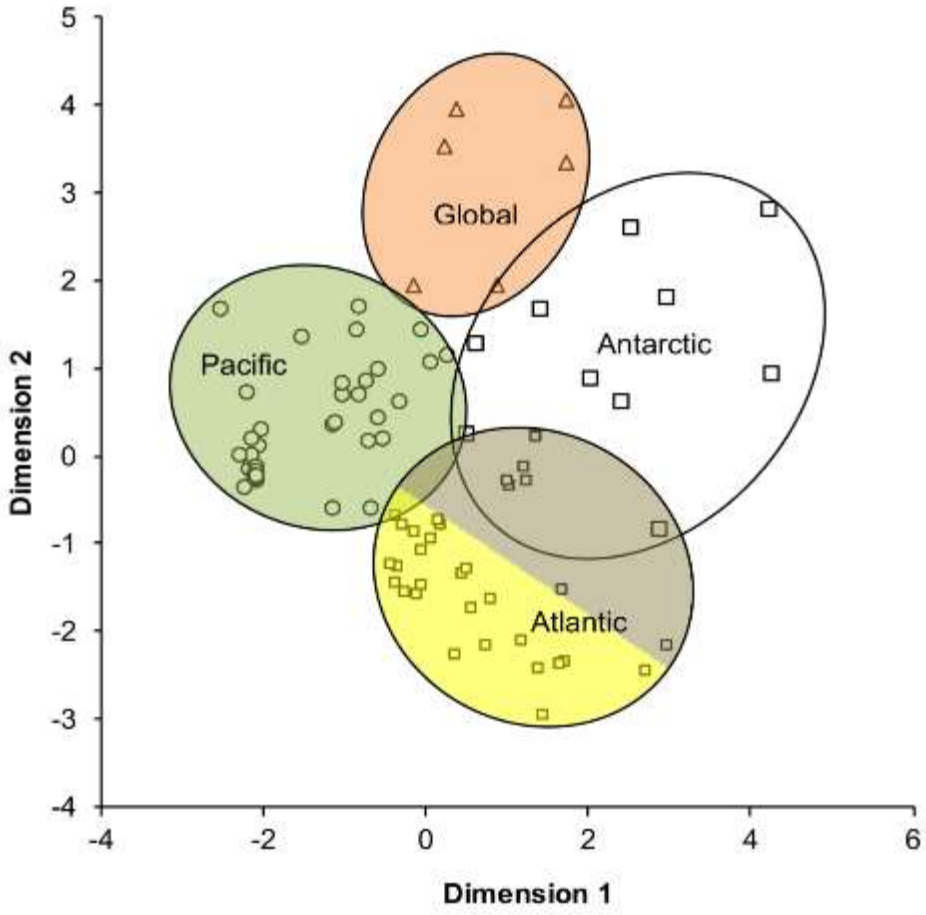


Figure 3.6. Relationship among egg colour, development mode, and ocean basin in lecithotrophic echinoderms. Dimension 1 = FAMD component with greatest variance. Dimension 2 = FAMD component with 2nd greatest variance. Colour of each ellipse reflects predominate egg colour family/ies (orange, yellow, green, brown). Symbol shape indicates developmental mode: circle = pelagic, large square = externally brooded, small square = internally brooded, triangle = no associated developmental mode. Test indicates associated ocean basin (global = distribution in Atlantic and Pacific, including Indo-Pacific, Ocean basins; *N* = 87, Appendix F).

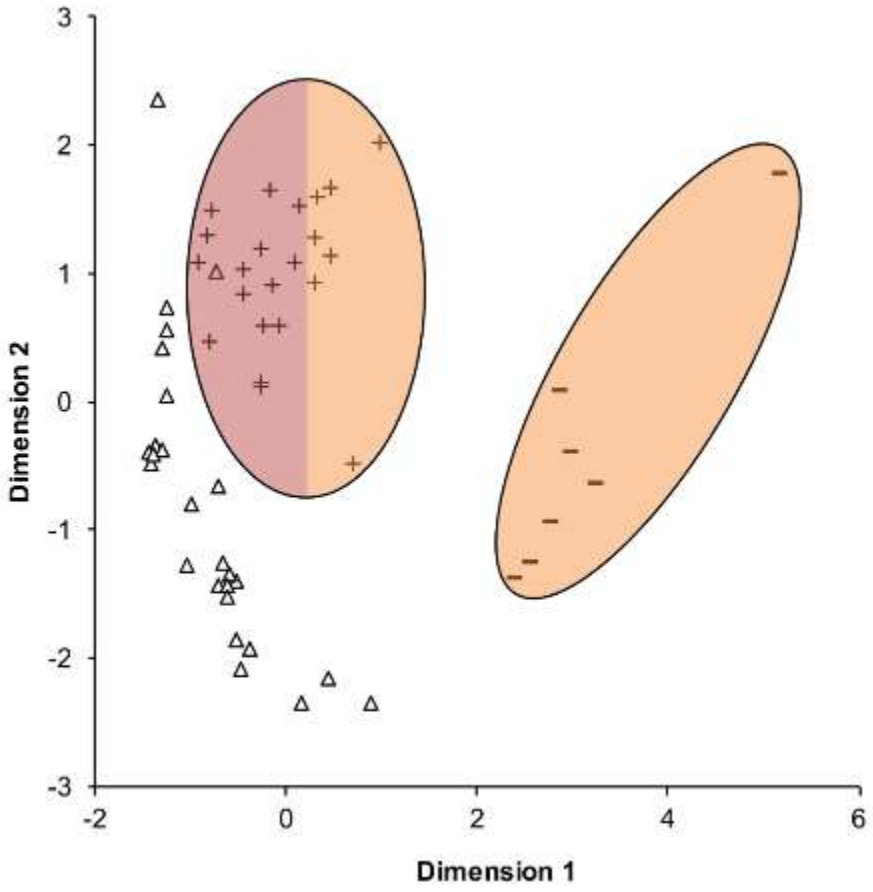


Figure 3.7. Relationship between egg colour, development mode and egg buoyancy in lecithotrophic echinoderms. Dimension 1 = FAMD component with greatest variance. Dimension 2 = FAMD component with 2nd greatest variance. Colour of each ellipse reflects predominate egg colour family/ies (red, orange). Symbol shape indicates buoyancy: - = negative buoyancy, + = positive buoyancy, and triangle = no clustered buoyancy (N = 56, Appendix G).

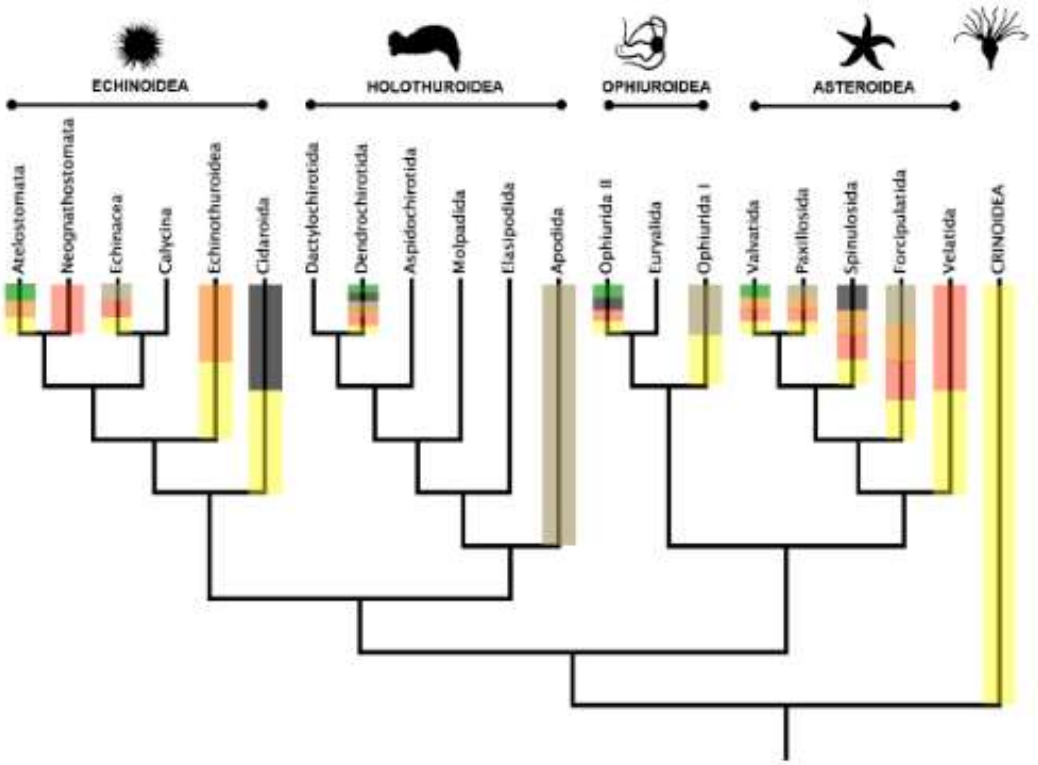


Figure 3.8. Phylogenetic distribution of dominant egg colour families (red, orange, brown, yellow, green, grey) among lecithotrophic echinoderms (N = 126 records). Colours are ordered by first appearance from bottom (more primitive) to top (more derived). Phylogeny modified from previously published accounts (Kerr and Kim, 2001; Kroh and Smith 2010; Mah and Blake 2012; O'Hara *et al.* 2014).

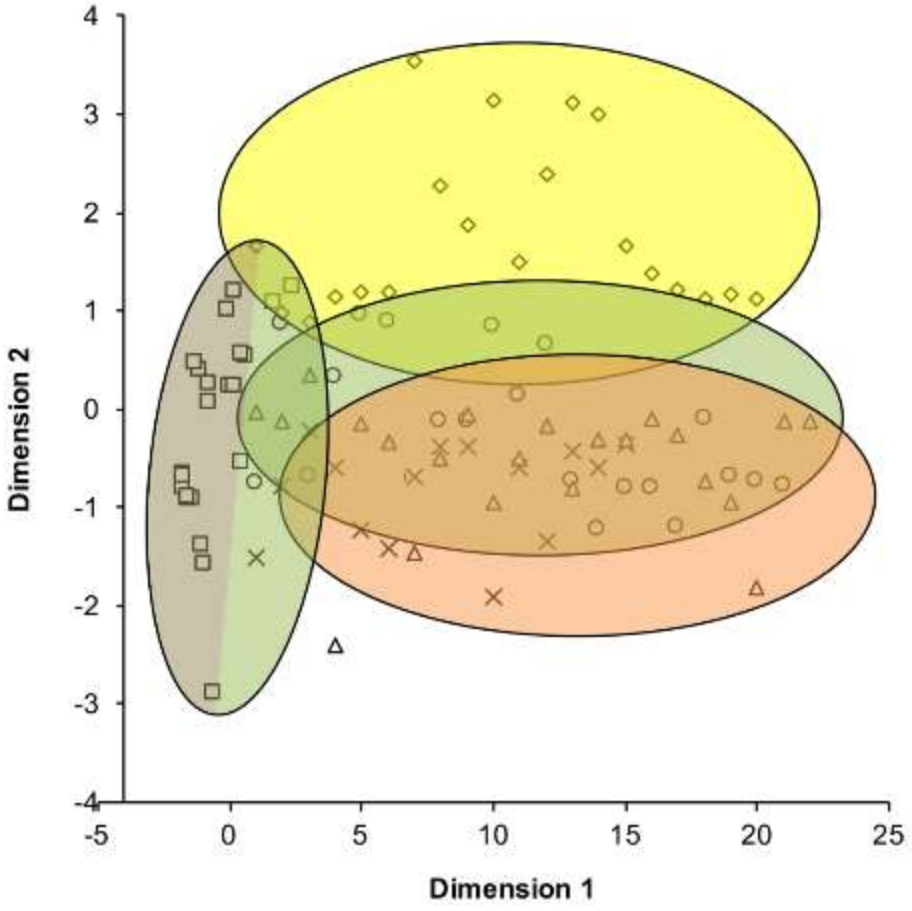


Figure 3.9. Relationship between egg colour and taxonomic class in lecithotrophic echinoderms, independent of development mode. Dimension 1 = FAMD component with greatest variance. Dimension 2 = FAMD component with 2nd greatest variance. Colour of each ellipse reflects predominate egg colour family/ies (orange, brown, yellow, green). Symbol shape indicates class, circle = Ophiuroidea, square = Holothuroidea, diamond = Echinoidea, triangle = Asteroidea, cross = no associated phylogenetic class (N = 103, Appendix H).

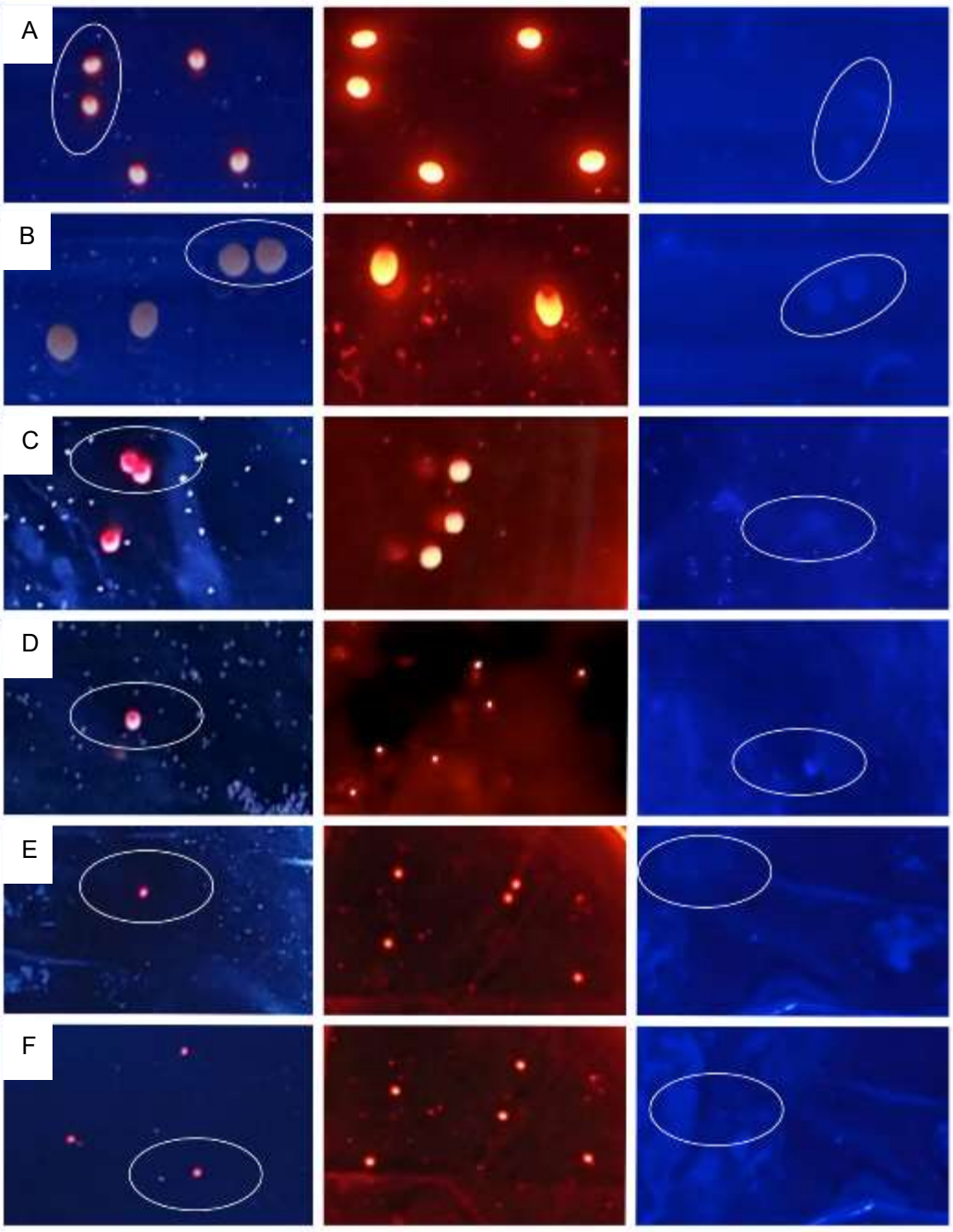

A
B
C
D
E
F

Figure 3.10. Lecithotrophic echinoderm eggs viewed under various light colours/wavelengths at 300 lux: white (left panels), red (middle panels), and blue (right panels). Results shown for the sea stars A) *Henricia sanguinolenta* (egg size 1.5 mm), B) *Henricia lisa* (1.5 mm), C) *Crossaster papposus* (0.9 mm) and D) *Solaster endeca* (0.9 mm); and the sea cucumbers E) *Cucumaria frondosa* (0.65 mm) and F) *Psolus fabricii* (0.6 mm). Eggs were floating on the water surface at the time of imaging. Circles on the photos highlight the location of some of the eggs. Images not to scale. Methods: Freshly collected eggs were exposed successively to white light (present at surface, λ = 440-650), red light (present at surface to 5 m, λ = 650 nm), and blue light (present at surface to 150 m, λ = 440 nm) using a Fuloon 12V 5050 RGB light emitting diode (LED) lamp. While wavelength varied, light intensity was standardized to near-surface values (~300 lux) typical of spring conditions when most spawning and larval development occurs. Images under each light condition were taken using an Olympus TG-2 digital camera for subsequent RBG measurements in Adobe Photoshop software. Unedited images (for a minimum of 50 eggs per species) were compared from each light condition and each tested species to assess the visibility of eggs. Data were compiled using eggs from at least two spawning events (involving >2 males and females) for species with pelagic development, and from a minimum of 2 clutches for brooding species.

Chapter 4. Ontogenetic Shifts in Swimming Capacity of Echinoderm Propagules: A Comparison of Planktotrophic and Lecithotrophic Species

A version of this chapter was published in the journal Marine Biology in March 2017 (Vol. 164, page 43)

4.1. Abstract

While developmental strategies can modulate the dispersal and recruitment of marine benthic species, the significance and drivers of propagule motility throughout ontogeny remain incompletely understood. Species with lecithotrophic (non-feeding) development are rarely studied, despite their predominance in some taxa, including echinoderms. Quantification of the swimming capacity (i.e. speed and trajectory) of early life-history stages and its variability with environmental factors is required to improve the ability to predict population connectivity and assess trade-offs associated with complex life histories. In general, lecithotrophic larvae of echinoderms are ascribed weak swimming abilities relative to planktotrophic larvae, although explicit measures are scarce. Here, we explored selected metrics of swimming capacity in four co-occurring species of North Atlantic echinoderms displaying different types of pelagic development: planktotrophs represented by the sea star *Asterias rubens* and the sea urchin *Strongylocentrotus droebachiensis*, and lecithotrophs represented by the sea star *Crossaster papposus* and the sea cucumber *Cucumaria frondosa*. Swimming was characterized in still water based on

the horizontal speed and path straightness of early life-history stages, from late blastula (hatched embryo) to late-stage larva. we tested the hypotheses that swimming capacity of propagules increases with progression through developmental stages and with increasing seawater temperature. Swimming speed increased with ontogeny in two of the four species (*A. rubens* and *C. papposus*) and with temperature in all species, although the effects of temperature were not uniform across life stages. The fastest swimming speeds across all species and temperatures were recorded in lecithotrophic propagules (i.e. max speed 1.2 mm s^{-1} in the brachiolaria of *C. papposus*), whereas propagules of species with planktotrophic development displayed faster relative speeds (body lengths s^{-1}). Relative speeds increased with temperature in all tested species except *C. papposus*. Swimming paths typically increased in straightness from early to later developmental stages, and also became straighter with increased temperature in most species, except in *C. papposus* where they became more circular and complex. In general, planktotrophic and lecithotrophic propagules had similar swimming capacities when tested at the same level of increased temperature, though several stage-specific differences were detected; propagules of species with planktotrophic development had greater relative speeds at the gastrula stage and greater path-corrected speeds at the larval stage. Swimming paths and swimming speeds were similar between propagules of species with planktotrophic development and lecithotrophic development, suggesting that phylogenetically-conserved, ontogenetic patterns of swimming capacity (seen here between two sea stars) may supersede the contribution of larval nutritional mode.

4.2. Introduction

Marine animals have evolved diverse developmental strategies that not only shape their reproductive success but also determine their settlement, recruitment and dispersal potential (Pechenik 1999, Thorson 1949). Complex, biphasic life histories, with sessile or sedentary adults and pelagic propagules (embryos and larvae), are common among benthic species (Pechenik 1999, Strathmann 1993). Pelagic larvae can be broadly characterized based on their nutritional requirements during development as either planktotrophic/feeding or lecithotrophic/non-feeding (Poulin *et al.* 2001). Propagules of species with planktotrophic development are typically smaller and neutrally or negatively buoyant, whereas pelagic lecithotrophic propagules are usually larger and positively buoyant (Chia *et al.* 1984, Emlet 1994).

Though size varies greatly due to development mode (e.g. propagule diameter varying by as much as fifteen-fold), all species with a planktonic stage share non-feeding embryonic stages that have the ability to swim using cilia or muscular contraction (Emlet 1983, Moore 2003, Staver and Strathmann 2002). Overall, species with ciliated propagules are classified as "weakly swimming" (e.g. echinoderms, molluscs; <1-10 mm s^{-1}) relative to those that rely on appendages and muscles for propulsion (e.g. polychaetes, crustaceans; >5-30 mm s^{-1}; Grunbaum and Strathmann 2003, Strathmann and Grünbaum 2006). While the swimming speeds of ciliated propagules are generally lower than currents, even small modifications (e.g. twofold increase in speed) can influence small-scale interactions with the benthos that may modulate the capture of food, encounters

with predators, settlement and ultimately, recruitmen t(Abelson and Denny 1997, Gross *et al.* 1992, North *et al.* 2008).

Propagule locomotion is primarily controlled by morphology and the mechanics of propulsion (Clay and Grunbaum 2010). Therefore, changes in morphology and behaviour that occur throughout ontogeny are expected to influence the swimming abilities of propagules. There is compelling evidence that even the most basal taxa (Porifera, Cnidaria) are sensitive to abiotic and biotic cues (Leys *et al.* 2002, Pawlik 1992, Tamburri *et al.* 1996). Yet, several authors have noted a scarcity of stage-specific studies of swimming capacity, particularly in response to environmental conditions (Metaxas 2001, Metaxas and Saunders 2009, Morgan 2014). Comparative studies involving more than one species are also extremely rare.

Many studies that examine the motility of marine ciliated propagules have focused on one or two species to assess the combined influence of flow and swimming on vertical positioning of larvae in the water column for the purpose of estimating transport over medium to large spatial scales (m to km; Metaxas 2001, Metaxas and Saunders 2009, Roy *et al.* 2012b, Sameoto *et al.* 2010). Studies of propagule swimming mechanisms in species with planktotrophic development have been conducted mainly in Mollusca (e.g. Arshavsky *et al.* 1993, Childress and Dudley 2004) and Echinodermata (e.g. Emlet 1983, Strathmann and Grunbaum 2006). Small-scale studies of horizontal swimming (that include horizontal components) are prevalent in some phyla (e.g. Porifera, Maldonado 2006; Annelida, Butman *et al.* 1988) but relatively limited in others (e.g. Mollusca, Chan *et al.* 2013; Echinodermata, Chan 2012, McDonald 2004, Mogami *et al.* 1988). In phyla

with different nutritional modes, studies that quantify the motility of lecithotrophic propagules are also scarce (Emlet 1994, Kelman and Emlet 1999, McEuen and Chia 1991). This is especially true in Echinodermata where lecithotrophy is predominant (estimated as 68% of all species, Uthicke *et al.* 2009). Addressing this will be of particular importance in temperate and cold-water ecosystems where species with lecithotrophic propagules developing in the plankton are equally abundant to species with planktotrophic propagules (Marshall *et al.* 2012).

Pelagic lecithotrophic echinoderm propagules have been ascribed weak swimming capacities, based on the assumption that their large size and positive buoyancy can impede swimming (Emlet 1994). The absence of feeding could exert a strong influence on swimming behaviour in lecithotrophic propagules, which are not constrained by dependence on external sources of nutrition and generally experience less predation; either through morphological incompatibility (Mercier *et al.* 2013a) or through predator rejection (Iyengar and Harvell 2001). The few studies dedicated to locomotion in ciliated propagules of lecithotrophic echinoderms have documented swimming in a sea star (*Pteraster tesselatus*, 1.0-1.7 mm s^{-1}; Kelman and Emlet 1999), a sea cucumber (*Psolus chitonoides*, 1.4 mm s^{-1}; McEuen and Chia 1991), a brittle star (*Ophioderma brevispinum*, 0.3 mm s^{-1}; Webb 1989) and discussed the general constraints of cilia band placement to swimming potential (Emlet 1994). Positively buoyant propagules may swim with or against the buoyant force (Emlet 1994), which makes assessment of "vertical" swimming capacities complicated in lecithotrophs. However, the combination of buoyancy and swimming in *P. tesselatus* and *P. chitonoides* was shown to generate vertical movement

that was faster than reported in planktotrophic echinoderms (Kelman and Emlet 1999, McEuen and Chia 1991).

In an effort to provide explicit comparisons between developmental strategies and generate novel empirical data of potential use in dispersal and connectivity models, we explored the swimming capacity of embryos and larvae in four common and co-occurring species of North Atlantic echinoderms; planktotrophs represented by the sea star *Asterias rubens* and the sea urchin *Strongylocentrotus droebachiensis*, and lecithotrophs by the sea star *Crossaster papposus* and the sea cucumber *Cucumaria frondosa*. An initial study was conducted to gather species-specific data and test the hypothesis that swimming capacity increases with ontogenetic development at the scale of the propagule. We hypothesized that swimming speed would increase chronologically from early to late developmental stages due to changes in propagule size, shape and competency. Because temperature is known to influence the swimming of ciliated propagules through physiology and the viscosity changes of water (Chan *et al.* 2011, Kashenko 2007, Podolsky and Emlet 1993), we also tested the hypothesis that stage-wise swimming capacity would be positively correlated with temperature. Finally, we tested the assumption that planktotrophs exhibit greater swimming capacity than lecithotrophs under similar conditions (Chia *et al.* 1984, Emlet 1994). Differences in morphology among these propagule types is likely to affect swimming.

4.3. Materials and Methods

4.3.1. Animal collections, maintenance and spawning

Adults of *Asterias rubens* (5-10 cm radius), *Crossaster papposus* (5-10 cm radius), *Strongylocentrotus droebachiensis* (5-8 cm test diameter), and *Cucumaria frondosa* (15-20 cm contracted body length) were collected by SCUBA between 10-20 m depth in southeast Newfoundland (eastern Canada). Specimens of all species were housed in 375-L tanks provided with flow-through seawater (approx. 60 L h^{-1}) at ambient temperatures ranging from 0-5 °C, salinities ranging from 34-36 psu, light intensities ranging daily from 5-450 lux (mean = 300 lux), and natural photoperiod (see Mercier and Hamel 2010 for details).

This study was undertaken in the spring 2014 and 2015, during the natural spawning periods of the focal species (Mercier and Hamel 2010). Cultures of *C. papposus* and *C. frondosa* were started following natural spawning events in February and March, by gently skimming the positively buoyant fertilized oocytes from the surface of the tanks. Cultures of *A. rubens* and *S. droebachiensis* were started in May. Though experimental trials were conducted at different times for each species, utmost care was taken to ensure continuity of experimental protocols across life stages and species. Gonads of female *A. rubens* were surgically collected from mature individuals and were treated with a solution of 0.1 µM 1-Methyladenine to promote final oocyte maturation (Dorée *et al.* 1976). Spawning was initiated in *S. droebachiensis* by injecting 1-2 mL of 0.5M KCl into the coelomic cavity (Meidel and Yund 2001). A minimum of three males

and five females were used to generate cultures with sufficient genetic diversity. Fertilization of mature oocytes was performed using a dilution of approximately 10,000 spermatozoa mL^{-1} as per Byrne *et al.* 2010 in both *A. rubens* and *S. droebachiensis* as this was an optimal concentration to promote 80-90% fertilization success and reduce the potential for polyspermy.

Embryos and larvae (generally referred to as propagules) were cultured under conditions chosen to reflect the ambient temperature experienced in nature during the spawning season and early development, and standard culture conditions for the planktotrophs(Meidel *et al.* 1999, Meidel and Yund 2001). Standard rearing techniques were used for each species (Meidel *et al.* 1999 for *A. rubens* and *S. droebachiensis*, and Hamel and Mercier 1996 for *C. frondosa and C. papposus*). Propagules of *C. papposus,* and *C. frondosa* were raised in 1.5 L vessels at 1-3°C (matching the ambient conditions during natural spawning) and approximately 0.1 L h^{-1} flow-through conditions. Propagules of *A. rubens* were obtained a little later in the spring when the ocean temperature was higher; the cultures were performed at 10°C (static conditions) to match standard culture conditions. Once the larvae began to feed, (pluteus stage *S. droebachiensis*, late bipinnaria stage *A. rubens*), cultures were fed with a commercial mix of live algae (Phytofeast Live, Reef Nutrition) at a density of 1000 cells mL^{-1} (concentration per Meidel *et al.* 1999). Planktotrophs consistently spent proportionally less time in the embryonic phase (11% in *A. rubens*; 13% in *S. droebachiensis*) than the lecithotrophs (57% in *C. frondosa*, 53% in *C. papposus*) relative to total development time (from egg to final larval stage). All trials were performed on propagules obtained

inside the same breeding season. In the case of multiple spawning events in the same season (only relevant for *C. frondosa*), cohorts were tested separately. However, there were no statistical differences among tested locomotory and morphological parameters so they were pooled for subsequent analyses.

4.3.2. Experimental protocols

Developmental stages were tested when ~80% of individuals in culture had reached that stage (Gemmill 1914, 1920, Hamel and Mercier 1996, Meidel *et al.* 1999). Focal stages included the late blastula and gastrula of all species, early brachiolaria in *Asterias rubens*, four-armed pluteus in *Strongylocentrotus droebachiensis*, brachiolaria in *Crossaster papposus*, and early pentactula in *Cucumaria frondosa*. Early time points in the most advanced larval stages (e.g. early brachiolaria, four-armed pluteus) were favoured over pre-competent forms to minimize the potential influence of settlement appendages on swimming that may occur during transition between the pelagic and benthic phases (near settlement). To this effect, early pentactulae of *C. frondosa* were tested prior to the emergence of the primary podia, as this results in a shift from swimming to crawling. Though *S. drobachiensis* passes through later-stage pluteii forms before undergoing metamorphosis, four-armed pluteii were chosen here as this is the stage commonly used by investigators working on other aspects of larval swimming in this species (e.g. under turbulent flows, Roy *et al.* 2012b).

Swimming capacity metrics were measured at temperatures representative of ambient culture conditions, 1-3°C for *C. papposus* and *C. frondosa* and 10°C for *A.*

rubens and *S. droebachiensis*, as well as an elevated temperature of 15°C. Selected temperatures were within the natural range of each species (*A. rubens* 5-20°C, Saranchova and Flyachinskya 2001, Villalobos *et al.* 2006; *S. droebachiensis* 0-24°C, Pearce *et al.* 2005, Roller and Stickle 1994; *C. frondosa* 0-15°C, Hamel and Mercier 1996; *C. papposus* 0-15°C, Reitzel *et al.* 2004). Experimental temperature values were confirmed prior to each trial using a glass thermometer and infrared gun (n = 3 measurements per trial).

A light intensity of 300 lux was selected, as this represented the mean ambient light level experienced by the propagules in culture vessels and represents mid-range light intensity measured in the surface waters of coastal Newfoundland during spring and summer (10 lux-1100 lux, Puvanendran and Brown 1998). A Fuloon 12V 5050 RGB light emitting diode (LED) lamp was used for all experimental trials, set directly over the experimental vessel to avoid a light gradient that could promote phototaxis in the horizontal plane. During the short duration of the trial, all propagules appeared to stay in the upper half of the experimental vessel. Background shade (white or black) did not affect swimming speed at any developmental stage (as determined during preliminary experiments) so white backgrounds were used to enhance propagule visibility during monitoring.

At the commencement of each trial, propagules were gently transferred into small glass dishes (6-10 cm diameter, 2 cm high) and allowed to swim undisturbed for 5 min. This time frame was sufficient for propagules to recover from the transfer procedure and return to normal swimming behaviour as per preliminary experiments. To determine an

appropriate acclimation length, we examined swimming propagules from 0 to 5 min after being transferred to experimental dishes. Most propagules swam immediately after being placed in the dish and seemed to be resilient to gentle manipulations. Five minutes was sufficient for all propagules to resume swimming as normal (trajectory and speed) after the transfer and is a comparable time frame to other studies of marine larvae that found a period of <5min more than sufficient for propagules to recover from transfer protocols (Forward and Costlow 1974). In fact, many studies begin tracking behaviour immediately after transfer (e.g., Maldonado *et al.* 2003, Metaxas and Young 1998, Pennington and Emlet 1986). Following the acclimation period, video recordings were taken for 5 min (30 fps, Olympus TG-1 Camera) and the resulting footage was later analysed with the software ImageJ (see method below). Three replicate trials were performed for each stage in each species, and this resulted in a total of 15-30 individual propagules per stage per species. The effect of replicate was statistically tested (see below) to ensure differences among life stages were not obscured by temporal replication.

As a control, recordings were also taken of unhatched propagules (early developing embryos) to correct for any passive surface drift that may occur from convection currents. Unhatched propagules (still inside the fertilization envelope) are useful in this capacity because they are comparable to newly hatched blastulae in size and buoyancy, but they are not motile (devoid of cilia). Therefore, it can be assumed that any displacement of non-motile embryos is purely due to background water movements in the horizontal plane. These mean drift currents were very small (0.001-0.01 mm s^{-1}) and were negligible (<5%) at all stages except the blastula of *A. rubens*, in which passive

displacement could represent up to 15% of the total horizontal displacement. Nevertheless, natural drift speeds obtained from unhatched propagules were subtracted from mean speeds to account for passive movement in all treatments.

4.3.3. Particle tracking and swimming capacity metrics

The MOSAIC particle tracking algorithm in ImageJ [http://mosaic.mpi-cbg.de/?q=downloads/imageJ] was used to analyze swimming at each tested life-stage (Chenouard *et al.* 2014). The 2D horizontal paths of swimming propagules were exported as x-y coordinates and converted into displacement data over one second intervals for the duration of the trials. We acknowledge that measuring only the horizontal component of swimming in propagules of species with planktotrophic development can be an underestimation of their true swimming capacity. However, lecithotrophic propagules exhibit limited vertical movement, and therefore, only the horizontal plane can be considered to make meaningful conclusions about nutritional mode differences. Propagules were excluded from the analysis if they collided with each other, or with the edge of the dish.

Four metrics were used to quantify swimming capacity. (1) Mean absolute speed (mm s^{-1}) was calculated as the average of distance travelled per one-second interval over the length of the trial. Absolute speed is the most commonly reported metric of swimming in the literature and provides a standard for comparisons. (2) Mean relative speed was calculated as the mean number of body lengths travelled per second (BL s^{-1}), where body length was the longest axis in asymmetrical propagules measured from images (Epp and

Lewis 1984). Standardizing speed by size to generate relative speed is useful for making comparisons among propagules of different sizes and shapes, such as the ones in the present study. (3) Net to gross displacement ratio (NGDR, an index of path shape) was calculated as the average ratio between net (displacement) and total distance travelled over 20 s intervals for the duration of the trial. NGDR is a measure of path complexity where values close to 1 indicate a relatively straight path and values near 0 indicate a complex path (Metaxas 2001). NGDR values typically plateaued before the end of swimming trials. (4) Path-corrected speed (PCS, mm s^{-1}) was calculated to incorporate the influence of swimming speed and path on propagule movement, allowing for holistic comparisons among stages and species. PCS was obtained by multiplying swimming speed by NGDR as a proxy for mean displacement per unit of time.

4.3.4. Statistical analysis

For each species, a nested analysis of variance (ANOVA) was used to test the effect of life stage and replicate trial number on all propagule swimming metrics under ambient conditions: mean absolute speed, mean relative speed, net to gross displacement ratio and path corrected speed, within species. For this analysis, replicate was nested within stage. Two-way ANOVA was used to test the combined effect of increased temperature and stage on the four metrics of propagule swimming capacity for each species. The same test was used for interspecific comparisons of swimming metrics and stages at 15°C. Tukey post-hoc tests were conducted on statistically significant ANOVA

models. All statistical analyses were conducted and assumptions verified using SigmaPlot statistical software at $\alpha = 0.05$.

4.4. Results

4.4.1. Swimming capacity throughout ontogeny under ambient conditions

4.4.1.1. Swimming speed

Among propagules of species with planktotrophic development, absolute swimming speed increased significantly from one developmental stage to the next in *A. rubens* ($F_{2,38} = 34.4$, $p < 0.01$; Fig. 4.1A), whereas it plateaued at the gastrula stage in *S. droebachiensis* ($F_{2,56} = 10.1$, $p < 0.01$; Fig. 4.1B). On average, the brachiolaria of *A. rubens* exhibited the fastest absolute swimming speed (0.48 mm s^{-1}), representing a tenfold increase compared to the blastula (0.04 mm s^{-1}), whereas values were more constant (0.19-0.34 mm s^{-1}) among life stages of *S. droebachiensis*.

Among lecithotrophic propagules, mean absolute swimming speed increased significantly with ontogeny in *C. papposus* ($F_{2,43} = 33.3$, $p < 0.01$; Fig. 4.1C) but not in *C. frondosa* ($F_{2,43} = 1.9$, $p = 0.17$; Fig. 4.1D). On average, the fastest swimming stage in *C. papposus* was the late larval stage (brachiolaria), with an absolute swimming speed of 0.78 mm s^{-1}. In contrast, the fastest stage of *C. frondosa* was the gastrula; with an absolute swimming speed of 0.21 mm s^{-1}.

When accounting for body length, relative swimming speed increased with ontogeny in *A. rubens* from 0.25 to 1.50 BL s^{-1} ($F_{2,38} = 21.8$, $p < 0.01$; Fig. 4.1E) but decreased in *S. droebachiensis* from the blastula/gastrula (1.20 BL s^{-1}) to the pluteus stage

(0.30 BL s^{-1}; $F_{2,56} = 39.3$, $p < 0.01$; Fig. 4.1F). Relative swimming speed in *C. papposus* increased significantly from 0.20 to 0.70 BL s^{-1} ($F_{2,43} = 15.5$, $p < 0.001$; Fig. 4.1G) but remained stable at 0.20-0.30 BL s^{-1} in *C. frondosa* ($F_{2,43} = 3.0$, $p = 0.064$; Fig. 4.1H).

4.4.1.2. Swimming trajectories

Among planktotrophs, net to gross displacement ratio (NGDR) increased significantly with ontogeny in *A. rubens* (from 0.57 to 0.81, Fig. 4.1I; $F_{2,38} = 5.1$, $p = 0.015$) and in *S. droebachiensis* (from 0.28 to 0.63, Fig. 4.1J; $F_{2,56} = 4.1$, $p = 0.028$). Paths became visibly straighter in both species with sequential life stages (Fig. 4.2A, B). Similar increases in NGDR with ontogeny were detected in the lecithotrophs *C. frondosa* (0.52-0.75, Fig. 4.1L; $F_{2,43} = 3.4$, $p = 0.049$) and *C. papposus* (0.44-0.82, Fig. 4.1K; $F_{2,43} = 8.9$, $p < 0.001$). The swimming paths also straightened in the late life stages of both species, although the pattern was more evident in *C. papposus* (Fig. 4.2C, D).

4.4.1.3. Path-corrected speed (PCS)

When both speed and path straightness (NGDR) were combined quantitatively, PCS exhibited uniform ontogenetic trends among planktotrophs; it plateaued after an increase from blastula to gastrula in *A. rubens* (from 0.02 to 0.39 mm s^{-1}, Fig. 4.1M) and *S. droebachiensis* (from 0.05 to 0.19 mm s^{-1}; Fig. 4.1N). In contrast, PCS followed different ontogenetic trends among lecithotrophs. In *C. papposus*, PCS increased ontogentically from the blastula to the brachiolaria (0.07 to 0.64 mm s^{-1}; Fig. 4.1O). In contrast, PCS did not change with ontogeny in *C. frondosa* (0.09-0.11 mm s^{-1}, Fig. 4.1P).

PCS cam be used over a 1-hour period to estimate effective displacement (horizontal). This provides a general method of comparison under standard conditions. The highest effective displacement among tested propagules was 2.3 m h^{-1}, in the brachiolaria of *C. papposus*. The next highest values were 1.4 and 0.7 m h^{-1}, in the brachiolaria of *A. rubens* and the gastrula of *S. droebachiensis*, respectively. Displacement was consistent among life stages of *C. frondosa* with values around 0.3 m h^{-1}.

4.4.2. Temperature effects on propagule swimming capacity

A within-stage analysis in the planktotrophs showed that propagules of *A. rubens* and *S. droebachiensis* responded slightly differently to increased water temperature. In *A. rubens*, only relative swimming speed increased significantly when propagules were tested at 15°C ($p = 0.014$). In contrast, absolute swimming speed (Fig. 4.3A; $p < 0.01$), relative swimming speed (Fig. 4.3C; $p = 0.026$) and path corrected speed (Fig. 4.3G; $p = 0.032$) were higher for *S. droebachiensis* propagules exposed to 15°C. No differences in the NGDR index of straightness were detected for either species (Fig. 4.3E).

At the within-stage level in lecithotrophs, not all tested propagules of *C. papposus* and *C. frondosa* responded the same to the increase in water temperature. While absolute speed, relative speed and PCS of *C. frondosa* propagules (Fig. 4.3B, D, H) increased significantly ($p < 0.01$) at 15°C, the NGDR index of straightness (Fig. 4.3F; $p < 0.01$) and PCS (Fig. 4.3H; $p = 0.011$) of *C. papposus* decreased significantly.

