**Dissolved organic matter influences the timing of embryonic development
of the purple sea urchin, *Strongylocentrotus purpuratus*.**

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by
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TITLE: Dissolved organic matter influences the timing of embryonic development of the purple sea urching, *Strongylocentrotus purpuratus*.

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ABSTRACT

Dissolved organic matter influences the timing of embryonic development of the purple sea urchin, *Strongylocentrotus purpuratus*.

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Marine dissolved organic matter (DOM) comprises one of the largest carbon reservoirs on earth and has long been considered a potential energy source for marine invertebrates. The importance of DOM transport has been adequately demonstrated for unicellular organisms, where DOM can meet 100% of an organisms energy needs, but the effects of DOM uptake for marine metazoans are less well understood. In this study, three general areas involving the influence of DOM transport to marine invertebrates were explored. First, we assessed the effects of using seawater exposed to high intensity ultraviolet radiation (UVR) on the study organism; embryos of the purple sea urchin, *Strongylocentrotus purpuratus*. This was important because we used seawater treated in this way to create water types used in the experiments. Exposing seawater to high intensity UVR oxidizes (and functionally removes) DOM in the seawater. Second, the influence of the presence of DOM on the timing of embryonic development was examined for embryos of *S. purpuratus*. Specifically, the time of cell division and the time of hatching were determined for embryos in seawater with and without DOM. Finally, the ability of DOM to moderate the negative effect of UV-exposure on time of cell division was assessed. To make these comparisons experiments were performed using three water types: FSW (0.22 micron filtered seawater), DOM-depleted seawater

(UV oxidized 0.22 micron filtered seawater), and DOM-enriched seawater (UV oxidized 0.22 micron filtered seawater enriched with labile DOM). In the first experiment, batches of embryos in the three water types were either exposed or not exposed to ultra-violet radiation and the time of first cell division was compared for embryos across the six treatments. In the second experiment, batches of embryos were placed in the same three water types and the time of first cell division and the time of hatching were quantified. From these experiments several results were generated. First, seawater exposed to high intensity UVR did not influence the timing of development of embryos of *S. purpuratus*. Embryos in water exposed to high intensity UVR (DOM-enriched and DOM-depleted seawater) hatched at similar times and completed first cell division at times similar to embryos in water not exposed to high intensity UVR (FSW). Next, we found that the influence of the presence of DOM on the development timing of *S. purpuratus* embryos depended on the event that was examined. The time of first cell division was not affected by the presence of DOM but the time of hatching was. Embryos in water with dissolved organic matter hatched on average 86 minutes later than embryos in water without DOM. Potentially, embryos in seawater without DOM speed up development to more quickly reach the point that they can feed on particulates. Lastly, the presence of DOM did not influence UVR-induced cleavage delay. The percent cleavage delay was not significantly different for embryos in seawater with (DOM-enriched) and without (DOM-depleted) DOM. In addition to the experiments, all studies in the literature that examine the realized effects of DOM transport were analyzed to ascertain when the manifestation of DOM uptake is most likely to occur. From these results, it appears that the effects of DOM transport are most likely to manifest after the life stage in which the majority of uptake

occurred. If DOM transport has an affect within a life stage it is most likely to manifest as moderation of biomass loss or maintenance of endogenous reserves. With the addition of the experimental results from this study to the information already in the literature we begin to more fully grasp the importance of DOM transport to *S. purpuratus*. DOM influences the time of hatching, biomass, arm length, and stomach size of the species; results that highlight the importance of examining multiple affects of DOM transport for a single species. In conclusion, future research should look for multiple effects of the presence of DOM both within and across life stages (for a single species) to better understand the importance of DOM to marine invertebrates.

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CHAPTER 1

Introduction

INTRODUCTION

Marine dissolved organic matter (DOM) comprises one of the largest carbon reservoirs on Earth with a carbon content of approximately 7×10^{11} kgs. This is close to the amount of carbon in atmospheric carbon dioxide, 7.5×10^{11} kgs, and as such may play an important role in the global carbon cycle (Hedges 1992). The uptake (transport) of free amino acids, monomeric sugars, and fatty acids has been well documented for a variety of marine organisms (for example, Stephens and Schinske 1961, Ferguson 1982), but utilization upon uptake is less well understood. While the importance of DOM uptake has been adequately shown for unicellular organisms, in which DOM can meet 100% of an organisms energy needs (Crawford et al. 1974), utilization by metazoans has received much less attention. Of the studies that have examined the effects of DOM uptake by marine invertebrates on parameters such as growth, survival, and reproduction there have been mixed results (for example Johnson and Wendt 2007, Manahan and Crisp 1982).

Bulk concentrations of DOM can range from 35-100 μM in marine waters. Components of the general DOM pool can be categorized based on their biological availability. At one end of the range are refractory DOM molecules, which are unavailable to organisms, are usually of low molecular weight, and turnover on time scales of thousands of years (Benner et al. 1992). Refractory DOM comprises a much larger percentage of total DOM than labile DOM. Labile DOM molecules are readily available to organisms, can be high molecular weight or low molecular weight compounds (Benner et al. 1992), have high fluxes (Furhman and Ferguson 1986), and make up 3-10% of the bulk DOM pool. Natural concentrations of labile DOM usually range from 1-4 μM (Benner 2003) and the labile DOM pool is often composed of neutral

1 amino acids and monomeric sugars. In addition, while the actual concentrations of labile
2 DOM in the water column at any one time may seem low the turnover rates can be very
3 high thus increasing its importance to marine organisms.

4 DOM is produced and consumed through several mechanisms. Production
5 mechanisms include extracellular release by phytoplankton, phytoplankton release via
6 bacteria and virus induced cell lysis, solubilization of particles, bacterial transformation
7 and release, and release and excretion by zooplankton (Carlson 2002). Consumptive
8 mechanisms include uptake by heterotrophic bacterioplankton, marine invertebrates and
9 photoheterotrophic microalgae, phototransformation, and sorption onto particles (Carlson
10 2002). Of these mechanisms, “sloppy grazing” by macro zooplankton accounts for the
11 majority of DOM production while uptake by heterotrophic bacterioplankton accounts for
12 the majority of DOM consumption (Carlson 2002). Of the identified consumption
13 mechanisms uptake by marine invertebrates is of importance for this study.

14 Metazoans remove free amino acids, monomeric sugars, and fatty acids from their
15 surroundings via active transport protein channels in the epidermis (Jørgenson 1976). It
16 has also been shown that uptake is not the result of associated bacteria (Manahan et al.
17 1980). Past research on DOM has adequately demonstrated uptake by both adult and
18 larval marine invertebrates. Of approximately 31 marine phyla, 12 have been tested for
19 DOM uptake (Figure 1). Of these, uptake has been shown for six phyla at naturally
20 occurring concentrations (Ferguson 1982) and for five additional phyla at unnaturally
21 high concentrations (Stephens and Schinske 1961, Jaeckle and Manahan 1989). Thus, of
22 12 phyla tested, DOM uptake has been shown for 11 with only the Arthropods not
23 exhibiting uptake (Ferguson 1982, Stephens and Schinske 1961). Though uptake of DOM

1 has been adequately demonstrated, there is a paucity of data on the putative ecological
2 benefits of uptake.