Overall, in interspecific comparisons across developmental modes, planktotrophs and lecithotrophs had similar absolute swimming speeds at 15°C ($F_{3,6} = 0.007$ $p = 0.93$). The fastest recorded speeds, across all species and temperatures, remained for the brachiolaria stage of *C. papposus* (1.2 mm s^{-1}). Gastrulae of planktotrophic species exhibited faster relative speeds than gastrulae of lecithotrophic species (1.65 BL s^{-1} faster, $F_{2,6} = 56.73$, $p<0.001$), but no other differences in relative speeds were detected. Planktotrophs also tended to have higher path corrected speeds at the larval stage (0.07 mm s^{-1} faster, $F_{2,6} = 5.43$, $p = 0.033$; with the exception of brachiolaria of *C. papposus*). No differences in NGDR were detected among the development modes at any stage ($F_{3,6} = 2.28$, $p = 0.13$).

4.5. Discussion

The active swimming behaviours of pelagic propagules are believed to serve various roles, i.e. prevent larvae from sinking, facilitate access to micro-environments, gas exchange and enhance settlement near the benthos (Clay and Grünbaum 2011). Speeds in the range of 0.1-30.0 mm s^{-1} have previously been reported for ciliated propagules from basal taxa such as Porifera (Maldonado 2006) and Cnidaria (Harii *et al.* 2002, Mileikovsky 1973), as well as more derived taxa such as Bryozoa (Wendt 2000), Mollusca (Chia *et al.* 1984) and Echinodermata (Chia *et al.* 1984, Podolsky and Emlet 1993). Direct comparisons of swimming speed values across studies are complicated by inherent differences in life stage and nutritional mode of focal propagules, experimental scale/conditions and direction of displacement (e.g. vertical swimming rates can be

influenced by gravity, buoyancy and flow). Results for echinoderms to date have chiefly been obtained for late-stage ciliated larvae of species with planktotrophic development, either in still-water vertical columns (Metaxas 2001, Metaxas and Saunders 2009), under various flow regimes (Roy *et al.* 2012b) or combining data from both horizontal and vertical planes (Rebolledo and Emlet 2015). Planktotrophic propagules have been in focus partly because they are easy to culture under laboratory conditions (Wray *et al.* 2004), they are commonly used in aquaculture (Liu *et al.* 2016, Loor *et al.* 2016, Mos *et al.* 2011) and their cilia serve both as feeding and locomotory structures (Strathmann 1971, Strathmann and Grunbaum 2006). Previous reports of swimming speeds in the bipinnaria of *Asterias rubens*, and pluteii of *Strongylocentrotus droebachiensis* and *Dendraster excentricus* are similar to swimming speed values measured in the present study at comparable stages (i.e. 0.1-0.5 mm s^{-1}; Civelek *et al.* 2013, McDonald 2012, Roy *et al.* 2012b). Increases in swimming speed with increasing water temperature were also reported, i.e. 0.2 to 0.5 mm s^{-1} in *S. droebachiensis* (Daigle and Metaxas 2012).

The present multi-species study of swimming capacity showed that the absolute swimming speeds of planktotrophs and lecithotrophs were surprisingly similar when tested under ambient conditions (respective culture temperatures), and at 15°C. The similarities at respective ambient conditions are of particular interest, given that the lecithotrophs were cultured and tested at a colder temperature, but often swam as fast as the planktotrophs which were raised and tested at a higher temperature. This suggests that lecithotrophic propagules might swim faster than planktotrophic propagules if they were all raised at a similar temperature (i.e. either 3 or 10°C). Such studies would be invaluable

for interspecific comparisons. However, the logistics of raising and testing multiple species under identical ambient conditions are likely why few multi-species studies exist.

Despite similarities in absolute speeds and trajectories, some planktotrophic propagules displayed faster relative swimming speeds (body lengths s^{-1}) than their lecithotrophic counterparts. This trend was notable at the gastrula stage, which is more fusiform in lecithotrophs than in the planktotrophs. While this shape may have evolved to reduce projected area and drag of lecithotrophic gastrulae, similar to the faster swimming speeds seen among cyprid versus nauplius larvae in crustaceans (Walker 2004), it apparently does not completely offset the effects of large size. The higher relative speeds of *A. rubens* and *S. droebachiensis* gastrulae could relate to increased risk of predation, previously documented in embryonic stages of planktotrophs (Mercier *et al.* 2013a). Though faster swimming larvae may encounter more predators (Gerritsen and Strickler 1977), the combination of complex paths and fast swimming speeds may allow gastrulae to escape after an encounter with predators as not all predators may be able to track a chaotically swimming propagule. Increased water temperature could also help the gastrulae of planktotrophic species escape from predators more effectively than other life stages, as the proportional increase in relative swimming speed from ambient to 15°C was the highest. However, predation rates may also vary with water temperature, as viscosity will decrease as temperature increases.

Relative speeds and other metrics of swimming capacity were similar among the focal species at early embryonic stages (late blastula), despite marked differences in propagule length (0.2-0.3 mm vs. 0.6-0.8 mm) and rearing temperature. Relative speeds

were higher among late-stage larvae of *A. rubens* and early stages of *S. droebachiensis*, but the brachiolaria of *C. papposus* displayed on average faster absolute speeds than all other tested life stages and species under both ambient and 15°C conditions. This was in stark opposition to our initial hypothesis that the propagules of planktotrophic species would swim faster than those species with lecithotrophic propagules in the horizontal plane. Over the past 20 years, other assumptions about lecithotrophic propagules have been revisited; they were recognized to disperse as far (Young *et al.* 1997) and spend as long in the pelagic region as planktotrophs (Mercier *et al.* 2013b), and have greater control over settlement site selection (Marshall and Keough 2003). Models have also revealed that positively buoyant propagules, such as most lecithotrophic larvae, can disperse further from adult habitats than neutrally buoyant or passive particles (Koehl 2005).

Even though absolute and relative swimming speeds varied, changes in swimming trajectories with progression through ontogeny were conserved across all four species. Propagules transitioned from circular swimming patterns in embryonic stages to expansive rectilinear paths that covered more ground per unit of time in more advanced larval stages. Overall, lecithotrophic propagules tended to swim with more complex paths than planktotrophs (on average they have lower NGDR values). This may provide an advantage by allowing these propagules to encounter less predators relative to planktotrophs, but without the cost of not finding as much food; a challenge faced primarily by planktotrophs (Visser and Kiørboe 2006). Generally, propagule paths displayed both clockwise and counter clockwise loops throughout the trials, which

reflects the variability reported in ciliated propagules of echinoderms (Chia *et al.* 1984). A capacity to change the direction of rotation while swimming emerged in sea star larvae, i.e. the brachiolaria of *A. rubens* and *C. papposus*. Such an ability was previously described as a backflip, representing body flexion rather than changes in cilia beating direction or speed (Strathmann 1971).

Effective horizontal displacement values (based on path-corrected speed) in the order of 0.5-2.5 m h^{-1} were recorded here, which may seem limited relative to locally strong mixing and currents. However, the fact that active propulsion has been maintained by pelagic propagules indicates that it serves a purpose. Dispersive abilities in planktotrophs are at least partially controlled by their position in the water column during development (Roy *et al.* 2012b, Sameoto *et al.* 2010). In contrast, most pelagic lecithotrophic propagules remain in the upper meters of the water column during much of their development, as a consequence of their positive buoyancy (Emlet 1994). The similarity of swimming trajectories in the horizontal plane among tested planktotrophs and lecithotrophs could be evidence of phylogenetically-conserved patterns of locomotion that supersede nutritional mode differences. Complex paths that cover both horizontal and vertical planes (as seen in planktotrophs) could expose propagules to different currents and flow environments as greater pelagic surface area is covered between path end points than relatively straighter paths (Chan 2012). In contrast, rectilinear (straight) trajectories in both horizontal (seen here) and vertical planes could promote rapid access to areas of different flow regimes at small scales (cm – m), especially near the benthos (Walters *et al.* 1999).

The path corrected speeds were the highest for the brachiolaria larvae of *A. rubens* and *C. papposus*, a life stage designed for substrate selection and settlement (Barker 1977, Byrne and Barker 1991). Furthermore, the increasingly rectilinear swimming trajectories exhibited by the larval stages of most species studied here could correspond to the onset of an exploration phase documented in several competent and pre-competent larvae of echinoderms (Barker 1977, Byrne and Barker 1991, Hamel and Mercier 1996). The importance of swimming trajectories to understanding swimming behaviour in the water column and near the benthos close to settlement has also been confirmed in lecithotrophic coral larvae (Pizarro and Thomason 2008). Together, these findings suggest that speed alone may not be a reliable predictor of swimming capacity in benthic invertebrate propagules, and that swimming trajectories need to also be considered.

While some recent studies have opted to parameterize propagules as passive particles for model simplification (e.g. Fenberg *et al.* 2015, Myksvoll *et al.* 2014, Salama *et al.* 2013, Wood *et al.* 2014), the value of stage-specific and species-specific capacities and behaviours is increasingly being emphasized (Morgan 2014, Pringle *et al.* 2014, Robins *et al.* 2013). Interspecific comparisons of propagule behaviour, like those undertaken here, represent a growing trend to examine multiple stages and species on the same playing field. However, efforts are still challenged by the difficulty of modeling dispersal < 2 m above the sea floor, by incomplete understanding of ontogenetic changes in locomotory abilities, fundamental buoyancy/shape differences between species with planktotrophic or lecithotrophic propagules (Metaxas and Saunders 2009, Robins *et al.* 2013), and by the relative scarcity of data on lecithotrophic propagules. These gaps in

methodology and knowledge have limited the development of biophysical dispersal models with universal applicability.

Propagule dispersal has two components: passive dispersal as a result of large scale oceanographic processes (e.g. currents, fronts, mixing) and active dispersal as a result of swimming behaviours (e.g. movement changes, taxis). We acknowledge that the swimming data generated here is unlikely to inform large-scale dispersal phenomena of these species. However, active swimming by propagules may impact the outcome of dispersal predictions at smaller scales. Interestingly, relatively subtle speed changes (e.g. a doubling) were shown to alter dispersal predictions (on scales of m – km) more strongly in weakly swimming ciliated propagules than other larval types (e.g. Morgan 2014, Robins *et al.* 2013). To this effect, horizontal swimming speeds of propagules may interact with currents and facilitate predictions of vertical as well as the horizontal displacement of propagules. Swimming speed data collected here are similar to those previously reported for echinoderm propagules, and therefore, may be useful to modellers as a starting point; after accounting for fundamental composition and buoyancy differences between planktotrophic and lecithotrophic propagules. Navigation of pelagic ciliated propagules over various spatial scales is currently incomplete (Kingsford *et al.* 2002, Scheltema 1986), warranting further investigation of the drivers and mechanisms of shifts in their swimming trajectories.

Propagule swimming and behavioural patterns may be driven primarily by location (pelagic development) since the need to detect and avoid unfavourable environments is a ubiquitous selective pressure, independent of nutritional mode and

morphology. The degree of propagule sensitivity to abiotic factors (salinity, light, and temperature) and biotic cues in the water column has been well studied in planktotrophs such as *A. rubens* and *S. droebachiensis* (Civelek *et al.* 2013, Metaxas 2001, Roy *et al.* 2012a). However, similar studies have not yet been conducted with lecithotrophic echinoderm propagules, although numerous studies exist for other lecithotrophic propagules in Porifera and Cnidaria (Collin *et al.* 2010, Holst and Jarms 2006, Jacobs *et al.* 2007). The use of small-scale studies (including detailed examinations of swimming mechanisms and sensory responses) in concert with large-scale population-based studies could help improve our understanding of the persistence of benthic marine animals with diverse types of pelagic development.

4.6 Acknowledgements

The authors wish to thank Memorial University Field Services for animal collections. The authors also wish to thank two anonymous reviewers and K. Gamperl (Memorial University) for constructive comments on the manuscript. This work was completed with funding from a Natural Sciences and Engineering Research Council Discovery Grant (#311406) and Canadian Foundation for Innovation Grant (#11231) issued to A. Mercier and an NSERC CGS-D Award to E. Montgomery.

4.7. References

Abelson, A., and M. Denny. 1997. Settlement of marine organisms in flow. *Annual Review of Ecology and Systematics* **28**:317-339.

Arshavsky, Y. I., G. Orlovsky, Y. V. Panchin, A. Roberts, and S. Soffe. 1993. Neuronal control of swimming locomotion: analysis of the pteropod mollusc *Clione* and embryos of the amphibian *Xenopus*. *Trends in Neurosciences* **16**:227-233.

Barker, M. 1977. Observations on the settlement of the brachiolaria larvae of *Stichaster australis* (Verill) and *Coscinasterias calamaria* (Gray)(Echinodermata: Asteroidea) in the laboratory and on the shore. *Journal of Experimental Marine Biology and Ecology* **30**:95-108.

Butman, C. A., J. P. Grassle, and E. J. Buskey. 1988. Horizontal swimming and gravitational sinking of *Capitella* sp. I (Annelida: Polychaeta) larvae: implications for settlement. *Ophelia* **29**:43-57.

Byrne, M., and M. Barker. 1991. Embryogenesis and larval development of the asteroid *Patiriella regularis* viewed by light and scanning electron microscopy. *Biological Bulletin* **180**:332-345.

Byrne, M., N. Soars, P. Selvakumaraswamy, S. A. Dworjanyn, and A. R. Davis. 2010. Sea urchin fertilization in a warm, acidified and high pCO_2 ocean across a range of sperm densities. *Marine Environmental Research* **69**:234-239.

Chan, K. Y. 2012. Biomechanics of larval morphology affect swimming: Insights from the sand dollars *Dendraster excentricus*. *Integrative and Comparative Biology* **52**:458-469.

Chan, K. Y. K., D. Grünbaum, and M. J. O'Donnell. 2011. Effects of ocean-acidification-induced morphological changes on larval swimming and feeding. *Journal of Experimental Biology* **214**:3857-3867.

Chan, K. Y. K., H. Jiang, and D. K. Padilla. 2013. Swimming speed of larval snail does not correlate with size and ciliary beat frequency. *PLoS One* **8**:e82764.

Chenouard, N., I. Smal, F. De Chaumont, M. Maška, I. F. Sbalzarini, Y. Gong, J. Cardinale, C. Carthel, S. Coraluppi, and M. Winter. 2014. Objective comparison of particle tracking methods. *Nature Methods* **11**:281.

Chia, F. S., J. Buckland, and C. M. Young. 1984. Locomotion of marine invertebrate larvae - A review. *Canadian Journal of Zoology* **62**:1205-1222.

Childress, S., and R. Dudley. 2004. Transition from ciliary to flapping mode in a swimming mollusc: Flapping flight as a bifurcation in Re. *Journal of Fluid Mechanics* **498**:257-288.

Civelek, C. V., R. M. Daigle, and A. Metaxas. 2013. Effects of temperature on larval swimming patterns regulate vertical distribution relative to thermoclines in *Asterias rubens*. *Journal of Experimental Marine Biology and Ecology* **445**:1-12.

Clay, T. W., and D. Grunbaum. 2010. Morphology-flow interactions lead to stage-selective vertical transport of larval sand dollars in shear flow. *Journal of Experimental Biology* **213**:1281-1292.

Clay, T. W., and D. Grünbaum. 2011. Swimming performance as a constraint on larval morphology in plutei. *Marine Ecology Progress Series* **423**:185-196.

Collin, R., A. S. Mobley, L. B. Lopez, S. P. Leys, M. C. Diaz, and R. W. Thacker. 2010. Phototactic responses of larvae from the marine sponges *Neopetrosia proxima* and *Xestospongia bocatorensis* (Haplosclerida: Petrosiidae). *Invertebrate Biology* **129**:121-128.

Daigle, R. M., and A. Metaxas. 2012. Modeling of the larval response of green sea urchins to thermal stratification using a random walk approach. *Journal of Experimental Marine Biology and Ecology* **438**:14-23.

Dorée, M., P. Guerrier, and N. J. Leonard. 1976. Hormonal control of meiosis: Specificity of the 1-methyladenine receptors in starfish oocytes. *Proceedings of the National Academy of Sciences of the United States of America* **73**:1669-1673.

Emlet, R. B. 1983. Locomotion, drag and the rigid skelton of larval echinoderms. *Biological Bulletin* **164**:433-445.

Emlet, R. B. 1994. Body form and patterns of ciliation in nonfeeding larvae of echinoderms - Functional solutions to swimming in the plankton. *American Zoologist* **34**:570-585.

Epp, R. W., and W. M. Lewis. 1984. Cost and speed of locomotion for rotifers. *Oecologia* **61**:289-292.

Fenberg, P. B., B. A. Menge, P. T. Raimondi, and M. M. Rivadeneira. 2015. Biogeographic structure of the northeastern Pacific rocky intertidal: the role of upwelling and dispersal to drive patterns. *Ecography* **38**:83-95.

Forward, R., and J. Costlow. 1974. The ontogeny of phototaxis by larvae of the crab *Rhithropanopeus harrisii*. *Marine Biology* **26**:27-33.

Gemmill, J. F. 1914. The development and certain points in the adult structure of the starfish *Asterias rubens*. *Philosophical Transactions of the Royal Society B: Biological Sciences* **205**:213-294.

Gemmill, J. F. 1920. The development of the starfish *Crossaster papposus*. *Quarterly Journal of Microscopical Science* **64**:155-187.

Gerritsen, J., and J. R. Strickler. 1977. Encounter probabilities and community structure in zooplankton: a mathematical model. *Journal of the Fisheries Board of Canada* **34**:73-82.

Gross, T. F., F. E. Werner, and J. E. Eckman. 1992. Numerical modeling of larval settlement in turbulent bottom boundary layers. *Journal of Marine Research* **50**:611-642.

Grunbaum, D., and R. R. Strathmann. 2003. Form, performance, and trade-offs in swimming and stability of armed larvae. *Journal of Marine Research* **61**:659-691.

Hamel, J.-F., and A. Mercier. 1996. Early development, settlement, growth, and spatial distribution of the sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea). *Canadian Journal of Fisheries and Aquatic Sciences* **53**:253-271.

Harii, S., H. Kayanne, H. Takigawa, T. Hayashibara, and M. Yamamoto. 2002. Larval survivorship, competency periods and settlement of two brooding corals, *Heliopora coerulea* and *Pocillopora damicornis*. *Marine Biology* **141**:39-46.

Holst, S., and G. Jarms. 2006. Substrate choice and settlement preferences of planula larvae of five Scyphozoa (Cnidaria) from German Bight, North Sea. *Marine Biology* **151**:863-871.

Iyengar, E. V., and C. D. Harvell. 2001. Predator deterrence of early developmental stages of temperate lecithotrophic asteroids and holoturoids. *Journal of Experimental Marine Biology and Ecology* **264**:171-188.

Jacobs, D. K., N. Nakanishi, D. Yuan, A. Camara, S. A. Nichols, and V. Hartenstein. 2007. Evolution of sensory structures in basal metazoa. *Integrative and Comparative Biology* **47**:712-723.

Kashenko, S. D. 2007. Adaptive responses of embryos and larvae of the heart-shaped sea urchin Echinocardium cordatum to temperature and salinity changes. *Russian Journal of Marine Biology* **33**:381-390.

Kelman, D., and R. B. Emlet. 1999. Swimming and buoyancy in ontogenetic stages of the cushion star *Pteraster tesselatus* (Echinodermata : Asteroidea) and their implications for distribution and movement. *Biological Bulletin* **197**:309-314.

Kingsford, M. J., J. M. Leis, A. Shanks, K. C. Lindeman, S. G. Morgan, and J. Pineda. 2002. Sensory environments, larval abilities and local self-recruitment. *Bulletin of Marine Science* **70**:309-340.

Leys, S. P., T. W. Cronin, B. M. Degnan, and J. N. Marshall. 2002. Spectral sensitivity in a sponge larva. *Journal of Comparative Physiology A* **188**:199-202.

Liu, W., C. Pearce, R. McKinley, and I. Forster. 2016. Nutritional value of selected species of microalgae for larvae and early post-set juveniles of the Pacific geoduck clam, *Panopea generosa*. *Aquaculture* **452**:326-341.

Loor, A., D. Ortega, C. Lodeiros, and S. Sonnenholzner. 2016. Early life cycle and effects of microalgal diets on larval development of the spiny rock-scallop, *Spondylus limbatus* (Sowerby II, 1847). *Aquaculture* **450**:328-334.

Maldonado, M. 2006. The ecology of the sponge larva. *Canadian Journal of Zoology* **84**:175-194.

Maldonado, M., M. Durfort, D. A. McCarthy, and C. M. Young. 2003. The cellular basis of photobehavior in the tufted parenchymella larva of demosponges. *Marine Biology* **143**:427-441.

Marshall, D. J., and M. J. Keough. 2003. Variation in the dispersal potential of non-feeding invertebrate larvae: the desperate larva hypothesis and larval size. *Marine Ecology Progress Series* **255**:145-153.

Marshall, D. J., P. J. Krug, E. K. Kupriyanova, M. Byrne, and R. B. Emlet. 2012. The biogeography of marine invertebrate life histories. *Annual Review of Ecology and Systematics* **43**:97-114.

McDonald, K. 2004. Patterns in early embryonic motility: Effects of size and environmental temperature on vertical velocities of sinking and swimming echinoid blastulae. *Biological Bulletin* **207**:93-102.

McDonald, K. A. 2012. Earliest ciliary swimming effects vertical transport of planktonic embryos in turbulence and shear flow. *Journal of Experimental Biology* **215**:141-151.

McEuen, F. S., and F. S. Chia. 1991. Development and metamorphosis of two psolid sea cucumbers, *Psolus chitonoides* and *Psolidium bullatum*, with a review of reproductive patterns in the family Psolidae (Holothuroidea: Echinodermata). *Marine Biology* **109**:267-279.

Meidel, S. K., R. E. Scheibling, and A. Metaxas. 1999. Relative importance of parental and larval nutrition on larval development and metamorphosis of the sea urchin *Strongylocentrotus droebachiensis*. *Journal of Experimental Marine Biology and Ecology* **240**:161-178.

Meidel, S. K., and P. O. Yund. 2001. Egg longevity and time-integrated fertilization in a temperate sea urchin (*Strongylocentrotus droebachiensis*). *Biological Bulletin* **201**:84-94.

Mercier, A., E. J. Doncaster, and J.-F. Hamel. 2013a. Contrasting predation rates on planktotrophic and lecithotrophic propagules by marine benthic invertebrates. *Journal of Experimental Marine Biology and Ecology* **449**:100-110.

Mercier, A., and J.-F. Hamel. 2010. Synchronized breeding events in sympatric marine invertebrates: Role of behavior and fine temporal windows in maintaining reproductive isolation. *Behavioral Ecology and Sociobiology* **64**:1749-1765.

Mercier, A., M. A. Sewell, and J.-F. Hamel. 2013b. Pelagic propagule duration and developmental mode: Reassessment of a fading link. *Global Ecology and Biogeography* **22**:517-530.

Metaxas, A. 2001. Behaviour in flow: perspectives on the distribution and dispersion of meroplanktonic larvae in the water column. *Canadian Journal of Fisheries and Aquatic Sciences* **58**:86-98.

Metaxas, A., and M. Saunders. 2009. Quantifying the "bio-" components in biophysical models of larval transport in marine benthic invertebrates: Advances and pitfalls. *Biological Bulletin* **216**:257-272.

Metaxas, A., and C. M. Young. 1998. Behaviour of echinoid larvae around sharp haloclines: effects of the salinity gradient and dietary conditioning. *Marine Biology* **131**:443-459.

Mileikovsky, S. A. 1973. Speed of active movement of pelagic larvae of marine bottom invertebrates and their ability to regulate their vertical position. *Marine Biology* **23**:11-17.

Mogami, Y., C. Oobayashi, T. Yamaguchi, Y. Ogiso, and S. A. Baba. 1988. Negative geotaxis in sea urchin larvae: A possible role of mechanoreception in the late stages of development. *Journal of Experimental Biology* **137**:141-156.

Moore, S. W. 2003. Scrambled eggs: Mechanical forces as ecological factors in early development. *Evolution & Development* **5**:61-66.

Morgan, S. G. 2014. Behaviorally mediated larval transport in upwelling systems. *Advances in Oceanography* **2014**:1-18.

Mos, B., K. L. Cowden, S. J. Nielsen, and S. A. Dworjanyn. 2011. Do cues matter? Highly inductive settlement cues don't ensure high post-settlement survival in sea urchin aquaculture. *PLoS One* **6**:e28054.

Myksvoll, M. S., K.-M. Jung, J. Albretsen, and S. Sundby. 2014. Modelling dispersal of eggs and quantifying connectivity among Norwegian coastal cod subpopulations. *ICES Journal of Marine Science* **71**:957-969.

North, E. W., Z. Schlag, R. Hood, M. Li, L. Zhong, T. Gross, and V. S. Kennedy. 2008. Vertical swimming behavior influences the dispersal of simulated oyster larvae in a coupled particle-tracking and hydrodynamic model of Chesapeake Bay. *Marine Ecology Progress Series* **359**:99-115.

Pawlik, J. R. 1992. Chemical ecology of the settlement of benthic marine invertebrates. *Oceanography and Marine Biology* **30**:273-335.

Pearce, C. M., S. W. Williams, F. Yuan, J. D. Castell, and S. Robinson. 2005. Effect of temperature on somatic growth and survivorship of early post‐settled green sea urchins, *Strongylocentrotus droebachiensis* (Müller). *Aquaculture Research* **36**:600-609.

Pechenik, J. A. 1999. On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. *Marine Ecology Progress Series* **177**:269-297.

Pennington, J. T., and R. B. Emlet. 1986. Ontogenetic and diel vertical migration of a planktonic echinoid larva, *Dendraster excentricus*: Occurence, causes, and probable consequences. *Journal of Experimental Marine Biology and Ecology* **104**:69-95.

Pizarro, V., and J. Thomason. 2008. How do swimming ability and behaviour affect the dispersal of coral larvae. 464-467 in *Proceedings of the 11th International Coral Reef Symposium, Fort Lauderdale, Florida, 7*.

Podolsky, R. D., and R. B. Emlet. 1993. Separating the effects of temperature and viscosity on swimming and water movement by sand dollar larvae (*Dendraster excentricus*). *Journal of Experimental Biology* **176**:207-222.

Poulin, E., S. V. Boletzky, and J.-P. Feral. 2001. Combined ecological factors permit classification of developmental patterns in benthic marine invertebrates: A discussion note. *Journal of Experimental Marine Biology and Ecology* **257**:109-115.

Pringle, J. M., J. E. Byers, P. Pappalardo, J. P. Wares, and D. Marshall. 2014. Circulation constrains the evolution of larval development modes and life histories in the coastal ocean. *Ecology* **95**:1022-1032.

Puvanendran, V., and J. A. Brown. 1998. Effect of light intensity on the foraging and growth of Atlantic cod larvae: interpopulation difference. *Marine Ecology Progress Series* **167**:207-214.

Rebolledo, A. P., and R. B. Emlet. 2015. The parachute function of the hull in eggs of *Mopalia kennerleyi* (Chitonida: Mopaliidae), and swimming of its larvae through ontogeny. *Invertebrate Biology* **134**:31-37.

Reitzel, A. M., B. G. Miner, and L. R. McEdward. 2004. Relationships between spawning date and larval developmental time for benthic marine invertebrates: A modelling approach. *Marine Ecology Progress Series* **280**:13-23.

Robins, P. E., S. P. Neill, L. Giménez, S. R. Jenkins, and S. K. Malham. 2013. Physical and biological controls on larval dispersal and connectivity in a highly energetic shelf sea. *Limnology and Oceanography* **58**:505-524.

Roller, R. A., and W. B. Stickle. 1994. Effects of adult salinity acclimation on larval survival and early development of *Strongylocentrotus droebachiensis* and *Strongylocentrotus pallidus* (Echinodermata: Echinoidea). *Canadian Journal of Zoology* **72**:1931-1939.

Roy, A., A. Metaxas, and R. M. Daigle. 2012a. Changes in vertical distribution and aggregative behaviour in response to population density for larval sea urchins (*Strongylocentrotus droebachiensis*) and sea stars (*Asterias rubens*). *Marine Ecology* **33**:194-204.

Roy, A., A. Metaxas, and T. Ross. 2012b. Swimming patterns of larval *Strongylocentrotus droebachiensis* in turbulence in the laboratory. *Marine Ecology Progress Series* **453**:117-127.

Salama, N., C. Collins, J. Fraser, J. Dunn, C. Pert, A. Murray, and B. Rabe. 2013. Development and assessment of a biophysical dispersal model for sea lice. *Journal of Fish Diseases* **36**:323-337.

Sameoto, J. A., T. Ross, and A. Metaxas. 2010. The effect of flow on larval vertical distribution of the sea urchin, *Strongylocentrotus droebachiensis*. *Journal of Experimental Marine Biology and Ecology* **383**:156-163.

Saranchova, O. L., and L. P. Flyachinskya. 2001. The influence of salinity on early ontogeny of the mussel *Mytilus edulis* and the starfish *Asterias rubens* from the White Sea. *Russian Journal of Marine Biology* **27**:87-93.

Scheltema, R. S. 1986. On dispersal and planktonic larvae of benthic invertebrates: an eclectic overview and summary of problems. *Bulletin of Marine Science* **39**:290-322.

Staver, J. M., and R. R. Strathmann. 2002. Evolution of fast development of planktonic embryos to early swimming. *Biological Bulletin* **203**:58-69.

Strathmann, R. R. 1971. The feeding behaviour of planktotrophic echinoderm larvae: Mechanisms, regulation, and rates of suspension feeding. *Journal of Experimental Marine Biology and Ecology* **6**:109-160.

Strathmann, R. R. 1993. Hypotheses on the origins of marine larvae. *Annual Review of Ecology and Systematics* **24**:89-117.

Strathmann, R. R., and D. Grunbaum. 2006. Good eaters, poor swimmers: compromises in larval form. *Integrative and Comparative Biology* **46**:312-322.

Strathmann, R. R., and D. Grünbaum. 2006. Good eaters, poor swimmers: compromises in larval form. *Integrative and Comparative Biology* **46**:312-322.

Tamburri, M. N., C. M. Finelli, D. S. Wethey, and R. K. Zimmer-Faust. 1996. Chemical induction of larval settlement behavior in flow. *Biological Bulletin* **191**:367-373.

Thorson, G. 1949. Reproductive and larval ecology of marine bottom invertebrates. *Biological Reviews* **25**:1-45.

Uthicke, S., B. Schaffelke, and M. Byrne. 2009. A boom-bust phylum? Ecological and evolutionary consequences of density variations in echinoderms. *Ecological Monographs* **79**:3-24.

Villalobos, F. B., P. A. Tyler, and C. M. Young. 2006. Temperature and pressure tolerance of embryos and larvae of the Atlantic seastars *Asterias rubens* and *Marthasterias glacialis* (Echinodermata: Asteroidea): potential for deep-sea invasion. *Marine Ecology Progress Series* **314**:109-117.

Visser, A. W., and T. Kiørboe. 2006. Plankton motility patterns and encounter rates. *Oecologia* **148**:538-546.

Walker, G. 2004. Swimming speeds of the larval stages of the parasitic barnacle, *Heterosaccus lunatus* (Crustacea: Cirripedia: Rhizocephala). *Journal of the Marine Biological Association of the UK* **84**:737-742.

Walters, L. J., G. Miron, and E. Bourget. 1999. Endoscopic observations of invertebrate larval substratum exploration and settlement. *Marine Ecology Progress Series* **182**:95-108.

Webb, C. M. 1989. Larval swimming and substrate selection in the brittle star *Ophioderma brevispinum* 217 in *Reproduction, Genetics and Distributions of Marine Organisms: 23rd European Marine Biology Symposium* Olsen & Olsen, School of Biological Sciences, University of Wales, Swansea.

Wendt, D. E. 2000. Energetics of larval swimming and metamorphosis in four species of *Bugula* (Bryozoa). *Biological Bulletin* **198**:346-356.

Wood, S., C. Paris, A. Ridgwell, and E. Hendy. 2014. Modelling dispersal and connectivity of broadcast spawning corals at the global scale. *Global Ecology and Biogeography* **23**:1-11.

Wray, G. A., C. Kitazawa, and B. Miner. 2004. Culture of echinoderm larvae through metamorphosis. *Methods in Cell Biology* **74**:75-86.

Young, C. M., M. A. Sewell, P. A. Tyler, and A. Metaxas. 1997. Biogeographic and bathymetric ranges of Atlantic deep-sea echinoderms and ascidians: The role of larval dispersal. *Biodiversity and Conservation* **6**:1507-1522.

4.8. Figures

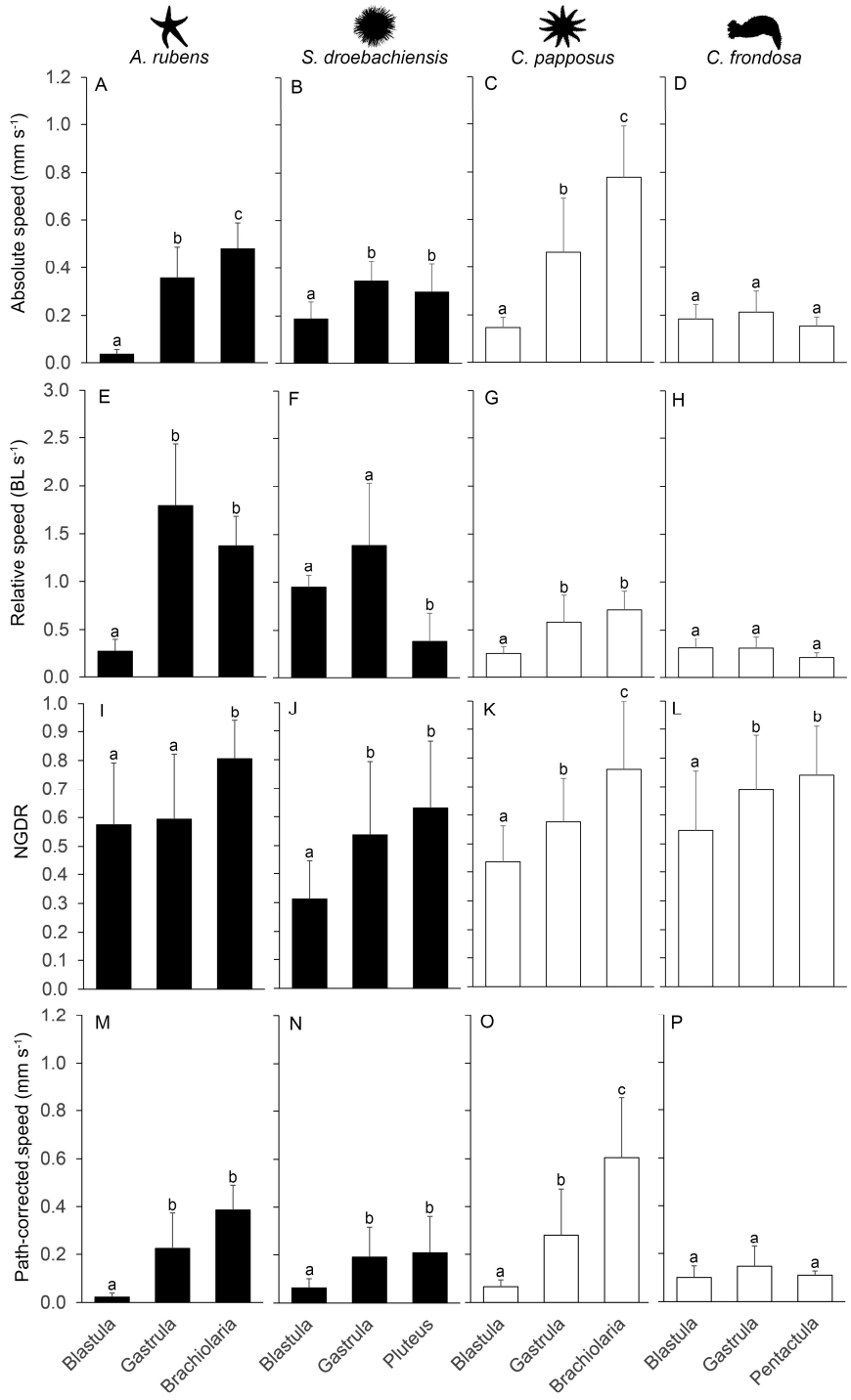

A. rubens
S. droebachiensis
C. papposus
C. frondosa
Absolute speed (mm s-1)
Relative speed (BL s-1)
NGDR
Path-corrected speed (mm s-1)
Blastula
Gastrula
Brachiolaria
Pluteus
Pentactula

Figure 4.1. Swimming capacity of echinoderm propagules under ambient conditions. (A, E, I, M) *A. rubens* at 10℃; (B, F, J, N) *S. droebachiensis* at10℃; (C, G, K, O) *C. papposus* at1-3℃*;* (D, H, L, P) *C. frondosa* at1-3℃. Black bars (left panels) represent planktotrophic species and white bars (right panels) represent lecithotrophic species. Values reported are means ± SD, n = 10-15 individuals per stage. Letters over the bars indicate statistically significant differences.

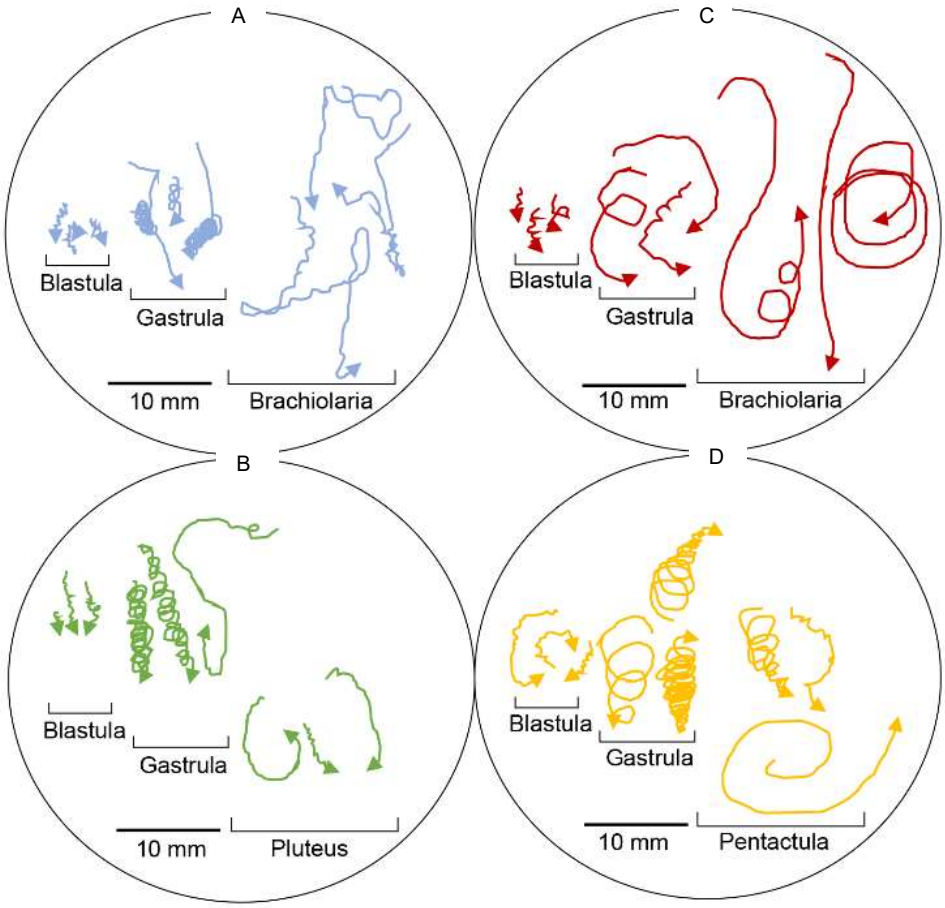


Figure 4.2. Swimming paths of echinoderm propagules under ambient conditions. A) *A. rubens* at 10°C; B) *S. droebachiensis* at10°C; C) *C. papposus* at1-3°C*;* D) *C. frondosa* at1-3°C. Paths represent typical swimming trajectories of propagules over 300s and are scaled relative to size of the circle (arena).