3 If DOM uptake is important for growth, survival, and reproduction of marine
4 invertebrates it is likely that the manifestation of benefits will occur during larval stages.
5 Larvae have greater surface area to volume ratios than adults, potentially making DOM
6 uptake and utilization more efficient, and in many cases larvae have to survive through
7 metamorphosis on endogenous reserves or DOM. Studies in the literature tentatively
8 support this idea. Of the two studies found examining the influence of DOM transport on
9 adult invertebrates one did not find effect while the other did. Ferguson (1980) placed
10 groups of adult *Echinaster* in DOM-depleted and DOM-enriched seawater and found no
11 pathological effects. There were no differences in behavior, ciliary activity, level of
12 mucous production, organ size indices, or biochemical composition of the integument for
13 starfish in the DOM-enriched, DOM-depleted, or control conditions. Contrary to this,
14 Shick (1975) found several effects of DOM uptake on the cnidarian *Aurelia aurita*.
15 Scyphistomae were kept in treatments for 56 days before parameters were assessed.
16 Polyps in artificial seawater without DOM were less likely to strobilate, began
17 strobilation later, produced fewer ephyrae, and fewer healthy ephyrae than polyps in
18 artificial seawater with natural concentrations of either alanine, glycine, or glucose added
19 to it. There was no difference in percent survival or total buds produced for polyps in all
20 conditions. Though mixed results have been found for adult invertebrates, the ecological
21 benefits of DOM uptake seem to be more conclusive for larvae where the majority of
22 studies have found effect of transport.

1 The studies of the utilization of DOM by invertebrate larvae can be categorized
2 based on the type of parameters assessed. Categories include effects on structure,
3 biomass, survival, and the occurrence of metamorphic events. Structure seems to be the
4 most commonly assessed parameter type for studies examining the utilization of DOM by
5 invertebrates. Manahan and Crisp (1982) found that larvae of the oyster *C. gigas* when
6 exposed to glycine, alanine, or glucose do not show increases in shell length relative to
7 starved controls. However, larvae in the DOM-enriched treatments had healthier looking
8 tissues than larvae in the starved treatments. Shilling (1995) showed that the enrichment
9 of seawater with different types of DOM can cause the postoral arms of the sea urchins
10 *Strongylocentrotus purpuratus* and *Lytechinus pictus* larvae to be significantly shorter
11 than the arms of larvae in un-enriched seawater. Lastly, Johnson and Wendt (2007)
12 demonstrated that reduction in lophophore size associated with the prolonged larval
13 swimming of the bryozoan *Bugula neritina* can be partially offset by the presence of
14 DOM.

15 Experiments have also examined the influence of DOM transport on biomass,
16 survival, and the occurrence of metamorphic events. Maintenance of biomass has been
17 demonstrated for embryos and non-feeding larvae both in the lab and field. Animals for
18 which positive effects have been found include larvae of the mollusk *Mya arenaria*
19 (Gustafson 1980), trochophore larvae of the red abalone *Haliotis rufescens* (Jaeckle and
20 Manahan 1992), embryos and larvae of the purple sea urchin *S. purpuratus* (Shilling and
21 Manahan 1990, Shilling and Bosch 1994, Shilling 1995) and embryos of the antarctic
22 seastar *Odontaster validus* (Shilling and Bosch 1994). Increased survival of larvae in
23 DOM-enriched water has only been shown for one species. Gustafson (1980)

1 demonstrated that *M. arenaria* larvae have greater survivorship when in the presence of
2 DOM. Finally, *B. neritina* larvae are more likely to complete metamorphosis after a
3 prolonged swimming period, if the swimming occurred in DOM-enriched seawater
4 (Johnson and Wendt 2007). Though several types of factors have been assessed in many
5 species the ecological benefits of uptake of DOM are still largely not understood.
6 Parameters such as timing of development and non-nutritive environmental stresses have
7 not been explored.

8 To better understand the importance of uptake of labile DOM to marine
9 invertebrates, the timing of developmental events was compared for embryos of *S.*
10 *purpuratus* in seawater with and without DOM. *S. purpuratus* was used because embryos
11 and larvae uptake amino acids and monomeric sugars (Manahan 1986), uptake prevents
12 biomass loss in both the lab (Shilling and Manahan 1990) and the field (Shilling and
13 Bosch 1994), larvae show a morphological response to the presence of DOM (Shilling
14 1995) and exposure to ultraviolet radiation (UVR) has been shown to delay the first
15 cellular division of embryos (Rustad 1971, Campanale 2009, Goschke 2005). Assessment
16 of UV-induced cleavage delays allows for the determination of the ability of DOM to
17 alleviate the negative consequences of a non-nutritive environmental stress. To make
18 these comparisons, embryos were reared in three different water types; DOM-depleted,
19 DOM-enriched, and filtered seawater (FSW). To create DOM-depleted seawater,
20 naturally occurring labile DOM in seawater was oxidized and thereby functionally
21 removed by exposing filtered seawater to high intensity ultraviolet irradiation (Johnson
22 and Wendt 2006). To create the DOM-enriched water type, known qualities and
23 quantities of DOM were added to the DOM-depleted seawater. FSW was included as a

1 water type in the experiments to test for potential adverse effects of the methodology
2 used to remove DOM from seawater. While intense UVR has been shown to reduce and
3 thereby functionally remove labile DOM from seawater (Beattie et al. 1961; Armstrong
4 et al. 1966, Armstrong and Tibbits 1968; Johnson and Wendt 2006) and several studies
5 have demonstrated that the treated water is safe for organisms (Johnson and Wendt
6 2006), there is still some debate on the issue. For example, Alam and Ohgaki (2004) and
7 Lund and Hongve (1994) both demonstrated a biocidal activity of ultraviolet irradiated
8 seawater on freshwater microbes. Similarly, Gjessing and Kalquist (1991) found an
9 algicidal effect of UV-radiated water that contained humic substances.

10 To address these general questions, two distinct experiments were performed. In
11 the first experiment, the time of completion of the first cellular division was determined
12 for embryos either exposed or not exposed to UVR while in the three water types. This
13 experiment allowed us to address the following questions: 1) Does the presence of DOM
14 influence the rate of embryonic development through the time of the first cellular
15 division? 2) Does exposure to UVR influence the rate of embryonic development
16 through the time of first cell division? and 3) Does the presence of DOM moderate the
17 influence of UV-exposure on the time of first cell division of embryos? In the second
18 experiment, the time of completion of the first cellular division and the time of hatching
19 were determined for embryos in the three water types. This experiment allowed the
20 following questions to be addressed: 1) Does the presence of DOM influence the rate of
21 development through the time of hatching? 2) Is the time of cell division predictive of the
22 time of hatching? 3) Does using high intensity UVR to oxidize and thereby functionally
23 remove DOM create seawater which influences the timing of embryonic development?

CHAPTER TWO

Do DOM and UV light influence the time of first cell division of embryos of the purple sea urchin, *Strongylocentrotus purpuratus*?

1 **INTRODUCTION**

2 DOM is one of the largest carbon reservoirs on Earth and may be a very important
3 nutritional source for marine organisms. The uptake and utilization of amino acids,
4 monomeric sugars, and fatty acids (the components of labile DOM) have been well
5 documented for unicellular organisms, where DOM can meet up to 100% of an
6 organisms energy needs (Crawford et al. 1974). While uptake of DOM has also been well
7 documented for multi-cellular organisms (for example, Stephens and Schinske 1961,
8 Ferguson 1982), the realized energetic benefits to metazoans are largely unknown (Wendt
9 and Johnson 2006).

10 Ultraviolet radiation (UVR), light in the wavelength range of 280-400nm, also
11 plays a major role in the marine environment. It penetrates to depths of 1 to 30 meters
12 depending on the transparency of the seawater (Jerlov 1950, Karentz and Lutz 1990,
13 Smith et al. 1992, Smith and Baker 1979, Booth and Morrow 1997, Adams et al. 2001)
14 with penetration depth correlating inversely with the amount of organics in the water
15 column. Where UVR reaches, it affects both physical and biological components of
16 marine ecosystems. Absorption of UVR by different chemical components of seawater
17 can lead to the production of hydrogen peroxide (Abele-Oeschger et al. 1997, Abele et al.
18 1999) and a change in the acidity of the water (Gjesiing and Källqvist 1991) and the
19 effects of UVR on marine organisms are well documented (Karentz 1994). UVR can
20 damage organisms in several different ways. UVB radiation (280-320nm) can cause the
21 formation of pyrimidine dimers (Harm 1980), photoadducts (Mitchell and Karentz 1993),
22 and DNA-protein cross links (Tevini 1993) while UVA (320-400nm) causes the
23 formation of potentially damaging reactive oxygen species (Tyrell 1991). These damages

1 can occur throughout an organism's life, but the planktonic eggs, embryos, and larvae of
2 marine invertebrates are more susceptible to the effects of ultraviolet (UV) exposure
3 because of their small size and lack of protective coverings (Adams and Shick 1996,
4 Hader et al. 1998, Sinha and Hader 2002).

5 In this experiment, we examined the influence of both UVR and DOM on the
6 timing of early embryonic development of the purple sea urchin, *Strongylocentrotus*
7 *purpuratus*. To do this the time of first cell division was compared for embryos either
8 exposed or not exposed to natural levels of UV light while in three different water types;
9 DOM-depleted, DOM-enriched, and FSW. FSW was 0.22 micron filtered seawater,
10 DOM-depleted seawater was UV oxidized 0.22 micron filtered seawater, and DOM-
11 enriched seawater was UV oxidized 0.22 micron filtered seawater enriched with labile
12 DOM. Specifically, the following questions were addressed: 1) Does exposure to UVR
13 influence the time first cell division? 2) Does the presence of DOM influence the time of
14 cell division? and 3) Does the presence of DOM moderate UV-induced delays in time of
15 cell division?

16 *S. purpuratus* was used as the study organism because embryonic development is
17 synchronous, embryos and larvae are able to transport DOM (Manahan 1986), and
18 transport can prevent biomass loss associated with the non-feeding planktotrophic
19 embryos and early larvae both in the lab (Shilling and Manahan 1990) and the field
20 (Shilling and Bosch 1994). In addition, with past studies already demonstrating an
21 influence of UVR on embryos (Adams and Shick 2001, Rustad 1971, Giese 1939 have
22 demonstrated that embryos exposed to UVR take longer to reach the completion of first
23 cell division) we were able to determine if the presence of DOM would moderate these

1 delays. Of the few studies that examine the realized energetic benefit of DOM transport
2 to marine metazoans none have examined at the ability of uptake to moderate
3 environmentally induce damages. Here, the demonstrated maintenance of endogenous
4 reserves due to transport of DOM may allow *S. purpuratus* to either prevent UV damages
5 or better utilize repair mechanisms that can fix the damages associated with exposure to
6 UV light. Embryos with greater biomass may procure less UV damage because of the
7 physical blocking effects of greater size or density and similarly and may also utilize their
8 energy stores to increase the rate of repair mechanisms.

1 **METHODS**

2 **Glassware Preparation**

3 Glassware was placed in 10% HCL solution for 24 hours then rinsed several times
4 with distilled water. It was covered with aluminum foil and heated at a temperature of
5 500°C for four hours to combust residual DOM. To prevent contamination, the glassware
6 remained covered until it was used.

7 **Preparation of Seawater**

8 Seawater was collected at the Center for Coastal Marine Sciences (Avila Beach,
9 California, USA) and filtered using glass fiber filters (Fisherbrand) and 0.22 micron filter
10 paper (GE Water and Process Technologies). To remove DOM, 100mls of filtered
11 seawater were added to each of ten UV transparent, fused-quartz test tubes. The test tubes
12 were placed into a UV oxidizer (Ace Glass #7900) and subjected to the exposure of a
13 1200 Watt UV lamp (Ace Glass #7825-40) emitting light in the range of 222-366 nm for
14 18 hours. The irradiance value was 000W/m². Previous experimentation has shown that
15 irradiation reduces total organic carbon (TOC) levels to about 10% of their initial levels
16 thereby functionally removing labile DOM and that treated water has no adverse effect
17 on larvae (Johnson and Wendt 2006).

18 **Seawater Treatments**

19 Three different seawater treatments were used; UV oxidized 0.22 micron filtered
20 seawater (DOM-depleted), UV oxidized 0.22 micron filtered seawater enriched with
21 labile DOM (DOM-enriched), and 0.22 micron filtered seawater (FSW). This experiment
22 examined the time of completion of first cleavage for embryos across two factors; water
23 type and UVR exposure. For this experiment the DOM-enriched water type contained

0.75 μ M each of glucose, glycine, alanine, aspartic acid, leucine, methionine, phenalanine, and valine; all of which have been shown to be transported by marine invertebrates. The concentrations used were based on naturally occurring concentrations of labile DOM (Benner 2002)

Animal Collection, Maintenance, and Spawning

S. purpuratus adults were collected in the Santa Barbara channel during the months of June, July, and April. Sea urchins were held in a 125-gallon tank equipped with an Eco-wheel© filtration assembly (Aquatic Engineers, Camby, IN). Water temperature was 14°C and they were fed once weekly with large amounts of *Macrocystis pyrifera*. Prior to the induction of spawning, the sea urchins were sprayed with a dilute alcohol solution and then rinsed with DOM-depleted seawater. To induce spawning, adults were injected with 1ml of 0.5M KCl using an 18 gauge needle attached to a 10 ml syringe. Injections were made through the peritoneal membrane and targeted at the coelomic cavity. To collect eggs, dry females were inverted over beakers filled with DOM-depleted seawater. Sperm was collected from male urchins using a micropipetter and was stored at 4°C. Five individual trials were performed, with each trial utilizing one parent pair.

Comparison of time of completion of first cell division across two factors

To determine the ability of DOM to influence development timing and alter the negative consequences of environmental stress, the time of completion of the first cell division was determined for embryos exposed and not exposed to UVR while in DOM-enriched, DOM-depleted, and FSW. A half milliliter of egg/DOM-depleted water mixture was added to 95 ml of DOM-enriched, DOM-depleted or filtered seawater. To fertilize

1 the eggs, 5uL of sperm was added to 12.5ml of DOM-depleted water and 4ml of this
2 solution was added to the beakers containing the eggs and treatment water. The number
3 of eggs fertilized was checked to ensure that fertilization was complete (100%). After
4 this, 50 ml of fertilized egg/water mixture from each beaker was decanted into 2, 250ml
5 finger bowls yielding three pairs of finger bowls with each pair containing the same water
6 type. The finger bowls were then placed in a tray containing 12°C water. One bowl from
7 each water type was placed underneath UV-opaque acrylic Plexiglass UF3 (Arkema, 50%
8 transmission at 400nm) and the other was placed underneath UV-transparent acrylic
9 Plexiglass G-UVT (Arkema, 50% transmission at 290 nm). Eighteen minutes after
10 fertilization the embryos were placed under 4, UVA-340 lamps (Q-Panel Lab Products)
11 (as in Adams and Shick ,2001) for 60 minutes giving an irradiance of 000W/m².
12 Temperature was maintained at ~ 12°C and the bowls were stirred periodically to prevent
13 clumping of embryos.