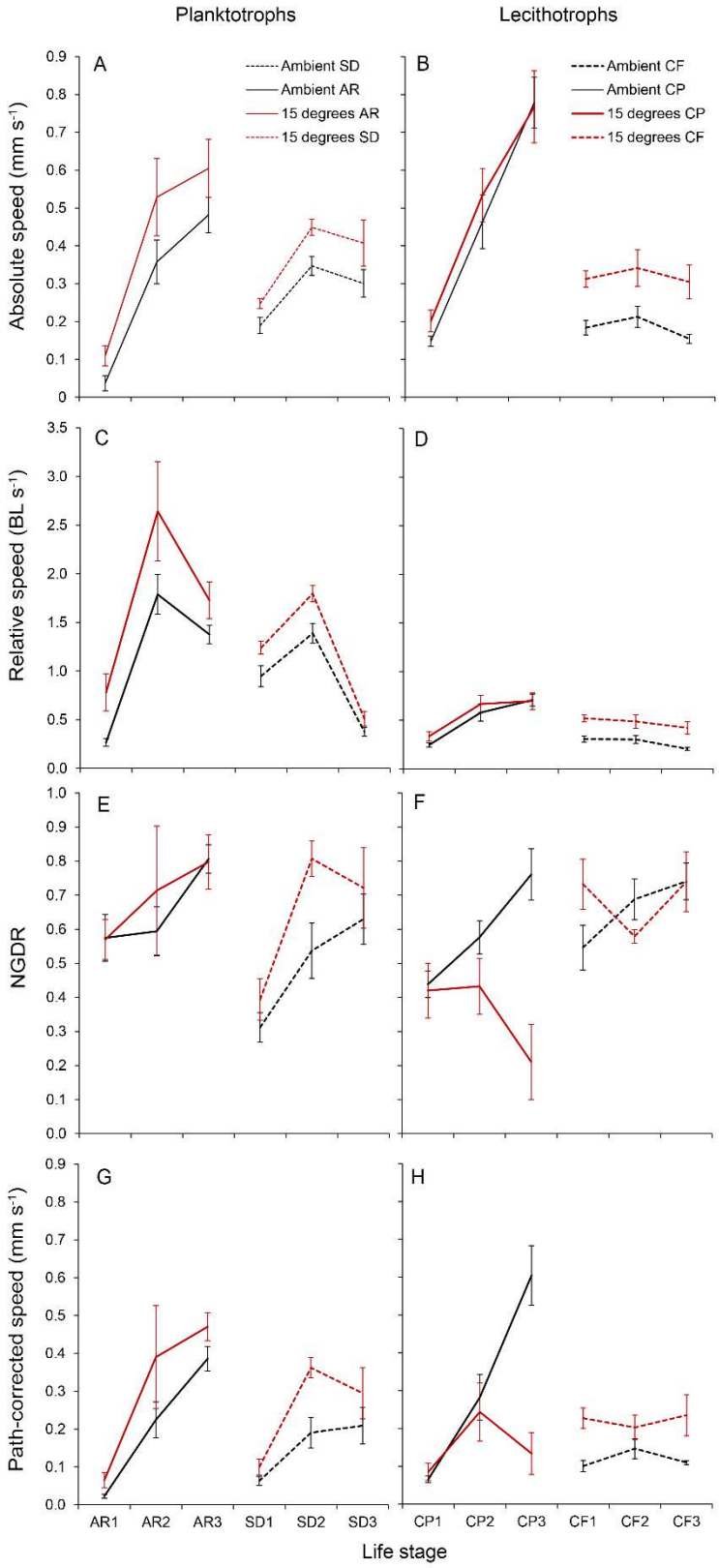

Planktotrophs
Lecithotrophs
A
B
C
D
E
F
G
H
Ambient SD
Ambient AR
15 degrees AR
15 degrees SD
Ambient CF
Ambient CP
15 degrees CP
15 degrees CF
Absolute speed (mm s-1)
Relative speed (BL s-1)
NGDR
Path-corrected speed (mm s-1)
AR1
AR2
AR3
SD1
SD2
SD3
CP1
CP2
CP3
CF1
CF2
CF3
Life stage

Figure 4.3. Mean swimming capacity (± SE) of echinoderm propagules under ambient conditions (black lines) and warm conditions (red lines). Trial temperatures (in ℃) are indicated to the right of each line. Left panels show planktotrophic species (A, C, E, G) and right panels show lecithotrophic species (B, D, F, H). Lines indicate mean values for each life stage (n = 5-15 individuals per stage). 1 = blastula, 2 = gastrula, 3 = a more-advanced larval stage that is species-specific (see Figure 4.1)

Chapter 5. Ontogenetic Variation in Photosensitivity of Developing Echinoderm Propagules

This chapter is being prepared for submission to a scientific journal.

5.1. Abstract

Swimming behaviours and sensory abilities of early pelagic stages play a prominent role in the life history and ecology of sessile/sedentary benthic species, with implications for settlement, recruitment and dispersal. Light is a particularly important driver of navigational behaviour in the ocean, as a signal of key habitat characteristics (e.g., depth, shelter). Work to date on phototaxis has largely focused on planktotrophic larvae that feed during development, and much less on the larger lecithotrophic larvae that rely on maternal provisions (yolk). It remains unclear how responses to light might differ among ciliated propagules of different sizes and nutritional modes. The present study explored if/how phototactic responses are modulated by ontogeny (from embryo to larva), nutritional mode and light colour in ciliated propagules using four co-occurring species of echinoderms: the sea stars *Asterias rubens* (planktotroph) and *Crossaster papposus* (lecithotroph), the sea urchin *Strongylocentrotus droebachiensis* (planktotroph) and the sea cucumber *Cucumaria frondosa* (lecithotroph). Two types of behavioural responses to stimuli (white, red, and blue light) were examined, 1) taxis when the light stimulus was placed at one end of the chamber (net movement towards or away from the light stimulus) and 2) activity level, using a suite of swimming metrics, under uniform

illumination. All four species consistently displayed some level of photosensitivity to white light. While responses varied interspecifically, there was a general transition from predominately positive to predominately negative phototaxis with ontogeny. When the stimulus was red or blue light, planktotrophs modified their phototactic responses in a species- and stage-specific manner, while lecithotrophs displayed heterogeneous taxis responses without a clear net direction. Swimming speeds displayed stage and species-specific variation under constant red or blue light, but swimming trajectories were consistently straighter under red light, resulting in greater displacement. Taken together, the results indicate that propagules of different species respond to light stimuli in distinctive stage-wise manners. Interestingly, ontogenetic patterns appear to be largely conserved in lecithotrophs and to differ more markedly among species and light colours in planktotrophs, though additional species will need to be examined to confirm these patterns. Further investigations of species-specific responses to light might help clarify its roles, in combination with factors such as buoyancy and gravity, in the ecology of propagules of benthic invertebrates.

5.2. Introduction

Light patterns undergo marked vertical changes in the ocean, in that light intensity is reduced (Dickey *et al.* 2011) and longer wavelengths (700-650 nm, red) are rapidly filtered out (McFarland 1986) with increasing depth. The combination of these two features produces a multi-faceted gradient that organisms can detect and respond to (Nilsson 2009). As such, light is an important driver of behaviour and vertical distribution in marine animals (Jékely 2009, Jékely *et al.* 2008, Taylor 1984, Thorson 1964). For benthic species with a complex pelagobenthic life history (whereby intermediate larval forms develop in the water column before returning to the benthos), the naturally-occurring light gradient in the ocean can act as a navigational cue to help propagules detect where they are in the water column and to direct larvae towards settlement sites for the completion of metamorphosis and recruitment (Thorson 1964). Knowledge of sensory behaviour from an ontogenic perspective, can therefore, shape our understanding of small-scale and large-scale species distributions (Anil *et al.* 2010).

Studies on swimming behaviour of marine propagules in response to light cues have been conducted in all major phyla, chiefly at the larval stage. Marine larvae are diverse in form (e.g., ciliated or bearing swimming appendages) and nutritional mode (planktotrophic, relying on external nutrients during development vs. lecithotrophic, yolk-sustained), but share the ability to respond to light during development (Jékely 2009, Jékely *et al.* 2008, Thorson 1964). Basal phyla, such as Porifera (lecithotrophic) and Cnidaria (mainly lecithotrophic), have simple ciliated ellipsoid larvae that alter their swimming patterns in response to light of variable intensity and colour (e.g. Porifera,

Collin *et al.* 2010, Leys *et al.* 2002, Leys and Degnan 2001; Cnidaria, Holst and Jarms 2006, Mundy and Babcock 1998, Svane and Dolmer 1995). In sponge larvae, the apical cilia flex in the presence of light, generally resulting in photonegative swimming behaviour (Collin *et al.* 2010). Planula larvae of cnidarians display more variable responses, showing either photonegative or photopositive behaviour depending on light colour and other environmental factors (Mundy and Babcock 1998). In addition, the swimming behaviours and eventual settlement patterns of planulae have also been shown to be affected by light cues in several species (Pizarro and Thomason 2008, Svane and Dolmer 1995, Tran and Hadfield 2013).

In more derived phyla, studies of phototactic responses have so far centered on planktotrophic larvae, and often excluded the obligatory non-feeding early embryonic stages. Ciliated planktotrophic larvae of Mollusca (Barile *et al.* 1994, Miller and Hadfield 1986), Annelida (Butman *et al.* 1988, McCarthy *et al.* 2002, Young and Chia 1982b), Bryozoa (Wendt 2000) and Echinodermata (Pennington and Emlet 1986) display species-specific patterns of phototaxis that often vary from intermediate to pre-competent larval stages. Patterns of phototaxis are particularly well described among larvae of Arthropoda that posses swimming appendages (Latz and Forward 1977, Shirley and Shirley 1988). Phototactic behaviours in all types of planktotrophic propagules are often presumed to be related to feeding habits and positioning in the water column (e.g., vertical position of barnacle larvae, *Verruca floridana* and *Paralepas pedunculate*, is controlled by light intensity and ontogeny; Bingham and Young 1993). Photosensitivity may also assist the settlement phase and ensure recruitment into the adult population (Jékely 2009).

Knowledge of propagule photosensitivity in lecithotrophic species belonging to derived phyla is quite limited, with the exception of phototaxis among tadpole larvae (phylum Urochordata; McHenry and Strother 2003, Svane and Dolmer 1995, Svane and Young 1989, Vazquez and Young 1998). Overall, phototactic behaviours in phyla with representatives that may develop through either ciliated lecithotrophic or planktotrophic larvae (e.g., Echinodermata) require further attention. Key questions include: Do pelagic lecithotrophic propagules in mixed-mode phyla possess the same degree of sensory ability as planktotrophic propagules and, if so, does it vary with ontogeny?

The present study explores the responses of four species of echinoderms (across three taxonomic classes) to light of various colours throughout early ontogeny, from newly-hatched embryo to late larva. Echinoderms provide a useful framework for studies of phototaxis, as photosensitive cells have been identified in the planktotrophic larvae of one of the five extant classes, and such larvae display taxis in response to light. However, studies of phototaxis in echinoderms have focused primarily on shallow-water adults (e.g., ophiuroids, Hendler 1984; asteroids, Yoshida and Ohtsuki 1968, Yoshida *et al.* 1984; Echinoids, Adams 2001, Domenici *et al.* 2003, Yoshida *et al.* 1984), although phototaxis in the deep sea has also been reported in one species of echinoid (Salazar 1970). Assessments of phototaxis in echinoderm larvae are less common, and historically have overlapped with studies of vertical migration patterns (Fox 1925, Haney 1988, Pennington and Emlet 1986, Roy *et al.* 2012) or settlement preferences in late-stage planktotrophic larvae (Metaxas *et al.* 2008, Mladenov and Chia 1983). Beyond some anecdotal records (e.g. McEuen and Chia 1991), no dedicated studies of phototaxis or

sensory behaviour exist for any lecithotrophic echinoderm larvae. In addition, previous studies of phototaxis have traditionally focused on a single species, whereas comparative studies of species with different development patterns and phylogenies are scarce.

The hypotheses underlying the present study were that: (1) all echinoderm propagules would exhibit behavioural responses to light (of varied colour); (2) that these responses would shift with early ontogeny, independently of nutritional mode; but that (3) planktotrophic larvae would be generally more sensitive to light cues, since they are reported to utilize light cues to facilitate feeding and daily vertical movements in the water column (Pennington and Emlet 1986). Phototactic responses to light of different colour/wavelength (white, red, blue) were characterized based on two aspects of swimming behaviour: (i) taxis (net movement towards/away from a light stimulus) and (ii) swimming metrics under constant light intensity (increase/decrease in speed, more/less straight paths). The focus was on *how* propagules were swimming under different light colours, as speed alone may not be a robust measure of behaviour in weakly swimming ciliated propagules (See Chapter 4 and Hansen *et al.* 2010, Montgomery *et al.* 2017).

5.3. Materials and Methods

5.3.1. Animal collections and maintenance

Asterias rubens (5-10 cm radius), *Crossaster papposus* (5-10 cm radius), *Strongylocentrotus droebachiensis* (5-8 cm test diameter), and *Cucumaria frondosa* (15-20 cm contracted body length) were collected by SCUBA between 10-20 m depth along

the Avalon Peninsula in Southeastern Newfoundland (eastern Canada; 46.640416 N, -52.686534 W). Specimens were housed in 375-L tanks provided with running seawater (~60 L h^{-1}) at ambient temperatures ranging from 0-5 °C, and a natural photoperiod where light intensities ranged daily from 5-450 lux (mean = 300 lux; Mercier and Hamel 2010, Montgomery *et al.* 2017 and Chapter 4).

5.3.2. Spawning induction and culture maintenance

This study was undertaken in the spring of 2014 and 2015, during the natural spawning periods of the focal species (Mercier and Hamel 2010). Gonads of *A. rubens* were surgically collected from mature females and were treated with a solution of 0.1 µM 1-Methyladenine to promote final oocyte maturation (Dorée *et al.* 1976). Spawning was initiated in *S. droebachiensis* by injecting 1-2 mL of 0.5 mol L^{-1} KCl into the coelomic cavity (Meidel and Yund 2001). Fertilization of mature oocytes was performed using a dilution (~ 10,000 spermatozoa mL^{-1}; Byrne *et al.* 2010) in both *A. rubens* and *S. droebachiensis* as this was the optimal concentration to promote 80-90% fertilization success, and reduce the potential for polyspermy. Gametes from a minimum of five females and three males were used to generate cultures. Fertilized oocytes of *C. papposus* and *C. frondosa* were collected following natural spawning events involving multiple males and females, by gently skimming them from the surface of the tanks (as they are positively buoyant).

Propagules were cultured in natural seawater, at the ambient temperature experienced in nature during the spawning season and early development. Standard

rearing techniques were used for each species (*A. rubens* and *S. droebachiensis,* Meidel *et al.* 1999; *C. frondosa and C. papposus,* Hamel and Mercier 1996; see Montgomery *et al.* 2017 and Chapter 4). Lecithotrophic propagules of *C. papposus,* and *C. frondosa* were raised at 1-3 °C (1.5-L vessels, approx. 0.1 L hr^{-1} flow-through conditions) matching the ambient conditions during natural spawning. Propagules of *A. rubens* were obtained a little later in the spring when the ocean temperature was higher; these cultures were maintained at 10°C (static conditions). Once feeding larval stages were reached (pluteus *S. droebachiensis*, late bipinnaria *A. rubens*), cultures were fed with a commercial mix of algae (Phytofeast Live, Reef Nutrition, at a density of 1000 cells mL^{-1}; see Chapter 4 and Meidel *et al.* 1999, Montgomery *et al.* 2017). All trials were performed on propagules obtained inside the same breeding season. In the case of multiple spawning events in the same season (only relevant for *C. frondosa*), cohorts were tested separately. However, there were no statistical differences among tested parameters so they were pooled for subsequent analyses.

5.3.3. Experimental protocols

Developmental stages were tested when 80% of individuals in culture had reached that stage (Gemmill 1914, 1920, Hamel and Mercier 1996, Meidel *et al.* 1999). Sensory responses were tested in the late blastula and gastrula of all species. Species-specific larval forms were also tested, including the bipinnaria and brachiolaria stages for *Asterias rubens*, the prism and pluteus stages for *Strongylocentrotus droebachiensis*, the early brachiolaria and late brachiolaria stages of *Crossaster papposus*, and the vitellaria and

early pentactula stages of *Cucumaria frondosa*. Early pentactula of *C. frondosa* were tested before primary podia emerged (Hamel and Mercier 1996) because after this time point, locomotion shifts from swimming with cilia to crawling with appendages. Trials were performed under their respective ambient culture conditions (described earlier).

Propagules were tested under white (λ = 440-650), red (λ = 650) and blue (λ = 440) light colours at fixed intensity (300 lux, i.e., with illumination from above) or with the light source placed at the side of the dish (light intensity varying from approximately 300 lux to 5 lux across the dish; see below). Light colours were chosen as a proxy for near surface (white, red) and several meters below surface (blue) conditions given the rapid loss of long wavelengths in North Atlantic waters in the first few meters (Figure 5.1). For fixed light intensity trials, 300 lux was selected, as this represented the mean ambient light level experienced by the propagules in the culture vessels. In the fixed light trials, the lamp was mounted 10 cm above the experimental dish. To measure / asses phototaxis, the lamp was mounted 5 cm above the surface of the dish on one side. The distance from one side of the dish to the other was far enough to reduce the light intensity to close to zero (5 lux). A Fuloon 12V 5050 RGB light emitting diode (LED) lamp was used for all experimental trials. Background colour (white or black) did not affect responses at any developmental stage (as determined during preliminary experiments) so black backgrounds were used to enhance the effectiveness of light gradients.

We chose to examine phototaxis at the level of the propagules in the horizontal plane to minimize the potential confounding effects of nutritional mode differences (buoyancy) and gravity. At the onset of each individual trial, propagules were gently

pipetted into small glass dishes (6-10 cm diameter, 2 cm high) and allowed to swim undisturbed for 5 min with no directional light source. This time frame was sufficient for propagules to return to normal swimming behaviour (preliminary experiments; Chapter 4, Montgomery *et al.* 2017). Stable temperatures were maintained during trials using an ice bath. Following the acclimation period, experimental light conditions were established. Video recordings were taken (using an Olympus TG-1 camera) for 5 min and the resulting footage was later analysed with the software ImageJ (see method below). Three replicate trials were performed for each stage in each species, for a total of 10-30 propagules per stage per species. As a control for passive drift, recordings were also taken of unhatched propagules (eggs/embryos still inside the fertilization envelope) under each set of experimental light conditions (Montgomery *et al.* 2017 and Chapter 4). Unhatched propagules are comparable to newly hatched blastulae in size and buoyancy, but have no cilia, and thus, do not exhibit any autonomous/active behaviour.

5.3.4. Particle tracking

The MOSAIC particle tracking algorithm in ImageJ [http://mosaic.mpi-cbg.de/?q=downloads/imageJ] was used to analyze swimming responses and directionality at each tested life stage (See Chapter 4 and Chenouard *et al.* 2014, Montgomery *et al.* 2017). The 2D horizontal paths of swimming propagules were exported as x-y coordinates and converted into displacement data over one second intervals for the duration of the trials. Propagules were excluded from the analysis if they collided with each other, or with the edge of the dish. For all treatments, natural drift

obtained from unhatched propagules were subtracted from mean speeds to account for passive movement in the horizontal plane. Initial and final coordinates of propagules were used for subsequent taxis analyses.

5.3.5 Sensory response metrics

Sensory responses were broken into two main categories: (1) *taxis* (directionality) and (2) *activity level* (magnitude of response). Propagule taxis was determined in trials where the light source was placed to one side of the dish; it was scored based on propagule direction of movement as photopositive (moving towards light stimulus), photonegative (moving away from light stimulus), or neutral (net displacement towards or away from the light source of less than five body lengths). The term heterogeneous taxis was used to describe trials where a mixture of photopositive, photonegative and neutral responses were observed with no clear majority (i.e., larvae were swimming randomly). Beginning and end values of *x y* coordinates were defined relative to the light source for taxis calculations and subsequent statistical analyses (see below).

Activity level was defined as mean absolute swimming speed (mm s^{-1}) calculated as the average of instantaneous speeds obtained from the distance travelled in each one-second interval (see Montgomery *et al.* 2017 and Chapter 4). Activity level was quantified in trials of uniform light intensity to assess the degree of propagule response to the three tested light colours. Net-to-gross displacement ratio (NGDR) was also determined in conjunction with mean absolute swimming speed to quantify swimming patterns independent of direction. NGDR was calculated (see Montgomery *et al.* 2017

and Chapter 4) as the ratio of propagule displacement to total distance travelled. Values closer to 1 indicate relatively straight paths whereas values closer to 0 reflect relatively circular paths. Here, NGDR was refined to be a running average of the ratio of net displacement to total distance travelled over 30 second intervals.

5.3.6. Statistical analysis

Chi-square tests were used to test phototaxis and directionality relative to randomly swimming propagules. N-1 corrected Chi-square tests (Campbell 2007) were performed when expected values were < 5. All directionality statistics were first conducted independently of light colour to assess the impact of light intensity separately from wavelength.

For each species, a nested analysis of variance (ANOVA) was used to test the effect of life stage and replicate trial number on activity level metrics under fixed light intensity conditions. Further, one-way and two-way ANOVAs were used to test the effect of life stage and nutritional mode on activity level (swimming speed, NGDR) and taxis under white, red, and blue light, within species. Statistical analyses were conducted using Sigma Plot and R statistical software at $\alpha = 0.05$. See appendices 4A-D for detailed statistical outputs.

5.4. Results

5.4.1. Phototaxis throughout early ontogeny

Table 5.1 shows the net response to the three light stimuli, based on statistical significance, for each stage-species combination, whereas Figure 5.2 provides a detailed overview of intra-stage variability in phototactic responses measured in the different propagules of each species.

Under white light, there was a general progression from heterogeneous or net positive phototaxis at early stages to net negative phototaxis at intermediate stages, and a uniform absence of net phototaxis in late larvae, in the four echinoderm-species tested. However, differences appeared to emerge among nutritional modes and interspecifically, as there was variation among individual propagules within each stage (Table 5.1, Fig. 5.2). General ontogenetic patterns in the planktotrophs, *A. rubens* and *S. droebachiensis,* were similar in terms of net phototactic response except at the gastrula stage: the blastulae exhibited heterogeneous phototaxis; the gastrulae became photopositive (Fig. 5.2A) and photonegative (Fig. 5.2E), respectively; the early larval stages (bipinnaria of *A. rubens*, prism *S. droebachiensis*) were chiefly photonegative, and the later larval stages (brachiolaria and pluteus, respectively) exhibited no net pattern of phototaxis (Fig. 5.2A, E). Propagules of the lecithotrophic species, *C. papposus* and *C. frondosa*, responded differently than the planktotrophs and differed at the blastula stage; photopositive in *C. papposus* and heterogeneous taxis in *C. frondosa*. These species demonstrated uniform responses afterward, including heterogeneous phototaxis in gastrulae, net photonegativity

in early larval stages (early brachiolaria of *C. papposus*, vitellaria of *C. frondosa*) and no clear net-phototaxis pattern in the final larval stages (late brachiolaria and pentactula, respectively) under white light (Fig. 5.2H, K).

Under red light, phototactic responses generally differed from those measured under white light, and effects varied among life stages (Table 5.1, Fig. 5.2). Furthermore, these changes were not uniform between the two planktotrophic species. In *A. rubens*, the net responses of embryos (blastula and gastrula) paralleled those obtained in white light, whereas larval stages displayed a different mixture of photopositive and photonegative behaviour. At the bipinnaria stage, *A. rubens* did not display net phototaxis but was photonegative at the brachiolaria stage (Fig. 5.2B). In *S. droebachiensis*, the opposite occurred, i.e., net behavioural changes induced by red light colour occurred at the embryonic stage, where the blastulae were heterogenous and the gastrulae had no clear response pattern (while prisms were photonegative and pluteii displayed heterogeneous phototaxis patterns; Fig. 5.2F). Among the lecithotrophs, *C. papposus* and *C. frondosa,* the red-light stimulus had no uniform effect, in that nearly all stages of both species displayed heterogeneous phototaxis (Fig. 5.2I, L). In general, *C. papposus* (Fig. 5.2I) tended to be more photonegative than *C. frondosa* (Fig. 5.2L) under red light. Moreover, the gastrulae of *C. frondosa* became largely unresponsive (displaying limited directional displacement away or towards the light stimulus; Fig. 5.2L).

Trials under blue light elicited a relatively uniform ontogenetic pattern in the net responses of planktotrophic species (*A. rubens*, *S. droebachiensis*), that differed from those measured in either white or red light (Fig. 5.2C, G). Blastulae of both species

showed net photonegative responses, while all later stages displayed heterogeneous phototaxis patterns. The net responses of lecithotrophic propagules to blue light also differed from those to white light and red light (Fig. 5.2J, M). In general, *C. frondosa* (Fig. 5.2M) were more photonegative than *C. papposus* (Fig. 5.2J), though within-stage variation was common. Most stages showed heterogeneous phototactic behaviours, whereas the late embryos and late larvae of *C. papposus* displayed a clear absence of movement relative to the light stimulus (Fig. 5.2J). The early brachiolaria of *C. papposus* and early pentactula of *C. frondosa* showed a tendency to be photopositive towards blue wavelength, although this was only significant in *C. papposus* (Fig. 5.2J, M; Table 5.1).

5.4.2. Activity level during phototactic responses

Activity level during phototactic responses (movement either away or toward the light stimulus) was first examined independently of light colour (Fig. 5.3). The response to a light stimulus generally had no effect on swimming speed, when all stages of all species were considered together, except at the bipinnaria stage of *A. rubens* and the gastrula stage of *S. droebachiensis*, which displayed greater speeds (0.66 ± 0.04 and 0.39 ± 0.02 mm s^{-1}, respectively) when moving towards the stimulus, during a photopositive response, than away from it, during a photonegative response (Fig. 5.3; $p = 0.025$ and 0.028, respectively). On the other hand, swimming trajectories (paths) generally varied across phototactic responses. Paths tended to be straighter in propagules swimming away or towards light (NGDR > 0.5, overall mean of 0.67; Fig. 5.4), relative to propagules that did not exhibit any net phototaxis (NGDR < 0.5, overall mean of 0.41; Fig. 5.4). This

trend was significant among several species-stage combinations, including the blastula and bipinnaria of *A. rubens*, the blastula and early and late brachiolaria of *C. papposus*, and the vitellaria of *C. frondosa*.

5.4.3. Activity level under uniform light levels

Activity levels during phototaxis varied across the three tested light colours. To account for differences in light intensity when the light source was placed at one side of the dish, propagules were further tested under uniform light levels to tease out the effect of light colour itself.

The propagules under study demonstrated various activity levels in the presence of white, red, and blue light of uniform intensity, based on swimming speeds and NGDR values. Propagules of *A. rubens* increased swimming speed with ontogeny under all light colours, whereas propagules of *S. droebachiensis* did not (Fig. 5.5). The fastest absolute speeds at the blastula stage were measured under blue light in *A. rubens* and *S. droebachiensis*, but the swimming capacity of the former species was only about one-third of the later (0.10 ± 0.01 vs.0.33 ± 0.05 mm s^{-1}, respectively; Fig. 5.5A, B). Differences in swimming capacity between *A. rubens* and *S. droebachiensis* also occurred at the intermediate gastrula stage, where *A. rubens* gastrulae swam faster under white than red or blue light (0.36 ± 0.04 vs 0.11 ± 0.01 and 0.05 ± 0.01 mm s^{-1}) but *S. droebachiensis* showed no swimming speed differences among tested light colours. In contrast, the early-larval stages (bipinnaria and prism, respectively) and late-larval stages

(brachiolaria and pluteus) of these species demonstrated similar swimming patterns (within stage); the fastest speeds were detected under red light (Fig. 5.5A, B).

Like planktotrophs, lecithotrophs (*C. papposus* and *C. frondosa*) exhibited contrasting ontogenetic patterns; propagules of *C. papposus* increased swimming speed with ontogeny, but propagules of *C. frondosa* did not (Fig. 5.5). Other aspects of their responses also differed. In *C. papposus*, blastulae swam slower under red light (0.095 ± 0.01 mm s^{-1}, $p = 0.007$) than either white (0.15 ± 0.01 mm s^{-1}) or blue light (0.14 ± 0.02 mm s^{-1}), whereas gastrulae swam faster under white light (0.46 ± 0.06 mm s^{-1}, $p< 0.001$) than either red or blue light (0.14 ± 0.05 and 0.32 ± 0.05 mm s^{-1}, respectively; Fig. 5.5C). Late brachiolaria stages of *C. papposus* exhibited different swimming speeds under all tested light colours; swimming the slowest under blue light (0.25 ± 0.06 mm s^{-1}, $p<0.001$) and the fastest under white light (0.78 ± 0.07 mm s^{-1}; Fig. 5.5C). All tested life stages of *C. frondosa* swam at similar speeds across the light colours (within stage; Fig. 5.5C, D).

NGDR values were frequently, but not always, lower (by an average of 20-50%) for propagules swimming under blue light than white or red light, indicating more circular swimming paths (overall mean of 0.49 vs. 0.64; Fig. 5.6). This trend was determined to be statistically significant in all stages of *A. rubens* (Fig. 5.6A), in the gastrula stage of *C. papposus* (Fig. 5.6C) and in the gastrula, vitellaria, and pentactula stages of *C. frondosa* (Fig. 5.6D). In contrast, paths were of similar straightness under red and blue light in the blastula stage of *S. droebachiensis* (Fig. 5.6B), and the blastula, early brachiolaria, and late brachiolaria of *C. papposus* (Fig. 5.6C).

5.5. Discussion

The propagules of all four species of echinoderms tested here showed clear responses to light (though not at all stages), based on their swimming speed and path under constant light of various colour, and on their net displacement relative to light. Comparisons across species and nutritional modes revealed both similarities and differences. (1) The overall phototactic responses to white light changed with ontogeny, from heterogeneous or positive phototaxis in early embryos, to net negative phototaxis in early larval stages, to heterogeneous responses in all late larvae. (2) Exposure to red and blue light elicited net phototactic behaviours in many propagules of planktotrophic species, with noted shifts in ontogenetic patterns relative to white light. In support of our initial hypotheses, a few life stages of lecithotrophic species were less responsive to red and blue light, relative to their planktotrophic counterparts, and displayed heterogeneous responses rather than clear net responses. (3) Within-stage phototactic responses to light (relative proportions of phototaxis direction) were variable under all light colours, and not consistent among tested species, even when net phototactic directions were the same. (4) Swimming speeds under uniform light varied across ontogeny and species. However, swimming paths tended to be straighter (NGDR values closer to 1) under uniform red light relative to uniform blue light in all species.

Photonegativity under white light has been reported in the larvae of other echinoderms; specifically, the planktotrophic pluteus of *Dendraster excentricus* (Pennington and Emlet 1986) and the lecithotrophic late-pentactula of *Psolus chitonoides* (Young and Chia 1982a). Both studies examined phototactic responses of early and late

larval stages in small-scale laboratory conditions, similar to the present study. However, *D. excentricus* was tested in a column, with a vertical light gradient. There are additional challenges associated with interpreting phototaxis results in vertical settings, because echinoderm propagules are highly sensitive to gravity, and display clear geotactic patterns in the absence of directional light from early embryonic stages to competent larvae (Mogami *et al.* 1988). In fact, the patterns observed by Pennington and Emlet (1986) were not replicable in the horizontal plane, indicating that more studies may be required to tease apart the influence of geotactic and phototactic stimuli, potentially by using top versus bottom lighting. A similar confounding relationship between gravity and light behavioural responses was also noted in studies of crustaceans (Latz and Forward 1977, Shirley and Shirley 1988) and annelids (McCarthy *et al.* 2002), which suggests that a hierarchy of cues may drive propagule navigational patterns in the water column (Jékely 2009, Jékely *et al.* 2008).

Patterns of phototaxis in the present study changed slightly when propagules were tested under red and blue light. In planktotrophs (*A. rubens* and *S. droebachiensis*) the clearest shift was detected after exposure to blue light, whereby early embryos became strongly photonegative. As for propagules of lecithotrophs (*C. frondosa* and *C. papposus*), they displayed either heterogeneous phototactic responses (random movements), were photopositive (in the case of early brachioaria of *C. papposus*) or did not have directional movement under red and blue light stimuli. Given the loss of red wavelengths in the first few meters of the ocean (McFarland 1986), surface behaviours might be expected to be more driven by the presence of red light (as part of white light)

and benthic behaviours to be driven by blue light (below certain depths, where red wavelengths have been filtered out). The phototactic patterns seen here under red light are consistent with studies of coral planulae (also lecithotrophic), which showed a reduction in phototaxis under red light compared to white light (Mundy and Babcock 1998). Certain species of larval reef fish also experience changes in their ability to detect red wavelengths as a result of a transition from surface to deeper waters with ontogeny (Shand 1993). Since lecithotrophic propagules are buoyant and do not need to locate food in surface waters, as planktotrophs do (Pechenik 1999), they may rely less on surface-related light cues. Alternatively, the absence of directional movement relative to a light cue might be interpreted to mean something else; e.g. that propagules have found suitable conditions (and do not need to move), that propagules are not sensitive to light, that another cue may be taking precedence, or that another behavioural parameter such as speed or path shape is being manipulated in response to the stimulus (Ettinger-Epstein *et al.* 2008, Pizarro and Thomason 2008).

The direction and speed of swimming have previously been used to holistically describe directional behavioural responses to stimuli such as light, salinity and temperature gradients (Civelek *et al.* 2013, Michalec *et al.* 2010, Pizarro and Thomason 2008, Richmond and Woodin 1996). Few differences in absolute swimming speeds were detected here amongst the different taxis directions, but swimming paths were generally straighter for clear photopositive and photonegative responses (relative to heterogeneous responses). Although speeds were generally similar amongst positive and negative responses to light, the combination of swimming speed and straighter paths increased the

dispersal potential of propagules that were swimming away and towards the stimulus. Taken together, these results support the hypothesis that speed alone is not an ideal measure of larval behaviour (See Chapter 4 and Montgomery *et al.* 2017, Pizarro and Thomason 2008). Other features of activity level are important, and need to be considered such as trajectory, width of helical path and feeding behaviours (in species with planktotrophic development, Hansen *et al.* 2010). To this end, one study found that coral settlement was more directly affected by swimming trajectory than changes in swimming speed (Pizarro and Thomason 2008). Differences in path straightness were also detected among the tested light colours when propagules swam under uniform light intensities. Even though speeds under blue light tended to be similar to the other tested light colours, paths were often more circular under uniform blue light. This variability in trajectory may facilitate different behaviours based on the external light environment, such as feeding or navigation.

Modifications of swimming direction and activity level in response to external stimuli are known to be related to settlement success in benthic marine invertebrates (Pizarro and Thomason 2008) and thus, may be particularly important close to the benthos. When pelagic propagules are first released, they need to escape the benthos quickly due to mortality risks associated with benthic predators and/or the lack of food in the case of planktotrophic species (Johnson and Shanks 2003, Mercier *et al.* 2013a, Pennington *et al.* 1986, Vaughn and Allen 2010). Early swimming probably evolved to facilitate this transition, especially in feeding propagules that are neutral or negatively buoyant (Staver and Strathmann 2002). At embryonic and early larval stages, positive

phototaxis may guide weakly swimming embryos towards the surface and away from the benthos, perhaps directing planktotrophic species towards the chlorophyll maximum layer where food would be present. In contrast, lecithotrophic propagules that depend on maternal lipids for energy typically float toward the surface soon after spawning (Prowse *et al.* 2009). This positive buoyancy, combined with the net attraction to light at early developmental stages seen here, and their natural swimming abilities (See Chapter 4 and Montgomery *et al.* 2017), likely drives early lecithotrophic propagules to remain close to the surface where water temperatures warm up in the spring (i.e., measured though the Atlantic Zone Monitoring Program at Station 27). Exposure to warmer conditions may act to speed the development of lecithotrophic and planktotrophic species that tend to be naturally slow developers under cold temperatures (Hamel and Mercier 1996, Hoegh-Guldberg and Pearse 1995, So *et al.* 2011). Maintaining a position in the upper regions of the ocean during development may also enhance the dispersal potential of propagules due to higher levels of turbulence and currents (Butler IV *et al.* 2011, Cowen and Sponaugle 2009, Mercier *et al.* 2013b, Robins *et al.* 2013). Following surface development, a shift to photonegativity could facilitate migration to the benthos for settlement, a requirement for all benthopelagic species regardless of nutritional mode (Hadfield 1986, Mundy and Babcock 1998, Pechenik 1999, Sulkin 1975, Svane and Dolmer 1995, Thorson 1964, Vazquez and Young 1998, Wendt 2000). This shift to photonegativity at early larval stages is relatively common (Thorson 1964); it has previously been reported in bryozoans (Ryland 1960), mussels (Bayne 1964), lobsters (Botero and Atema 1982) and barnacles (Lang *et al.* 1979), suggesting that conservation of this behavioural pattern may be

adaptive for marine larvae (Svane and Dolmer 1995). In planktotrophic species, the emergence of these patterns might also overlap with geotaxis, fasciliating daily vertical migrations to surface waters in the dark for food capture (Barile *et al.* 1994, Pennington and Emlet 1986).

At advanced larval stages, we observed a consistent lack of net phototaxis under white light in all four species. An ontogenetic shift away from clear phototactic patterns as larvae approach competency has been generally noted (Young 1999) and specifically documented in competent larvae of corals (e.g., *Montastraea annularis* and *M. faveolata*; Pizarro and Thomason 2008), bryozoans (e.g., *Bugula neretina*; Lynch 1947) and crabs (e.g., *Panopeus herbstii* and *Leptodius floridanus* Sulkin 1975). This heterogeneity is likely temporary, and may in fact be a consequence of the cellular reorganization that occurs before and during metamorphosis (Young 1999), or the result of asynchronous development that is common among echinoderms, where internal structures may be more/less advanced than external features used for identification (Cameron and Fankboner 1989, Hamel and Mercier 1996, Parks *et al.* 1989). The fully-developed pentactula of *C. frondosa* (a more advanced pre-competent stage than tested here, that possesses primary podia for crawling) display clear photonegative responses, similar to the behaviour of early juveniles of this species (Pers. Obs. 2015, 2017; B. Gianasi, E. Montgomery). However, the early pentactula tested here (devoid of primary podia) did not exhibit a net phototactic swimming direction. This was similar to the results of Young and Chia (1982a), who reported neutral phototaxis in the late vitellaria/ early pentactula

of *Psolsus chitonoides,* but clear negative phototaxis in the mature, crawling pentactula and post-settled juvenile.

Taken together, these data suggest that propagule behaviours and preferences are time-sensitive and complex, consistent with pivotal stages in morphogenesis that occur from hatching to settlement. While the developmental patterns of some taxa do not easily permit comparisons of embryos and larvae (e.g., some molluscs and bryozoans have a protected embryonic phase and pelagic larvae), incorporation of embryonic stages into behavioural studies of larvae, where possible, will only enhance phylogenetically-relevant comparisons of ontogenetic patterns.

5.6. Acknowledgements

The authors wish to thank Memorial University Field Services for animal collections. The authors also wish to thank K. Gamperl (Memorial University) for constructive comments on the manuscript. This work was completed with funding from a Natural Sciences and Engineering Research Council Discovery Grant (#311406) and Canadian Foundation for Innovation Grant (#11231) issued to A. Mercier and an NSERC CGS-D Award to E. Montgomery.

5.7. References

Adams, N. L. 2001. UV radiation evokes negative phototaxis and covering behavior in the sea urchin *Strongylocentrotus droebachiensis*. *Marine Ecology Progress Series* **213**:87-95.

Anil, A. C., L. Khandeparker, D. V. Desai, L. V. Baragi, and C. A. Gaonkar. 2010. Larval development, sensory mechanisms and physiological adaptations in acorn barnacles with special reference to *Balanus amphitrite*. *Journal of Experimental Marine Biology and Ecology* **392**:89-98.

Barile, P. J., A. W. Stoner, and C. M. Young. 1994. Phototaxis and vertical migration of the queen conch (*Strombus gigas*) veliger larvae. *Journal of Experimental Marine Biology and Ecology* **183**:147-162.

Bayne, B. 1964. The responses of the larvae of *Mytilus edulis* L. to light and to gravity. *Oikos*:162-174.

Bingham, B. L., and C. M. Young. 1993. Larval phototaxis in barnacles and snails associated with bathyal sea urchins. *Deep Sea Research Part I: Oceanographic Research Papers* **40**:1-12.

Botero, L., and J. Atema. 1982. Behavior and substrate selection during larval settling in the lobster *Homarus americanus*. *Journal of Crustacean Biology* **2**:59-69.

Butler IV, M. J., C. B. Paris, J. S. Goldstein, H. Matsuda, and R. K. Cowen. 2011. Behavior constrains the dispersal of long-lived spiny lobster larvae. *Marine Ecology Progress Series* **422**:223-237.

Butman, C. A., J. P. Grassle, and E. J. Buskey. 1988. Horizontal swimming and gravitational sinking of *Capitella* sp. I (Annelida: Polychaeta) larvae: implications for settlement. *Ophelia* **29**:43-57.

Byrne, M., N. Soars, P. Selvakumaraswamy, S. A. Dworjanyn, and A. R. Davis. 2010. Sea urchin fertilization in a warm, acidified and high pCO_2 ocean across a range of sperm densities. *Marine Environmental Research* **69**:234-239.

Cameron, J. L., and P. V. Fankboner. 1989. Reproductive biology of the commercial sea cucumber *Parastichopus californicus* (Stimpson)(Echinodermata: Holothuroidea). II. Observations on the ecology of development, recruitment, and the juvenile life stage. *Journal of Experimental Marine Biology and Ecology* **127**:43-67.

Campbell, I. 2007. Chi squared and Fisher Irwin tests of two by two tables with small sample recommendations. *Statistics in Medicine* **26**:3661-3675.

Chenouard, N., I. Smal, F. De Chaumont, M. Maška, I. F. Sbalzarini, Y. Gong, J. Cardinale, C. Carthel, S. Coraluppi, and M. Winter. 2014. Objective comparison of particle tracking methods. *Nature Methods* **11**:281.

Civelek, C. V., R. M. Daigle, and A. Metaxas. 2013. Effects of temperature on larval swimming patterns regulate vertical distribution relative to thermoclines in *Asterias rubens*. *Journal of Experimental Marine Biology and Ecology* **445**:1-12.

Collin, R., A. S. Mobley, L. B. Lopez, S. P. Leys, M. C. Diaz, and R. W. Thacker. 2010. Phototactic responses of larvae from the marine sponges *Neopetrosia proxima* and *Xestospongia bocatorensis* (Haplosclerida: Petrosiidae). *Invertebrate Biology* **129**:121-128.

Cowen, R. K., and S. Sponaugle. 2009. Larval dispersal and marine population connectivity. *Marine Science* **1**.

Dickey, T. D., G. W. Kattawar, and K. J. Voss. 2011. Shedding new light on light in the ocean. *Physics Today* **64**:44-49.

Domenici, P., D. Gonzalez-Calderon, and R. Ferrari. 2003. Locomotor performance in the sea urchin *Paracentrotus lividus*. *Journal of the Marine Biological Association of the UK* **83**:285-292.

Dorée, M., P. Guerrier, and N. J. Leonard. 1976. Hormonal control of meiosis: Specificity of the 1-methyladenine receptors in starfish oocytes. *Proceedings of the National Academy of Sciences of the United States of America* **73**:1669-1673.

Ettinger-Epstein, P., S. Whalan, C. N. Battershill, and R. de Nys. 2008. A hierarchy of settlement cues influences larval behaviour in a coral reef sponge. *Marine Ecology Progress Series* **365**:103-113.

Fox, H. M. 1925. The effect of light on the vertical movement of aquatic organisms. *Biological Reviews* **1**:219-224.

Gemmill, J. F. 1914. The development and certain points in the adult structure of the starfish *Asterias rubens*. *Philosophical Transactions of the Royal Society B: Biological Sciences* **205**:213-294.

Gemmill, J. F. 1920. The development of the starfish *Crossaster papposus*. *Quarterly Journal of Microscopical Science* **64**:155-187.

Hadfield, M. G. 1986. Settlement and recruitment of marine invertebrates: A perspective and some proposals. *Bulletin of Marine Science* **39**:418-425.