14 **Sampling and Counting**

15 Cultures were sampled by first stirring the bowls to ensure an even distribution of
16 embryos throughout the water column and then by removing 450ul of treatment
17 water/embryo solution. All samples were preserved in 5% formalin solution. For time of
18 first cleavage sampling, the first samples were taken at 80 minutes post-fertilization.
19 Samples were then taken every 10 minutes for the following 2 hours (or until 100%
20 cleavage was observed in all treatments). All samples were counted by placing 300µl of
21 each sample onto a 1ml Sedgewick Rafter counting cell. The cleavage state of
22 approximately 200 embryos from each sample were identified and recorded.

1 **Analysis**

2 From the raw data, the percentage of embryos cleaved at each sampling point was
3 calculated. These data were then used to calculate the time at which 50% of embryos
4 were cleaved in each of the six treatments. The percent cleavage delay (Adams and other
5 references) was also calculated to determine the delay in time of first cell division due to
6 UVR exposure. Two analyses were completed. Significance of differences among means
7 was determined using a two-way analysis of variance (ANOVA) for embryos exposed to
8 UVR. An ANOVA was also used to compare the average percent cleavage delay for
9 embryos in DOM-depleted, DOM-enriched, and FSW. In both analyses, a square root
10 transformation was performed to normalize data. Data in each analysis were normal,
11 linear, and variance was homogenous

RESULTS

Time of completion of first cell division

There were no significant differences in the mean time ($\pm$ S.E.) of completion of first cellular division for embryos of *Strongylocentrotus purpuratus* in different water types ($p=0.992$, Fig. 1, Table 1). Embryos not exposed to UVR and in filtered seawater, DOM-enriched water, and DOM-depleted water took 126.2 (± 17.4), 125.0 (± 13.3), and 127.2 (± 11.8) minutes to complete the first cell division, respectively.

DOM moderation of UV-induced delay

Embryos in all water types completed first cleavage faster in the absence of ultraviolet radiation exposure. On average, exposure to ultraviolet radiation caused embryos to complete first cell division 40.3 minutes after embryos not exposed to UVR thus slowing early development by 33% (Fig. 1, Fig.2). Water type did not, on average, significantly affect the intensity of UV induced cleavage delay (Table 2, $p=0.981$, Fig. 2). The percent cleavage delay for embryos in FSW, DOM-enriched water and DOM-depleted water was 34.1 (± 8.0), 32.8(± 7.04), and 31.8(± 7.1) percent, respectively.

1 **DISCUSSION**

2 The timing of early embryonic development of the sea urchin, *S. purpuratus*, is
3 strongly influenced by exposure to UVR (Fig. 1, Fig.2). Embryos that were exposed to
4 natural levels of UV light took approximately 40 minutes (32%) longer to complete first
5 cell division than embryos not exposed to UVR. These results were expected as past
6 studies have demonstrated that embryos of both *S. purpuratus* and the green sea urchin,
7 *S. droebachiensis*, sustain damages from UV light which cause first cleavage to be
8 delayed (Goschke 2005, Campanale 2009, Giese 1964, Adams and Shick 1996). The
9 ecological implications of this demonstrated delay are largely unknown but are most
10 likely to be significant if the delay causes the timing of other, later developmental events,
11 such as hatching, developing the ability to feed, or metamorphosis, to be postponed.

12 Hatching is an important event because embryos of the purple sea urchin are not
13 actively motile until they hatch approximately 24 hours after fertilization (Strathman
14 1987). Before this, embryos are unable to change their position to avoid negative
15 environmental conditions, are unable to actively escape predation, and cannot orient
16 themselves in the water column to avoid transport offshore (Vance 1973). The
17 development of the ability to feed is also an important event for, *S. purpuratus*. Embryos
18 and larvae are unable to feed on particulates until the larvae develop arms and stomachs
19 approximately five days after fertilization (Strathman 1987). If the UV-induced delay of
20 first cell division causes larvae to reach the feeding point later, then larvae must survive
21 on endogenous reserves for a longer period of time.

22 Finally, metamorphosis, the time at which larvae settle out of the water column, is
23 an important event because it marks the end of the embryonic and larval life stages; life

1 stages which typically suffer from very high degrees of mortality relative to others. In
2 fact, it has been suggested that at least 99% of planktotrophic larvae die prior to
3 metamorphosis (Vance 1973). In addition, for individuals that survive until
4 metamorphosis the detrimental effects associated with a prolonged larval stage have been
5 well documented (Pechenik and Cerulli 1991, Pechenik 2006). For example, Wendt
6 (1996) demonstrated that larvae of the bryozoan *Bugula neritina* develop into juveniles
7 with significantly smaller lophophores when metamorphosis was prolonged. In future
8 studies, the timing of later developmental events should be determined for embryos
9 exposed to UVR to ascertain the potential ecological implications of the UV-induced
10 delay.

11 Contrary to what was expected, the results of this experiment show that the
12 presence of DOM does not influence the time of cell division of the purple sea urchin
13 (Fig. 1). Though *S. purpuratus* embryos and larvae transport DOM (Manahan 1986) and
14 the transport can cause endogenous reserves to remain steady (Shilling and Manahan
15 1990, Shilling and Bosch 1994), embryos in seawater with and without DOM completed
16 the first cellular division in the same amount of time. Though affects of DOM on
17 development timing were not found here they may exist but occur later in the life stage
18 when more time has passed allowing for the transport and assimilation of larger amounts
19 of sugars and amino acids. In the next experiment the influence of DOM on the time of
20 hatching was explored as hatching is an event that occurs towards the end of embryonic
21 development and has potentially greater ecological significance.

22 The results of this experiment also suggest that the presence of DOM does not
23 alleviate the delay in developmental timing associated with exposure to UVR for

1 embryos of *S. purpuratus* (Table 2, Fig. 2). The amount of UV-induced delay did not
2 change for embryos in the presence of monomeric sugars and amino acids. Though
3 unlikely, these results may have been obtained because of experimental error. The DOM
4 in the seawater was exposed to UVR along with the embryos and studies have shown that
5 DOM can be oxidized by UVR exposure (Beattie et al. 1961; Armstrong et al. 1966,
6 Armstrong and Tibbits 1968; Johnson and Wendt 2006). However, in this experiment we
7 used natural amounts of UV radiation (4 W for 60 minutes, irradiation=0000W/m²) and
8 in experiments where complete oxidation occurs very high amounts of UV radiation are
9 used (1600 W for 18 hrs). In the future, the concentration of DOM should be quantified
10 after the embryo exposure period to show that it is still present.

11 The other and more probable explanation for these results is that the cellular
12 mechanisms responsible for cell division are not dependent on DOM. Embryos can
13 reduce UV-induced percent cleavage delay by either preventing UV damages or
14 increasing the rate at which damages are repaired and it was hypothesized that embryos
15 in the presence of DOM (which have been shown to have greater biomass (Shilling and
16 Manahan 1990)) would better utilize these mechanisms. Embryos with greater biomass
17 may incur fewer damages because of their larger sizes (or greater densities) and when
18 damages are accrued they may utilize energy stores to increase the rate of repair but the
19 results of this experiment suggest that this is not the case. Possibly, the additional
20 biomass added by embryos in the presence of DOM is not a significant barrier to UV-
21 light causing embryos in the presence and absence of DOM to sustain similar levels of
22 UV-induced damages. In addition, it's likely that the repair of damages is very important

- 1 for the survival of embryos and because of this damages are fixed at a maximum rate
- 2 regardless of the amount biomass.