Hamel, J.-F., and A. Mercier. 1996. Early development, settlement, growth, and spatial distribution of the sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea). *Canadian Journal of Fisheries and Aquatic Sciences* **53**:253-271.

Haney, J. F. 1988. Diel patterns of zooplankton behavior. *Bulletin of Marine Science* **43**:583-603.

Hansen, B. W., H. H. Jakobsen, A. Andersen, R. Almeda, T. M. Pedersen, A. M. Christensen, and B. Nilsson. 2010. Swimming behavior and prey retention of the polychaete larvae *Polydora ciliata* (Johnston). *Journal of Experimental Biology* **213**:3237-3246.

Hendler, G. 1984. Brittlestar color‐change and phototaxis (Echinodermata: Ophiuroidea: Ophiocomidae). *Marine Ecology* **5**:379-401.

Hoegh-Guldberg, O., and J. S. Pearse. 1995. Temperature, food availability, and the development of marine invertebrate larvae. *American Zoologist* **35**:415-425.

Holst, S., and G. Jarms. 2006. Substrate choice and settlement preferences of planula larvae of five Scyphozoa (Cnidaria) from German Bight, North Sea. *Marine Biology* **151**:863-871.

Jékely, G. 2009. Evolution of phototaxis. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364**:2795-2808.

Jékely, G., J. Colombelli, H. Hausen, K. Guy, E. Stelzer, F. Nédélec, and D. Arendt. 2008. Mechanism of phototaxis in marine zooplankton. *Nature* **456**:395-399.

Johnson, K. B., and A. L. Shanks. 2003. Low rates of predation on planktonic marine invertebrate larvae. *Marine Ecology Progress Series* **248**:125-139.

Lang, W. H., R. B. Forward Jr, and D. C. Miller. 1979. Behavioral responses of *Balanus improvisus* nauplii to light intensity and spectrum. *Biological Bulletin* **157**:166-181.

Latz, M. I., and R. B. Forward. 1977. The effect of salinity upon phototaxis and geotaxis in a larval crustacean. *Biological Bulletin* **153**:163-179.

Leys, S. P., T. W. Cronin, B. M. Degnan, and J. N. Marshall. 2002. Spectral sensitivity in a sponge larva. *Journal of Comparative Physiology A* **188**:199-202.

Leys, S. P., and B. M. Degnan. 2001. Cytological basis of photoresponsive behavior in a sponge larva. *Biological Bulletin* **201**:323-338.

Lynch, W. F. 1947. The behavior and metamorphosis of the larva of *Bugula neritina* (Linnaeus): experimental modification of the length of the free-swimming period and the responses of the larvae to light and gravity. *Biological Bulletin* **92**:115-150.

McCarthy, D. A., R. B. Forward Jr, and C. M. Young. 2002. Ontogeny of phototaxis and geotaxis during larval development of the sabellariid polychaete *Phragmatopoma lapidosa*. *Marine Ecology Progress Series* **241**:215-220.

McEuen, F. S., and F. S. Chia. 1991. Development and metamorphosis of two psolid sea cucumbers, *Psolus chitonoides* and *Psolidium bullatum*, with a review of reproductive patterns in the family Psolidae (Holothuroidea: Echinodermata). *Marine Biology* **109**:267-279.

McFarland, W. N. 1986. Light in the sea-Correlations with behaviors of fishes and invertebrates. *American Zoologist* **26**:389-401.

McHenry, M., and J. Strother. 2003. The kinematics of phototaxis in larvae of the ascidian *Aplidium constellatum*. *Marine Biology* **142**:173-184.

Meidel, S. K., R. E. Scheibling, and A. Metaxas. 1999. Relative importance of parental and larval nutrition on larval development and metamorphosis of the sea urchin *Strongylocentrotus droebachiensis*. *Journal of Experimental Marine Biology and Ecology* **240**:161-178.

Meidel, S. K., and P. O. Yund. 2001. Egg longevity and time-integrated fertilization in a temperate sea urchin (*Strongylocentrotus droebachiensis*). *Biological Bulletin* **201**:84-94.

Mercier, A., E. J. Doncaster, and J.-F. Hamel. 2013a. Contrasting predation rates on planktotrophic and lecithotrophic propagules by marine benthic invertebrates. *Journal of Experimental Marine Biology and Ecology* **449**:100-110.

Mercier, A., and J.-F. Hamel. 2010. Synchronized breeding events in sympatric marine invertebrates: Role of behavior and fine temporal windows in maintaining reproductive isolation. *Behavioral Ecology and Sociobiology* **64**:1749-1765.

Mercier, A., M. A. Sewell, and J.-F. Hamel. 2013b. Pelagic propagule duration and developmental mode: Reassessment of a fading link. *Global Ecology and Biogeography* **22**:517-530.

Metaxas, A., R. E. Scheibling, M. C. Robinson, and C. M. Young. 2008. Larval development, settlement, and early post-settlement behavior of the tropical sea star *Oreaster reticulatus*. *Bulletin of Marine Science* **83**:471-480.

Michalec, F. G., S. Souissi, G. Dur, M. S. Mahjoub, F. G. Schmitt, and J. S. Hwang. 2010. Differences in behavioral responses of *Eurytemora affinis* (Copepoda, Calanoida) reproductive stages to salinity variations. *Journal of Plankton Research* **32**:805-813.

Miller, S. E., and M. G. Hadfield. 1986. Ontogeny of phototaxis and metamorphic competence in larvae of the nudibranch *Phestilla sibogae* Bergh (Gastropoda: Opisthobranchia). *Journal of Experimental Marine Biology and Ecology* **97**:95-112.

Mladenov, P. V., and F. Chia. 1983. Development, settling behaviour, metamorphosis and pentacrinoid feeding and growth of the feather star *Florometra serratissima*. *Marine Biology* **73**:309-323.

Mogami, Y., C. Oobayashi, T. Yamaguchi, Y. Ogiso, and S. A. Baba. 1988. Negative geotaxis in sea urchin larvae: A possible role of mechanoreception in the late stages of development. *Journal of Experimental Biology* **137**:141-156.

Montgomery, E. M., J.-F. Hamel, and A. Mercier. 2017. Ontogenetic shifts in swimming capacity of echinoderm propagules: A comparison of species with planktotrophic and lecithotrophic larvae. *Marine Biology* **164**:43.

Mundy, C. N., and R. C. Babcock. 1998. Role of light intensity and spectral quality in coral settlement: Implication for depth-dependent settlement? *Journal of Experimental Marine Biology and Ecology* **223**:235-255.

Nilsson, D.-E. 2009. The evolution of eyes and visually guided behaviour. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364**:2833-2847.

Parks, A. L., B. W. Bisgrove, G. A. Wray, and R. A. Raff. 1989. Direct development in the sea urchin *Phyllacanthus parvispinus* (Cidaroidea): Phylogenetic history and functional modification. *Biological Bulletin* **177**:96-109.

Pechenik, J. A. 1999. On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. *Marine Ecology Progress Series* **177**:269-297.

Pennington, J. T., and R. B. Emlet. 1986. Ontogenetic and diel vertical migration of a planktonic echinoid larva, *Dendraster excentricus*: Occurence, causes, and probable consequences. *Journal of Experimental Marine Biology and Ecology* **104**:69-95.

Pennington, J. T., S. S. Rumrill, and F.-S. Chia. 1986. Stage-specific predation upon embryos and larvae of the Pacific sand dollar, *Dendraster excentricus*, by 11 species of common zooplanktonic predators. *Bulletin of Marine Science* **39**:234-240.

Pizarro, V., and J. Thomason. 2008. How do swimming ability and behaviour affect the dispersal of coral larvae. 464-467 in *Proceedings of the 11th International Coral Reef Symposium, Fort Lauderdale, Florida, 7*.

Prowse, T. A., I. Falkner, M. A. Sewell, and M. Byrne. 2009. Long-term storage lipids and developmental evolution in echinoderms. *Evolutionary Ecology Research* **11**:1069-1083.

Richmond, C. E., and S. A. Woodin. 1996. Short-term fluctuations in salinity: Effects on planktonic invertebrate larvae. *Marine Ecology Progress Series* **133**:167-177.

Robins, P. E., S. P. Neill, L. Giménez, S. R. Jenkins, and S. K. Malham. 2013. Physical and biological controls on larval dispersal and connectivity in a highly energetic shelf sea. *Limnology and Oceanography* **58**:505-524.

Roy, A., A. Metaxas, and R. M. Daigle. 2012. Changes in vertical distribution and aggregative behaviour in response to population density for larval sea urchins (*Strongylocentrotus droebachiensis*) and sea stars (*Asterias rubens*). *Marine Ecology* **33**:194-204.

Ryland, J. 1960. Experiments on the influence of light on the behaviour of polyzoan larvae. *Journal of Experimental Biology* **37**:783-800.

Salazar, M. H. 1970. Phototaxis in the deep-sea urchin *Allocentrotus fragilis* (Jackson). *Journal of Experimental Marine Biology and Ecology* **5**:254-264.

Shand, J. 1993. Changes in the spectral absorption of cone visual pigments during the settlement of the goatfish *Upeneus tragula*: the loss of red sensitivity as a benthic existence begins. *Journal of Comparative Physiology A* **173**:115-121.

Shirley, S. M., and T. C. Shirley. 1988. Behavior of red king crab larvae: phototaxis, geotaxis and rheotaxis. *Marine and Freshwater Behaviour and Physiology* **13**:369-388.

So, J. J., S. Uthicke, J.-F. Hamel, and A. Mercier. 2011. Genetic population structure in a commercial marine invertebrate with long-lived lecithotrophic larvae: *Cucumaria frondosa* (Echinodermata: Holothuroidea). *Marine Biology* **158**:859-870.

Staver, J. M., and R. R. Strathmann. 2002. Evolution of fast development of planktonic embryos to early swimming. *Biological Bulletin* **203**:58-69.

Sulkin, S. D. 1975. The influence of light in the depth regulation of crab larvae. *Biological Bulletin* **148**:333-343.

Svane, I., and P. Dolmer. 1995. Perception of light at settlement: A comparative study of two invertebrate larvae, a scyphozoan planula and a simple ascidian tadpole. *Journal of Experimental Marine Biology and Ecology* **187**:51-61.

Svane, I., and C. M. Young. 1989. The ecology and behaviour of ascidian larvae. *Oceanography and Marine Biology* **27**:45-90.

Taylor, M. H. 1984. Lunar synchronization of fish reproduction. *Transactions of the American Fisheries Society* **113**:484-493.

Thorson, G. 1964. Light as an ecological factor in the dispersal and settlement of larvae of marine bottom invertebrates. *Ophelia* **1**:167-208.

Tran, C., and M. G. Hadfield. 2013. Localization of sensory mechanisms utilized by coral planulae to detect settlement cues. *Invertebrate Biology* **132**:195-206.

Vaughn, D., and J. D. Allen. 2010. The peril of the plankton. *Integrative and Comparative Biology* **50**:552-570.

Vazquez, E., and C. M. Young. 1998. Ontogenetic changes in phototaxis during larval life of the Ascidian *Polyandrocarpa zorritensis Journal of Experimental Marine Biology and Ecology* **231**:267-277.

Wendt, D. E. 2000. Energetics of larval swimming and metamorphosis in four species of *Bugula* (Bryozoa). *Biological Bulletin* **198**:346-356.

Yoshida, M., and H. Ohtsuki. 1968. The phototactic behavior of the starfish, *Asterias amurensis* Lütken. *Biological Bulletin* **134**:516-532.

Yoshida, M., N. Takasu, and S. Tamotsu. 1984. Photoreception in echinoderms. Pp. 743-771 in *Photoreception and Vision in Invertebrates* Springer.

Young, C., and F.-S. Chia. 1982a. Factors controlling spatial distribution of the sea cucumber *Psolus chitonoides*: Settling and post-settling behavior. *Marine Biology* **69**:195-205.

Young, C. M. 1999. Marine invertebrate larvae. *Encyclopedia of Reproduction*:89-97.

Young, C. M., and F.-S. Chia. 1982b. Ontogeny of phototaxis during larval development of the sedentary polychaete, *Serpula vermicularis* (L.). *Biological Bulletin* **162**:457-468.

5.8. Tables and Figures

Table 5.1. Net phototactic response of focal echinoderm propagules under light gradients of the three light colours tested.

Type	Planktotrophs								Lecithotrophs							
Species	*A. rubens*				*S. droebachiensis*				*C. papposus*				*C. frondosa*			
Stage	Blastula	Gastrula	Bipinnaria	Early brachiolaria	Blastula	Gastrula	Prism	Pluteus	Blastula	Gastrula	Early brachiolaria	Late brachiolaria	Blastula	Gastrula	Vitellaria	Early pentactula
White[1]	x	+	–	x	x	–	–	x	+	x	–	x	x	x	–	x
Red[1]	x	+	x	–	x	x	–	x	x	x	x	x	x	0	x	x
Blue[1]	–	x	x	x	–	x	x	x	x	0	+	0	x	x	x	x
P-values																
White	0.86	0.002	0.031	0.66	0.31	0.039	0.030	0.53	0.020	0.17	0.044	0.60	0.26	0.17	0.075	0.82
Red	0.14	0.039	0.27	0.015	0.035	0.20	0.030	0.69	0.14	0.87	0.69	0.72	0.27	0.045	0.23	0.67
Blue	0.015	0.06	0.30	0.58	0.044	0.20	0.72	0.17	0.90	0.030	0.004	0.007	0.37	0.61	0.27	0.38

[1] x = heterogeneous taxis (propagules exhibited a mix of displacement patterns, towards or away from stimulus); + = positive taxis (net displacement of most propagules towards stimulus), – = negative taxis (net displacement of most propagules away from stimulus), 0 = no taxis relative to stimulus (most propagules maintained their initial position).

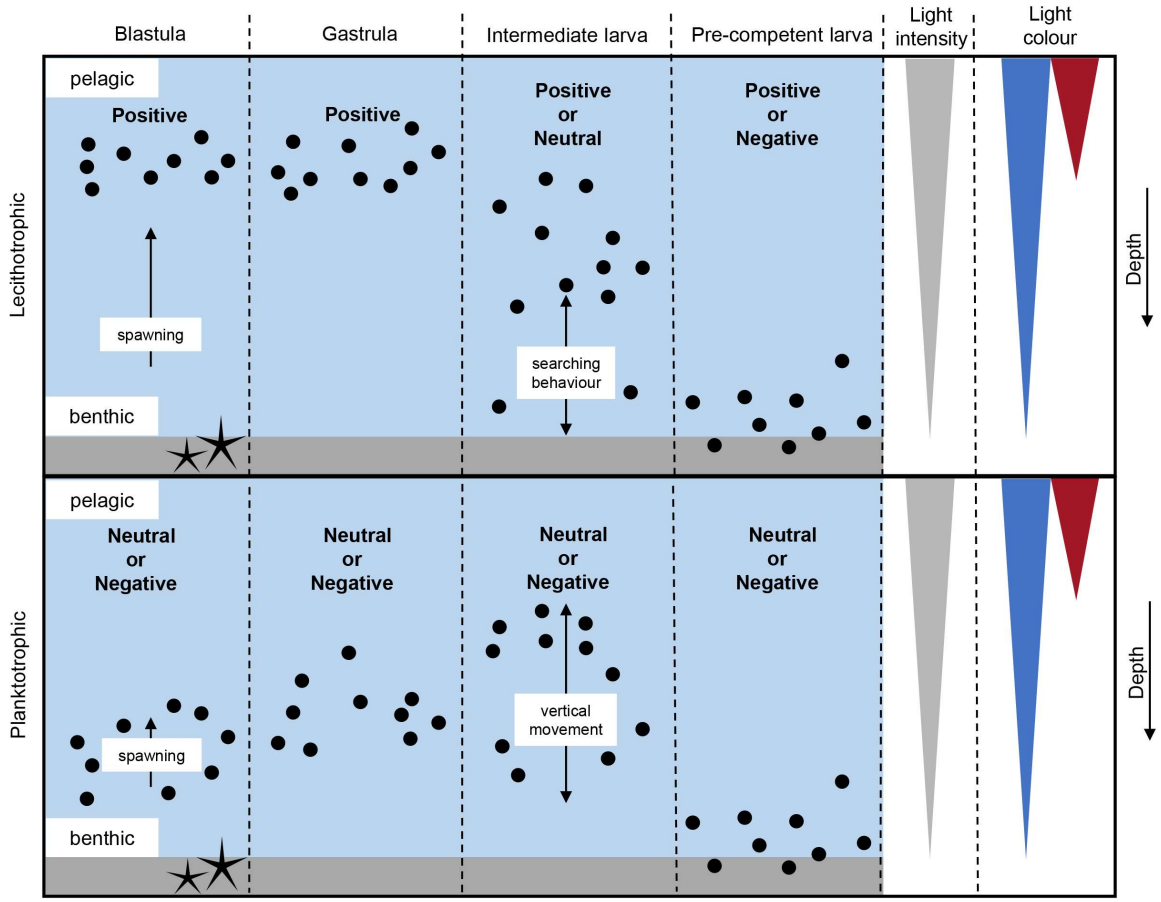


Figure 5.1. Typical vertical profile of theoretical lecithotrophic (top panel) and planktotrophic (bottom panel) species with pelagic larvae. Buoyancy is indicated for each life stages; positive (floating), neutral (neither floating nor sinking) or negative (sinking). Competency is defined as the period during which larvae are capable of undergoing settlement / metamorphosis. Searching behaviour refers to the swimming patterns exhibited by many larvae close to the benthos as they identify suitable settlement sites. Planktotrophic larvae also undergo daily vertical movements as they follow their phytoplankton prey. The width of the triangles indicates the relative amount of total light intensity and light colour present with increasing depth in coastal waters (typical of the NW Atlantic).

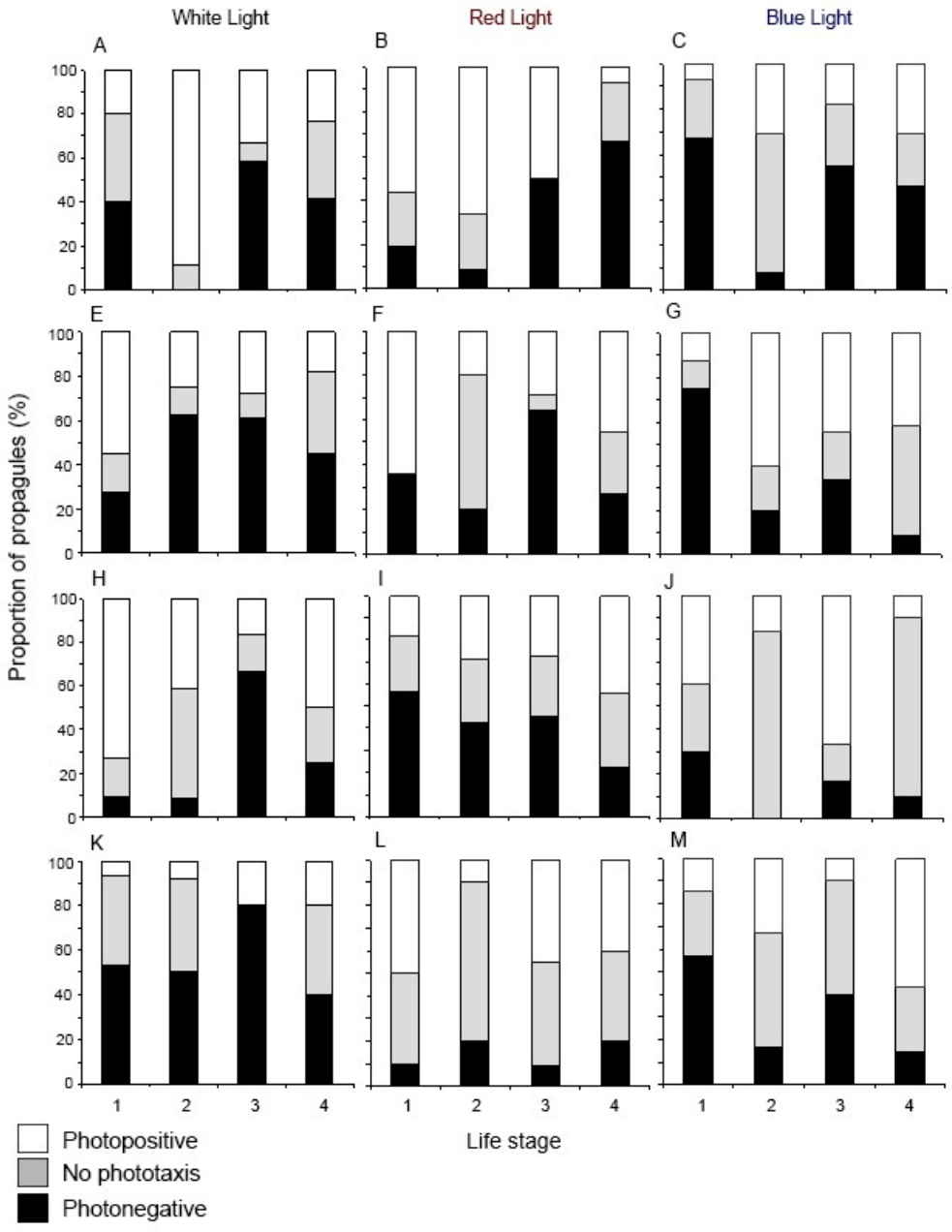

White Light
Red Light
Blue Light
A
B
C
E
F
G
H
I
J
K
L
M
Proportion of propagules (%)
0
20
40
60
80
100
1
2
3
4
Life stage
Photopositive
No phototaxis
Photonegative

Figure 5.2. Phototactic response of echinoderm propagules when exposed to a point source of white, red or blue light. A-C) *A. rubens* E-G) *S. droebachiensis* H-J) *C. papposus* K-M) *C. frondosa*. Life stages are as follow: (1) blastula; (2) gastrula; (3) early larva (bipinnaria in *A. rubens*, prism in *S. droebachiensis*, early-brachiolaria in *C. papposus*, vitellaria in *C. frondosa*), and (4) pre-competent larva (brachiolaria in *A. rubens*, pluteus in *S. droebachiensis*, late-brachiolaria in *C. papposus*, early-pentactula in *C. frondosa*). Net phototaxis at each life stage is summarized in Table 5.1. Photopositive = net displacement of propagules towards stimulus; photonegative = net displacement of propagules away from stimulus; no phototaxis = no taxis relative to stimulus (propagules maintained their initial position).

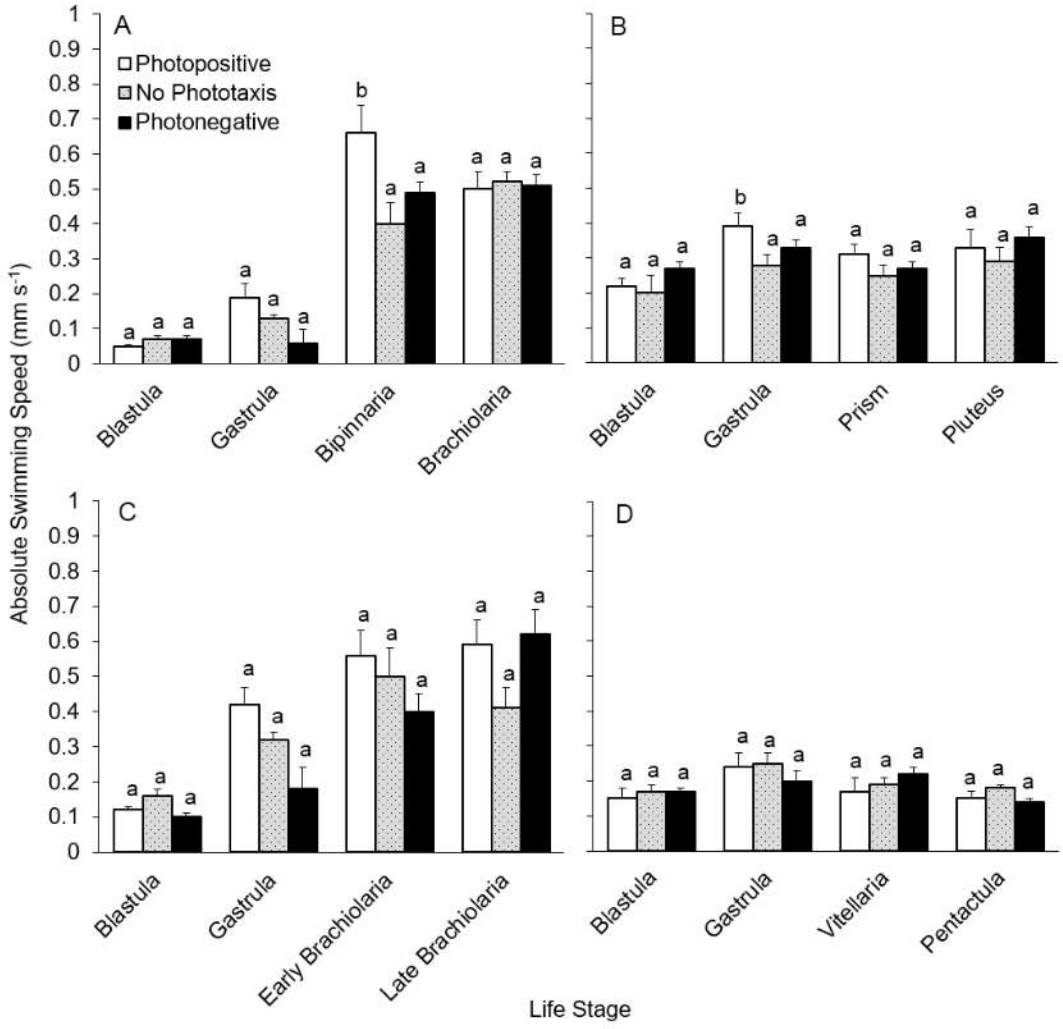


Figure 5.3. Absolute swimming speed in mm s^{-1} of echinoderm propagules exhibiting either positive, negative or neutral phototaxis, independent of light colour (Mean ± SE, n = 8-20). A) *Asterias rubens*. B) *Strongylocentrotus droebachiensis*. C) *Crossaster papposus*. D) *Cucumaria frondosa*. Lower-case letters indicate statistical groupings within each life stage.

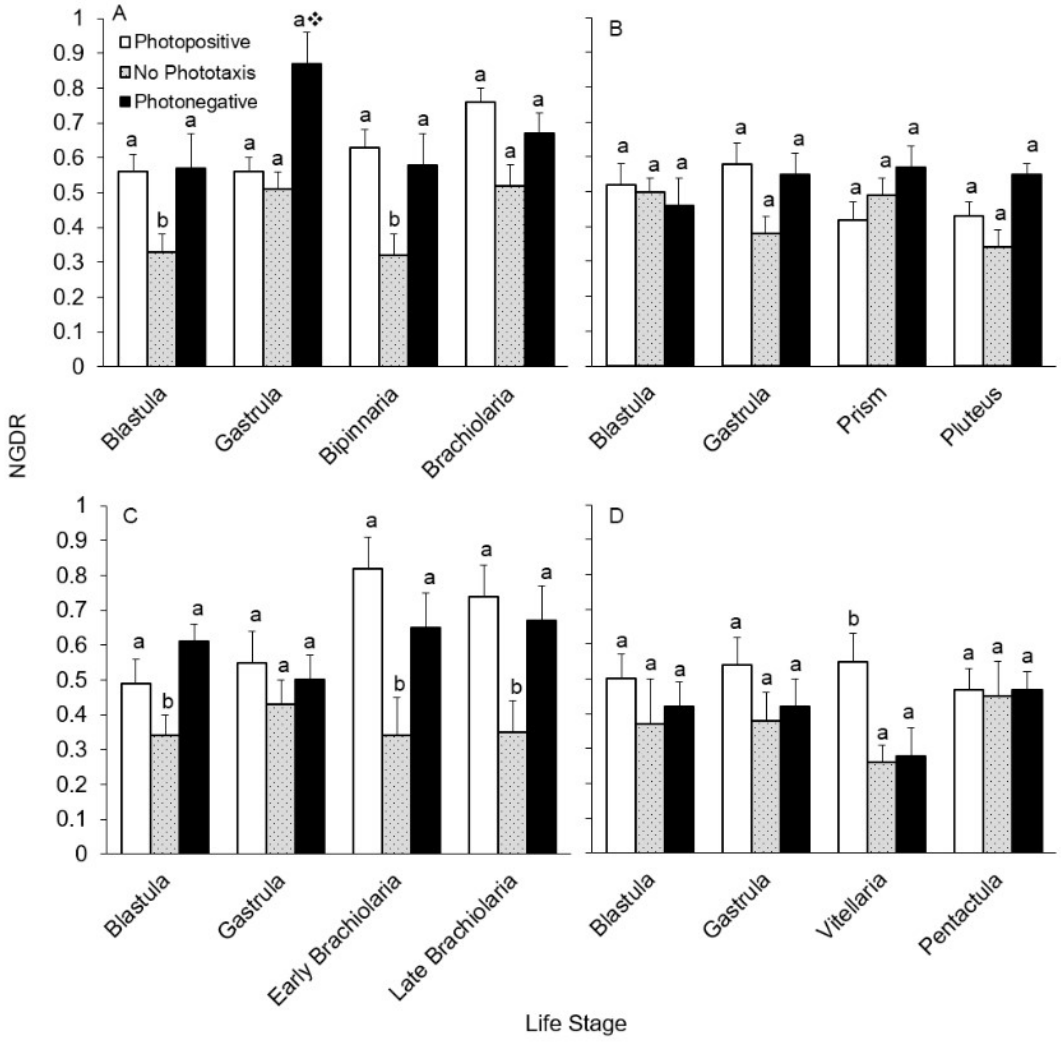


Figure 5.4. Net-to-gross displacement ratio (NGDR) (Mean ± SE, n = 2-20) of echinoderm propagules swimming towards (photopositive) or away from the light stimulus (photonegative), or showing no directionality (neutral), independent of light colour. A) *Asterias rubens*. B) *Strongylocentrotus droebachiensis*. C) *Crossaster papposus*. D) *Cucumaria frondosa*. Lower-case letters indicate statistical groupings within in each life stage. ❖ = though the category appears statistically different, too few individuals were in this category to detect any differences in an ANOVA (n=2).

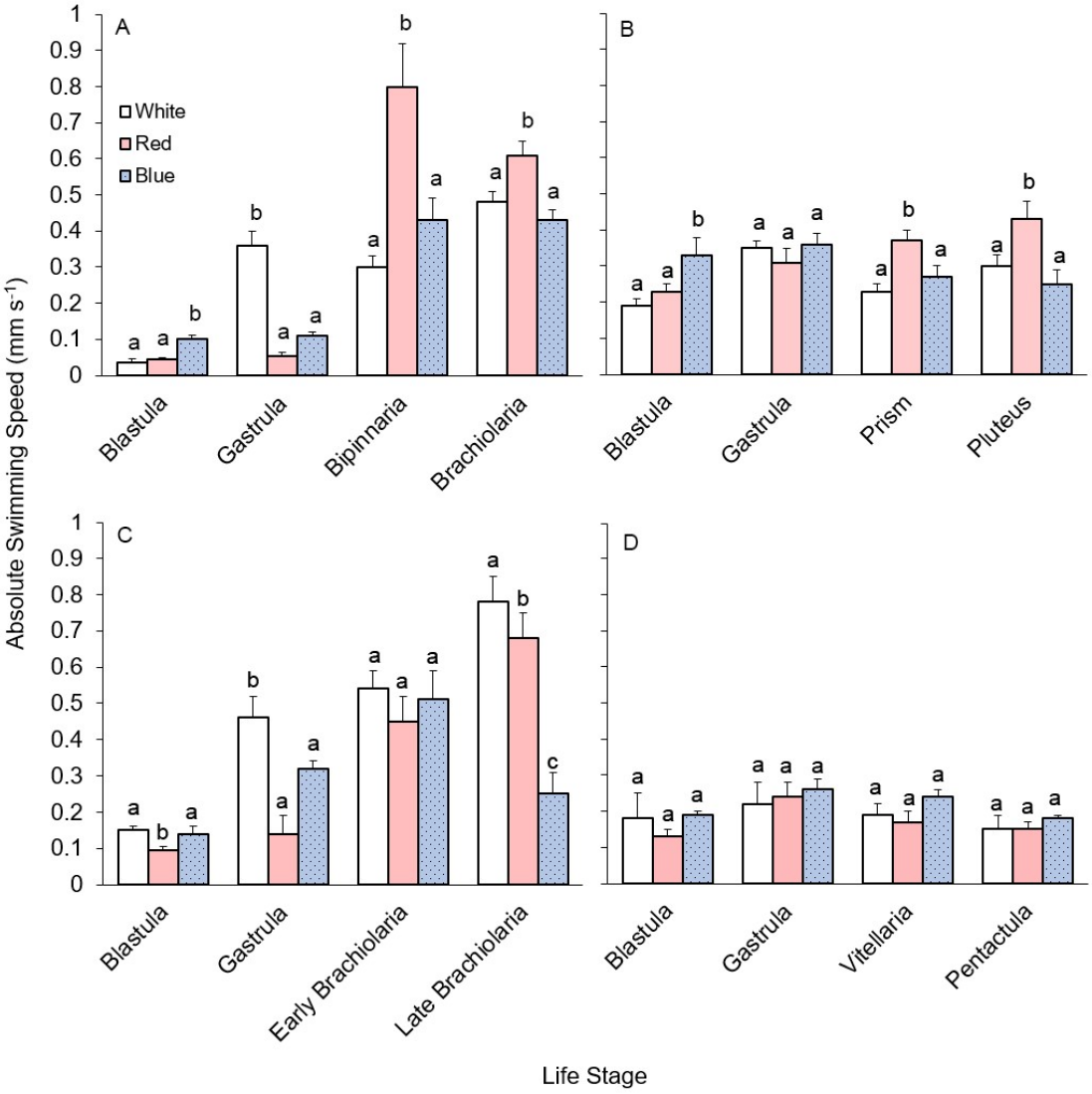


Figure 5.5. Absolute swimming speed (in mm s^{-1}) of echinoderm propagules exposed to a constant level of white, red and blue light from above (Mean ± SE, n = 8-20). A) *Asterias rubens*. B) *Strongylocentrotus droebachiensis*. C) *Crossaster papposus*. D) *Cucumaria frondosa*. Lower-case letters indicate statistical groupings within in each life stage.

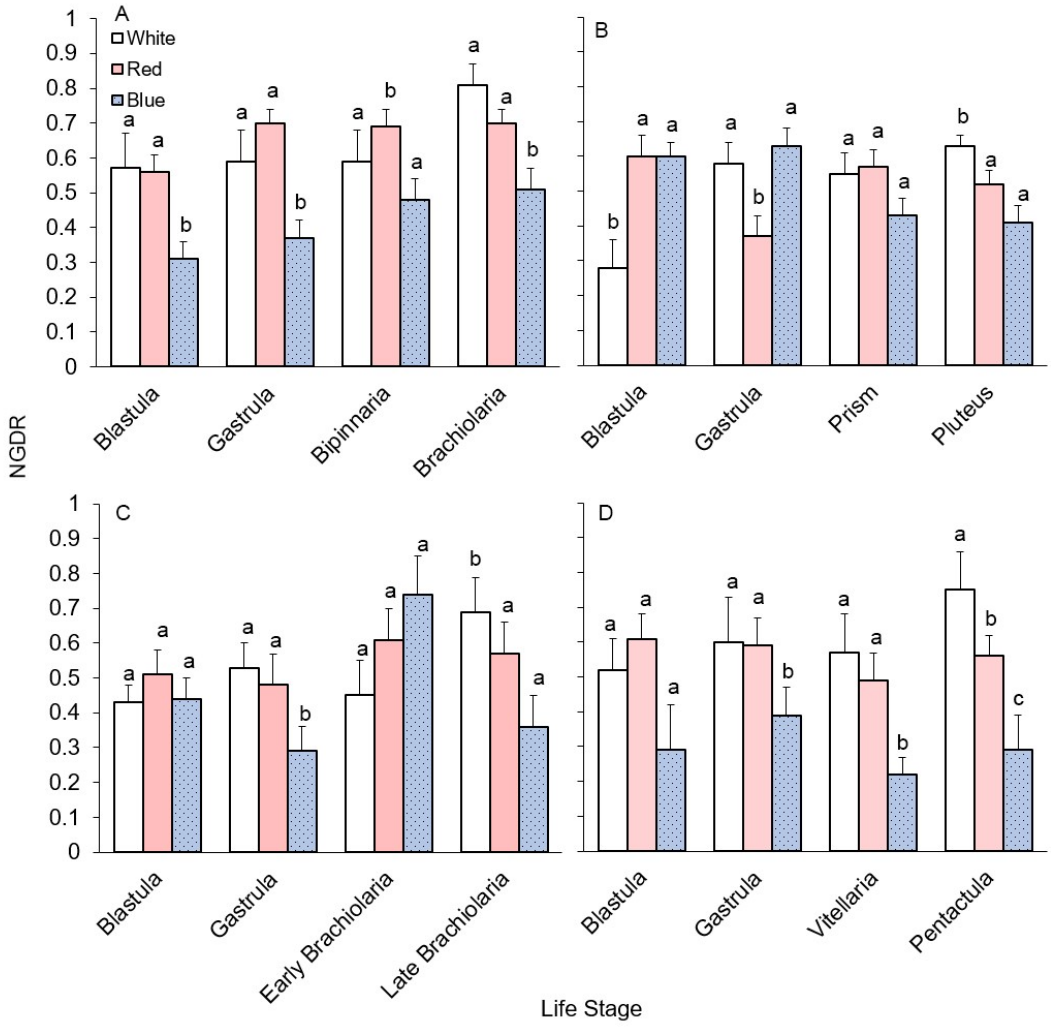


Figure 5.6. Net-to-gross displacement ratio (NGDR) of echinoderm propagules exposed to a constant level of white, red and blue light from above (Mean ± SE, n = 8-20). A) *Asterias rubens*. B) *Strongylocentrotus droebachiensis*. C) *Crossaster papposus*. D) *Cucumaria frondosa*. Lower case letters indicate statistical groupings within each life stage.

Chapter 6. Critical Assessment of Swimming Capacity, Sensory Ability and Nutritional Mode in Marine Propagules

A manuscript comprised of a version of this chapter combined with Chapter 2 is being prepared for submission to a scientific journal.

6.1. Is Propagule Nutritional Type a Good Predictor of Swimming Capacity?

In Chapter 4, I showed similar swimming capacity among planktotrophic and lecithotrophic echinoderm propagules under ambient conditions. In Chapter 5, I showed that changes in activity level (through a combination of swimming speed and trajectory) in response to different light colours were present in both planktotrophic and lecithotrophic echinoderm propagules. A logical follow-up is to assess whether these patterns are conserved across other marine invertebrate taxa with ciliated propagules. Several excellent reviews already discuss swimming among planktotrophic propagules (Chia *et al.* 1984, Koehl and Reidenbach 2007), no study has ever explicitly tested or examined the differences between planktotrophic and lecithotrophic propagules across multiple phyla.

6.2. Dataset Collection and Statistical Analyses

To test the hypothesis that ciliated planktotrophic and lecithotrophic propagules swim similarly, relative to body length, I gathered swimming speed data of pelagic ciliated propagules across five major marine phyla (Porifera, Cnidaria, Annelida, Mollusca, Echinodermata; n = 67 complete records). Phylum Porifera (sponges) and

Cnidaria (corals and sea anemones) are predominantly lecithotrophic while Annelida, Mollusca and Echinodermata have a mixture of planktotrophic and lecithotrophic species. Records were collected from the scientific literature, including journal papers, theses and reports. Metrics collected included: nutritional mode, phylogeny, propagule diameter and mean swimming speed. Two-way ANOVA and Factorial Analysis of Mixed Data (FAMD) were used to test the relationship among all collected metrics. Statistical analyses were performed in Sigma Plot and R statistical software.

6.2.1. Phylum, not nutritional mode, has a greater effect on swimming speed

On average, planktotrophic propagules (diameter = 271 ± 31 μm) were smaller than lecithotrophic propagules (512 ± 57 μm) across the dataset, though differences in egg size were not conserved within all tested taxa. However, no significant differences existed in absolute swimming speeds between planktotrophic and lecithotrophic propagules (ANOVA $p = 0.87$; Table 6.2). Interestingly, there were phylum-based differences that emerged in the two-way ANOVA that warranted further analyses ($p < 0.001$; Table 6.2). Two clusters were obtained from the FAMD model. Planktotrophic echinoderm propagules were smaller on average (FAMD $p = 0.037$, Table 6.3) and swam slower than the average (FAMD $p = 0.025$). In complete contrast, poriferan propagules (lecithotrophic) were larger on average (FAMD $p = 0.015$) and swam faster than other propagules (FAMD $p < 0.001$).

Taken together, results show that ciliated planktotrophic and lecithotrophic propagules generally swim at comparable speeds when all phyla are considered together.

This provides evidence that challenges previous assumptions to the effect that the large size and high lipid content of lecithotrophic propagules constrain their locomotory abilities(Emlet 1994). The phylum-based differences in swimming speed seen between echinoderms are poriferans suggest that, in fact, the opposite may be true; that some lecithotrophs may swim faster than average planktotrophs. There is a marked size difference between planktotrophic echinoderm and poriferan propagules. The larger size of poriferan propagules may yield an advantage as increased surface area could result in increased numbers of cilia and potentially increased propulsion. It is also worth noting that planktotrophic echinoderm propagules use a completely different morphological strategy; they often possess calcified elements and appendages that affect their density and interaction with fluid, in contrast to the simple prolate spheroid shape of poriferan propagules. Finally, the pelagic propagule duration (PPD) of sponges is typically much shorter than that of echinoderm propagules (1-7 days vs. 1-2 months). Swimming is an energetically costly process. Species with a long PPD need to conserve enough energy to undergo metamorphosis, so it is logical that they might not have the same capacity for other energetically expensive processes such as swimming. Testing the relationship between PPD and swimming speed in other taxa could yield interesting results, but unfortunately more PPD data are first needed, especially for lecithotrophs.

6.2.2. Speed scales with size in planktotrophs, but not in lecithotrophs

Absolute swimming speed decreased with increasing propagule length among planktotrophic propagules in the dataset (Fig. 6.2). In contrast, there was no relationship

between propagule length and absolute swimming speed among lecithotrophic propagules (Fig. 6.2).

Taken together, this suggests that planktotrophic propagules may be more constrained by size than lecithotrophic propagules. A previous study of planktotrophic species found a decrease in vertical swimming speed with increasing propagule length (McDonald and Grunbaum 2010). While large larval size may increase the quality and survival of the juvenile (Emlet and Hoegh-Guldberg 1997, Phillips 2002), there may be a trade-off between swimming ability and size for planktotrophic propagules. Swimming is an energetic demand. Larger propagules may need more energy to swim at comparable speeds relative to smaller individuals. Since food is a limiting factor for the growth and development of planktotrophic propagules, larger propagules may not have enough resources to compete. In contrast, swimming speeds may be affected by something other than size in lecithotrophic propagules, which do not require external nutrition to complete metamorphosis. Cilia length, organization and body shape (Emlet 1994) are a few mechanical reasons why certain lecithotrophic propagules swim faster than others. The level of sensory and behavioural complexity among propagules may also result in different swimming speeds under different environmental conditions. To this effect, phylogenetic constraints on propagule form and behaviour may influence swimming more strongly than nutritional mode differences among species. Therefore, patterns among diverse taxa ideally need to be examined under uniform conditions to prevent bias.

6.3. Sensory Ability and Swimming Ability Co-Occur in Marine Propagules with Different Development Modes

Several reviews of sensory abilities and responses (particularly in regards to settlement cue detection and the interaction with biofilm) exist in the literature (Hadfield 2011, Kingsford *et al.* 2002). Kingsford *et al.* 2002 reported a trend showing increasing swimming speed proportional to increasing sensory structures / abilities in phyla ranging from Porifera to Chordata. However, the study did not consider morphological or developmental differences among propagules, or ontogeny. In general, discussion of sensory abilities among lecithotrophic propagules is limited for phyla that have evolved multiple modes of development (e.g. Mollusca, Annelida, Echinodermata).

In Chapter 5, I showed that planktotrophic echinoderm propagules displayed clearer net-phototactic patterns more readily than lecithotrophic echinoderm propagules under coloured light. This was not totally surprising given evidence that nervous elements in echinoderm larvae are closely associated with feeding cilia bands, and the loss of these bands in lecithotrophic propagules has resulted in a reduction in nervous complexity as larvae (Byrne *et al.* 2001, Lacalli *et al.* 1990). Certain types of propagules may also be more/less sensitive to specific wavelengths of light at different time points in their development. Since lecithotrophic propagules do not feed, their sensitivity to environmental cues could be different than planktotrophs, though more thourough study is warrented before any definitive conclusions can be made.

6.4. Why Have Sensory and Locomotory Functions Been Maintained?

Clearly, lecithotrophs have retained locomotory and sensory capabilities for a reason. These features would presumably not be maintained if propagules were entirely passive with no drivers to retain control over their navigation at any point in their early life. The ability to detect and respond to cues may supersede any trade-offs associated with variations in life-history strategies in ciliated marine propagules. Such strategies (e.g. nutritional mode, parental protection) could have evolved to tackle other risks associated with the biphasic life style and baseline sensory abilities and response patterns would exist because they are fundamental for propagule survival, settlement and eventual recruitment back into adult populations. Developing pelagic propagules have three separate tasks to accomplish: 1) escape from the benthos, 2) survive in the water column and 3) settle back on the benthos. The greatest variation in sensory behaviours and capabilities might be expected to exist while propagules are in the water column, since some species require external nutrition to complete metamorphosis.

Studies that examine behaviour from a comparative and ontogenetic perspective will be critical to assess the advantages and disadvantages of different propagule adaptions. In Chapter 5, there was a general progression from undetermined taxis or photopositivity at early life stages to photonegativity at early larval stages. The timing of ontogenetic shifts in phototaxis are likely species-specific, and may help support life-history diversity and variable pelagic duration. Strong phototactic responses may be adaptive in planktotrophs (due to their need to migrate to the surface for food), and this could explain why light sensitivity emerged in the earliest marine animals.

6.5. Considerations and Future Directions

Phyla with multiple development modes (e.g. Annelida, Mollusca, Echinodermata) and species with alternating development modes (i.e. poecilogonous species; Krug 2009) are extremely valuable for comparative studies, but are relatively under-exploited in behavioural work. The marine gastropod *Alderia modesta* is a good example of a poecilogonous species with alternating modes of development; switching between pelagic planktotrophy and pelagic lecithotrophy. Larvae of both nutritional modes were shown to swim with complex paths early in their ontogeny that became straighter as larvae grew (Krug *et al.* 2012). This pattern was similar to the trends in NDGR seen among echinoderm propagules in Chapter 4. However, lecithotrophic larvae of *A. modesta* swam downward four times faster than their planktotrophic counterparts and explored the substrate significantly more. Such behavioural differences could have ramifications for settlement and eventual recruitment success. Future studies using poecilogonous species could provide insight into the trade-offs of maternal investment and offspring success.

6.6. References

Byrne, M., P. Cisternas, and D. Koop. 2001. Evolution of larval form in the sea star genus *Patiriella*: conservation and change in the larval nervous system. *Development, Growth & Differentiation* **43**:459-468.

Chia, F. S., J. Buckland, and C. M. Young. 1984. Locomotion of marine invertebrate larvae - A review. *Canadian Journal of Zoology* **62**:1205-1222.

Emlet, R. B. 1994. Body form and patterns of ciliation in nonfeeding larvae of echinoderms - Functional solutions to swimming in the plankton. *American Zoologist* **34**:570-585.

Emlet, R. B., and O. Hoegh-Guldberg. 1997. Effects of egg size on postlarval performance: experimental evidence from a sea urchin. *Evolution*:141-152.

Hadfield, M. G. 2011. Biofilms and marine invertebrate larvae: what bacteria produce that larvae use to choose settlement sites. *Annual Review of Marine Science* **3**:453-470.

Kingsford, M. J., J. M. Leis, A. Shanks, K. C. Lindeman, S. G. Morgan, and J. Pineda. 2002. Sensory environments, larval abilities and local self-recruitment. *Bulletin of Marine Science* **70**:309-340.

Koehl, M., and M. A. Reidenbach. 2007. Swimming by microscopic organisms in ambient water flow. *Experiments in Fluids* **43**:755-768.

Krug, P. J. 2009. Not my "type": Larval dispersal dimorphisms and bet-hedging in opisthobranch life histories. *Biological Bulletin* **216**:355-372.

Krug, P. J., D. Gordon, and M. R. Romero. 2012. Seasonal polyphenism in larval type: rearing environment influences the development mode expressed by adults in the sea slug *Alderia willowi*. *Integrative and Comparative Biology* **52**:161-172.

Lacalli, T., T. Gilmour, and J. West. 1990. Ciliary band innervation in the bipinnaria larva of *Piaster ochraceus*. *Philosophical Transactions of the Royal Society B: Biological Sciences* **330**:371-390.

McDonald, K. A., and D. Grunbaum. 2010. Swimming performance in early development and the "other" consequences of egg size for ciliated planktonic larvae. *Integrative and Comparative Biology* **50**:589-605.

Phillips, N. E. 2002. Effects of nutrition‐mediated larval condition on juvenile performance in a marine mussel. *Ecology* **83**:2562-2574.

6.7. Tables and Figures

Table 6.1. Mean diameter and swimming speed summarized across the two nutritional modes and five phyla featured in the dataset.

Factor		Mean diameter (mm ± SE)	Mean swimming speed (mm s^{-1} ± SE)
Nutritional mode	Planktotrophic (P)	271 ± 31	1.3 ± 0.4
	Lecithotrophic (L)	512 ± 57	10.6 ± 2.8
Phylum	Porifera	596 ± 85	30.1 ± 4.3
	Cnidaria	352 ± 99	5.7 ± 4.1
	Annelida	(L) 300 ± 15	0.7 ± 0.3
		(P) 328 ± 74	1.6 ± 1.0
	Mollusca	(L) 170 ± 53	1.8 ± 0.7
		(P) 162 ± 38	3.1 ± 0.9
	Echinodermata	(L) 750 ± 22	0.6 ± 0.5
		(P) 279 ± 34	0.4 ± 0.2

Table 6.2. Summary of results from an ANOVA testing the effect of phylum and nutritional mode on swimming speed in ciliated propagules (n = 67)

Factor	**df**	**F-stat**	**P-value**
Phylum	4	45.2	<0.001
Nutritional mode	1	0.03	0.86
Phylum × Nutritional mode	2	0.07	0.93

Table 6.3. Summary of results from an FAMD testing the relationship among phylum, propagule size, propagule swimming speed, and nutritional mode in the dataset

Hierarchical Clusters		**P-value**
1	Echinodermata	<0.001
	Planktotrophic	<0.001
	Size < Mean	0.025
	Speed < Mean	0.037
2	Porifera	<0.001
	Lecithotrophic	<0.001
	Size > Mean	<0.001
	Speed > Mean	0.015

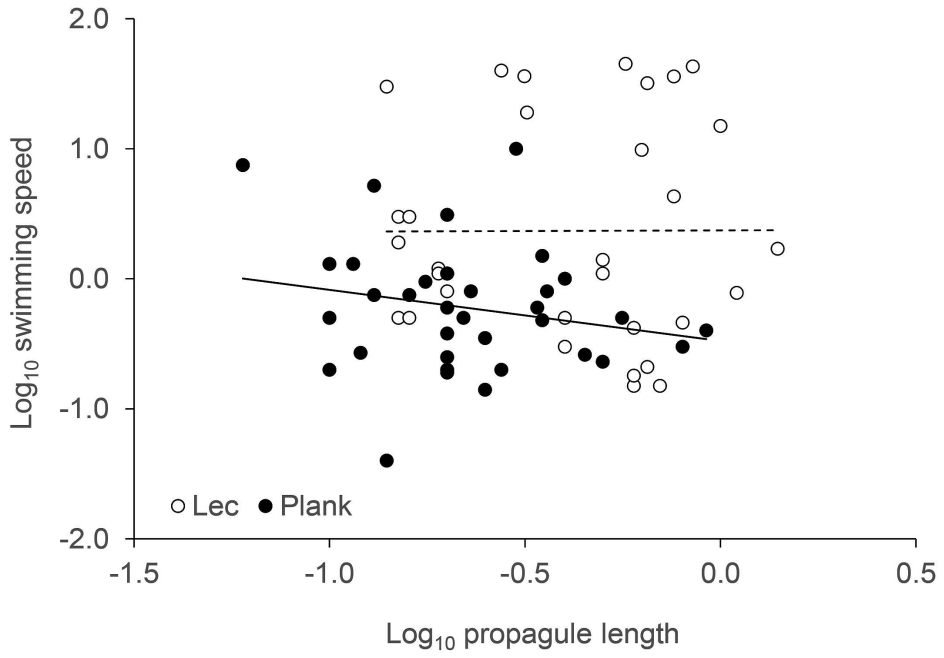


Figure 6.1. Swimming speed versus propagule length in lecithotrophic and planktotrophic invertebrate propagules of various phyla (Porifera, Cnidaria, Mollusca, Annelida, Echinodermata). Points represent mean values for individual species. Black circles indicate planktotrophic species and white circles indicate lecithotrophic species. *n* = 66 total. Planktotrophs: y =-0.39x – 0.48; Lecithotrophs: y = 0.0097x + 0.37.

Chapter 7. Conclusions and Future Directions

7.1. Main Findings and Contributions to the Field

7.1.1. Propagule phenotypes

Maternal investment controls the size, shape, pigment level and buoyancy of eggs through nutrient deposition (Prowse *et al.* 2008). For lecithotrophic species that rely only on this source of maternal nutrition (i.e. on yolk) for development, the quality of maternal supply is critical (Falkner *et al.* 2006, Prowse *et al.* 2008). Lecithotrophic propagules are typically larger and yolky, relative to other types of propagules (planktotrophic, facultative planktotrophic; Pechenik 1999, Strathmann 2007). Individuals with high levels of lipids (such as those found in lecithotrophic propagules) are susceptible to UV damage (Blount 2004) and require antioxidant protection such as pigments. In contrast, planktotrophic eggs are generally pale or translucent; while some have colour upon their initial release (e.g. *Ophiocoma* spp. from Panama and Australasia have red-coloured eggs; Maria Byrne pers. com. 2016), the colour is lost as development begins. Overall, despite the functional importance of pigments and the colour diversity present in the lecithotrophic eggs of some taxa (e.g. Mollusca, Echinodermata), the ecological relevance of egg colour variation in the marine environment remains surprisingly unexplored.

Through my PhD work, I found a strong link between colour intensity (brightness) and development location (brooded vs. pelagic) among lecithotrophic echinoderms. Colour shade (red vs. green) seemed to relate to geographic location. Most of the work in

the field of egg colour to date has been conducted in terrestrial animals, and chiefly in birds.

7.1.2. Swimming capacity

Marine propagules have traditionally been considered passive particles with little to no control over their dispersal destiny (noted byMetaxas and Saunders 2009). However studies (at both large and small scales) have demonstrated that minute (sometimes as little as two-fold) changes in speed can have a significant impact on dispersal and settlement processes (Abelson and Denny 1997, Pizarro and Thomason 2008). Since most propagules are predicted to move autonomously at slower speeds than most ocean currents, variability in propagule swimming presumably has the greatest impact close to the benthos in the viscous sublayer of the boundary layer (mm above the benthos; Gross *et al.* 1992, Wildish 2009). Yet, intriguingly, early life stages and positively buoyant propagules confined to the pelagic environment have maintained swimming abilities.

Most studies to date have focused on planktotrophic propagules since they are easy to maintain in the laboratory (Wray *et al.* 2004), are common globally and swim vertically as well as horizontally (McDonald 2012, Mileikovsky 1973, Pennington and Emlet 1986). Due to their large size and positive buoyancy, lecithotrophic propagules were thought to be weaker swimmers than planktotrophs (Emlet 1994). This constraint could have consequences for phyla that have a predominance of lecithotrophic species (e.g. ~68% of species in Echinodermata, Uthicke *et al.* 2009). However, I showed here that the absolute swimming speeds and consequent autonomous dispersal potential of

North Atlantic planktotrophic and lecithotrophic propagules was surprisingly similar. Differences only emerged when swimming speeds were calculated relative to body size. The planktotrophic propagules tended to travel further relative to their body length when compared to lecithotrophic propagules. This was the first study to date that compared in detail the swimming speed and swimming trajectories of co-occurring echinoderm propagules displaying different life-history strategies.

7.1.3. Sensory behaviour

Sensory behaviour evolved early in the animal tree of life (Jacobs *et al.* 2007). Pelagic larvae of the most basal marine phyla (Porifera, Cnidaria) have predictable and repeatable behavioural patterns in response to external stimuli (Leys *et al.* 2002, Mundy and Babcock 1998). Sensory responses can include: taxis relative to directional stimuli, and changes in activity level. Taxis can indicate preferred conditions and therefore is not only informative for ecological studies but also for optimizing aquaculture practices. Shifts in activity level such as variation in locomotory output and patterns can be reliably quantified and used as a proxy for the degree of organism's response when exposed to a cue.

It appears especially advantageous for pelagic propagules to be able to detect and respond to their external environment since specialized sensory cells and behaviours have been maintained from basal marine phyla like Porifera to more derived phyla such as Echinodermata and Chordata. This is particularly true for "universal" stimuli such as light, which undergoes natural depth-dependent gradients in the photic zone, and drives

cyclic processes in both adults and propagules. Previous studies of photosensitivity in phyla displaying multiple development modes (e.g. Mollusca, Annelida, Echinodermata) have been centered on planktotrophic species (for examples, see Barile *et al.* 1994, Jékely *et al.* 2008, McCarthy *et al.* 2002). To address this gap, I tested the sensitivity of co-occurring echinoderm planktotrophic and lecithotrophic propagules to light. The lecithotrophic propagules generally displayed less net-tactic behaviour in response to light but still changed their locomotory output (speed and trajectory) in response to variable light colour. This was not totally surprising as lecithotrophic propagules in this group may have reduced nervous system development associated with their loss of feeding capabilities relative to other types of propagules (e.g. planktotrophs). However, the maintenance of some photosensitivity and swimming abilities in lecithotrophic propagules suggests that they are fundamental for survival during pelagic development.

7.2. General Conclusions and Future Research

7.2.1. Marine propagule morphology reflects development mode, geographic location and phylogeny

Patterns of echinoderm egg colour intensity and diversity seen in Chapter 3 were detected among species with developmental and geographic differences. In the case of development mode differences, variation in egg colour *intensity* showed a clear relationship with development location and the degree of parental care. Pigments are expensive for mothers to produce, and if there is no need for UV protection, camouflage or signaling, a reduction in pigment deposition in eggs is a logical consequence (such as

in internally-brooded eggs). These patterns also likely apply to eggs that develop in capsules in other phyla, such as the pale cream eggs of the gastropod *Buccinum scalariforme* (Appendix 1). However, the driver(s) of egg colour *variation* was much less clear. Strong biogeographical patterns exist, but these cannot yet be explained by any clear environmental, genetic or physiological features.

Basic knowledge of pigments exists for adult echinoderms, but little work has been done to identify the corresponding pigments in echinoderm eggs, or in other phyla that also possess egg-colour variation (e.g., Mollusca, Annelida, Cnidaria). Functional tests of pigment efficiency have also not been conducted, making it difficult to assess the adaptive value of certain pigments over others. Clusters of closely related species with very different colour pigments and geographic distributions provide an excellent framework to begin to fill some of these gaps. The sea cucumber genus *Cucumaria* could be a good place to start; it has representatives in both the Atlantic (e.g. *C. frondosa*, red eggs) and Pacific (e.g. *C. frondosa japonica, C. miniata*, green eggs) that exhibit similar adult shapes and propagule sizes. Pigment types could be compared among propagules and adults of this genus while limiting the potential confounding factor of phylogenetic diversity. A thorough study of variation in egg colour within species would also be of interest.

7.2.2. Swimming capacity and patterns are more closely dependent on phylogenetic and ontogenetic traits than on larval nutritional mode

Marine propagules possess incredible morphological diversity, which is particularly prominent among different nutritional modes; planktotrophic propagules are small and more or less transparent whereas lecithotrophic propagules are larger and yolky. On the one hand, planktotrophic and lecithotrophic echinoderm propagules swam more similar than previously thought. On the other hand, phylogenetic and ontogenic differences significantly modulated swimming capacity. Relative to strong local mixing and currents, the swimming speeds reported here for all propagules would be almost negligible, raising several critical questions including: why have swimming abilities been maintained in marine propagules and are they only effective under low-flow conditions, such as in the benthic boundary layer or extremely protected coastal areas? If so, why would buoyant propagules and early stages (embryos) that dwell near the surface retain any swimming capacities?

There is often a disconnect between what is observed in the laboratory versus what can be tested in field. Even for well-studied species such as oysters, mussels and sea urchins, science is only beginning to develop the tools and understanding necessary to bridge the gap between small and large scale data. Processes such as dispersal, connectivity, settlement and recruitment are expected to be directly affected by changing ocean temperature and chemistry (Byrne and Przeslawski 2013). To improve predictions and models, the study of life histories in the ocean cannot be restricted to only one scale. Therefore, studies at the scale of the propagule and at the scale of oceans will be equally

critical. Since even the most basic understanding of lecithotrophic propagules in the context of changing oceans is limited, special attention will be required to include representatives with this type of development in future studies.

7.2.3. Sensory behaviour is prevalent among marine propagules but infrequently studied

All planktotrophic and lecithotrophic echinoderm propagules tested here showed some degree of sensitivity to light intensity and colour. Swimming behaviours were also affected by increased water temperature. The ability to detect and respond to sensory stimuli such as light, chemicals, gravity, temperature and pressure is a feature of early marine animals, and may still be fundamental to the dispersion and settlement of modern marine propagules.

Despite the importance of sensory behaviour to critical biological processes in the marine environment, work to date has focused primarily on planktotrophic species. Lecithotrophic species warrant further investigation, especially in taxonomic groups containing both planktotrophic and lecithotrophic representatives. Sensory behaviour data generated from such studies can be utilized for several key applications in the fields of aquaculture, ecotoxicology and climate change (see Chapter 2). While survival data can be used as an indication of the severity of exposure to a toxicant or environmental change, the information provided is limited since it reflects only one time-point. Alternatively, an organism's behaviour may be adversely affected by exposure that can result in reduced fitness or death over the long term. Baseline data of swimming behaviours under ambient conditions (like those generated here) are therefore critical for facilitating comparisons

under other sets of conditions including predicted climate change scenarios. The integrated response(s) of propagules to a hierarchy of stimuli also needs to be further explored as different combinations of cues are likely involved in guiding propagules throughout the development process from egg to juvenile.

7.3. Limitations and Recommendations

Lecithotrophic propagules are as important as planktotrophic propagules, especially in phyla like Echinodermata where they comprise the majority of extant species (~68%, Uthicke *et al.* 2009). I have shown here that preconceptions regarding the locomotory constraints of lecithotrophic propagules need to be re-evaluated since they appear to swim just as fast as planktotrophs. Future studies should explore these patterns in other regions and other taxa with mixed development modes such as Mollusca and Annelida.

The many phyla with mixed development modes (planktotrophy and lecithotrophy) and species that produce multiple propagule types (poecilogony) represent an untapped resource for comparative studies in the field of larval ecology (Knott and McHugh 2012). Poecilogonous species are particularly interesting, since questions of behaviour and development can be directly attributed to nutritional mode differences among different propagule types, rather than to phylogenetic differences. Comparative work on propagule behaviour has already begun in two poecilogonous species: the annelid *Capitella* sp. (Butman *et al.* 1988) and the sea slug *Alderia willowi* (Krug *et al.* 2012). However, there is no echinoderm equivalent with poecilogony that has been

examined in such a context. The deep-water sea star *Henricia lisa* produces both pelagic lecithotrophic propagules and externally brooded propagules (Mercier and Hamel 2008). These two types of propagules have two different colours (cream and dark yellow), but still develop similarly (Mercier and Hamel 2008). It would be interesting to compare locomotory capacity and behaviour between these two types of propagules, since the brooded propagules can swim freely when removed from underneath the body of the mother, just like their pelagic counterparts (pers. obs. 2014). Future studies of this type could also be used to compare species from different habitats (deep sea versus shallows), and test whether local environmental conditions (e.g. light and pressure) affect the swimming capacity and behaviour of developing propagules differently. The snail genus *Buccinum* could also be useful for comparisons of deep versus shallow, as congeneric species can be found at a wide range of depths with similar modes of reproduction (see Appendix 1 for a description of a deep-sea representative).

With the possible exception of corals, there is a shortage of lecithotrophic representatives in the larval ecology literature (especially marked for echinoderms), which could be due to several reasons. Firstly, lecithotrophic propagules are more difficult to maintain in the laboratory under standard culturing protocols, which involve raising propagules in closed, or semi-closed seawater systems under set incubation temperatures. Due to the large (and often buoyant) nature of lecithotrophic propagules, culture densities need to be lower than for their planktotrophic counterparts, and the risks of propagules getting stuck to culture walls or to each other during development are much higher among lecithotrophs (pers. obs. 2014). Lecithotrophy is also common in temperate

and polar regions (Marshall *et al.* 2012), which means that adults and propagules grow very slowly relative to tropical species. The long generation times in cold-water species make long-term studies a challenge, and are a limitation to reproductive studies in the context of changing oceans. Work involving the intersection of maternal effects, propagule behaviour and changing ocean chemistry in cold-water species is critical for the future, but is logistically complex relative to tropical species. Finally, there are fewer commercial lecithotrophs so less attention has been paid to them overall. Taken together, these limitations suggest that although studies on lecithotrophic species are important, more work needs to be done to improve the way researchers work with them and consider their value in the global ocean community.

7.4. References

Abelson, A., and M. Denny. 1997. Settlement of marine organisms in flow. *Annual Review of Ecology and Systematics* **28**:317-339.

Barile, P. J., A. W. Stoner, and C. M. Young. 1994. Phototaxis and vertical migration of the queen conch (*Strombus gigas*) veliger larvae. *Journal of Experimental Marine Biology and Ecology* **183**:147-162.

Blount, J. D. 2004. Carotenoids and life-history evolution in animals. *Archives of Biochemistry and Biophysics* **430**:10-15.

Butman, C. A., J. P. Grassle, and E. J. Buskey. 1988. Horizontal swimming and gravitational sinking of *Capitella* sp. I (Annelida: Polychaeta) larvae: implications for settlement. *Ophelia* **29**:43-57.

Byrne, M., and R. Przeslawski. 2013. Multistressor impacts of warming and acidification of the ocean on marine invertebrates' life histories. *Integrative and Comparative Biology* **53**:582-596.

Emlet, R. B. 1994. Body form and patterns of ciliation in nonfeeding larvae of echinoderms - Functional solutions to swimming in the plankton. *American Zoologist* **34**:570-585.

Falkner, I., M. Byrne, and M. A. Sewell. 2006. Maternal provisioning in *Ophionereis fasciata* and *O. schayeri*: brittle stars with contrasting modes of development. *Biological Bulletin* **211**:204-207.

Gross, T. F., F. E. Werner, and J. E. Eckman. 1992. Numerical modeling of larval settlement in turbulent bottom boundary layers. *Journal of Marine Research* **50**:611-642.

Jacobs, D. K., N. Nakanishi, D. Yuan, A. Camara, S. A. Nichols, and V. Hartenstein. 2007. Evolution of sensory structures in basal metazoa. *Integrative and Comparative Biology* **47**:712-723.

Jékely, G., J. Colombelli, H. Hausen, K. Guy, E. Stelzer, F. Nédélec, and D. Arendt. 2008. Mechanism of phototaxis in marine zooplankton. *Nature* **456**:395-399.

Knott, K. E., and D. McHugh. 2012. Introduction to symposium: poecilogony-A window on larval evolutionary transitions in marine invertebrates. *Integrative and Comparative Biology* **52**:120-127.

Krug, P. J., D. Gordon, and M. R. Romero. 2012. Seasonal polyphenism in larval type: rearing environment influences the development mode expressed by adults in the sea slug *Alderia willowi*. *Integrative and Comparative Biology* **52**:161-172.

Leys, S. P., T. W. Cronin, B. M. Degnan, and J. N. Marshall. 2002. Spectral sensitivity in a sponge larva. *Journal of Comparative Physiology A* **188**:199-202.

Marshall, D. J., P. J. Krug, E. K. Kupriyanova, M. Byrne, and R. B. Emlet. 2012. The biogeography of marine invertebrate life histories. *Annual Review of Ecology and Systematics* **43**:97-114.

McCarthy, D. A., R. B. Forward Jr, and C. M. Young. 2002. Ontogeny of phototaxis and geotaxis during larval development of the sabellariid polychaete *Phragmatopoma lapidosa*. *Marine Ecology Progress Series* **241**:215-220.

McDonald, K. A. 2012. Earliest ciliary swimming effects vertical transport of planktonic embryos in turbulence and shear flow. *Journal of Experimental Biology* **215**:141-151.

Mercier, A., and J.-F. Hamel. 2008. Depth-related shift in life history strategies of a brooding and broadcasting deep-sea asteroid. *Marine Biology* **156**:205-223.

Metaxas, A., and M. Saunders. 2009. Quantifying the "bio-" components in biophysical models of larval transport in marine benthic invertebrates: Advances and pitfalls. *Biological Bulletin* **216**:257-272.

Mileikovsky, S. A. 1973. Speed of active movement of pelagic larvae of marine bottom invertebrates and their ability to regulate their vertical position. *Marine Biology* **23**:11-17.

Mundy, C. N., and R. C. Babcock. 1998. Role of light intensity and spectral quality in coral settlement: Implication for depth-dependent settlement? *Journal of Experimental Marine Biology and Ecology* **223**:235-255.

Pechenik, J. A. 1999. On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. *Marine Ecology Progress Series* **177**:269-297.

Pennington, J. T., and R. B. Emlet. 1986. Ontogenetic and diel vertical migration of a planktonic echinoid larva, *Dendraster excentricus*: Occurence, causes, and probable consequences. *Journal of Experimental Marine Biology and Ecology* **104**:69-95.

Pizarro, V., and J. Thomason. 2008. How do swimming ability and behaviour affect the dispersal of coral larvae. 464-467 in *Proceedings of the 11th International Coral Reef Symposium, Fort Lauderdale, Florida, 7*.

Prowse, T. A. A., M. A. Sewell, and M. Byrne. 2008. Fuels for development: evolution of maternal provisioning in asterinid sea stars. *Marine Biology* **153**:337-349.

Strathmann, R. R. 2007. Three functionally distinct kinds of pelagic development. *Bulletin of Marine Science* **81**:167-179.

Uthicke, S., B. Schaffelke, and M. Byrne. 2009. A boom-bust phylum? Ecological and evolutionary consequences of density variations in echinoderms. *Ecological Monographs* **79**:3-24.

Wildish, D. 2009. Benthic boundary layer effects. *Elements of Physical Oceanography: A Derivative of the Encyclopedia of Ocean Sciences*:199.

Wray, G. A., C. Kitazawa, and B. Miner. 2004. Culture of echinoderm larvae through metamorphosis. *Methods in Cell Biology* **74**:75-86.

Appendices

Appendix 1 The deep sea neogastropod *Buccinum scalariforme*: Reproduction, development, and growth

---

A version of this manuscript was published in the journal Deep-Sea Research Part 1 in January 2017 (Vol. 119, page 24).

A.1.1. Abstract

Specimens of the neogastropod *Buccinum scalariforme* (60-70 mm shell height) collected between 700 and 1450 m depth along the continental slope of eastern Canada were kept for 4 years in mesocosm settings. Their mating, spawning and development were assessed, thereby generating the first complete life cycle account of a deep-sea gastropod. Egg laying occurred in March and September, with a total of 9 egg masses laid in 2013 and 2015, coinciding with periods of maximum deposition of particulate organic matter (phytodetritus). Oviposition lasted 2-3 days and the female protected the egg mass for ~3 more days until it had hardened. Typically, egg masses contained 50-75 egg capsules, each measuring 5-8 mm in diameter. A capsule contained between 100-150 spherical eggs (300-500 µm) of which ≤ 50 developed into embryos. Potential fecundity calculated from the entire egg mass at spawning was between 1500-2250 propagules; it drastically decreased over ~4-5 months of development to an effective fecundity of 30-50 juveniles emerging from the mass (0-2 juveniles per capsule). Development went through early embryonic stages in ~15 days and reached the trocophore in 15-21 days, followed by intracapsular veliger larva (480 µm) and intracapsular pediveliger (~1000 µm) after

~90 days. Completion of development relied on oophagy and adelphophagy. The juveniles hatched at a shell height of 1-2 mm and consumed the capsule membrane. Over 2.5 years, they reached a maximum size of ~8-10 mm shell height at an average of 9.8 μm day^{-1}. Estimations indicate that *B. scalariforme* could require between 20 and 50 years to reach maximum adult size. Large gastropods like *B. scalariforme* are among the most abundant motile benthic invertebrates of the bathyal zone of eastern Canada. Knowledge of their reproductive biology constitutes a first step in assessing their vulnerability and resilience to ever growing anthropogenic pressures, including fisheries, and oil/gas exploration and exploitation.

A.1.2. Introduction

The superfamily Buccinoidea is the most geographically widespread and ecologically diverse clade within the Neogastropoda (Harasewych and Kantor, 2004). These predatory and scavenging molluscs have radiated since the Early Cretaceous to occupy a breadth of benthic marine habitats, from tropical to polar regions and from intertidal to hadal depths (Clarke, 1962; Tracey et al., 1993). In the shallow coastal waters of eastern Canada, neogastropods form the vast majority of the large, active mollusc species, including members of the genera *Colus*, *Neptunea*, *Beringius*, *Plicifusus*, *Volutopsius*, and *Buccinum* (Brunel et al., 1998). Neogastropods remain the most abundant large mollusc species collected on sandy and muddy substrates in deep waters, especially in bathyal regions between 500-1500 m depth (MacDonald et al., 2010; Rowe et al., 1982). Despite the abundance of neogastropods in several regions of the deep sea, key features of their biology and reproductive strategies remain poorly studied.

It has been suggested that breeding occurs year-round in the abyssal gastropod *Benthonella tenella* in the Northwest Atlantic (Rex et al., 1979). Similar reproductive patterns were suspected to characterize the neogastropod *Colus jeffreysianus* and the trochid *Calliotropis ottoi* from the Northeast Atlantic. Gametogenesis appears to be a continuous process with oocytes in all stages found in the ovaries year round (Colman and Tyler, 1988a; Colman et al., 1986a). Olabarria and Ramirez-Llodra (2004) indicated that the shallow-water gastropods *Amphissa acutecostata* and *Gymnobela subaraneosa* from the Northeast Atlantic exhibited a quasi-continuous production of oocytes, suggesting release of a small number of oocytes all year long. Colman et al. (1986b)

studied the larval shell morphology of several species of deep-sea neogastropods from the Northeast Atlantic to distinguish feeding and non-feeding development. Similar studies were conducted by Bouchet and Waren (1979) on bathyal and abyssal gastropods. Gustafson et al. (1991) analyzed the contents of gastropod capsules collected from hydrothermal vent fields and Gustafson and Lutz (1994) reviewed the life-history traits of molluscs from chemosynthetic environments. The review by Gage and Tyler (1991) indicated that planktotrophic development in gastropod snails became more prevalent with depth, from 25% of species at depths <1 km to 50% at depths >4 km. However, data on developmental patterns that would help understand the ecology, evolution, and distribution of deep-water gastropods were said to be scarce (Bouchet and Warén, 1994); a statement that still stands today.

Unlike the limited number of publications dedicated to deep-sea gastropods, literature on the reproductive biology of shallow-water gastropods, and Buccinidae in particular, has been gathering since the early 1900s (e.g. Gendron, 1992; Ilano et al., 2004; Kideys et al., 1993; Lamy, 1928; Martel et al., 1986a; 1986b; Portmann, 1925, 1930; Smith and Thatje, 2013). Neogastropods undergo a period of intracapsular development, after which propagules either hatch as free-swimming larvae or as crawling juveniles (Fretter and Graham, 1994; Miloslavich and Dufresne, 1994; Pechenik, 1979; Rivest, 1983). Forms of intracapsular food sources include nurse eggs (Gallardo and Garrido, 1987; Smith and Thatje, 2013) to be consumed by one or more larvae (Penchasziadeh, 1976; Smith and Thatje, 2013), yolk-filled oocytes (generally larger than 800 μm) (Borzone, 1995) and/or provision of nutrients in the intracapsular fluid

(Miloslavich, 1996; Moran, 1999; Ojeda and Chaparro, 2004). Oogenesis, copulation and egg laying were documented by Staiger (1951) and Martel et al. (1986a) in *B. undatum* and by Ito (1978) in *B. kinukatsugi* and *B. miyauchii*. Miloslavich and Dufresne (1994) described the development of *B. cyaneum* from eastern Canada, whereas Ilano et al. (2003) examined the reproductive cycle, and size at sexual maturity of *Buccinum isaotakii.* Finally, Ilano et al. (2004) described copulation, development and fecundity in *B. isaotakii* from Japan.

Buccinum scalariforme Moller 1842 is currently recognized to occur from subtidal to bathyal depths in west Greenland, Iceland, the Arctic, eastern and western Canada, as well as Maine and Alaska (USA) (Gofas, 2004). To our knowledge, nothing is known about its biology. The present study explored the reproductive habits, including egg laying, development and growth, of *B. scalariforme* collected at bathyal depths off eastern Canada, from slope habitats that are under growing pressures from the fishing and petroleum industries.

A.1.3. Material and Methods

A.1.3.1. Collection and maintenance

A variety of marine invertebrates were collected as by-catch during multispecies research surveys conducted by Fisheries and Oceans Canada (DFO) on the CCGS *Teleost* in Fall 2011 and 2013 off Newfoundland, eastern Canada (48°52'N: 45°51'W) between 700 and 1450 m depth. Collection of deep-sea species in the fall and early winter ensures that bottom temperature in the bathyal zone roughly matches surface temperature (~1-6 °C). Surveys followed a stratified random sampling design with a Campellen 1800 trawl

towed for 15 minutes on ~1.4 km of seafloor (gear opened and closed at depth). The density of *Buccinum scalariforme* was estimated from the trawls in which they were present (n=37) and for three depth ranges: 700-900 m, >900-1100 and >1100 m. All individuals collected were counted and measured. Some specimens were kept alive (n=12 in 2011 and n=37 in 2013) aboard the ship in 2000 L tanks supplied with running seawater pumped from the ocean, equivalent to ~75 water changes per day. Individuals from all depths adapted well to captive conditions.

The live gastropods (60-80 mm maximum shell height measured along the central axis) were held at the Ocean Sciences Centre (Memorial University) in flow-through tanks (350-800 L) for continuous monitoring. A minimum of 10 individuals were maintained together in each tank. All tanks were darkened and supplied with running unfiltered seawater at a rate ~50 L h^{-1}. They were filled with a thick layer of soft sediment (12-15 cm), boulders (~10 cm in diameter) and a few pebbles (<2 cm diameter). An in-line chilling unit (Universal Marine Industries, 5 hp) was used to keep the running seawater suitably cold during warmer months, from July to October (<7ºC). Overall, the laboratory conditions were set to mimic those found in the native habitat of *B. scalariforme* as closely as possible. The mesocosms in which *B. scalarifome* were kept also hosted numerous species collected simultaneously, i.e. the sea anemones *Hormatia* spp., *Urticina* sp. and *Bolecera* sp., the solitary cup coral *Flabellum alabastrum*, several sea stars (*Henricia lisa*, *Ceramaster granularis*, *Hippasteria phrygiana*, *Leptychaster*, *Poroniomorpha* spp.), basket stars, gastropods (*Stephanasterias albula*, *Boreotrophon*

clathratus, *Neptunea* spp., *Colus* spp, *Apporhais* sp., *Beringius* spp. and other *Buccinum*) as well as polychaetes and small bivalves.

The temperature in the tanks was recorded with a temperature-light logger HOBO Pendant (UA-002-64), and was consistent with seasonal fluctuations in this area of the Canadian coast at depths down to 600–800 m (0-5°C; DFO, 2009). The annual primary productivity (data from DFO Station 27) and load of suspended matter (monthly rate of detrital matter deposition) was obtained from concurrent and previous studies conducted in the same laboratory (Hamel et al., 2010; Mercier et al., 2011), and was consistent with observations during the study.

A.1.3.2 Behaviour and development

Monitoring of gastropods (behaviour and social interactions) occurred at regular intervals, generally 2 or 3 times a week (sometimes more often when more activities were noted). Individuals were scored either as pairing, mating, laying eggs or guarding their egg masses. Survival rates of adults over the whole study were noted.

The duration of egg laying, the time spent close to the egg mass after laying, the number and types of interactions with congeners and other species present in the tanks were also described. The size of the spawning individuals and of their egg masses, as well as the number of capsules per mass were recorded. I use the term egg mass here to describe the entire cluster of egg capsules (individual pouches filled with developing propagules) held together by a proteinaceous membrane. Predatory events on egg masses were scored when a predator remained static over the mass, and/or when its proboscis was inserted in the mass (gastropods) and/or its stomach was everted (sea stars). Predators

where thereafter moved to a distant part of the mesocosm (1-2 m away), to preserve the egg mass. Recurrent attempts at predation (by the same individual) were also recorded.

Measurements of whole egg masses were done underwater (they were never removed from the holding tank). Groups of 5 capsules from each monitored egg mass were sampled at regular intervals, opened and examined under a microscope to take photos and measurements. For each of these capsules, the total number and development stage of all propagules were recorded and their Feret diameter or length (n=2-20 ind. per life stage) noted. The proportion of nurse cells and non-developing embryos was also established. The potential fecundity, i.e. the number of propagules at the onset of the development, was established from 3 capsules per mass, and the effective fecundity, i.e. the number of juveniles emerging from the egg mass, was documented from 3 egg masses.

Photographs and measurements were taken under a Nikon SMZ1500 stereomicroscope attached to a Nikon DXM1200F digital camera using the imaging software Simple PCI (v. 6.0), and with a Leica M205A stereomicroscope using the Leica Application Suite X (LASX) software.

A.1.3.3. Feeding

The diet of adults (60-80 mm shell height) and juveniles (free living; 1-8 mm shell height) was established by offering various potential prey on an opportunistic basis (when available in the laboratory and from periodic deep-sea samplings). Assessments of prey acceptance, with minimal disturbance, were made every two days. Positive feeding was scored if the proboscis was inserted into the prey or a prey fragment was being brought to

the mouth or if the food item disappeared (presumably eaten by the gastropods). Small pieces of mussel (*Mytilus edulis*) tissue and urchin gonads were used as food.

A.1.3.4. Growth

Size measurements were collected from the juveniles over 30 months, based on maximum shell height along the vertical axis, and used to estimate time necessary to reach adult size, which was determined to be 60-80 mm shell height, based on specimens collected for this study (n = 27). As growth to the adult size could not be monitored, estimates of the age at full size were made using curve fitting over data obtained for the first 2.5 years, as per Hirst and Forster (2013).

A.1.4. Results and Discussion

The reproductive biology and life cycle of the deep-sea neogastropod *Buccinum scalariforme* closely followed the general patterns described in shallow-water congeners. Feeding habits, egg laying and development stages were generally conserved. The main differences included lower effective fecundity per capsule and per egg mass and a much slower growth rate under laboratory conditions than in other *Buccinum* species. Moreover, findings gathered here provide the first evidence of seasonal reproduction in a deep-sea gastropod.