3

4

5 **Figure 1.** The time of first cell division for batches of embryos in three water types

6 (DOM-depleted, DOM-enriched and filtered seawater) that were either exposed or not

7 exposed to natural levels of UVR (mean $\pm$ 1 SE, N=5). The time of first cell division

8 was not significantly altered by DOM ($p > 0.05$) but was significantly altered by exposure

9 to UVR ($p < 0.05$). The presence of DOM did not alter the intensity of the UV-induced

10 delay in time of first cell division ($p > 0.05$).

Figure 2. The percent cleavage delay do to UV-exposure for batches of embryos in three water types (DOM-depleted, DOM-enriched and filtered seawater) (mean $\pm$ 1 SE, N=5). Percent cleavage delay was not significantly altered by water type ($p>0.05$).

Table 1. ANOVA table for differences in time of cell division for embryos in three different water types and not exposed to UVR.				
SOURCE	D.F.	S.S.	F-test	P-value
Water Type	2	0.038	0.01	0.99
Error	12	22.834		

Table 2. ANOVA table for differences in percent cleavage delay for embryos in three different water types.				
SOURCE	D.F.	S.S.	F-test	P-value
Water Type	2	0.075	0.02	0.981
Error	12	23.738		

CHAPTER THREE

**Does DOM influence the time of hatching of embryos of the purple sea urchin,
Strongylocentrotus purpuratus.**

1 **INTRODUCTION**

2 In this experiment the influences of the presence of dissolved organic matter
3 (DOM) on the timing of embryonic development, the safety to embryos of using high
4 intensity UVR to remove DOM from seawater, and the variation in the timing of
5 developmental events were examined for the purple sea urchin *Strongylocentrotus*
6 *purpuratus*.

7 **DOM and the timing of embryonic development**

8 The influences of the presence of dissolved organic matter (DOM) is important
9 because the amount of carbon in oceanic DOM is only slightly less than the amount of
10 carbon in the atmosphere (Carlson 2002) thus representing a very large potential energy
11 source for marine organisms. The uptake of fatty acids, monosaccharides, and amino
12 acids (the components of DOM) has been well documented in the literature but the
13 realized energetic benefits of transport are not well understood. While DOM uptake has
14 been demonstrated to account for up to 100% of a microorganism's energy needs
15 (Crawford 1976), the effects on growth, survival, and reproduction of metazoans are not
16 well known (Wendt and Johnson 2006, Johnson and Wendt 2007). Of the studies found
17 examining the realized energetic benefits of DOM transport only one explored the timing
18 of developmental events. Shick (1975) found that polyps of the cnidarian, *Aurelia aurita*,
19 strobilate earlier in artificial seawater enriched with DOM relative to polyps in artificial
20 seawater without DOM.

21 The timing of early developmental events is critical to the survival of marine
22 invertebrates with 99% of embryos and larvae not surviving until metamorphosis (Vance
23 1973). In addition, for those that survive until metamorphosis there are heavy costs

1 associated with a prolonged embryonic/larval period (Pechenik 2006). Embryos and
2 larvae of many marine invertebrates are unable to feed on particulates until
3 metamorphosis or at least several days after formation of the zygote. For example, *S.*
4 *purpuratus* larvae are unable to feed on particulates until approximately five days after
5 fertilization (Strathman 1987). Though the early life stages are perilous for marine
6 invertebrates they also serve a very fundamental ecological and evolutionary role. As
7 many species are sessile or very slow moving as adults, the mobile early life stages are
8 beneficial for long distance dispersal and creating gene flow between adult populations.

9 In this experiment, we sought to determine whether the presence of DOM
10 influences the time of early embryonic development of *S. purpuratus*. More specifically,
11 the following questions were addressed: 1) Does the presence of DOM influence the time
12 of first cell division?, 2) Does the presence of DOM influence the time of hatching?, and
13 3) Does the presence of DOM influence the relationship between time of first cell
14 division and time of hatching? To do this we compared the time of first cell division and
15 time of hatching of embryos swimming in DOM-enriched, DOM-depleted, and filtered
16 seawater. FSW was 0.22 micron filtered seawater, DOM-depleted seawater was UV
17 oxidized 0.22 micron filtered seawater, and DOM-enriched seawater was UV oxidized
18 0.22 micron filtered seawater enriched with labile DOM.

19 **Effects of using high-intensity UVR treated seawater on animals**

20 In addition to these questions, the methodology allowed us to assess the effects on
21 study organisms of using high intensity UV light to oxidize DOM in seawater. This
22 method is important because it functionally removes DOM from seawater which can be
23 very difficult to accomplish. In fact, few past studies actually compare the performance

1 of marine invertebrates in seawater with and without DOM because of the difficulties
2 associated with its removal. For example, Shick (1975) sought to compare several
3 parameters for the scyphozoan *Aurelia aurita* in seawater with and without DOM by
4 using artificial seawater either enriched or not enriched with amino acids but artificial
5 seawater has since been shown to contain significant amounts of DOM (Johnson pers.
6 comm.). Though very effective at removing DOM from seawater (Beattie et al. 1961,
7 Armstrong et al. 1966, Johnson and Wendt 2006), UV-induced photo-oxidation can cause
8 the production of chemicals such as hydrogen peroxide and free radicals (Gerringa et al.
9 2004, Palenik 1991, Abele et al. 1999, Abele-Oeshger et al. 1997, Gjessing and Kalquist
10 1991) which are potentially harmful to organisms. Studies examining the effects of UV
11 irradiated water on organisms have found mixed results. For example, Alam and Ohgaki
12 (2004) and Lund and Hongve (1994) demonstrated a biocidal activity of UV-irradiated
13 water on freshwater microbes. Similarly, Gjessing and Kalquist (1991) found that treated
14 water had an algicidal effect. Contrarily, Hamilton and Carlucci (1966) found that growth
15 curves for the marine pseudomonad, *Skeletonema costatum*, and chrysophyte,
16 *Monochrysis lutheri*, were similar for organisms in UV irradiated seawater and seawater
17 controls, though on one occasion the chrysophyte had a slightly lower growth rate in the
18 treated water. They also found that the carbon-14 dioxide uptake rate of the marine
19 centric diatom, *Cyclotella nana* was usually but not always the same for organisms in
20 treated and untreated water. Finally Johnson and Wendt (2007) demonstrated that the
21 respiration rates of larvae of *Haliotis rufescens*, *Artemia* sp., *Bugula neritina*, and *S.*
22 *purpuratus* are not affected by UV-induced photo-oxidation of seawater. We were able to
23 contribute to this body of knowledge by comparing the time of early developmental

1 events of *S.purpuratus* embryos in UV treated and non-treated seawater. The
2 aforementioned treatments, DOM-depleted seawater (UV-irradiated seawater), DOM-
3 enriched seawater (UV-irradiated seawater with 4uM DOM added) and filtered seawater
4 (FSW), were used so that the labile DOM concentrations in the filtered seawater
5 treatment, which should be in the natural range of 1-4uM (Benner 2002), would fall
6 between the concentrations in the DOM-enriched and DOM-depleted conditions. This
7 allowed for the determination of any negative effects of the method while accounting for
8 any effects of the presence of DOM. Specifically, the methodology allowed the following
9 questions to be addressed: 1) Does using high intensity UV light to oxidize DOM in
10 seawater create treated water that alters the time of first cell division of embryos of *S.*
11 *purpuratus*? and 2) Does using high intensity UV light to oxidize DOM in seawater
12 create treated water that alters the hatch time of embryos of *S. purpuratus*?