Buccinum scalariforme occurred in trawls conducted over soft bottoms (muddy and/or sandy, sometimes mixed with scattered boulders) between 700 and 1450 m depth along the continental slope off Northeast Newfoundland (eastern Canada). The species had already been reported from eastern Canada (Brunel et al., 1998), and is commonly collected at bathyal depths during DFO surveys (unpublished data). Density estimates of

B. scalariforme were maximal between 900-1100 m (0.12 ± 0.09 individuals m^{-2}, n=16 trawls). Only 3 individuals were collected >1100 m (n=7 trawls), whereas between 0.04 and 0.09 individuals m^{-2} were recorded from 700-900 m (n=25 trawls). However, these densities likely represent an underestimation, as the gear and mesh used were not designed to collect benthic invertebrates. In the mesocosms, *B. scalariforme* typically occurred on the surface of the muddy substrate and sometimes buried almost completely in it, with only the siphon visible. Some specimens were recorded on hard substrata (including the vertical walls of the tanks), especially during the egg-laying season (see below). These field and laboratory observations suggest that *B. scalariforme* requires mixed substrata of mud/sand and scattered hard surfaces (e.g. boulders) to complete its life cycle.

Food items, including mussel tissues, urchin gonads and dead shrimps were readily accepted by adults of *B. scalariforme* in the mesocosms. Gut content analysis was performed by cracking the shell to remove the intact soft body; the stomach was located and slit open with a scalpel. The contents were flushed with a jet of ethanol using a pipette and analyzed under the microscope. Examination of stomach contents indicated that adults also ingest particulate organic matter (POM) as well as polychaete worms (probably burried in the substrate). Juveniles (1-8 mm shell height) were observed to feed on both mussel and urchin tissues. These findings suggest that *B. scalariforme* is a detritivore and opportunistic scavenger, similar to other buccinid species reported by Smith and Thatje (2013).

Copulation was never directly observed during the monitoring periods and egg laying occurred up to a year post collection, indicating that either copulation was not noticed or sperm storage occurred from copulation at depth before collection. Delay between copulation and egg laying in neogastropods is known to be between 2 and 4 months in *Nucella lamellosa* (Stickle, 1975) and 1 month in *B. isaotakii* (Ilano et al., 2004), suggesting that it is common for sperm to be stored for several months in gastropods. Individuals of *B. scalariforme* always laid egg masses on vertical surfaces and did so individually (Fig 1a), not displaying the gregarious egg-laying behaviour reported in shallow-water species like *B. undatum* (Smith and Thatje, 2013). Three different females were confirmed to have laid 3 of the 7 egg masses released between March 2014 and March 2015 (see below for egg-laying timing). The remaining egg masses could have been released by the same, or different females (not confirmed). In *B. scalariforme*, each egg-laying bout lasted 2-3 days, after which the female guarded the mass for another ~3 more days before leaving it, which is shorter than the 9-11 days mentioned by Smith and Thatje (2013) for the shallow-water *B. undatum*. Egg masses of *B. scalariforme* ranged from 40-60 mm wide and 20-30 mm thick and were mostly spherical in shape. The freshly laid capsules were soft, and it took 2-3 days for the mass to harden. At first capsules contents looked whitish with space between propagules (Fig 1b) compared to older masses in which content appeared yellow and visibly packed (Fig 1c). Older capsules (several weeks old; with embryos in the trocophore stage) contained well-defined nurse cells of various shapes that created a compact mass appearance (Fig 2). The number of egg capsules per egg mass varied between 50-75, slightly less than the

100 capsules of *B. cyaneum* (Miloslavich and Dufresne, 1994) and 80-150 capsules of *B. undatum* (Smith and Thatje, 2013). Each capsule measured 5-8 mm in diameter and were plano-convex in shape with the flat side toward the substrate (Figure 1a). Each individual capsule overlapped 2 or 3 previously laid capsules. Overall, the egg capsules contained between 100-150 entities (including nurse eggs), again less than reports of 300-1000 for *B. cyaneum* from eastern Canada (Miloslavich and Dufresne, 1994) and of ~1000 for *B. undatum* (Fretter and Graham, 1984; Smith and Thatje, 2013; Valentinsson, 2002). Here, between 0 and 2 fully developed *B. scalariforme* juveniles emerged from each capsule, and total effective fecundity per mass/female was 30-50 juveniles. The relative effective fecundity of *B. scalariforme* was lower than the 10 crawling juveniles per capsule reported in the shallow-water *B. cyaneum* by Miloslavich and Dufresne (1994), but similar to the number observed in the deep-sea *Colus jeffreysianus* collected at 2200 m, whereby only one juvenile emerged per capsule (Colman and Tyler, 1988b). Overall, between 0.6 and 2% of the eggs developed into juveniles in *B. scalariforme*, which is similar to other buccinids for which the percentage of developing eggs varies from 0.2 to 2% (Ilano et al., 2004; Martel et al., 1986a; Miloslavich and Dufresne, 1994; Portmann, 1925; Smith and Thatje, 2013; Valentinsson, 2002). Low effective fecundity might be common in deep-sea neogastropods, due to limited food resources.

Notably, completion of development in *B. scalariforme* was determined to partly rely on adelphophagy (consumption of sibling embryos). Juveniles near hatching were seen to consume siblings in the same capsule and, sometimes, hatched juveniles fed on other capsules and their unhatched juveniles (i.e. fecundity of some capsules was

therefore zero). Moreover, empty broken shells found in several of the capsules examined were interpreted as further evidence of adelphophagy, which may be common in this species. Adelphophagy was also suggested to occur in *B. undatum* by Fretter and Graham (1984), although this conclusion was debated (Smith and Thatje, 2013), despite being common in other gastropods such as *Crucibilum quiriquinae* (Veliz et al., 2001), *Crepidula coquimbensis* (Brante et al., 2009) and *Trophon geversianus* (Cumplido et al., 2011).

Two predators, a sea star and a gastropod, were attracted by the newly laid egg masses of *B. scalariforme* in the mesocosms. On two occasions, predation on the egg masses was confirmed by removing the sea star *Stephanasterias albula* to reveal its everted stomach (Fig 1d). This species was repetitively seen to attempt consumption of egg masses when they were still soft, despite the presence of the guarding mother, after which time no more predation attempts were recorded. Females of *B. scalariforme* were seen to use their foot in an attempt to repel sea star predators and to cover newly-laid egg masses completely. This suggests that the female remains close to the egg mass during the most vulnerable developmental period. On the other hand, the neogastropod *Boreotrophon clathratus* was observed to prey on the egg masses (proboscis introduced in capsules) over the whole development period (Fig 1e). When confirmed, the predation was interrupted to preserve the egg mass. In general, capsules found along the margin of the mass contained approximately 50% fewer eggs compared to capsules in the middle of the mass, and they also had a rougher surface texture. As described in *B. cyaneum*

(Miloslavich and Dufresne, 1994), the less fecund capsules might be used as a defence mechanism against predation by opportunistic species.

Some years ago, Scheltema (1994) stipulated that there was no evidence for any periodicity in reproduction related to seasonal fluctuations in productivity or other parameters among deep-sea molluscs. However, in the present study, *B. scalariforme* laid egg capsules twice a year (spring and fall) apparently following a seasonal pattern. More precisely, three egg masses were laid in March 2014, two in September 2014 and another two in March 2015. Egg laying has been reported in fall and early winter in the shallow-water *B. cyaneum* from eastern Canada (Miloslavich and Dufresne, 1994), as well as in *B. undatum* from the Irish Sea (Kideys et al., 1993), whereas mating reportedly occurs in spring in *B. undatum* from the Northwest Atlantic (Himmelman and Hamel, 1993). The deep-sea neogastropod *Colus jeffreysianus* from 2200 m in the Northeast Atlantic was suggested to exhibit a continuous gametogenesis and no post-spawning individuals were ever found (Colman et al., 1986a). Similar partial evidence was gathered for the deep-sea gastropods *Calliotropis ottoi* (Colman and Tyler, 1988a), *Benthonella tenella* (Rex et al., 1979), and *Amphissa acutecostata* and *Gymnobela subaraneosa* (both from the Northeast Atlantic) (Olabarria and Ramirez-Llodra, 2004).

The fact that egg laying in *B. scalariforme* occurred twice a year points to a factor that varies on a biannual basis. In the spring and fall periods when *B. scalariforme* laid their eggs, the temperature was 1°C and 6.5 °C, respectively, suggesting that this factor is not the proximate spawning cue. Similarly, various temperatures were noted during egg-laying in *B. undatum*; Martel et al. (1986a) mentioned 2-3°C in the Northwest Atlantic

and Hancock (1967) and Kideys et al. (1993) mentioned 9°C in the Northeast Atlantic. The seasonal input of deposited organic matter, in the form of down-fluxes of phytoplankton and zooplankton blooms from the surface layers, might provide a cue for oogenesis in *B. scalariforme*. The amount of deposited material in the tanks increased drastically following the spring and fall blooms of surface primary production, from <1 mm $month^{-1}$ m in most months to 5.5 mm $month^{-1}$ and 4 mm $month^{-1}$ in spring and fall, respectively. The diet of adult *B. scalariforme* included deposited particulate matter and infaunal invertebrates mixed with the layer of fresh sediment (e.g. polychaete worms). This seasonal deposition could thus provide energy to fuel the final stages of oogenesis (vitellogenesis) and might explain why spawning was confined to these two periods. Deposited matter was suspected to enhance gametogenesis and be used as spawning cue in other-deep sea species from the same region (Mercier et al., 2011; Sun et al., 2011). Fecundity metrics measured in *B. scalariforme* were low compared to other buccinids (shallow-water species), supporting the idea that reproductive output is spread over two breeding seasons, instead of one major annual spawning. Although it could not be confirmed, each spawning bout might only have involved partial gamete release.

The greyish oocytes of *B. scalariforme* were ~300 µm in diameter (Table 1; Fig 3a) with a surface showing a mosaic of yolky spheres (Fig 3a insert). Cleavage was holoblastic, spiral and unequal (Fig 3b), creating large yolky macromeres at the vegetal pole and numerous small micromeres at the animal pole (Fig 3b). Embryos were seen at different stages of development up to day 21 post laying (Fig 3d, e, f). Very few trocophores were detected inside different capsules in the interval between days 15 and

21 (Fig 3g). As the number of egg masses was limited, the precise number of days over which propagules remain in the trocophore stage was thus not established with any precision; it may be because this stage is of short duration, as reported by Smith and Thatje (2013). The trocophore developed into a veliger after about 45 days. Nurse cells were clearly visible inside the digestive tract of late veligers (Fig 3h, i; 4a, b). After 90 days, intracapsular pediveligers were the dominant stage and also displayed nurse cells in their digestive tracts (Fig 4c, d). Nurse cells seem to be ingested whole at the veliger stage, as their shape could be seen through the transparent body wall (Fig 3h, i; 4a), as mentioned by Smith and Thatje (2013) in *B. undatum* and by Rivest (1983) in *Searlesia dira*. However, in *B. scalariforme* the nurse cells became less recognizable, except by the color of the digestive tract, in more advanced stages including pediveliger (Fig 4c, d) suggesting their more rapid digestion and/or damage during ingestion. Hatching occurred after ~4-5 months (Fig 4e, f, g), a delay similar to the 3-5 months reported in the shallow cold-water neogastropod *B. undatum* studied in the Irish Sea (Kideys et al., 1993; Smith and Thatje, 2013), but shorter than the 9 months reported in *B. cyaneum* from eastern Canada and the 6-7 months documented in *B. isaotakii* from Japan (Ilano et al., 2004) suggesting that deep-sea species do not always require more time than shallow-water species for development. The hatching process in *B. scalariforme* took up to 10 days for a single egg mass, illustrating possible asynchrony in development among propagules or egg capsules, as observed in *B. undatum* (Smith and Thatje, 2013). Hatching juveniles had transparent yellowish shells, distinct head, foot, tentacles and eyes, as well as an operculum, and they measured 1.5 mm shell height (Fig 4e, f). Hatching was described to

occur through radular scraping of the capsule in *B. undatum* (Smith and Thatje, 2013), which might be similar in *B. scalariforme*, but could not be confirmed. After hatching, the juveniles consumed the capsule from which they emerged.

The average growth rate of *B. scalariforme* was around 9.8 µm d^{-1} from the eggs to the largest juveniles recorded (~9 mm), which is slow compared to neogastropods from tropical shallow waters, like *Babylonia areolata* at ~100 µm day^{-1} (Chaitanawisuti and Kritsanapuntu, 1997) and *Babylonia formosae habei* at ~49 µm day^{-1} (Chen et al, 2005). Heude-Berthelin et al. (2011) mentioned that growth parameters may differ as a function of water temperature or food availability, which could be enough to explain the slower growth recorded in a deep-sea species like *B. scalariforme*. Juveniles of *B. scalariforme* emerged from the egg mass with two whorls (after ~120 days of development) and developed a third whorl after 180 days (Fig 4h). They finally developed a fourth whorl when they reached 6-9 mm shell height after 2.5 years (Fig 5a, b, c). Adult individuals had seven whorls and measured between 60-80 mm shell height. Based on data for the first 2.5 years of growth, between 20 and 50 years would be required to reach the maximum shell height, using fits of linear, power and quadratic curves (Table 2, Fig 6). It cannot be ruled out that growth would significantly increase in the fourth or subsequent years or that laboratory conditions constrained growth rates in some way, although deep-sea species are notoriously slow growing (e.g. Hamel et al., 2010, Mercier et al., 2015). Values found here are slower than reported for shallow-water *B. undatum* (held at temperatures between 10-15°C) that may reach up to 33 mm shell length in 14 months under laboratory conditions (Nasution and Roberts, 2005). The colder water temperature

at which *B. scalariforme* was kept in the present study (consistent with its native habitat) may explain, at least in part, its slower growth rate relative to shallow-water gastropods.

Long-term monitoring of *B. scalariforme* in mesocosm settings showed that it still reproduced successfully after a change of habitat, i.e. from native deep-sea conditions to laboratory conditions (atmospheric pressure). While predation pressure on freshly laid egg masses seemed to be high, this particular threat is likely lower in its unconstrained native habitat. Relatively low fecundity and slow growth in *B. scalariforme* may increase vulnerability to anthropogenic pressures and climate-induced fluctuations that are anticipated to affect deep-sea ecosystems in the near future. However, the present study testifies to the general adaptability of gastropods with encapsulated development, which have been very successful at colonizing a diversity of terrestrial, freshwater and marine habitats.

A.1.5. References

Borzone, C.A., 1995. Ovicapsulas de prosobranquios (Mollusca: Gastropoda) de una playa arenosa expuesta del sur de Brasil. Iheringia, Ser. Zool. 79, 47-58.

Bouchet, P., Waren, A., 1979. Planktotrophig larval development in deep-water gastropods. Sarsia 64 (1-2), 37-40.

Bouchet, P., Warén, A., 1994. Ontogenetic migration and dispersal of deep-sea gastropod larvae. Reproduction, larval biology, and recruitment of the deep-sea benthos. Columbia University Press, New York, 98-117.

Brante, A., Fernández, M., Viard, F., 2009. Limiting factors to encapsulation: the combined effects of dissolved protein and oxygen availability on embryonic growth and survival of species with contrasting feeding strategies. J. Exp. Biol. 212 (14), 2287-2295.

Brunel, P., Bossé, L., Lamarche, G., 1998. Catalogue of the marine invertebrates of the Estuary and Gulf of Saint Lawrence. In: Canada, F.a.O. (Ed.), Can. Spe. Publ. Fish. Aqua. Sci. No. 126, p. 405.

Chaitanawisuti, N., Kritsanapuntu, A., 1997. Laboratory spawning and juvenile rearing of the marine gastropod: Spotted Babylon, *Babylonia areolata* Link 1807 (Neogastropoda: Buccinidae), in Thailand. Oceano. Lit. Rev. 11 (44), 1351.

Chen, Y., Ke, C.H., Zhou, S.Q. and Li, F.X., 2005. Effects of food availability on feeding and growth of cultivated juvenile *Babylonia formosae habei* (Altena & Gittenberger 1981). Aqua. Res. 36 (1), 94-99.

Clarke, A.H., 1962. On the composition, zoogeography, origin and age of the deep-sea mollusk fauna. Deep-Sea Res.: Oceanogr. Abstr. 9 (7-10), 291-306.

Colman, J.G., Tyler, P.A., 1988a. Observations on the reproductive biology of the deep-sea trochid *Calliotropis ottoi* (Philippi). J. Molluscan Stud. 54 (2), 239-242.

Colman, J.G., Tyler, P.A., 1988b. The egg capsules of *Colus jeffreysianus* (Fischer, 1868) (Prosobranchia: Neogastropoda) from the Rockall Trough, North East Atlantic. Sarsia 73 (2), 139-145.

Colman, J.G., Tyler, P.A., Gage, J.D., 1986a. The reproductive biology of *Colus jeffreysianus* (Gastropoda: Prosobranchia) from 2200 m in the N.E. Atlantic. J. Molluscan Stud. 52 (1), 45-54.

Colman, J.G., Tyler, P.A., Gage, J.D., 1986b. Larval development of deep-sea gastropods (Prosobranchia: Neogastropoda) from the Rockall Trough. J. Mar. Biol. Assoc. U. K. 66 (4), 951–965.

Cumplido, M., Pappalardo, P., Fernández, M., Averbuj, A., Bigatti, G., 2011. Embryonic development, feeding and intracapsular oxygen availability in *Trophon geversianus* (Gastropoda: Muricidae). J. Molluscan Stud., eyr025.

DFO, 2009. 2008 State of the ocean: Physical oceanographic conditions in the Newfoundland and Labrador region DFO. Can. Sci. Advis. Sec. Sci. Advis. Rep. 2009/057.

Fretter, V., Graham, A., 1984. The prosobranch molluscs of Britain and Denmark: Part 8-Neogastropoda. J. Molluscan Stud. Supplement 15, 435-556.

Fretter, V., Graham, A., 1994. British prosobranch molluscs: Their functional anatomy and ecology (revised and updated). Ray Society, London.

Gage, J.D., Tyler, P.A., 1991. Deep-sea biology: a natural history of organisms at the deep-sea floor. Cambridge University Press, Cambridge.

Gallardo, C.S., Garrido, O.A., 1987. Nutritive egg formation in the marine snails *Crepidula dilatata* and *Nucella crassilabrum*. Int. J. Inver. Rep. Dev. 11 (3), 239-254.

Gendron, L., 1992. Determination of the size at sexual maturity of the waved whelk *Buccinum undatum* Linnaeus, 1758, in the Gulf of St. Lawrence, as a basis for the establishment of a minimum catchable size. J. Shell. Res. 11 (1), 1-7.

Gofas, S., 2004. *Buccinum scalariforme* Møller, 1842. MolluscaBase (2016). Accessed Jul 2016 through: World Register of Marine Species at http://www.marinespecies.org/aphia.php?p=taxdetails&id=138875.

Gustafson, R.G., Lutz, R.A., 1994. Molluscan life history traits at deep-sea hydrothermal vents and cold methane/sulfide seeps. In: Young, C.M., Eckelbarger, K.J. (Eds.), Reproduction, larval biology, and recruitment of the deep-sea benthos. Columbia University Press, NY, pp. 76-97.

Gustafson, R.G., Littlewood, D.T.J., Lutz, R.A., 1991. Gastropod egg capsules and their contents from deep-sea hydrothermal vent environments. Biol. Bull. 180 (1), 34-55.

Hamel, J.-F., Sun, Z., Mercier, A., 2010. Influence of size and seasonal factors on the growth of the deep-sea coral *Flabellum alabastrum* in mesocosm. Coral Reefs 29, 521-525.

Hancock, D., 1967. Whelks. Laboratory Leaflet (new series) No. 15. Ministry of Agriculture, Fisheries, and Food, Fisheries Laboratory, Burnham on Crouch, Essex, England.

Harasewych, M.G., Kantor, Y.I., 2004. The deep-sea Buccinoidea (Gastropoda: Neogastropoda) of the Scotia Sea and adjacent abyssal plains and trenches. The Nautilus 118 (1), 1-42.

Heude-Berthelin, C., Hégron-Macé, L., Legrand, V., Jouaux, A., Adeline, B., Mathieu, M., Kellner, K., 2011. Growth and reproduction of the common whelk *Buccinum undatum* in West Cotentin (Channel), France. Aquat. Living Resour. 24 (3), 317-327.

Himmelman, J.H., Hamel, J.-R., 1993. Diet, behaviour and reproduction of the whelk *Buccinum undatum* in the northern Gulf of St. Lawrence, eastern Canada. Mar. Biol. 116 (3), 423-430.

Hirst, A.G., Forster, J., 2013. When growth models are not universal: evidence from marine invertebrates. Proc. R. Soc. Lond. B 280 (1768).

Ilano, A.S., Fujinaga, K., Nakao, S., 2003. Reproductive cycle and size at sexual maturity of the commercial whelk *Buccinum isaotakii* in Funka Bay, Hokkaido, Japan. J. Mar. Biol. Assoc. U. K. 83 (06), 1287-1294.

Ilano, A.S., Fujinaga, K., Nakao, S., 2004. Mating, development and effects of female size on offspring number and size in the neogastropod *Buccinum isaotakii* (Kira, 1959). J. Molluscan Stud. 70 (3), 277-282.

Ito, H., 1978. Observations on the spawning of sea snails, two species of the genus *Buccinum*, *B. kinukatsugi* and *B. miyauchii*. Bulletin of the Hokkaido Regional Fisheries Research Laboratory. Bulletin of the Hokkaido, Regional Fisheries Research Laboratory, Fisheries Agency 43, 69-77.

Kideys, A.E., Nash, R.D.M., Hartnoll, R.G., 1993. Reproductive cycle and energetic cost of reproduction of the neogastropod *Buccinum undatum* in the Irish Sea. J. Mar. Biol. Assoc. U. K. 73 (02), 391-403.

Lamy, É., 1928. La ponte chez les gastéropodes prosobranches. J. Conchyliol. 72, 41-79.

MacDonald, I.R., Bluhm, B.A., Iken, K., Gagaev, S., Strong, S., 2010. Benthic macrofauna and megafauna assemblages in the Arctic deep-sea Canada Basin. Deep-Sea Res. II 57 (1), 136-152.

Martel, A., Larrivee, D.H., Himmelman, J.H., 1986a. Behavior and timing of copulation and egg-laying in the neogastropod *Buccinum undatum*. J. Exp. Mar. Biol. Ecol. 96 (1), 27-42.

Martel, A., Larrivee, D.H., Klein, K.R., Himmelman, J.H., 1986b. Reproductive cycle and seasonal feeding activity of the neogastropod *Buccinum undatum*. Mar. Biol. 92 (2), 211-222.

Mercier, A., Sun, Z., Hamel, J.-F., 2011. Reproductive periodicity, spawning and development of the deep-sea scleractinian coral *Flabellum angulare*. Mar. Biol. 158, 371-380.

Mercier, A., Baillon, S. and Hamel, J.-F., 2015. Life history and feeding biology of the deep-sea pycnogonid *Nymphon hirtipes*. Deep-Sea Res. I 106, 1-8.

Miloslavich, P., 1996. Nurse-egg feeding prosobranchs: a comparative biochemical and electrophoretic analysis of eggs and hatchlings. Am. Malacol. Bull. 13, 37-46.

Miloslavich, P., Dufresne, L., 1994. Development and effect of female size on egg and juvenile production in the neogastropod *Buccinum cyaneum* from the Saguenay Fjord. Can. J. Fish. Aquat. Sci. 51 (12), 2866-2872.

Moran, A.L., 1999. Intracapsular feeding by embryos of the gastropod genus *Littorina*. Biol. Bull. 196 (3), 229-244.

Nasution, S., Roberts, D., 2005. Laboratory trials on the effects of different diets on growth and survival of the common whelk, *Buccinum undatum*, L. 1758, as a candidate species for aquaculture. Aquac. Int. 12 (6), 509-521-521.

Ojeda, J.A., Chaparro, O.R., 2004. Morphological, gravimetric, and biochemical changes in *Crepidula fecunda* (Gastropoda: Calyptraeidae) egg capsule walls during embryonic development. Mar. Biol. 144 (2), 263-269.

Olabarria, C., Ramirez-Llodra, E., 2004. Reproductive strategies of two deep-sea gastropod species from the Porcupine Seabight (Northeast Atlantic). Mar. Biol. 145 (3), 541-549.

Pechenik, J.A., 1979. Role of encapsulation in invertebrate life histories. Am. Nat. 114 (6), 859–870.

Penchasziadeh, P.E., 1976. Reproduccion de gastropodos prosobranquios del Atlantico Surocïcidental. El genero *Trophon*. Physis A 35 (90), 69-76.

Portmann, A., 1925. Der einfluss der nähreier auf die larvenentwicklung von buccinum und purpura. Zoomorphology 3 (4), 526-541.

Portmann, A., 1930. Die larvennieren von *Buccinum undatum* L. . Z. Zellforsch. Mikrosk. Anat. 18, 401-410.

Rex, M.A., Van Ummersen, C.A., Turner, R.D., 1979. Reproductive pattern in the abyssal snail *Benthonella tenella* (Jeffreys). Reproductive ecology of marine invertebrates. University of South Carolina Press Columbia, pp. 173-188.

Rivest, B.R., 1983. Development and the influence of nurse egg allotment on hatching size in *Searlesia dira* (Reeve, 1846) (Prosobranchia: Buccinidae). J. Exp. Mar. Biol. Ecol. 69 (3), 217-241.

Rowe, G.T., Polloni, P.T., Haedrich, R.L., 1982. The deep-sea macrobenthos on the continental margin of the northwest Atlantic Ocean. Deep-Sea Res. A 29 (2), 257-278.

Scheltema, R.S., 1994. Adaptations for reproduction among deep-sea benthic molluscs: An appraisal. In: Young, C.M., Eckelbarger, K.J. (Eds.), Reproduction, larval biology, and recruitment of the deep-sea benthos. Chapter 3, Columnbia University Press, NY, pp. 44-75.

Smith, K.E., Thatje, S., 2013. Nurse egg consumption and intracapsular development in the common whelk *Buccinum undatum* (Linnaeus 1758). Helgol. Mar. Res. 67 (1), 109.

Staiger, H., 1951. Cytologische und morphologische Untersuchungen zur Determination der Nähreier bei Prosobranchiern. Cell Tissue Res. 35 (5), 495-549.

Stickle, W.B., 1975. The reproductive physiology of the intertidal prosobranch *Thais lamellosa* (Gmelin). II. Seasonal changes in biochemical composition. Biol. Bull. 148 (3), 448-460.

Sun, Z., Hamel, J.-F., Mercier, A., 2011. Planulation, larval biology, and early growth of the deep-sea soft corals *Gersemia fruticosa* and *Duva florida* (Octocorallia: Alcyonacea). Invertebr. Biol. 130 (2), 91-99.

Tracey, S., Todd, J.A., Erwin, D.H., 1993. Mollusca: Gastropoda. The Fossil Record 2 (845), 131-167.

Valentinsson, D., 2002. Reproductive cycle and maternal effects on offspring size and number in the neogastropod *Buccinum undatum* (L.). Mar. Biol. 140 (6), 1139-1147.

Veliz, D., Guisado, C., Winkler, F., 2001. Morphological, reproductive, and genetic variability among three populations of *Crucibulum quiriquinae* (Gastropoda: Calyptraeidae) in northern Chile. Mar. Biol. 139 (3), 527-534.

A.1.6. Tables and Figures

Table 1. Development of Buccinum scalariforme at a temperature of 5-6°C and a salinity of 35 psu (n=3-20 individuals per life stage). Values in italics are unconfirmed.

Life Stage	Diameter / Shell Length Mean (SD) (µm)	Age (days)	Corresponding Image
Egg	320 (73)	1	Fig. 3a
Embryos	418 (82)	*2*-21	Fig. 3b, d-f
Trocophore	345 (10)	*15*-21	Fig. 3g
Veliger	481 (66)	*21*-45	Fig. 3h, i
Late Veliger	587 (59)	~45[a]	Fig. 4a
Pediveliger	1073 (31)	~90	Fig. 4c, d
Hatched Juveniles	1420 (184)	~120	Fig. 4e, f
1-year Juveniles	3240 (733)	~365	-
2.5-year Juveniles	9000 (879)	~915	Fig. 5a-c

[a] From the late veliger onward, sampling intervals were too long to estimate the duration of a stage.

Table 2. Estimated age at adult size in *Buccinum scalariforme* based on different maximum sizes and curve fitting models.

Maximum size (mm)	Age determined by curve models (days)			Age determined by curve models (years)		
	Linear	Power	Quadratic	Linear	Power	Quadratic
60	6287.36	15583.91	6506.88	17.2	42.7	17.8
70	7338.86	18181.23	7597.42	20.1	49.8	20.8
80	8390.36	20778.55	8687.96	23.0	56.9	23.8
Mean	7338.86	18181.23	7597.42	20.1	49.8	20.8

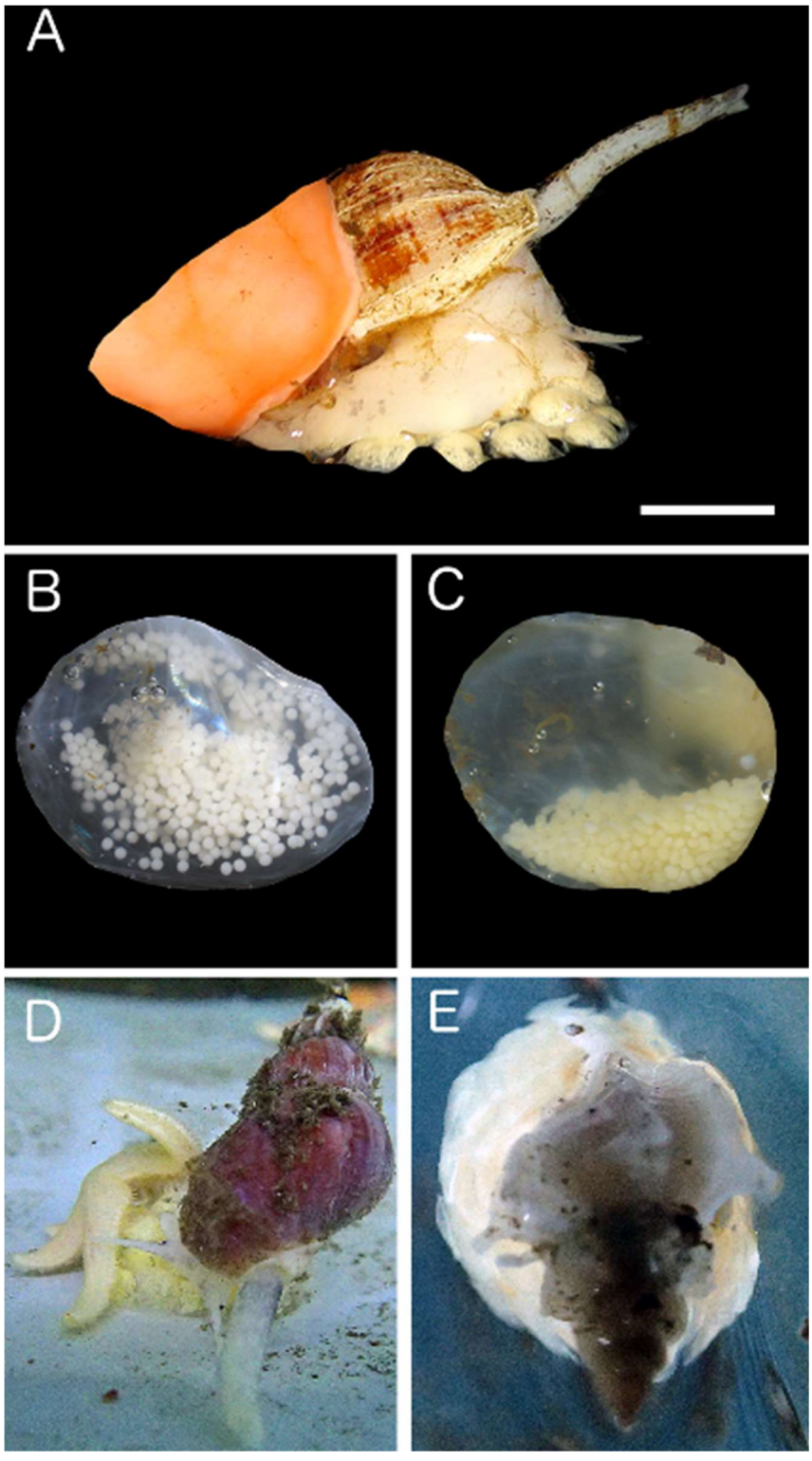

A
B
C
D
E

Fig 1. Spawning behaviour in *B. scalariforme* (A) Adult depositing egg mass. (B) Newly-laid egg mass with visible propagules in the capsule. (C) 2-week old egg mass with propagules accumulating at the bottom of the capsule still guarded by the female. (D) Deep-water sea star *Stephanasterias albula* with everted stomach over the capsules. (E) The neogastropod *Boreotrophon clathratus* feeding on egg mass. Scale bar represents 3 cm in A, 250 μm in B-C, 2 cm in D, 1 cm in E.

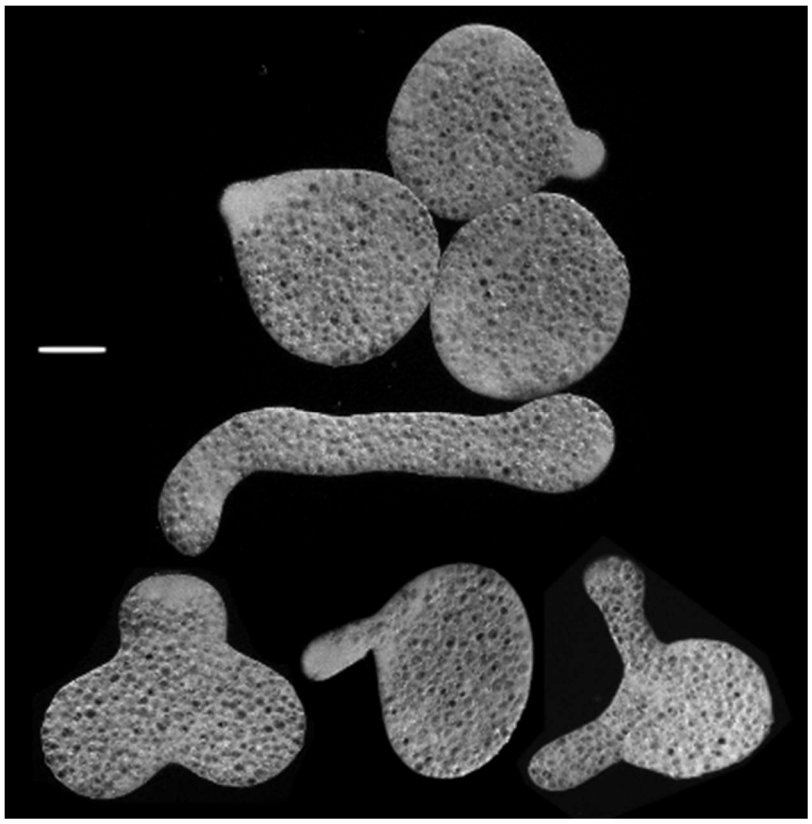


Fig 2. Diversity of nurse cell shapes found in egg-mass capsules of *B. scalariforme* concurrent with embryonic and early larval stages (trocophores). Scale bar represents 150 μm.

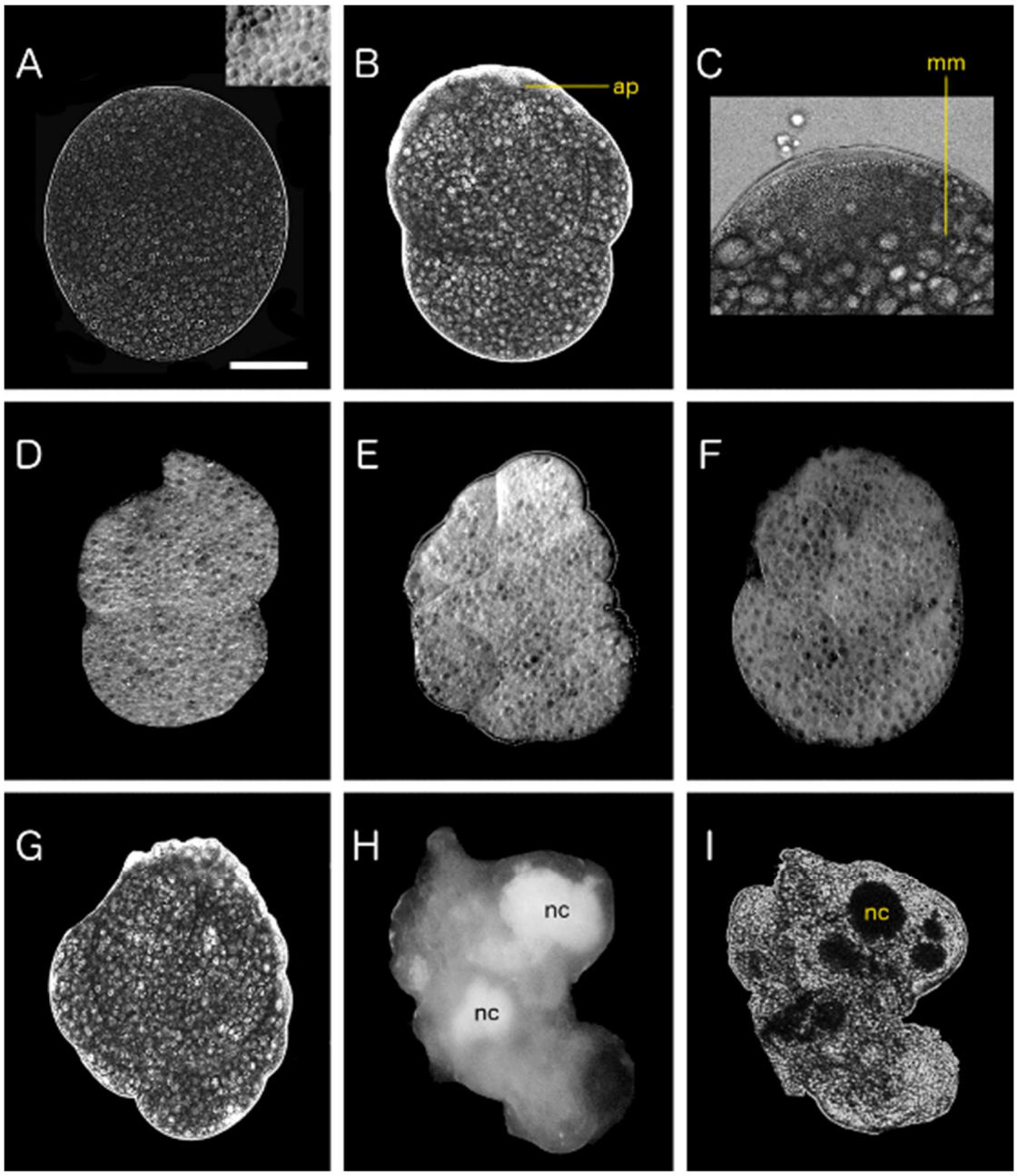

A
B
ap
C
mm
D
E
F
G
H
nc
nc
I
nc

Fig 3. Early development of *B. scalariforme* (for kinetic see Table 1). (A) Fertilized egg, insert shows lipid droplets on the egg surface. (B) Early embryos (C) showing the micromeres. (D-F) Late embryos. (G) Trocophore. (H-I) Veliger. Labels *ap* represent the animal pole, *mm* represent the micromeres, *nc* represent the nurse cells. Scale bar represents 85 μm in A-B, 30 μm in C, 95 μm in D, 100 μm in E-G, 110 μm in H-I.

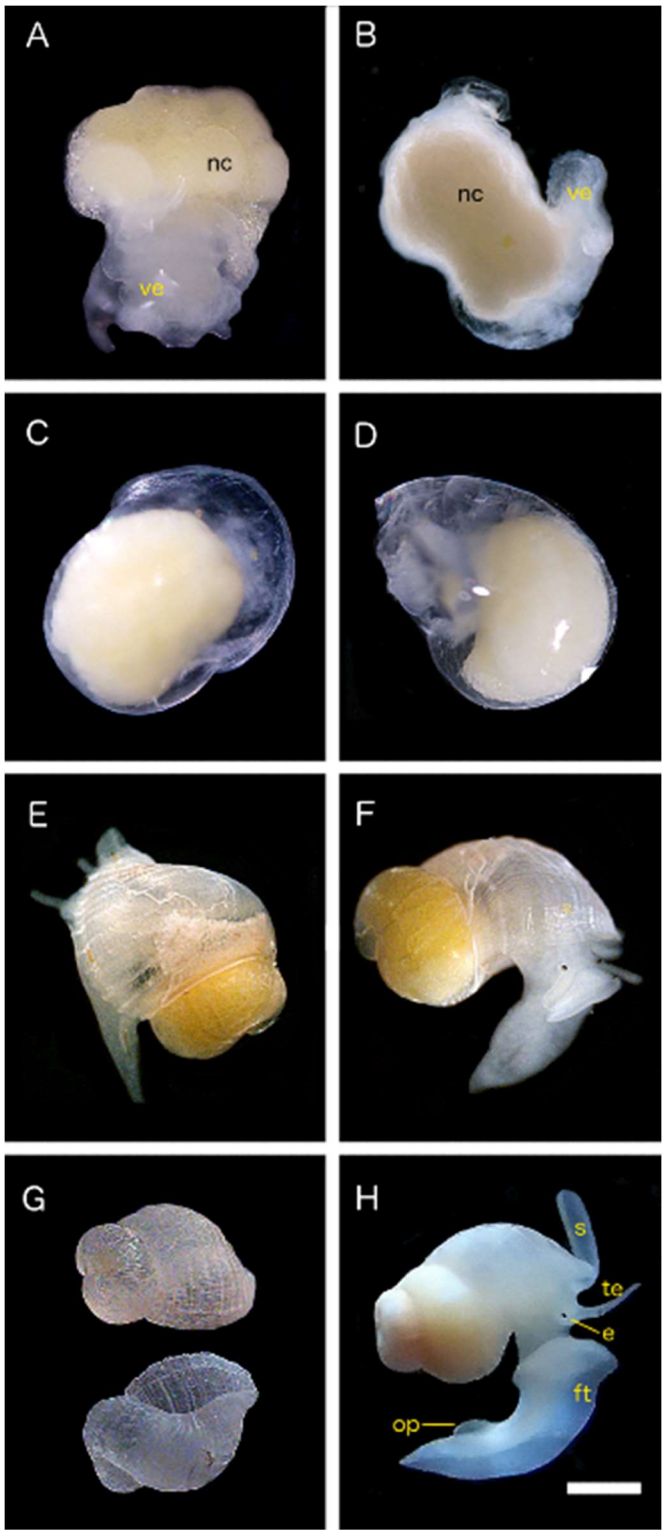

A
nc
ve
B
nc
ve
C
D
E
F
G
H
s
te
e
ft
op

Fig 4. Late development of *B. scalariforme* (for kinetic see Table 1). (A-B) Late veliger filled with nurse cells. (C-D) Pediveliger with early shell clearly visible. (E-G) Newly-hatched juvenile with 2 whorls. (H) Free-living juvenile with 3 whorls. Labels *e* represents the eyes, *ft* the foot, *s* the siphon, *op* the operculum, *te* the tentacles and *nc* the nurse cells. Scale bar represents 250 μm in A, 350 μm in B-D, 500 μm in E-F, 600 μm in G, 850 μm in H.