13 **Variation in the timing of developmental events for the purple sea urchin, *S.***
14 ***purpuratus***

15 In addition to these questions, the methodology allowed us to explore the basic
16 biology of *S. purpuratus* embryos. By determining the time of first cell division and the
17 time of hatching for the same groups of embryos we were able to compare the variation
18 around the timing of late embryonic developmental events to the variation around the
19 timing of early embryonic developmental events. In addition, the relationship between
20 the time of first cell division and the time of hatching was assessed to determine whether
21 the rate of development of early events is predictive of the timing of later events. These
22 are important questions because they add to the understanding of the integration of early
23 and late developmental processes.

1 **METHODS**

2 **Glassware Preparation**

3 Glassware was placed in 10% HCL solution for 24 hours then rinsed several times
4 with distilled water. It was covered with aluminum foil and heated at a temperature of
5 500°C for four hours to combust residual DOM. To prevent contamination, the glassware
6 remained covered until it was used.

7 **Preparation of Seawater**

8 Seawater was collected at the Center for Coastal Marine Sciences (Avila Beach,
9 California, USA) and filtered using glass fiber filters (Fisherbrand) and 0.22 micron filter
10 paper (GE Water and Process Technologies). To remove DOM, 100mls of filtered
11 seawater were added to each of ten UV transparent, fused-quartz test tubes. The test tubes
12 were placed into a UV-oxidizer (Ace Glass #7900) and subjected to the exposure of a
13 1200 Watt UV lamp (Ace Glass # 7825-40) emitting light in the range of 222-366 nm for
14 18 hours. The irradiance value was 0000Wm². Previous experimentation has shown that
15 irradiation reduces total organic carbon (TOC) levels to about 10% of their initial levels
16 thereby functionally removing labile DOM (Johnson and Wendt 2006).

17 **Seawater Treatments**

18 Three different seawater treatments were used; UV oxidized .22 micron filtered
19 seawater (DOM-depleted), UV oxidized .22 micron filtered seawater enriched with labile
20 DOM (DOM-enriched), and .22 micron filtered seawater (FSW). In this experiment, time
21 of completion of first cleavage and time of hatching were determined for embryos in
22 different water types. The DOM-enriched water type contained 1uM each of leucine,
23 methionine, phenalanine, and valine; all of which have been shown to be transported by

1 marine invertebrates. The concentrations used were based on naturally occurring
2 concentrations of labile DOM (Benner 2002).

3 **Animal Collection, Maintenance, and Spawning**

4 *S. purpuratus* adults were collected in the Santa Barbara channel during the
5 months of June, July, and April. Sea urchins were kept in a 125-gallon tank equipped
6 with an Eco-wheel© filtration assembly (Aquatic Engineers, Camby, IN). Water
7 temperature was 14°C and they were fed once weekly with large amounts of *Macrocystis*
8 *pyrifera*. Prior to the induction of spawning, the urchins were sprayed with a dilute
9 alcohol solution and then rinsed with DOM-depleted seawater. To induce spawning
10 adults were injected with 1ml of 0.5M KCl using an 18 gauge needle attached to a 10 ml
11 syringe. Injections were made through the peritoneal membrane and targeted at the
12 coelomic cavity. To collect eggs dry females were inverted over beakers filled with
13 DOM-depleted seawater. Sperm was collected from male urchins using a micropipetter
14 and was stored at 4°C. Five individual trials were performed, with each trial utilizing one
15 parent pair.

16 **Comparison of time of first cell division and time of hatching**

17 For each trial, 4 ml of a mixture of five µL of sperm and 12.5 mL of DOM-
18 depleted water was added to the washed and settled eggs. The number of eggs fertilized
19 was checked to ensure that fertilization was complete (100%). Five mL of fertilized eggs
20 were then added to each of 6 finger bowls. Two of the bowls contained FSW, two
21 contained DOM-enriched water and two contained DOM-depleted water. All bowls were
22 placed in an incubator set to 15°C. One dish of each water type was used for cleavage

time sampling while the other was used for hatching time sampling. For both sampling periods, the three bowls were removed together from the incubator, samples were taken, and the three bowls were placed together back in the incubator.

Sampling and Counting

Cultures were sampled by first stirring the bowls to ensure an even distribution of embryos throughout the water column and then by removing 450µl of treatment water/embryo solution. All samples were placed into 0.5ml microcentrifuge tubes containing 5% formalin solution. For time of first cleavage sampling, the first samples were taken at 80 minutes post-fertilization to determine the time at which embryos began cleaving. Samples were then taken every 10 minutes for the following 2 hours or until 60% cleavage was observed in all treatments. Though samples were not taken until 100% of embryos had cleaved in each dish, dishes were checked and at least 97% cleavage occurred in each. For time of hatching sampling, the first samples were taken at 20 hours post-fertilization. Samples were then taken every 30 minutes for the following 6 hours or until 50% of embryos were hatched. All samples were counted by placing 300µl of each sample onto a 1ml Sedgewick Rafter counting cell. The cleavage or hatched state of approximately 200 embryos from each sample were determined and recorded.

Analysis

For all analyses the raw data were processed to yield the mean time at which 50% of embryos were cleaved or hatched for embryos in all treatments.

1 DOM and the timing of embryonic development

2 Three simple regressions were performed to determine the relationship between
3 time of cell division and time of hatching for embryos in each water type (Table 1). A
4 multiple regression model was fit to determine whether the relationship between time of
5 hatching and time of cell division was significantly different for embryos in different
6 water types and to determine if there was a relationship between hatch time and time of
7 cell division while accounting for water type (Table 2). A General Linear Model was fit
8 with data from just the DOM-enriched and DOM-depleted water types to determine the
9 potential difference in mean time of embryonic hatching due to the presence of DOM
10 while accounting for time of cell division (Table 3). Another General Linear Model was
11 also fit with data from just the DOM-enriched and DOM-depleted water types to
12 determine the potential difference in mean time of cell division due to the presence of
13 DOM (Table 4).

14 Effects of using high-intensity UVR treated seawater on animals

15 A general linear model was fit with the time of first cell division data from the
16 DOM-enriched, DOM-depleted, and FSW treatments to determine whether embryos in
17 the non-UV treated seawater (FSW) have similar early embryonic development rates to
18 embryos in UV-treated seawater (DOM-enriched and DOM-depleted seawater) (Table 5).
19 A second general linear model was fit with the time of hatching data from all three water
20 types to determine if embryos in the non-UV treated seawater (FSW) have similar late
21 embryonic development rates to embryos in UV-treated seawater (DOM-enriched and
22 DOM-depleted seawater) (Table 6).

1 **Variation in the timing of developmental events for the purple sea urchin, *S.***

2 ***purpuratus***

3 The coefficients of variation were calculated and compared for the time of cell
4 division and the time of hatching to further explore embryonic development of the purple
5 sea urchin, *S. purpuratus* (Table 7). In addition, the multiple regression indicated the
6 degree of relatedness between time of first cell division and time of hatching when water
7 type was accounted for (Table 2).

RESULTS

DOM and the timing of embryonic development

Simple Regressions

For embryos in FSW the time of cell division was not related to the time of hatching ($p=0.173$) (Table 1, Fig. 1). Contrary to this, the hatch time for embryos in the DOM-depleted treatment was related to time of first cell division ($p=0.038$) and the model explained a large amount of variation ($R^2_{adj.} = 74.3\%$) (Table 1, Fig. 1). For embryos in DOM-enriched water, the relationship was also significant ($p=0.05$) and the model explained a majority of the variation in the data ($R^2_{adj.} = 68.2\%$) (Table 1, Fig. 1). Interestingly, it appears that the relationship between the timing of these two developmental events is stronger when embryos are swimming in water with the same amount of DOM among trials.

Multiple Regression Model

The overall model was significant ($p=0.004$) and explained a large amount of variation ($R^2_{adj.} = 71.2\%$), indicating that the time of hatching was related to the time of cell division when water type was considered (Table 2, Fig. 1). The interaction terms DOM-depleted * Time of Cell Division and DOM-enriched*Time of Cell Division were both significant ($p=0.006$ and $p=0.10$, respectively) indicating that they are significantly different from the interaction term of FSW * time of cell division. When the model was run with either the DOM-enriched or DOM-depleted interaction terms and the FSW interaction term, the coefficients for the DOM-enriched and DOM-depleted data were not significantly different from one another ($p=0.977$ and $p=0.977$), indicating that the relationship between the time of first of cell division and time of hatching is similar for

embryos in the two treatments. Interestingly, though the slopes of the regression lines were very similar for these treatments, the intercepts appeared to be different suggesting that a main effect on time of hatching could exist between the two water types, the analysis for which is described below.

General Linear Model: Time of Cell Division with DOM-depleted and DOM-enriched

Data

Water type did not influence the time of first cell division ($p=0.743$) (Table 4, Fig. 2).

General Linear Model: Time of Hatching with only DOM-depleted and DOM-enriched

Data

Water type was a significant predictor of time of hatching when the time of first cell division was accounted for ($p=0.010$); embryos in water with DOM (DOM-enriched water type) hatched significantly later than embryos in water without DOM (DOM-depleted water type) (Table 3, Fig. 3). In every trial except for one, embryos in DOM-enriched water hatched after embryos in DOM-depleted water (Fig. 4). On average ($\pm$ S.E.), embryos in the DOM-depleted water hatched 1324.7 ($\pm$ 46.0) minutes after fertilization while embryos in the DOM-enriched water hatched 1410.4 ($\pm$ 37.2) minutes after fertilization. Embryos in the presence of DOM took approximately 85.7 minutes or 6.5% longer to hatch than embryos in water without DOM suggesting that DOM slows the progression of development.

Safety of using high-intensity UVR treated seawater

General Linear Model: Time of first cell division

Similar to the results of the previous experiment, there were no significant differences in the time of completion of first cleavage for embryos in different water types ($p=0.9$) (Table 5, Fig. 5). Average time ($\pm$ S.E.) of completion of first cell division for embryos in FSW, DOM-depleted water, and DOM-enriched water were 127.1($\pm$ 6.1) minutes, 126.8 ($\pm$ 7.2) minutes, and 123.6 ($\pm$ 5.7) minutes respectively.

General Linear Model: Time of hatching

Similar to the results for the time of first cell division, there were no significant differences in the time of hatching for embryos in different water types ($p=0.1$) (Table 6, Fig. 6). Embryos in FSW, DOM-depleted water, and DOM-enriched water hatched at 1351.9 ($\pm$ 16.9) minutes, 1324.7 ($\pm$ 46) minutes, and 1410.4 ($\pm$ 37.2) minutes respectively.

Variation in the timing of developmental events for the purple sea urchin, *S.*

purpuratus

Coefficients of Variation

The coefficient of variation for time of cell division and time of hatching were 0.11 and 0.06 respectively. Thus the variation around the mean for time of cell division was ~2 times as great as that for time of hatching (Table 7).

DISCUSSION

DOM and the timing of embryonic development

The realized energetic benefits of the transport of DOM are not well understood for marine invertebrates. Of the studies examining the putative effects of uptake of free sugars, amino acids and fatty acids on the growth, survival, and reproduction of marine metazoans only one tested for effects on the timing of development (Shick 1975). In this experiment we found that the timing of late embryonic development is dependent on the presence of DOM (Fig. 3 and 4). Embryos in seawater with DOM hatched 85.7 minutes after embryos in water without DOM, when the time of cell division was accounted for. There are several possible explanations for this counterintuitive result. First, it has been shown that embryos and larvae of *S. purpuratus* maintain biomass despite the inability to feed on particulates an effect that has been determined to be a result the nutritional benefit of the presence of DOM (Shilling and Manahan 1990). Embryos in seawater without DOM may reach a critically low biomass and need to hatch sooner in order to begin feeding on particulates.

Second, the components of DOM have been shown to act as signaling molecules for embryos and larvae of *S. purpuratus* and *L. pictus*. Data show that embryos and larvae in seawater with DOM develop short arms and large stomachs while embryos in seawater without DOM develop long arms and small stomachs in an evolutionary trade off strategy (Shilling1995). In this experiment the lack of DOM may signal to embryos that there is little particulate food in the water column causing them to hasten development in order to more quickly begin acquiring a non-abundant resource. Further experimentation is needed to decipher the reason for the difference in hatch time due to the presence of

DOM. Performing a similar experiment with multiple concentrations of DOM may elucidate whether the embryos respond to the amount of DOM in the water column or simply the presence and absence of organic matter. In addition by coating particulates with DOM molecules (see) and testing for an effect of coated particles versus non-coated particulates we could determine whether the observed effect was due to signaling or biomass influences.

We also found in this experiment that the time of cell division was predictive of the time of hatching for embryos of *S. purpuratus* in the DOM-enriched and DOM-depleted conditions but not for embryos in FSW (Fig. 1). In addition, the relationship was not altered due to the presence of DOM. For embryos in the presence of DOM the time of hatching increased by 5 minutes for every 1 minute increase in the time of cell division while for embryos in water without DOM the time of hatching increased 5.6 minutes for every 1 minute increase in the time of cell division; a difference that was not significant when variation was accounted for (Fig. 1). While the relationship did not differ because of the presence of DOM it only exists if DOM is kept constant across trials; results that are understandable when we consider that the time of hatching was dependent on the presence of DOM. The amount of DOM in FSW was not quantified from trial-to-trial and probably fluctuated, thereby altering the time of hatching for embryos. This in turn caused embryos from FSW to not show a relationship between time of cell division and time of hatching. These results demonstrate the importance of understanding the effects of DOM to marine invertebrates as the relationship was only significant when the amount of DOM was held constant from trial to trial.

Safety of using high-intensity UVR treated seawater

A problem with studying the effects of the presence of DOM on marine invertebrates is the difficulty in removing DOM from seawater. Because of this, some studies have used either enrichment treatments (for example, Shilling and Manahan 1990), which do not address questions related to the absence of DOM, or have used methods to exclude DOM which were later shown to not actually do so (for example Shilling, 1995). In our experiment we sought to further test for the potentially adverse effects of one method of removing DOM, exposing seawater to high intensity UVR, by using filtered seawater as a treatment water type in addition to the DOM-enriched and DOM-depleted water conditions. From the results, it appears that the timing of embryonic development of the purple sea urchin, *S. purpuratus*, is not adversely affected by using high intensity UVR to oxidize DOM in seawater (Fig. 5 and Fig. 6). Embryos in the FSW treatment took approximately the same amount of time to complete the first cellular division and to reach their hatching point as embryos in the UVR exposed water types. As *S. purpuratus* is sensitive to environmental perturbations early in development this study provides evidence in support of using this methodology for studies of this nature and corroborates the findings of Johnson and Wendt (2007).