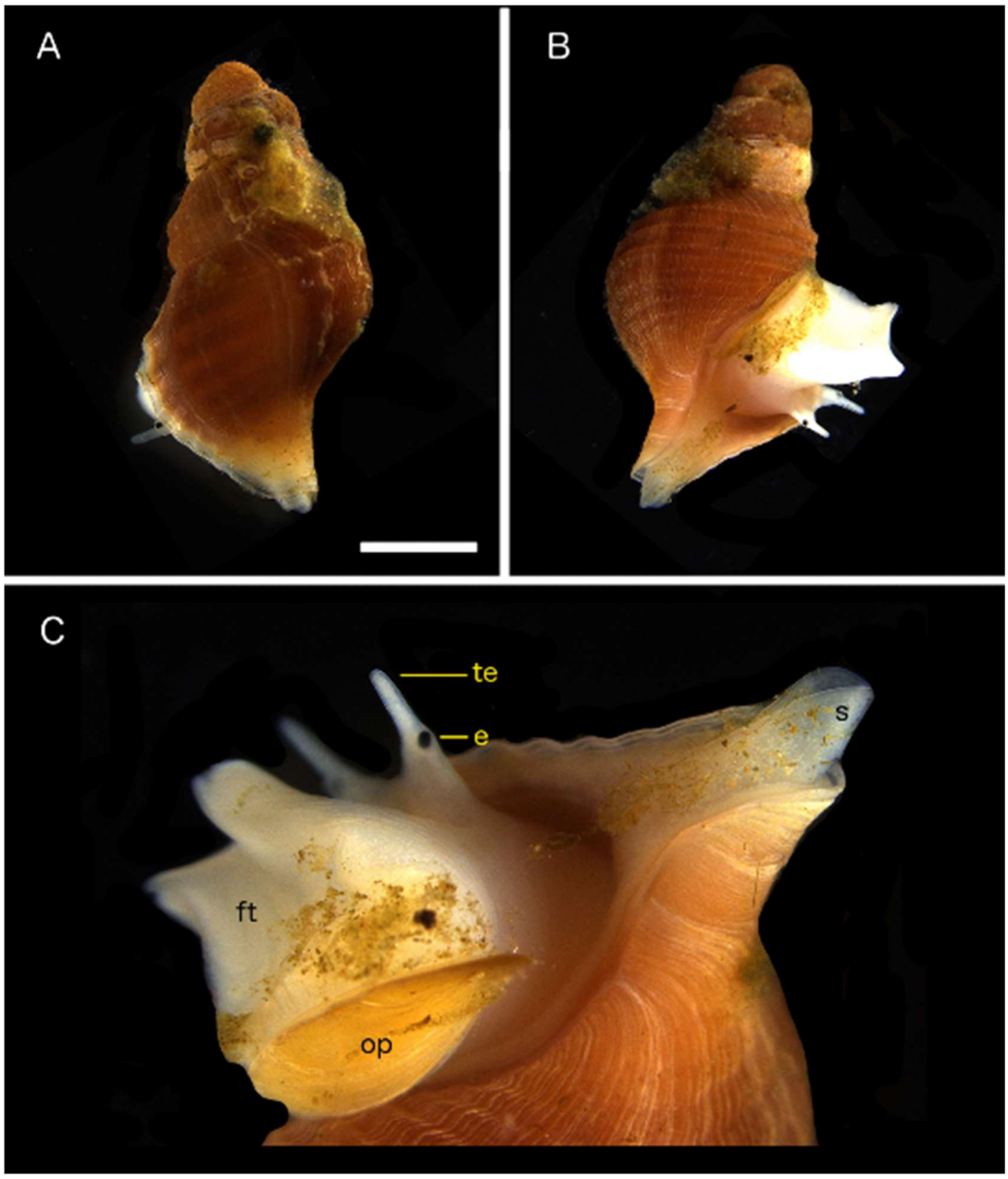


Fig 5. Juvenile of *B. scalariforme* at 2.5 years of age measuring around 8 mm shell height and having 4 whorls. Adults colour and features of the shell are visible; brown / red colouration patterns, inter-whorl ridges are well formed, sensory organs (eyes and tentacles) are well formed. (A) Ventral view and (B) dorsal view. (C) Close-up of the

eye-stalks, siphon and operculum. Labels *e* represent the eyes, *te* the tentacles, *ft* the foot, *op* the operculum, *s* the siphon. Scale bar represents 30 mm in A-B, 10 mm in C.

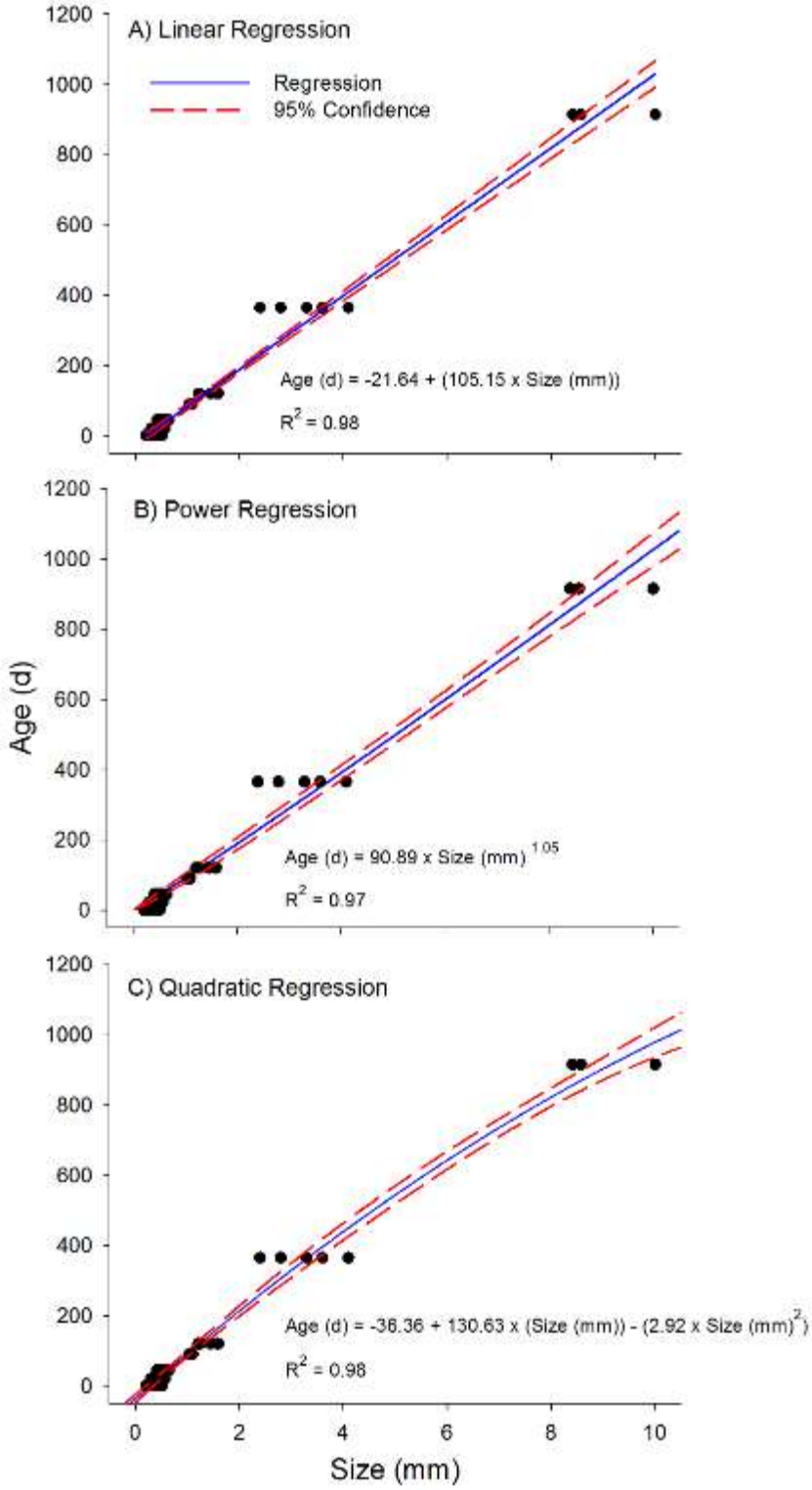

A) Linear Regression
Regression
95% Confidence
Age (d) = -21.64 + (105.15 x Size (mm))
R2 = 0.98
B) Power Regression
Age (d) = 90.89 x Size (mm) 1.05
R2 = 0.97
C) Quadratic Regression
Age (d) = -36.36 + 130.63 x (Size (mm)) - (2.92 x Size (mm)2)
R2 = 0.98
Age (d)
Size (mm)
0
200
400
600
800
1000
1200
2
4
6
8
10

Fig 6. Growth of *B. scalariforme* from fertilization until 2.5-year-old juvenile, with curve fits showing (A) linear, (B) power and (C) quadratic equations. Size (mm) corresponds to maximum shell height (n=3-20 measurements per life stage).

Appendix 2 Dispersal Model Dataset for Chapter 2

Dispersal model papers 2013-2015 obtained from Web of Science. The presence or absence of biological variables (behaviour, vertical migration, swimming, settlement preference) used in hypothetical or species-specific models are indicated.

Year	Author(s)	Animals	Behaviour	Vertical migration	Swimming	Settlement preference
2013	Salama et al.	sea lice	No	No	No	No
2013	Neo et al.	giant clams	Yes	No	No	No
2013	Simons et al.	none	No	No	No	No
2013	Robins et al.	none	Yes	No	Yes	Yes
2013	Paris et al.	corals	Yes	Yes	No	No
2013	Kough et al.	spiny lobster	Yes	Yes	No	No
2013	Coscia et al.	cockles	Yes	Yes	No	No
2013	Paris et al.	none	Yes	Yes	Yes	No
2013	Bidegain et al.	clams	Yes	Yes	Yes	Yes
2014	Wood et al.	corals	No	No	No	No

2014	Myksvoll et al.	cod	No	No	No	No
2014	Hoyer et al.	bivalve	No	No	No	No
2014	Soria et al.	none	No	No	No	No
2014	Staaterman & Paris	fish	Yes	No	Yes	No
2014	Crandall et al.	blue sea star	Yes	No	Yes	No
2014	Burgess et al.	none	Yes	No	No	Yes
2014	Cuif et al.	reef fish	Yes	No	No	Yes
2014	Wolankski & Kingsford	coral reef fish	Yes	No	Yes	Yes
2014	Adams et al.	none	Yes	Yes	No	No
2014	Puckett et al.	oyster	Yes	Yes	No	No
2015	Fenberg et al.	many	No	No	No	No
2015	Nickols et al.	none	No	No	No	No
2015	Wright et al.	many	No	No	No	No
2015	Qian et al.	coral	No	No	No	No
2015	Pfaff et al.	mix	No	No	No	No
2015	Teske et al.	gastropod	No	No	No	No

2015	Laurel et al.	fish	Yes	No	No	Yes
2015	Barbut et al.	fish	Yes	Yes	No	No
2015	Phelps et al.	fish	Yes	Yes	No	No
2015	Hilario et al.	many	Yes	Yes	Yes	No

Appendix 3 Dataset and Subset Tables for Chapter 3

Appendix 3A. Full dataset of lecithotrophic echinoderms used in Chapter 3

Full dataset of lecithotrophic echinoderms used in the study (N = 126). Data were collated for taxonomic grouping, embryonic development site, egg size, egg colour, egg colour intensity, egg buoyancy, adult size and geographic distribution (ocean basin).

Class[1]	Order[2]	Species	Dev Site[3]	Egg Size (μm)	Egg Colour	Egg Colour Intensity[4]	Egg Buoyancy[5]	Adult Size (cm)[6]	Ocean Basin[7]	Sources
As	For	*Anasterias antarctica*	EB	1810	yellow	bright	?	40	Ant	(Gil *et al.* 2011)
As	For	*Diplasterias brandti*	EB	2870	brown	pale	?	110	Ant	Image Search
As	For	*Diplasterias brucei*	EB	3400	orange	pale	-	250	Ant	(McClintock and Baker 1997a, Pearse *et al.* 1991)
As	For	*Leptasterias (Hexasterias) alaskensis*	EB	?	orange	bright	?	160	Pac	Image Search
As	For	*Leptasterias (Hexasterias) hexactis*	EB	800	orange	bright	-	50	Pac	Image Search
As	For	*Leptasterias (Hexasterias) polaris*	EB	850	orange	pale	-	300	Pac / Atl	(Hamel and Mercier 1995)

As	For	*Leptasterias aequalis (hexactis)*	EB	900	yellow	pale	?	60	Pac	(Bingham *et al.* 2004)
As	For	*Leptasterias groenlandica*	EB	900	brown	pale	?	60	Pac / Atl	NMNM Collections
As	For	*Leptasterias tenera*	EB	?	brown	pale	?	160	Atl	(Hendler and Franz 1982)
As	For	*Smilasterias multipara*	IB	1000	red	regular	-	40	Pac	(Komatsu *et al.* 2006)
As	Pax	*Astropecten gisselbrechti*	P	350	yellow	pale	-	80	Pac	(Komatsu and Nojima 1985)
As	Pax	*Astropecten latespinosus*	P	300	brown	pale	-	80	Pac	(Komatsu 1975, Nojima 1982)
As	Pax	*Ctenopleura fisheri*	P	465	brown	pale	0	100	Pac	(Komatsu 1982)
As	Pax	*Psilaster charcoti*	P	750	red	regular	?	300	Ant	(McClintock and Baker 1997a)
As	Pax	*Trophodiscus sp.*	EB	?	orange	regular	?	?	Pac	Image Search
As	Pax	*Trophodiscus sp.*	EB	?	red	bright	?	?	Pac	Image Search
As	Pax	*Trophodiscus sp.*	EB	?	yellow	pale	?	?	Pac	Image Search

As	Spi	*Echinaster (Othilia) echinophorus*	P	1150	grey	bright	+	70	Atl	(Atwood 1973)
As	Spi	*Echinaster (Othilia) echinophorus*	P	800	orange	regular	-	700	Atl	(Atwood 1973)
As	Spi	*Echinaster brasiliensis*	P	1000	brown	bright	?	80	Atl	(Nobre and Campos 2004)
As	Spi	*Echinaster graminicola*	P	850	orange	regular	?	80	Atl	(Campbell and Turner 1984)
As	Spi	*Echinaster luzonicus*	P	1000	red	regular	+	150	Pac	Charonia Webpage - Cached, gbri.org, accessed July 2015
As	Spi	*Henricia lisa*	EB	1100	grey	pale	-	100	Atl	(Mercier and Hamel 2008)
As	Spi	*Henricia lisa*	P	1100	yellow	regular	-	100	Atl	(Mercier and Hamel 2008)
As	Spi	*Henricia sanguinolenta*	EB	1000	orange	bright	-	150	Pac / Atl	(Mercier and Hamel 2008)
As	Val	*Aquilonastra burtoni*	P	500	green	pale	-	30	Ind	(Achituv and Sher 1991, James 1972)
As	Val	*Asterina gibbosa*	EB	400	yellow	bright	-	60	Atl	Image Search, (Haesaerts *et al.* 2006)
As	Val	*Asterina phylactica*	EB	550	orange	regular	-	15	Atl	Image Search, (Strathmann *et al.* 1984)

As	Val	*Crossaster papposus*	P	550	red	bright	+	300	Pac / Atl	(Gemmill 1920)
As	Val	*Cryptasterina hystera*	IB	440	green	regular	+	12	Pac	(Byrne 2005, Dartnall *et al.* 2003)
As	Val	*Cryptasterina pacifica*	P	400	orange	regular	+	20	Pac	(Dartnall *et al.* 2003)
As	Val	*Cryptasterina pentagona*	P	413	orange	regular	+	24	Pac	(Byrne 2006)
As	Val	*Fromia elegans*	P	2000	red	regular	+	120	Ind / Pac	Charonia Webpage – Cached, accessed July 2015
As	Val	*Fromia monilis*	P	1000	red	bright	+	100	Ind	Image Search, (Emlet 1994)
As	Val	*Hippasteria phrygiana*	P	450	orange	bright	+	400	Pac / Atl	(Baillon *et al.* 2011)
As	Val	*Iconaster longimanus*	P	1000	orange	regular	+	200	Ind / Pac	(Lane and Hu 1994)
As	Val	*Mediaster aequalis*	P	1000	orange	bright	+	200	Pac	(Birkeland *et al.* 1971)
As	Val	*Meridiastra calcar (Patiriella)*	P	415	green	regular	-	60	Pac	(Byrne and Anderson 1994)
As	Val	*Meridiastra gunnii (Patiriella)*	P	430	green	regular	+	40	Pac	(Byrne and Anderson 1994)

As	Val	*Meridiastra occidens*	P	400	green	regular	+	30	Pac	(Byrne 2006)
As	Val	*Meridiastra oriens*	P	400	green	regular	-	20	Pac	(Byrne 2006)
As	Val	*Nardoa novaecaledoniae*	P	1000	orange	regular	+	110	Pac	Charonia Webpage – Cached, accessed July 2015
As	Val	*Nardoa tuberculata*	P	1000	orange	regular	+	280	Pac	Charonia Webpage – Cached, accessed July 2015
As	Val	*Ophidiaster granifer*	P	600	orange	regular	+	100	Pac	(Yamaguchi and Lucas 1984)
As	Val	*Ophidiaster granifer*	P	600	orange	regular	-	100	Pac	(Yamaguchi and Lucas 1984)
As	Val	*Parvulastra exigua (Patiriella)*	EB	390	orange	regular	-	15	Ind / Pac	(Byrne and Anderson 1994)
As	Val	*Parvulastra vivipara (Patiriella)*	IB	150	orange	regular	?	30	Ant	(Prestedge 1998)
As	Val	*Perknaster fuscus*	P	1200	red	regular	?	300	Ant	(McClintock and Baker 1997a, McClintock and
As	Val	*Solaster endeca*	P	800	orange	bright	+	300	Pac / Atl	(Gemmill 1912)
As	Val	*Solaster stimpsoni*	P	1000	green	pale	+	400	Pac	(Strathmann 1987)

As	Val	*Tosia neossia*	EB	700	orange	regular	?	60	Ant	Image Search, (Naughton and O'Hara 2009)
As	Vel	*Pteraster abyssorum*	IB	?	yellow	regular	?	80	Atl	
As	Vel	*Pteraster militaris*	IB	1400	yellow	regular	?	120	Pac / Atl	(McClary and Mladenov 1990)
As	Vel	*Pteraster tesselatus*	P	1200	red	bright	+	150	Pac	(McEdward and Coulter 1987)
Cr	Art	*Antedon mediterranea*	EB	200	yellow	regular	?	?	Atl	(Barbaglio *et al.* 2012)
Cr	Cor	*Dorometra sesokonis*	EB	200	yellow	bright	?	30	Pac	(Obuchi *et al.* 2010)
Cr	Iso	*Metacrinus rotundus*	P	350	yellow	regular	+	600	Pac	(Nakano *et al.* 2005)
Ec	Cam	*Heliocidaris erythrogramma*	P	400	orange	regular	+	140	Ind / Ant	(Williams and Anderson 1975, Wray 1996)
Ec	Cam	*Holopneustes purpurescens*	P	580	brown	regular	+	80	Pac	(Morris 1995)
Ec	Cam	*Sterechinus sp.*	EB	?	brown	bright	?	?	Ant	Image Search
Ec	Cas	*Cassidulus mitis*		370	yellow	regular	0	25	Atl	(Contins and Ventura 2011)

Ec	Cid	Cidaroidea	EB	?	red	regular	?	?	Ant	Image Search
Ec	Cid	*Phyllacanthus imperialis*	P	510	yellow	regular	+	80	Ind / Pac	(Olson *et al.* 1993)
Ec	Cid	*Phyllacanthus parvispinus*	P	700	grey	pale	+	100	Pac	(Parks *et al.* 1989)
Ec	Cly	*Peronella japonica*	P	300	red	pale	-	60	Pac	(Okazaki and Dan 1954)
Ec	Ech	*Asthenosoma ijimai*	P	1200	orange	regular	+	130	Pac	(Amemiya and Tsuchiya 1979)
Ec	Ech	*Phormosoma placenta*	P	1100	yellow	regular	+	120	Atl	(Young and Cameron 1987)
Ec	Spa	*Abatus cavernosus*	EB	1400	yellow	regular	?	40	Ant	(Gil *et al.* 2009, Poulin and Feral 1996)
Ec	Spa	*Abatus cordatus*	EB	1300	orange	bright	-	30	Ant	(Magniez 1983, Schatt and Féral 1996)
Ec	Spa	*Brisaster latifrons*	P	350	green	regular	?	60	Pac	(Strathmann 1979)
Ho	Apo	*Leptosynapta clarki*	IB	250	brown	pale	-	50	Pac	(McEuen 1988, Sewell and Chia 1994)
Ho	Den	*Athyonidium chilensis*	P	360	brown	pale	?	150	Ant	(Guisado *et al.* 2012)

Ho	Den	*Cucumaria fallax (pallida)*	P	500	brown	pale	+	120	Pac / Atl	(Emlet 1994, McEuen 1988)
Ho	Den	*Cucumaria frondosa*	P	750	orange	bright	+	200	Atl	(Hamel and Mercier 1996)
Ho	Den	*Cucumaria frondosa japonica*	P	500	green	regular	+	300	Pac	(Tyurin and Drozdov 2002)
Ho	Den	*Cucumaria lubrica*	EB	900	red	regular	-	50	Pac	(Engstrom 1982)
Ho	Den	*Cucumaria miniata*	P	520	green	bright	+	250	Pac	(McEuen 1988)
Ho	Den	*Cucumaria piperata*	P	530	green	regular	+	120	Pac	(McEuen 1988)
Ho	Den	*Cucumaria pseudocurata*	EB	1000	grey	bright	-	30	Pac	(McEuen 1988, Rutherford 1973)
Ho	Den	Cucumariid *sp.*	IB	800	brown	pale	?	10	Pac	(O'Loughlin 1991)
Ho	Den	*Echinopsolus charcoti*	IB	1800	yellow	pale	?	60	Pac	(O'Loughlin 2000)
Ho	Den	*Eupentacta chronhjelmi (quinquesemita)*	P	300	green	regular	-	60	Pac	(Catalan and Yamamoto 1994)
Ho	Den	*Eupentacta fraudatrix*	P	340	green	regular	-	100	Pac	(Kashenko 2000)

Ho	Den	*Eupentacta quinquesemita*	P	400	green	regular	0	100	Pac	(McEuen 1988)
Ho	Den	*Neocnus sp.*	IB	600	yellow	regular	?	4	Pac	(O'Loughlin 1991)
Ho	Den	*Pentamera populifera*	P	370	green	regular	0	30	Pac	(McEuen 1988)
Ho	Den	*Pseudocnus (Pentactella) laevigata*	EB	1500	brown	regular	?	30	Ant	(O'Loughlin 2000)
Ho	Den	*Pseudocnus echinatus*	P	400	green	regular	-	?	Ind	(Emlet 1994, Ohshima 1921)
Ho	Den	*Pseudocnus lubricus*	EB	1050	yellow	regular	-	50	Pac	(McEuen 1988, Rutherford 1973)
Ho	Den	*Psolidiella nigra*	EB	600	yellow	pale	?	40	Pac	(O'Loughlin 2000)
Ho	Den	*Psolidium bidiscum*	P	300	yellow	regular	?	30	Pac	(Lambert 1997)
Ho	Den	*Psolidium bullatum*	P	330	yellow	bright	0	25	Pac	(McEuen 1988, McEuen and Chia 1991)
Ho	Den	*Psolus chitinoides*	P	625	red	bright	+	75	Pac	(McEuen 1988, McEuen and Chia 1991)
Ho	Den	*Psolus fabricii*	P	500	orange	bright	+	200	Pac / Atl	(Hamel *et al.* 1993)

Ho	Den	*Psolus phantapus*	P	450	red	regular	+	265	Atl	(Baillon *et al.* 2011)
Ho	Den	*Squamocnus aureoruber*	IB	?	brown	pale	?	10	Pac	Image Search
Ho	Den	*Stereoderma kirchsbergii*	P	?	green	regular	-	?	Pac	(Ohshima 1921)
Ho	Den	*Trachythyone nina*	IB	1800	brown	pale	?	14	Atl	(Mercier *et al.* 2010)
Ho	Ela	*Penilidia desbarresi*	IB	150	brown	pale	?	20	Atl	(Gebruk *et al.* 2013)
Ho	Mol	*Molpadia intermedia*	P	270	red	pale	-	400	Pac	(McEuen and Chia 1985)
Op	Oph	*Amphioplus abditus*	P	150	grey	bright	-	50	Atl	(Hendler 1977)
Op	Oph	*Amphiura carchara*	IB	450	yellow	pale	?	80	Pac	(Clark 1911, Hendler and Tran 2001)
Op	Oph	*Amphiura squamata*	IB	880	red	pale	?	5	Atl	(Byrne 1991)
Op	Oph	*Clarkcoma pulchra*	P	290	yellow	pale	-	120	Pac	(Falkner *et al.* 2015)
Op	Oph	*Ophiarthrum elegans*	P	380	green	regular	+	13	Pac	(Falkner *et al.* 2006)

Op	Oph	*Ophiarthrum pictum*	P	420	green	regular	?	30	Pac	(Hendler and Meyer 1982), Pers. Com. Maria Byrne
Op	Oph	*Ophioderma brevispina**	P	350	brown	regular	+	40	Atl	(Hendler and Littman 1986, Hendler and Tyler
Op	Oph	*Ophioderma brevispina**	P	350	green	regular	+	40	Atl	(Hendler and Littman 1986, Hendler and Tyler
Op	Oph	*Ophioderma brevispina**	P	350	yellow	regular	+	40	Atl	(Grave 1916)
Op	Oph	*Ophioderma rubicunda*	P	?	red	regular	?	20	Atl	(Hagman and Vize 2003, Hendler and Littman 1986)
Op	Oph	*Ophiolepis elegans*	P	250	yellow	regular	?	50	Atl	(Stancyk 1973)
Op	Oph	*Ophiomastix annulosa*	P	430	green	regular	?	150	Pac	Pers. Com. Maria Byrne
Op	Oph	*Ophiomastix caryophyllata*	P	200	green	regular	?	10	Pac	Pers. Com. Maria Byrne
Op	Oph	*Ophiomastix elegans*	P	200	green	regular	?	10	Pac	Pers. Com. Maria Byrne
Op	Oph	*Ophiomastix janualis*	P	200	green	regular	?	120	Pac	Pers. Com. Maria Byrne
Op	Oph	*Ophiomastix marshallensis*	P	220	green	regular	?	?	Pac	Pers. Com. Maria Byrne

Op	Oph	*Ophiomastix mixta*	P	335	green	regular	?	600	Pac	Pers. Com. Maria Byrne
Op	Oph	*Ophiomastix venosa*	P	500	green	regular	+	20	Ind/Pac	(Fourgon *et al.* 2005)
Op	Oph	*Ophionereis olivacea*	IB	480	orange	regular	-	10	Atl	(Byrne 1991)
Op	Oph	*Ophionereis schayeri*	P	240	brown	pale	-	150	Pac	(Selvakumaraswamy and Byrne 2000)
Op	Oph	*Ophiopeza spinosa*	IB	300	yellow	regular	?	70	Pac	(Byrne *et al.* 2008)
Op	Oph	*Ophioplocus japonicus*	P	300	red	regular	?	140	Pac	(Clark 1911, Komatsu and Shosaku 1993)
Op	Oph	*Ophiothrix oerstedii*	P	400	brown	pale	-	100	Atl	(Mladenov 1979)
Op	Oph	*Opiolepis paucispina*	IB	480	red	pale	-	20	Atl	(Byrne 1989)
Op	Oph	*Sigsbeia conifera*	IB	800	red	pale	-	10	Atl	(Byrne 1991)
Op	Phyr	*Gorgonocephalus caryi*	EB	220	orange	pale	?	140	Pac	(Patent 1970)

[1] As = Asteroidea, Cr = Crinoidea, Ec = Echinoidea, Ho = Holothuroidea, Op = Ophiuroidea
[2] Apo = Apodida, Cam = Camerodonta, Cid = Cidaroidea, Cly = Clypeasteroida, Den = Dendrochirotida. Ech = Echinothuroida, For = Forcipulatida, Iso = Isocrinida, Mol = Molpadida, Oph = Ophiurida, Pax = Paxillosida, Spa = Spatangoida, Spi = Spinulosida, Val = Valvatida, Vel = Velatida

[3] P = pelagic lecithotrophic, EB = externally brooded, IB = internally brooded
[4] Refer to Table 1 for definitions of egg colour intensity
[5] Egg buoyancy reported in the literature was categorized based on a previous comprehensive review of echinoderm larvae (Emlet 1994). (-) = Negative buoyancy, (+) = Positive buoyancy, (0) = Neutral buoyancy, (?) = No data
[6] Adult body size is diameter for Asteroidea, Ophiuroidea and Echinoidea, and length in Holothuroidea and Crinoidea
[7] Ant = Antarctic, Atl = Atlantic, Ind = Indian, Pac = Pacific
***** Egg colour in *Ophioderma brevispina* is ambiguous due to conflicting records, it was considered brown for the purpose of analysis as this was the most common shade reported.

Appendix 3B. Subset of lecithotrophic echinoderms (all variables)

Subset of lecithotrophic echinoderms used to test the hypothesis that egg colour is not randomly distributed among dataset variables ($N = 87$). Data are shown for embryonic development site, egg size, egg colour, egg colour intensity, adult size, class and geographic distribution (ocean basin).

Class[1]	Species	Development Site[2]	Egg Size	Egg Colour	Egg Intensity[3]	Adult Size[4]	Ocean Basin[5]
e	*Abatus cavernosus*	external	1400	yellow	regular	40	Ant
e	*Abatus cordatus*	external	1300	orange	bright	30	Ant
e	*Amphiura carchara*	internal	450	yellow	pale	80	Pac
o	*Amphiura squamata*	internal	880	red	pale	5	Atl
a	*Anasterias antarctica*	external	1810	yellow	bright	40	Ant
a	*Asterina gibbosa*	external	400	yellow	bright	60	Atl
h	*Asthenosoma ijimai*	planktonic	1200	orange	regular	130	Pac
a	*Astropecten gisselbrechti*	planktonic	350	yellow	pale	80	Pac
a	*Astropecten latespinosus*	planktonic	300	brown	pale	80	Pac
h	*Athyonidium chilensis*	planktonic	360	brown	pale	150	Ant
e	*Cassidulus mitis*	planktonic	367	yellow	regular	25	Atl
o	*Clarkcoma pulchra*	planktonic	290	yellow	pale	120	Pac
a	*Cryptasterina hystera*	internal	440	yellow	regular	24	Pac

a	*Cryptasterina pacifica*	planktonic	400	orange	regular	20	Pac
a	*Cryptasterina pentagona*	planktonic	413	orange	regular	24	Pac
a	*Ctenopleura fisheri*	planktonic	465	brown	pale	100	Pac
h	*Cucumaria frondosa japonica*	planktonic	500	green	regular	300	Pac
h	*Cucumaria miniata*	planktonic	520	green	bright	250	Pac
h	*Cucumaria piperata*	planktonic	530	green	regular	120	Pac
h	*Cucumariid sp.*	internal	800	brown	pale	10	Pac
a	*Diplasterias brandti*	external	2870	brown	pale	110	Ant
a	*Diplasterias brucei*	external	3400	orange	pale	250	Ant
a	*Echinaster brasiliensis*	planktonic	1000	brown	bright	80	Atl
a	*Echinaster graminicola*	planktonic	850	orange	regular	80	Pac
a	*Echinaster luzonicus*	planktonic	1000	red	regular	150	Pac
h	*Echinopsolus charcoti*	internal	1800	yellow	pale	60	Ant
h	*Eupentacta chronhjelmi*	planktonic	300	green	regular	60	Pac
h	*Eupentacta fraudatrix*	planktonic	340	green	regular	100	Pac
h	*Eupentacta quinquesemita*	planktonic	400	green	regular	100	Pac
o	*Gorgonocephalus eucnemis*	external	220	orange	pale	140	Atl
a	*Henricia lisa*	planktonic	1100	yellow	regular	100	Atl
a	*Henricia sanguinolenta*	external	1000	orange	bright	150	Pac / Atl

e	*Holopneustes purpurescens*	planktonic	580	brown	regular	80	Pac
a	*Iconaster longimanus*	planktonic	1000	orange	regular	200	Ind / Pac
a	*Leptasterias aequalis*	external	900	yellow	pale	60	Pac
a	*Leptasterias hexactis*	external	800	orange	bright	50	Pac
a	*Leptasterias polaris*	external	850	orange	pale	300	Pac / Atl
h	*Leptosynapta clarki*	internal	250	brown	pale	50	Pac
a	*Mediaster aequalis*	planktonic	1000	orange	bright	200	Pac
a	*Meridiastra calcar (Patiriella)*	planktonic	415	green	regular	60	Pac
a	*Meridiastra gunnii (Patiriella)*	planktonic	430	green	regular	40	Pac
a	*Meridiastra occidens*	planktonic	400	green	regular	30	Pac
a	*Meridiastra oriens*	planktonic	400	green	regular	20	Pac
h	*Molpadia intermedia*	planktonic	270	red	pale	400	Pac
a	*Nardoa novaecaledoniae*	planktonic	1000	orange	regular	110	Pac
a	*Nardoa tuberculata*	planktonic	1000	orange	regular	280	Pac
h	*Neocnus sp.*	internal	600	yellow	regular	5	Pac
a	*Neosmilaster georgianus*	internal	2170	brown	pale	70	Atl
o	*Ophiarthrum elegans*	planktonic	384	green	regular	13	Pac
o	*Ophiarthrum pictum*	planktonic	419	green	regular	30	Pac
a	*Ophidiaster granifer*	planktonic	600	orange	regular	100	Pac

a	*Ophidiaster granifer*	planktonic	600	orange	regular	100	Pac
o	*Ophioderma brevispina*	planktonic	350	brown	regular	40	Atl
o	*Ophioderma wahlbergii*	internal	250	yellow	regular	30	Atl
o	*Ophiolepis elegans*	planktonic	250	yellow	regular	50	Atl
o	*Ophiolepis paucispina*	internal	480	red	pale	20	Atl
o	*Ophiomastix annulosa*	planktonic	430	green	regular	150	Pac
o	*Ophiomastix caryophyllata*	planktonic	200	green	regular	10	Pac
o	*Ophiomastix elegans*	planktonic	200	green	regular	10	Pac
o	*Ophiomastix janualis*	planktonic	200	green	regular	120	Pac
o	*Ophiomastix mixta*	planktonic	335	green	regular	600	Pac
o	*Ophiomastix venosa*	planktonic	500	green	regular	20	Ind / Pac
o	*Ophionereis olivacea*	internal	400	orange	regular	3	Atl
o	*Ophionereis olivacea*	internal	480	orange	regular	10	Atl
o	*Ophionereis schayeri*	planktonic	240	brown	pale	150	Pac
o	*Ophiopeza spinosa*	internal	300	yellow	regular	70	Pac
o	*Ophiothrix oerstedii*	planktonic	400	brown	pale	100	Atl
a	*Parvulastra exigua (Patiriella)*	external	390	orange	regular	15	Ind / Pac
h	*Penilidia desbarresi*	internal	150	brown	pale	20	Atl
h	*Pentamera populifera*	planktonic	370	green	regular	30	Pac

a	*Perknaster fuscus*	planktonic	1200	red	regular	300	Ant
e	*Phormosoma placenta*	planktonic	1100	yellow	regular	120	Atl
e	*Poriocidaris purpurata*	planktonic	1500	brown	pale	30	Atl
h	*Pseudocnus laevigata*	external	1500	brown	regular	30	Ant
h	*Pseudocnus lubrica*	external	1050	yellow	regular	50	Pac
a	*Psilaster charcoti*	planktonic	750	red	regular	300	Ant
h	*Psolidiella nigra*	external	600	yellow	pale	40	Pac
h	*Psolidium bidiscum*	planktonic	300	yellow	regular	30	Pac
h	*Psolidium bullatum*	planktonic	330	yellow	bright	25	Pac
h	*Psolus chitinoides*	planktonic	625	red	bright	75	Pac
h	*Psolus fabricii*	planktonic	500	orange	bright	200	Pac / Atl
a	*Pteraster tesselatus*	planktonic	1200	red	bright	150	Pac
o	*Sigsbeia conifera*	internal	800	red	pale	10	Atl
a	*Solaster endeca*	planktonic	800	orange	bright	300	Pac / Atl
a	*Solaster stimpsoni*	planktonic	1000	green	pale	400	Pac
a	*Tosia neossia*	external	700	orange	regular	60	Ant
h	*Trachythyone nina*	internal	1800	brown	pale	14	Atl

[1] a = Asteroidea, e = Echinoidea, c = Crinoidea, h = Holothuroidea and o = Ophiuroidea

[2] Planktonic = pelagic lecithotrophic, External = externally brooded, Internal = internally brooded

[3] Refer to Table 1 for definitions of egg colour intensity

[4] Adult body size is diameter for Asteroidea, Ophiuroidea and Echinoidea, and length in Holothuroidea and Crinoidea

[5] Ant = Antarctic, Atl = Atlantic, Ind = Indian, Pac = Pacific

Appendix 3C. Subset of lecithotrophic echinoderms (buoyancy focus)

Subset of lecithotrophic echinoderms used to test whether egg buoyancy correlates with egg colour and development mode, independently of geographic location ($N = 56$). Data are shown for embryonic development site, egg size, egg colour, egg buoyancy, and adult size.

Species	Development Site[1]	Egg Size	Egg Colour	Buoyancy[2]	Adult Size[3]
Abatus cordatus	external	1300	orange	-	30
Aquilonastra burtoni	planktonic	500	green	-	30
Asterina phylactica	external	550	orange	-	15
Asthenosoma ijimai	planktonic	1200	orange	+	130
Astropecten latespinosus	planktonic	300	brown	-	80
Clarkcoma pulchra	planktonic	290	yellow	-	120
Crossaster papposus	planktonic	550	red	+	300
Cryptasterina hystera	internal	440	yellow	+	24
Cryptasterina pacifica	planktonic	400	orange	+	20
Cryptasterina pentagona	planktonic	413	orange	+	24
Cucumaria frondosa	planktonic	750	orange	+	200
Cucumaria frondosa japonica	planktonic	500	green	+	300
Cucumaria miniata	planktonic	520	green	+	250

Cucumaria piperata	planktonic	530	green	+	120
Diplasterias brucei	external	3400	orange	-	250
Echinaster echinophorus	planktonic	800	orange	-	700
Echinaster luzonicus	planktonic	1000	red	+	150
Eupentacta chronhjelmi	planktonic	300	green	-	60
Eupentacta fraudatrix	planktonic	340	green	-	100
Fromia elegans	planktonic	2000	red	+	120
Fromia ghardaqana	planktonic	1000	red	+	100
Heliocidaris erythrogramma	planktonic	400	orange	+	140
Henricia sanguinolenta	external	1000	orange	-	150
Hippasteria phrygiana	planktonic	450	orange	+	400
Holopneustes purpurescens	planktonic	580	brown	+	80
Iconaster longimanus	planktonic	1000	orange	+	200
Leptasterias hexactis	external	800	orange	-	50
Leptasterias polaris	external	850	orange	-	300
Mediaster aequalis	planktonic	1000	orange	+	200
Meridiastra calcar (Patiriella)	planktonic	415	green	-	60
Meridiastra gunnii (Patiriella)	planktonic	430	green	+	40
Meridiastra occidens	planktonic	400	green	+	30

Meridiastra oriens	planktonic	400	green	-	20
Metacrinus rotundus	planktonic	350	yellow	+	600
Nardoa novaecaledoniae	planktonic	1000	orange	+	110
Nardoa tuberculata	planktonic	1000	orange	+	280
Ophiarthrum elegans	planktonic	384	green	+	13
Ophidiaster granifer	planktonic	600	orange	-	100
Ophidiaster granifer	planktonic	600	orange	+	100
Ophioderma brevispina	planktonic	350	green	+	40
Ophiolepis paucispina	internal	480	red	-	20
Ophiomastix venosa	planktonic	500	green	+	20
Ophionereis olivacea	internal	480	orange	-	10
Ophionereis schayeri	planktonic	240	brown	-	150
Ophiothrix oerstedii	planktonic	400	brown	-	100
Parvulastra exigua (Patiriella)	external	390	orange	-	15
Phormosoma placenta	planktonic	1100	yellow	+	120
Phyllacanthus imperialis	planktonic	510	yellow	+	80
Pseudocnus echinatus	planktonic	400	green	-	40
Psolus chitinoides	planktonic	625	red	+	75
Psolus fabricii	planktonic	500	orange	+	200

Psolus phantapus	planktonic	450	red	+	265
Pteraster tesselatus	planktonic	1200	red	+	150
Sigsbeia conifera	internal	800	red	-	10
Solaster endeca	planktonic	800	orange	+	300
Solaster stimpsoni	planktonic	1000	green	+	400

[1] Planktonic = pelagic lecithotrophic, External = externally brooded, Internal = internally brooded

[2] Egg buoyancy reported in the literature was categorized based on a previous comprehensive review of echinoderm larvae (Emlet 1994). (-) = Negative buoyancy, (+) = Positive buoyancy, (0) = Neutral buoyancy, (?) = No data

[3] Adult body size is diameter for Asteroidea, Ophiuroidea and Echinoidea, and length in Holothuroidea and Crinoidea

Appendix 3D. Subset of lecithotrophic echinoderms (phylogeny focus)

Subset of lecithotrophic echinoderms used to test whether certain egg colours are phylogenetically linked in the four main extant classes ($N = 103$). Data are shown for taxonomic class, egg size, egg colour, and adult size.

Class	Species	Egg Size	Egg Colour	Adult Size[1]
Echinoidea	*Abatus cavernosus*	1400	yellow	40
Echinoidea	*Abatus cordatus*	1300	orange	30
Ophiuroidea	*Amphiura carchara*	450	yellow	80
Ophiuroidea	*Amphiura squamata*	880	red	5
Asteroidea	*Anasterias antarctica*	1810	yellow	40
Asteroidea	*Aquilonastra burtoni*	500	green	30
Asteroidea	*Asterina gibbosa*	400	yellow	60
Asteroidea	*Asterina phylactica*	550	orange	15
Echinoidea	*Asthenosoma ijimai*	1200	orange	130
Asteroidea	*Astropecten gisselbrechti*	350	yellow	80
Asteroidea	*Astropecten latespinosus*	300	brown	80
Holothuroidea	*Athyonidium chilensis*	360	brown	150
Echinoidea	*Cassidulus mitis*	367	yellow	25
Ophiuroidea	*Clarkcoma pulchra*	290	yellow	120

Asteroidea	*Crossaster papposus*	550	red	300
Asteroidea	*Cryptasterina hystera*	440	green	12
Asteroidea	*Cryptasterina hystera*	440	yellow	24
Asteroidea	*Cryptasterina pacifica*	400	orange	20
Asteroidea	*Cryptasterina pentagona*	413	orange	24
Asteroidea	*Ctenopleura fisheri*	465	brown	100
Holothuroidea	*Cucumaria fallax (pallida)*	500	brown	120
Holothuroidea	*Cucumaria frondosa*	750	orange	200
Holothuroidea	*Cucumaria frondosa japonica*	500	green	300
Holothuroidea	*Cucumaria lubrica*	900	red	50
Holothuroidea	*Cucumaria miniata*	520	green	250
Holothuroidea	*Cucumaria piperata*	530	green	120
Asteroidea	*Diplasterias brandti*	2870	brown	110
Asteroidea	*Diplasterias brucei*	3400	orange	250
Asteroidea	*Echinaster brasiliensis*	1000	brown	80
Asteroidea	*Echinaster echinophorus*	800	orange	700
Asteroidea	*Echinaster graminicola*	850	orange	80
Asteroidea	*Echinaster luzonicus*	1000	red	150
Holothuroidea	*Echinopsolus charcoti*	1800	yellow	60

Holothuroidea	*Eupentacta chronhjelmi*	300	green	60
Holothuroidea	*Eupentacta fraudatrix*	340	green	100
Holothuroidea	*Eupentacta quinquesemita*	400	green	100
Asteroidea	*Fromia elegans*	2000	red	120
Asteroidea	*Fromia ghardaqana*	1000	red	100
Ophiuroidea	*Gorgonocephalus eucnemis*	220	orange	140
Echinoidea	*Heliocidaris erythrogramma*	400	orange	140
Asteroidea	*Henricia lisa*	1100	yellow	100
Asteroidea	*Henricia sanguinolenta*	1000	orange	150
Asteroidea	*Hippasteria phrygiana*	450	orange	400
Echinoidea	*Holopneustes purpurescens*	580	brown	80
Asteroidea	*Iconaster longimanus*	1000	orange	200
Asteroidea	*Leptasterias aequalis*	900	yellow	60
Asteroidea	*Leptasterias groeanlandica*	900	brown	60
Asteroidea	*Leptasterias hexactis*	800	orange	50
Asteroidea	*Leptasterias polaris*	850	orange	300
Holothuroidea	*Leptosynapta clarki*	250	brown	50
Asteroidea	*Mediaster aequalis*	1000	orange	200
Asteroidea	*Meridiastra calcar (Patiriella)*	415	green	60

Asteroidea	*Meridiastra gunnii (Patiriella)*	430	green	40
Asteroidea	*Meridiastra occidens*	400	green	30
Asteroidea	*Meridiastra oriens*	400	green	20
Holothuroidea	*Molpadia intermedia*	270	red	400
Asteroidea	*Nardoa novaecaledoniae*	1000	orange	110
Asteroidea	*Nardoa tuberculata*	1000	orange	280
Asteroidea	*Neosmilaster georgianus*	2170	brown	70
Ophiuroidea	*Ophiarthrum elegans*	384	green	13
Ophiuroidea	*Ophiarthrum pictum*	419	green	30
Asteroidea	*Ophidiaster granifer*	600	orange	100
Asteroidea	*Ophidiaster granifer*	600	orange	100
Ophiuroidea	*Ophioderma brevispina*	350	brown	40
Ophiuroidea	*Ophioderma wahlbergii*	250	yellow	30
Ophiuroidea	*Ophiolepis elegans*	250	yellow	50
Ophiuroidea	*Ophiolepis paucispina*	480	red	20
Ophiuroidea	*Ophiomastix annulosa*	430	green	150
Ophiuroidea	*Ophiomastix caryophyllata*	200	green	10
Ophiuroidea	*Ophiomastix elegans*	200	green	10
Ophiuroidea	*Ophiomastix janualis*	200	green	120

Ophiuroidea	*Ophiomastix mixta*	335	green	600
Ophiuroidea	*Ophiomastix venosa*	500	green	20
Ophiuroidea	*Ophionereis olivacea*	400	orange	3
Ophiuroidea	*Ophionereis olivacea*	480	orange	10
Ophiuroidea	*Ophionereis schayeri*	240	brown	150
Ophiuroidea	*Ophiopeza spinosa*	300	yellow	70
Ophiuroidea	*Ophiothrix oerstedii*	400	brown	100
Asteroidea	*Parvulastra exigua (Patiriella)*	390	orange	15
Asteroidea	*Parvulastra vivipara (Patiriella)*	150	orange	30
Holothuroidea	*Penilidia desbarresi*	150	brown	20
Holothuroidea	*Pentamera populifera*	370	green	30
Asteroidea	*Perknaster fuscus*	1200	red	300
Echinoidea	*Phormosoma placenta*	1100	yellow	120
Echinoidea	*Phyllacanthus imperialis*	510	yellow	80
Holothuroidea	*Pseudocnus echinatus*	400	green	40
Holothuroidea	*Pseudocnus laevigata*	1500	brown	30
Holothuroidea	*Pseudocnus lubrica*	1050	yellow	50
Asteroidea	*Psilaster charcoti*	750	red	300
Holothuroidea	*Psolidiella nigra*	600	yellow	40

Holothuroidea	*Psolidium bidiscum*	300	yellow	30
Holothuroidea	*Psolidium bullatum*	330	yellow	25
Holothuroidea	*Psolus chitinoides*	625	red	75
Asteroidea	*Psolus fabricii*	500	orange	200
Holothuroidea	*Psolus phantapus*	450	red	265
Asteroidea	*Pteraster militaris*	1400	yellow	120
Asteroidea	*Pteraster tesselatus*	1200	red	150
Ophiuroidea	*Sigsbeia conifera*	800	red	10
Asteroidea	*Smilasterias multipara*	1000	red	40
Asteroidea	*Solaster endeca*	800	orange	300
Asteroidea	*Solaster stimpsoni*	1000	green	400
Asteroidea	*Tosia neossia*	700	orange	60
Holothuroidea	*Trachythyone nina*	1800	brown	14

[1] Adult body size is diameter for Asteroidea, Ophiuroidea and Echinoidea, and length in Holothuroidea and Crinoidea

Appendix 3E. Subset of lecithotrophic echinoderms (FAMD all factors)

Clusters from FAMD analysis in Fig. 3.5 - Testing all factors

Cluster	Factor		*P*-Value
I	Egg Colour	Green	<0.001
	Egg Intensity	Standard	<0.001
	Egg Size	< Mean	= 0.025
	Adult Size	> Mean	= 0.017
	Phylogeny		
	Ocean Basin	Pacific	<0.001
	Dev Site	Planktonic	<0.001
II	Egg Colour	Yellow	= 0.002
		Brown	<0.001
	Egg Intensity	Pale	<0.001
	Egg Size		
	Adult Size	< Mean	= 0.003
	Phylogeny	Ophiuroidea	= 0.02
	Ocean Basin	Atlantic	<0.001
	Dev Site	Internal	<0.001

III	Egg Colour	Orange	<0.001
	Egg Intensity	Bright	<0.001
	Egg Size	> Mean	<0.001
	Adult Size		
	Phylogeny	Asteroidea	= 0.003
	Ocean Basin	Global	<0.001
		Antarctic	<0.001
	Dev Site	External	<0.001

Appendix 3F. Subset of lecithotrophic echinoderms (FAMD geographic location)

Clusters from FAMD analysis in Fig. 3.6 - Testing geographic location.

Cluster	Factor		*P*-Value
I	Egg Colour	Green	<0.001
	Egg Intensity	Standard	<0.001
	Egg Size	< Mean	= 0.009
	Adult Size		
	Ocean Basin	Pacific	<0.001
	Dev Site	Planktonic	<0.001
II	Egg Colour	Yellow	= 0.001
		Brown	<0.001
	Egg Intensity	Pale	<0.001
	Egg Size		
	Adult Size	< Mean	= 0.004
	Ocean Basin	Atlantic	<0.001
	Dev Site	Internal	<0.001
III	Egg Colour	Orange	<0.001
	Egg Intensity	Bright	<0.001

	Egg Size		
	Adult Size	> Mean	= 0.02
	Ocean Basin	Global	<0.001
	Dev Site		
IV	Egg Colour		
	Egg Intensity		
	Egg Size	> Mean	<0.001
	Adult Size		
	Ocean Basin	Antarctic	<0.001
	Dev Site	External	<0.001

Appendix 3G. Subset of lecithotrophic echinoderms (FAMD buoyancy)

Clusters from FAMD analysis in Fig. 3.7 - Testing egg buoyancy.

Cluster	Factor		*P*-Value
I	Egg Colour	Orange	= 0.0011
	Development Site	External	<0.001
	Buoyancy	Negative	<0.001
	Egg Size	> Mean	= 0.005
	Adult Size		
II	Egg Colour	Red	<0.001
		Orange	<0.001
	Development Site	Planktonic	= 0.016
	Buoyancy	Positive	= 0.023
	Egg Size		
	Adult Size		

Appendix 3H. Subset of lecithotrophic echinoderms (FAMD phylogeny)

Clusters from FAMD analysis in Fig. 3.9 - Testing phylogenetic class, independently of development mode.

Cluster	Factor		*P*-Value
I	Egg Colour	Green	<0.001
	Class	Ophiuroidea	<0.001
	Egg Size	< Mean	<0.001
	Adult Size		
II	Egg Colour	Green	<0.001
	Class	Holothuroidea	<0.001
	Egg Size		
	Adult Size		
III	Egg Colour	Brown	<0.001
	Class	Holothuroidea	= 0.036
	Egg Size		
	Adult Size		
IV	Egg Colour	Yellow	<0.001
	Class	Echinoidea	<0.001
	Egg Size		

	Adult Size		
VI	Egg Colour	Orange	<0.001
	Class	Asteroidea	<0.001
	Egg Size	> Mean	= 0.002
	Adult Size		

Appendix 3I. General key to lecithotrophic echinoderm eggs / early embryos.

1a. Propagules are greenish and yolky_____ Planktonic (Pacific / Indian)

1b. Propagules are reddish / yellowish and yolky_____2

2a. Propagules are positively buoyant_____ Planktonic

2b. Propagules are neutral or negatively buoyant _____ 3

3a. Large adult size (> 10 cm) and / or small egg size (< 1 mm)_____ Planktonic

3b. Small adult size (< 10 cm) and /or large egg size (> 1 mm)_____4

4a. Propagules are brightly coloured or match adult colour _____ External Brooder

4b. Propagules are pale in colour _____ Internal Brooder

Appendix 3 References

Achituv, Y., and E. Sher. 1991. Sexual reproduction and fission in the sea star *Asterina burtoni* from the Mediterranean coast of Israel. *Bulletin of Marine Science* **48**:670-678.

Amemiya, S., and T. Tsuchiya. 1979. Development of the echinothurid sea urchin *Asthenosoma ijimai*. *Marine Biology* **52**:93-96.

Atwood, D. G. 1973. Larval development in the asteroid *Echinaster echinophorus*. *Biological Bulletin* **144**:1-11.

Baillon, S., J.-F. Hamel, and A. Mercier. 2011. Comparative study of reproductive synchrony at various scales in deep-sea echinoderms. *Deep Sea Research Part I: Oceanographic Research Papers* **58**:260-272.

Barbaglio, A., C. Turchi, G. Melone, C. Di Benedetto, T. Martinello, M. Patruno, M. Biggiogero, I. C. Wilkie, and M. D. C. Carnevali. 2012. Larval development in the feather star *Antedon mediterranea*. *Invertebrate Reproduction and Development* **56**:124-137.

Bingham, B. L., K. Giles, and W. B. Jaeckle. 2004. Variability in broods of the seastar *Leptasterias aequalis*. *Canadian Journal of Zoology* **82**:457-463.

Birkeland, C., F.-S. Chia, and R. R. Strathmann. 1971. Development, substratum selection, delay of metamorphosis and growth in the seastar, *Mediaster aequalis*. *Biological Bulletin* **141**:99-108.

Byrne, M. 1989. Ultrastructure of the ovary and oogenesis in the ovoviviparous ophiuroid *Ophiolepis paucispina* (Echinodermata). *Biological Bulletin* **176**:79-95.

Byrne, M. 1991. Reproduction, development and population biology of the Caribbean ophiuroid *Ophionereis olivacea,* a protandric hermaphrodite that broods its young. *Marine Biology* **111**:387-399.

Byrne, M. 2005. Viviparity in the sea star *Cryptasterina hystera* (Asterinidae)—conserved and modified features in reproduction and development. *Biological Bulletin* **208**:81-91.

Byrne, M. 2006. Life history diversity and evolution in the Asterinidae. *Integrative and Comparative Biology* **46**:243-254.

Byrne, M., and M. J. Anderson. 1994. Hybridization of sympatric *Patiriella* species (Echinodermata: Asteroidea) in New South Wales. *Evolution* **48**:564-576.

Byrne, M., P. Cisternas, and T. O'Hara. 2008. Brooding of pelagic-type larvae in *Ophiopeza spinosa*: reproduction and development in a tropical ophiodermatid brittlestar. *Invertebrate Biology* **127**:98-107.

Campbell, D. B., and R. L. Turner. 1984. *Echinaster graminicola*, a new species of spinulosid sea star (Echinodermata: Asteroidea) from the west coast of Florida. 167-178 in *Biological Society of Washington*.

Catalan, M. A. B., and M. Yamamoto. 1994. Annual reproductive cycle of the Japanese holothurian *Eupentacta chronhjelmi*. *Canadian Journal of Zoology* **72**:387-396.

Clark, H. L. 1911. North Pacific ophiurans in the collection of the United States National Museum. *Smithsonian Institution United States National Museum Bulletin* **75**:1-302.

Contins, M., and C. R. R. Ventura. 2011. Embryonic, larval, and post-metamorphic development of the sea urchin *Cassidulus mitis* (Echinoidea; Cassiduloida): an endemic brooding species from Rio de Janeiro, Brazil. *Marine Biology* **158**:2279-2288.

Dartnall, A. J., M. Byrne, J. Collins, and M. W. Hart. 2003. A new viviparous species of asterinid (Echinodermata, Asteroidea, Asterinidae) and a new genus to accommodate the species of pan-tropical exiguoid sea stars. *Zootaxa* **359**:1-14.

Emlet, R. B. 1994. Body form and patterns of ciliation in nonfeeding larvae of echinoderms - Functional solutions to swimming in the plankton. *American Zoologist* **34**:570-585.

Engstrom, N. A. 1982. Brooding behaviour and reproductive biology of a subtidal Puget Sound sea cucumber, *Cucumaria lubrica* (Clark, 1901) (Echinodermata: Holothuroidea). 447-450 in *International Echinoderm Conference*, A. Balkema, ed. Rotterdam, Tampa Bay.

Falkner, I., M. Byrne, and M. A. Sewell. 2006. Maternal provisioning in *Ophionereis fasciata* and *O. schayeri*: brittle stars with contrasting modes of development. *Biological Bulletin* **211**:204-207.

Falkner, I., M. A. Sewell, and M. Byrne. 2015. Evolution of maternal provisioning in ophiuroid echinoderms: characterisation of egg composition in planktotrophic and lecithotrophic developers. *Marine Ecology Progress Series* **525**:1.

Fourgon, D., I. Eeckhaut, D. Vaïtilingon, and M. Jangoux. 2005. Lecithotrophic development and metamorphosis in the Indo-West Pacific brittle star *Ophiomastix venosa* (Echinodermata: Ophiuroidea). *Invertebrate Reproduction and Development* **47**:155-165.

Gebruk, A. V., A. V. Rogacheva, D. L. Pawson, J.-F. Hamel, K. G. Macisaac, and A. Mercier. 2013. *Penilidia desbarresi* sp. nov.(Echinodermata: Holothuroidea: Elasipodida) from the upper slope of Newfoundland and re-description of *P. ludwigi* (von Marenzeller, 1893). *Marine Biology Research* **9**:1029-1036.

Gemmill, J. F. 1912. I. The development of the starfish *Solaster endeca* Forbes. *The Transactions of the Zoological Society of London* **20**:1-71.

Gemmill, J. F. 1920. The development of the starfish *Crossaster papposus*. *Quarterly Journal of Microscopical Science* **2**:155-187.

Gil, D. G., G. Escudero, and H. E. Zaixso. 2011. Brooding and development of *Anasterias minuta* (Asteroidea: Forcipulata) in Patagonia, Argentina. *Marine Biology* **158**:2589-2602.

Gil, D. G., H. E. Zaixso, and J. A. Tolosano. 2009. Brooding of the sub-Antarctic heart urchin, *Abatus cavernosus* (Spatangoida: Schizasteridae), in southern Patagonia. *Marine Biology* **156**:1647-1657.

Grave, C. 1916. *Ophiura brevispina* II. An embryological contribution and a study of the effect of yolk substance upon development and developmental processes. *Journal of Morphology* **27**:413-451.

Guisado, C., S. A. Carrasco, D. Diaz-Guisado, R. Maltrain, and H. Rojas. 2012. Embryonic development, larval morphology and juvenile growth of the sea cucumber *Athyonidium chilensis* (Holothuroidea: Dendrochirotida). *Revista de Biologia Marina y Oceanografia* **47**:65-73.

Haesaerts, D., M. Jangoux, and P. Flammang. 2006. Adaptations to benthic development: functional morphology of the attachment complex of the brachiolaria larva in the sea star *Asterina gibbosa*. *Biological Bulletin* **211**:172-182.

Hagman, D. K., and P. D. Vize. 2003. Mass spawning by two brittle star species, *Ophioderma rubicundum* and *O. squamosissimum* (Echinodermata: Ophiuroidea), at the Flower Garden Banks, Gulf of Mexico. *Bulletin of Marine Science* **72**:871-876.

Hamel, J.-F., J. H. Himmelman, and L. Dufresne. 1993. Gametogenesis and spawning of the sea cucumber *Psolus fabricii* *Biological Bulletin* **184**:125-143.

Hamel, J. F., and A. Mercier. 1995. Prespawning behaviour, spawning, and development of the brooding starfish *Lepasterias polaris*. *Biological Bulletin* **188**:32-45.

Hamel, J. F., and A. Mercier. 1996. Early development, settlement, growth, and spatial distribution of the sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea). *Canadian Journal of Fisheries and Aquatic Sciences* **53**:253-271.

Hendler, G. 1977. Development of *Amphioplus abditus* (Verrill)(Echinodermata: Ophiuroidea): I. Larval biology. *Biological Bulletin* **152**:51-63.

Hendler, G., and D. R. Franz. 1982. The biology of a brooding seastar, *Leptasterias tenera*, in Block Island Sound. *Biological Bulletin* **162**:273-289.

Hendler, G., and B. S. Littman. 1986. The ploys of sex: relationships among the mode of reproduction, body size and habitats of coral-reef brittlestars. *Coral Reefs* **5**:31-42.

Hendler, G., and D. L. Meyer. 1982. Ophiuroids flagrante delicto and notes on the spawning behavior of other echinoderms in their natural habitat. *Bulletin of Marine Science* **32**:600-607.

Hendler, G., and L. U. Tran. 2001. Reproductive biology of a deep-sea brittle star *Amphiura carchara* (Echinodermata: Ophiuroidea). *Marine Biology* **138**:113-123.

Hendler, G., and P. A. Tyler. 1986. The reproductive cycle of *Ophioderma brevispinum* (Echinodermata: Ophiuroidea). *Marine Ecology* **7**:115-122.

James, D. B. 1972. Note on the development of the asteroid *Asterina burtoni*. *Journal of the Marine Biological Association of India* **14**:883-884.

Kashenko, S. D. 2000. Combined effect of temperature and salinity on the development of the holothurian *Eupentacta fraudatrix*. *Russian Journal of Marine Biology* **26**:188-193.

Komatsu, M. 1975. On the development of the sea-star, *Astropecten latespinosus*. *Biological Bulletin*:49-59.

Komatsu, M. 1982. Development of the sea-star *Ctenopleura fisheri*. *Marine Biology* **66**:199-205.

Komatsu, M., and S. Nojima. 1985. Development of the seastar, *Astropecten gisselbrechti*. *Pacific Science* **39**:274-282.

Komatsu, M., P. M. O'Loughlin, B. Bruce, H. Yoshizawa, K. Tanaka, and C. Murakami. 2006. A gastric-brooding asteroid, *Smilasterias multipara*. *Zoological Science* **23**:699-705.

Komatsu, M., and T. Shosaku. 1993. Development of the brittle star, *Ophioplocus japonicus* I. *Zoological Science* **10**:295-306.

Lambert, P. 1997. *Sea Cucumbers of British Columbia, Southeast Alaska and Puget Sound.* UBC Press, Vancouver, British Columbia, Canada.

Lane, D. J. W., and J. M. L. Hu. 1994. *Abbreviated development in Iconaster longimanus (Möbius): planktonic lecithotrophy in a tropical goniasterid sea star.* Rotterdam & Brookfield.

Magniez, P. 1983. Reproductive cycle of the brooding echinoid *Abatus cordatus* (Echinodermata) in Kerguelen (Antarctic Ocean): changes in the organ indices, biochemical composition and caloric content of the gonads. *Marine Biology* **74**:55-64.

McClary, D. J., and P. V. Mladenov. 1990. Brooding biology of the sea star *Pteraster militaris* : Energetic and histological evidence for nutrient translocation to brookded juveniles. *Journal of Experimental Marine Biology and Ecology* **142**:183-199.

McClintock, J. B., and B. J. Baker. 1997a. Palatability and chemical defense of eggs, embryos and larvae of shallow-water antarctic marine invertebrates. *Marine Ecology Progress Series* **154**:121-131.

McClintock, J. B., and B. J. Baker. 1997b. A review of the chemical ecology of Antarctic marine invertebrates. *American Zoologist* **37**:329-342.

McEdward, L. R., and L. K. Coulter. 1987. Egg volume and energetic content are not correlated among sibling offspring of starfish: Implications for life-history theory. *Evolution* **41**:914-917.

McEuen, F. S. 1988. Spawning behaviors of northeast Pacific sea cucumbers (Holothuroidea: Echinodermata). *Marine Biology* **98**:565-585.

McEuen, F. S., and F.-S. Chia. 1985. Larval development of a molpadiid holothuroid, *Molpadia intermedia* (Ludwig, 1894) (Echinodermata). *Canadian Journal of Zoology* **63**:2553-2559.

McEuen, F. S., and F. S. Chia. 1991. Development and metamorphosis of two psolid sea cucumbers, *Psolus chitonoides* and *Psolidium bullatum*, with a review of reproductive patterns in the family Psolidae (Holothuroidea: Echinodermata). *Marine Biology* **109**:267-279.

Mercier, A., and J.-F. Hamel. 2008. Depth-related shift in life history strategies of a brooding and broadcasting deep-sea asteroid. *Marine Biology* **156**:205-223.

Mercier, A., D. L. Pawson, D. J. Pawson, and J.-F. Hamel. 2010. First record of the sea cucumber *Trachythyone nina* (Echinodermata: Holothuroidea) in Canadian waters, with a redescription of the species and notes on its distribution and biology. *Marine Biology Research* **6**:315-320.

Mladenov, P. V. 1979. Unusual lecithotrophic development of the Caribbean brittle star *Ophiothrix oerstedi*. *Marine Biology* **55**:55-62.

Morris, V. 1995. Apluteal development of the sea urchin *Holopneustes purpurescens* Agassiz (Echinodermata: Echinoidea: Euechinoidea). *Zoological Journal of the Linnean Society* **114**:349-364.

Nakano, H., S. Amemiya, T. Hibino, Y. Hara, and T. Oji. 2005. Development of the sea lily *Metacrinus rotundus*: Comparisons with feather stars. 41 in *11th International Echinoderm Conference* Taylor & Francis, Munich, Germany.

Naughton, K. M., and T. D. O'Hara. 2009. A new brooding species of the biscuit star *Tosia* (Echinodermata: Asteroidea: Goniasteridae), distinguished by molecular, morphological and larval characters. *Invertebrate Systematics* **23**:348-366.

Nobre, C., and L. Campos. 2004. Effect of salinity on the larval development of *Echinaster brasiliensis* Müller & Troschel, 1842 (Echinodermata: Asteroidea). 45-51 in *Echinoderms*, J. N. T. Heinzeller, ed., Muchen.

Nojima, S. 1982. Ecological studies of the sea star *Astropecten latespinosus* Meissner. II Growth rate and differences in growth pattern of immature and mature sea stars. *Publications Amakusa Marine Biological Laboratory* **6**:65-84.

O'Loughlin, P. M. 1991. Brooding and fission in shallow water echinoderms of southern Australia. 223-228 in *Biology of Echinodermata*, T. Yanagisawa, ed. Balkema.

O'Loughlin, P. M. 2000. A review of the cucumariid genus *Psolidiella* Mortensen (Echinodermata, Holothuroidea). *Memoirs of Museum Victoria* **58**:25-37.

Obuchi, M., Y. Fujita, Y. Nakano, T. Uehara, and T. Motokawa. 2010. Reproductive biology and early life history of the hermaphroditic feather star *Dorometra sesokonis* (Echinodermata: Crinoidea). *Marine Biology* **157**:1191-1201.

Ohshima, H. 1921. On the development of *Cucumaria echinata*. *Journal of Cell Science* **65**:173-246.

Okazaki, K., and K. Dan. 1954. The metamorphosis of partial larvae of *Peronella japonica* Mortensen, a sand dollar. *Biological Bulletin* **106**:83-99.

Olson, R. R., J. L. Cameron, and C. M. Young. 1993. Larval development (with observations on spawning) of the pencil urchin *Phyllacanthus imperialis*: A new intermediate larval form. *Biological Bulletin* **185**:77-85.

Parks, A. L., B. W. Bisgrove, G. A. Wray, and R. A. Raff. 1989. Direct development in the sea urchin *Phyllacanthus parvispinus* (Cidaroidea): Phylogenetic history and functional modification. *Biological Bulletin* **177**:96-109.

Patent, D. H. 1970. The early embryology of the basket star *Gorgonocephalus caryi* (Echinodermata, Ophiuroidea). *Marine Biology* **6**:262-267.

Pearse, J. S., J. B. McClintock, and I. Bosch. 1991. Reproduction of Antarctic benthic marine invertebrates: tempos, modes, and timing. *American Zoologist* **31**:65-80.

Poulin, E., and J.-P. Feral. 1996. Why are there so many species of brooding Antarctic echinoids? *Evolution*:820-830.

Prestedge, G. K. 1998. The distribution and biology of *Patiriella vivipara* (Echinodermata: Asteroidea: Asterinidae) a sea star endemic to southeast Tasmania. *Records-Australian Museum* **50**:161-170.

Rutherford, J. C. 1973. Reproduction, growth and mortality of the holothurian *Cucumaria pseudocurata*. *Marine Biology* **22**:167-176.

Schatt, P., and J.-P. Féral. 1996. Completely direct development of *Abatus cordatus*, a brooding schizasterid (Echinodermata: Echinoidea) from Kerguelen, with description of perigastrulation, a hypothetical new mode of gastrulation. *Biological Bulletin*:24-44.

Selvakumaraswamy, P., and M. Byrne. 2000. Reproduction, spawning, and development of 5 ophiuroids from Australia and New Zealand. *Invertebrate Biology* **119**:394-402.

Sewell, M. A., and F. S. Chia. 1994. Reproduction of the intraovarian brooding apodid *Leptosynapta clarki* (Echinodermata: Holothuroidea) in British Columbia. *Marine Biology* **121**:285-300.

Stancyk, S. E. 1973. Development of *Ophiolepis elegans* (Echinodermata: Ophiuroidea) and its implications in the estuarine environment. *Marine Biology* **21**:7-12.

Strathmann, M. F. 1987. *Reproduction and development of marine invertebrates of the northern Pacific coast: Data and methods for the study of eggs, embryos, and larvae.* University of Washington Press, Washington, USA.

Strathmann, R. R. 1979. Echinoid larvae from the northeast Pacific (with a key and comment on an unusual type of planktotrophic development). *Canadian Journal of Zoology* **57**:610-616.

Strathmann, R. R., M. F. Strathmann, and H. E. Roland. 1984. Does limited brood capacity link adult size, brooding, and simultaneous hermaphroditism? At with the starfish *Asterina phylactica*. *American Naturalist* **123**:796-818.

Tyurin, S. A., and A. L. Drozdov. 2002. Spermatozoon ultrastructure of two holothurian species of the genus *Cucumaria* (Dendrochirotida, Holothuroidea) from the sea of Japan. *Russian Journal of Marine Biology* **28**:61-64.

Williams, D. H. C., and D. T. Anderson. 1975. The reproductive system, embryonic development, larval development and metamorphosis of the sea urchin *Heliocidaris erythrogramma* (Val.) (Echinoidea : Echinometridae). *Australian Journal of Zoology* **23**:371-403.

Wray, G. A. 1996. Parallel evolution of nonfeeding larvae in echinoids. *Systematic Biology* **45**:308-322.

Yamaguchi, M., and J. S. Lucas. 1984. Natural parthenogenesis, larval and juvenile development, and geographical distribution of the coral reef asteroid *Ophidiaster granifer*. *Marine Biology* **83**:33-42.

Young, C. M., and J. L. Cameron. 1987. Laboratory and in situ flotation rates of lecithotrophic eggs from the bathyal echinoid *Phormosoma placenta*. *Deep-Sea Research Part I-Oceanographic Research Papers* **34**:1629-1639.

Appendix 4 Summary Tables of Statistical Results for Chapter 5

Appendix 4A. ANOVA summary table

Summary table of ANOVA testing whether ontogenetic stage, light colour (white, red, blue) and phototaxis (positive, negative, neutral) affected mean absolute swimming speed among focal echinoderm propagules

Species	Factor	*df*	F Stat	*P* value
A. rubens	Ontogeny	3	115.7	<0.001
	Colour	2	15.5	<0.001
	Taxis	2	0.08	0.78
	O x C	6	18.1	<0.001
	O x T	6	0.03	0.99
	C x T	4	0.07	0.93
	O x C x T	6	0.06	0.99
	Ontogeny[1]	3	104.7	<0.001
	Colour	2	15.2	<0.001
S. droebachiensis	Ontogeny	3	15.0	<0.001
	Colour	2	7.2	0.008
	Taxis	2	0.01	0.93
	O x C	6	4.1	<0.001
	O x T	6	0.4	0.74
	C x T	4	0.8	0.47
	O x C x T	6	1.5	0.20
	Ontogeny	3	11.4	<0.001
	Colour	2	4.6	0.03
C. papposus	Ontogeny	3	37.7	<0.001
	Colour	2	10.3	<0.001
	Taxis	2	0.2	0.67
	O x C	6	6.4	<0.001
	O x T	6	0.3	0.84
	C x T	4	0.2	0.83
	O x C x T	6	1.5	0.20
	Ontogeny	3	22.5	<0.001
	Colour	2	9.1	<0.001
C. frondosa	Ontogeny	3	3.7	0.028
	Colour	2	5.8	0.001
	Taxis	2	1.6	0.21
	O x C	6	0.8	0.58
	O x T	6	1.7	0.19
	C x T	4	0.4	0.72
	O x C x T	6	0.6	0.71

[1] In the case of significant interaction terms, each factor was analyzed independently

Appendix 4B. ANOVA summary table

Summary table of ANOVA testing whether phototaxis direction affected trajectory straightness among echinoderm propagules under different light gradients (white, red, blue)

Species	Factor	*df*	F Stat	*P* value
A. rubens	Ontogeny	3	10.4	<0.001
	Colour	2	12.1	<0.001
	Taxis	2	4.8	0.031
	O x C	6	1.7	0.09
	O x T	6	2.0	0.11
	C x T	4	0.2	0.83
	All	6	0.8	0.56
S. droebachiensis	Ontogeny	3	1.7	0.18
	Colour	2	1.0	0.39
	Taxis	2	32.0	<0.001
	O x C	6	0.5	0.79
	O x T	6	0.7	0.58
	C x T	4	1.2	0.30
	All	6	1.1	0.4
C. papposus	Ontogeny	3	2.8	0.045
	Colour	2	5.7	0.005
	Taxis	2	34.2	<0.001
	O x C	6	6.4	<0.001
	O x T	6	3.1	0.031
	C x T	4	6.5	0.002
	All	6	1.2	0.33
	Ontogeny[1]	3	2.6	0.047
	Colour	2	4.5	0.01
	Taxis	2	28.3	<0.001
C. frondosa	Ontogeny	3	1.6	0.20
	Colour	2	7.5	<0.001
	Taxis	2	0.8	0.37
	O x C	6	0.2	0.95
	O x T	6	0.9	0.47
	C x T	4	0.5	0.61
	All	6	1.9	0.08

[1] In the case of significant interaction terms, each factor was analyzed independently

Appendix 4C. ANOVA summary table

Summary table of ANOVA testing the effect of light colour (uniform intensity) on mean absolute swimming speed of echinoderm propagules

Species	Factor	*df*	F Stat	*P* value
A. rubens	Ontogeny	3	125.7	<0.001
	Colour	2	16.8	<0.001
	O x C	6	19.6	<0.001
	Ontogeny[1]	3	63.7	<0.001
	Colour	2	3.74	0.026
S. droebachiensis	Ontogeny	3	6.0	<0.001
	Colour	2	4.0	0.020
	O x C	6	4.8	<0.001
	Ontogeny	3	4.9	0.003
	Colour	2	3.2	0.043
C. papposus	Ontogeny	3	44.6	<0.001
	Colour	2	19.2	<0.001
	O x C	6	6.0	<0.001
	Ontogeny	3	28.3	<0.001
	Colour	2	8.0	<0.001
C. frondosa	Ontogeny	3	6.5	<0.001
	Colour	2	4.3	<0.001
	O x C	6	1.1	0.38

[1] In the case of significant interaction terms, each factor was analyzed independently

Appendix 4D. ANOVA summary table

Summary table of ANOVA testing the effect of light colour (uniform intensity) on mean path straightness of echinoderm propagules

Species	Factor	*df*	F Stat	*P* value
A. rubens	Ontogeny	3	6.3	<0.001
	Colour	2	21.5	<0.001
	O x C	6	1.9	0.08
S. droebachiensis	Ontogeny	3	2.2	0.10
	Colour	2	0.77	0.47
C. papposus	Ontogeny	3	3.1	0.032
	Colour	2	4.1	0.021
	O x C	6	3.2	0.006
	Ontogeny	3	2.8	0.046
	Colour	2	3.9	0.028
C. frondosa	Ontogeny	3	1.4	0.25
	Colour	2	18.3	<0.001
	O x C	6	0.5	0.84

[1] In the case of significant interaction terms, each factor was analyzed independently

Appendix 5 Dataset for Chapter 6

Summary table of swimming speeds across two nutritional modes and five representative marine phyla

Phylum	Class	Species	Nutrition	Life stage	Size (μm)	Speed (mm/s)
Annelida	Polychaeta	*Polydora ciliata*	P	Stage3	920	0.4
Annelida	Polychaeta	*Scoloplos armiger*	L	Meta	400	0.5
Annelida	Polychaeta	*Heteromastus filiformis*	P	Tro	100	0.5
Annelida	Polychaeta	*Polydora ciliata*	P	Stage1	220	0.5
Annelida	Polychaeta	*Polydora ciliata*	P	Stage2	560	0.5
Annelida	Polychaeta	*Heteromastus filiformis*	P	Meta	340	0.6
Annelida	Polychaeta	*Pholoe minuta*	P	Meta	360	0.8
Annelida	Polychaeta	*Scoloplos armiger*	L	Tro	200	0.8
Annelida	Polychaeta	*Nereis virens*	P	Meta	230	0.8
Annelida	Polychaeta	*Harmothoe imbricata*	P	Tro	200	1.1
Annelida	Polychaeta	*Anaitides mucosa*	P	Tro		1.2

Annelida	Polychaeta	*Eteome longa*	P	Tro		1.2
Annelida	Polychaeta	*Nephtys ciliata*	P	Meta		1.3
Annelida	Polychaeta	*Eulalia viridis*	P	Tro	350	1.5
Annelida	Polychaeta	*Pectinaria koreni*	P	Tro		1.7
Annelida	Polychaeta	*Anaitides maculata*	P	Tro		1.8
Annelida	Polychaeta	*Nephtys ciliata*	P	Tro		2.5
Annelida	Polychaeta	*Pectinaria koreni*	P	Meta		2.5
Annelida	Polychaeta	*Capitella capitata*	P	L.Tro	200	3.1
Annelida	Polychaeta	*Chone infundibulariformis*	P	Tro		3.3
Annelida	Polychaeta	*Capitella capitata*	P	Tro	130	5.2
Bryozoa		*Membranipora sp.*	L			1.9
Bryozoa		*Bugula sp.*	L			8
Cnidaria	Hydrozoa	*Thecaphora sp.*	L	Plan	600	0.42
Cnidaria	Anthozoa	*Lophelia pertusa*	L	Plan	150	0.5
Cnidaria	Anthozoa	*Oculina varicosa*	L	Plan	160	0.5

Cnidaria	Hydrozoa	*Aurelia aurita*	L	Plan		0.5
Cnidaria	Anthozoa	*Montastraea faveolata*	L	Plan	500	1.1
Cnidaria	Hydrozoa	*Aurelia aurita*	L	Plan		1.5
Cnidaria	Anthozoa	*Corallium rubrum*	L	Plan		1.5
Cnidaria	Anthozoa	*Oculina varicosa*	L	Plan	160	3
Cnidaria	Anthozoa	*Agaricia tenuifolia*	L	Plan		3.6
Cnidaria	Anthozoa	*Porites astreoides*	L	Plan	760	4.3
Cnidaria		*Fungia actiniformis*	L	Plan		5
Cnidaria		*Caryophyllia smithi*	L	Plan	140	30
Echinodermata	Asteroidea	*Asterias rubens*	P	Blas	140	0.04
Echinodermata	Echinoidea	*Hemicentrotus pulcherrimus*	P	Plut	250	0.14
Echinodermata	Asteroidea	*Crossaster papposus*	L	Blas	600	0.15
Echinodermata	Asteroidea	*Asterias rubens*	P	Blas	140	0.04
Echinodermata	Echinoidea	*Hemicentrotus pulcherrimus*	P	Plut	250	0.14
Echinodermata	Asteroidea	*Crossaster papposus*	L	Blas	600	0.15

Echinodermata	Holothuroidea	*Cucumaria frondosa*	L	Pen	700	0.15
Echinodermata	Holothuroidea	*Cucumaria frondosa*	L	Blas	600	0.18
Echinodermata	Echinoidea	*Strongylocentrotus droebachiensis*	P	Bra	200	0.19
Echinodermata	Ophiuroidea	*Amphiura filiformis*	P	Plut2	275	0.2
Echinodermata	Echinoidea	*Strongylocentrotus purpuratus*	P	Plut4	200	0.2
Echinodermata	Echinoidea	*Hemicentrotus pulcherrimus*	P	Gas	100	0.2
Echinodermata	Holothuroidea	*Cucumaria frondosa*	L	Gas	650	0.21
Echinodermata	Echinoidea	*Paracentrotus lividus*	P	Plut	500	0.23
Echinodermata	Ophiuroidea	*Amphiura filiformis*	P	Plut1	200	0.25
Echinodermata	Echinoidea	*Strongylocentrotus purpuratus*	P	Plut6	450	0.26
Echinodermata	Echinoidea	*Paracentrotus lividus*	P	Blas	120	0.27
Echinodermata	Ophiuroidea	*Ophioderma brevispinum*	L	Vit	400	0.3
Echinodermata	Echinoidea	*Strongylocentrotus droebachiensis*	P	Plut	800	0.3
Echinodermata	Echinoidea	*Strongylocentrotus droebachiensis*	P	Gas	250	0.35
Echinodermata	Asteroidea	*Asterias rubens*	P	Gas	200	0.38

Echinodermata	Asteroidea	*Crossaster papposus*	L	Gas	800	0.46
Echinodermata	Asteroidea	*Asterias rubens*	P	Bra	350	0.48
Echinodermata	Echinoidea	*Arbacia punctulata*	P	Plut4	130	0.75
Echinodermata	Asteroidea	*Crossaster papposus*	L	Bra	1100	0.78
Echinodermata	Echinoidea	*Arbacia punctulata*	P	Plut6	176	0.95
Echinodermata	Echinoidea	*Dendraster excentricus*	P	Plut	400	1