Variation in the timing of developmental events for the purple sea urchin, *S.*

purpuratus Embryos of *S. purpuratus* are a model system for developmental biology but there are still some facets of the species biology that are not well understood. While the time of certain developmental events such as first cell division and hatching are clearly documented information about the natural variation associated with the average

time of occurrence of such events is lacking. In this experiment, the coefficients of variation were calculated for the average time of completion of the first cellular division (19.49) and the average time of hatching (5.91) and there was approximately three times more variation around the mean for time of the first cell division (Table 7). These results were unexpected as the first cellular division occurred 118 minutes after fertilization on average while hatching occurred 1378 minutes post fertilization on average. With a greater amount of time for developmental trajectories to diverge from one another it seems counterintuitive that embryos of the species have greater relative variation in the time to reach first cell division than around the time of hatching. These results suggest that there are greater differences in developmental rates early in embryonic development and that upon completion of cell division embryos return to similar developmental trajectories. Embryos of the purple sea urchin may be more susceptible to environmental perturbations early in development which cause there to be more variation around the average time of cell division than the time of hatching. In addition, this may suggest that experiments examining the effects of a parameter on the time of cell division should be extended to include effects on the timing of later developmental events to better understand the real effects of the parameter on the ecology of the organism

Figure 2. Time of cell division for batches of embryos in water with (DOM-enriched) and without (DOM-depleted) DOM (mean $\pm$ 1 SE, N=5). The presence of DOM did not significantly influence the time of first cell division ($p>0.05$).

Figure 3. Time of hatching for embryos in seawater with (DOM-enriched) and without (DOM-depleted) DOM (mean $\pm$ 1 SE, N=5). The presence of DOM causes embryos to hatch significantly later ($p < 0.05$).

Figure 4. Time of hatching for batches of embryos from six different parent pairs (trial) in either DOM-enriched or DOM-depleted seawater. With the exception of embryos from trial 2, embryos from each parent pair took longer to hatch when in seawater with DOM present (DOM-enriched). Note that data points for trial 2 are superimposed.

Figure 5. Time of first cell division for batches of embryos in DOM-depleted, DOM-enriched, and filtered seawater (mean $\pm$ 1 SE, N=5). The time of first cell division was not significantly different for embryos in water exposed to high intensity ultra-violet radiation (DOM-depleted and DOM-enriched water types) relative to embryos in water not exposed to UVR ($p < 0.05$).

Figure 6. Time of hatching for batches of embryos in DOM-depleted, DOM-enriched, and filtered seawater (mean $\pm$ 1 SE, N=5). The time of hatching was not significantly different for embryos in water exposed to high intensity ultra-violet radiation (DOM-depleted and DOM-enriched water types) relative to embryos in water not exposed to UVR (FSW) ($p < 0.05$).

Table 1. Regression Tables for the relationship between the time of cell division and the time of hatching for embryos in DOM-enriched, DOM-depleted and FSW.

WATER TYPE	Predictor	Coeffecient	SE Coefficient	T-test	P-value	Regression Equation	R-squared
DOM DEPLETED	Constant	601.1	205.5	2.92	0.061	Hatch Time = 601 + 5.71(Time of Cell Division)	74.30%
	Time of Cell Division	5.707	1.611	3.54	0.038		
DOM ENRICHED	Constant	712.8	226.6	3.15	0.051	Hatch Time = 713 + 5.64(Time of Cell Division)	68.20%
	Time of Cell Division	5.641	1.824	3.09	0.054		
FSW	Constant	1606.8	143.8	11.17	0.002	Hatch Time = 1607 - 2.00(Time of Cell Division)	35.10%
	Time of Cell Division	-2.004	1.126	-1.78	0.173		

Table 2. Multiple regression model to determine if the relationship between time of cell division and time of hatching is dependent on water type.

Predictor	Coeffecient	SE Coefficient	T-test	P-value	R-squared
Constant	1606.8	208.3	7.71	0	71.20%
Time of Cell Division	-2.004	1.632	-1.23	0.25	
DOM-depleted Indicator	-1005.6	271.4	-3.71	0.005	
DOM-enriched Indicator	-893.9	298.3	-3	0.015	
DOM-depleted Indicator * Cell Division	7.711	2.126	3.63	0.006	
DOM-enriched Indicator * Cell Division	7.645	2.37	3.23	0.01	
Regression Equation					
Hatch Time= 1607 - 2.00 Time of Cell Division - 1006 DOM-depleted Ind. - 894 DOM-enriched Ind + 7.71 DOM-depleted Interaction + 7.65 DOM-enriched Interaction					

Table 3. ANOVA table for differences in time of hatching for embryos in two water types when time of cell division was accounted for.

SOURCE	D.F.	S.S.	F-test	P-value
Water Type	1	26395	12.51	0.01
Time Of Cell Division	1	55237	26.17	0.001
Error	7	14774		

Table 4. ANOVA table for differences in time of cell division for embryos in two water types.

SOURCE	D.F.	S.S.	F-test	P-value
Water Type	1	24.6	0.11	0.743
Error	8	1711.3		

Table 5. ANOVA table for differences in time of cell division for embryos in three water types.

SOURCE	D.F.	S.S.	F-test	P-value
Water Type	2	36.3	0.09	0.915
Error	12	2444.2		

Table 6. ANOVA table for differences in time of hatching for embryos in three water types when time of cell division was accounted for.

SOURCE	D.F.	S.S.	F-test	P-value
Water Type	2	24452	2.81	0.103
Time Of Cell Division	1	27868	6.4	0.028
Error	11	47877		

CHAPTER FOUR

Meta-analysis: The ecological effects of DOM transport are more likely to occur in life stages after which the majority of uptake occurs

INTRODUCTION

The epidermal transport of dissolved organic matter (DOM) by marine invertebrates has been well documented. Of the approximately thirty-one marine invertebrate phyla species from twelve phyla have been tested for DOM transport and members of only one phyla, the Arthropoda, did not take up DOM (Ferguson 1982, Stephens and Schinske 1961) (Table 1). For example, Stephens and Schinske (1961) tested for the uptake of glycine by thirty four species from eleven phyla and all species except the six species of Arthropods transported the amino acid. Though the transport of DOM has been shown for many species, very few studies have directly assessed the effects of uptake on ecological parameters such as survival, growth, and reproduction (Johnson and Wendt 2007). Of the eleven phyla and many species for which DOM uptake has been demonstrated, only ten species from four phyla have been directly tested to determine putative realized energetic benefits of transport.

If there is an effect of DOM transport it is likely that it is exhibited only at a certain point in an organism's life cycle. Wendt and Johnson (2006) suggested that the importance of DOM uptake might be manifested in latent effects. Latent effects are defined as "characteristics that originate in embryonic and larval experiences but become visible only in juvenile or adult stages" (Pechenik 2006). Johnson and Wendt (2007) demonstrated this when they found that lophophores of the ancestrulae of the bryozoan, *Bugula neritina*, were larger when larvae were swimming in DOM-enriched seawater prior to metamorphosis. The importance of DOM transport to marine invertebrates may manifest in latent effects as embryos and larvae need time to accumulate and store quantities of free amino acids, sugars, and fatty acids before effective utilization can

occur. Likewise, incorporation of absorbed DOM into developing structures may be time dependent. Extending this idea, the realized energetic benefits of the uptake of DOM may commonly be exhibited in any life stage after the stage in which the majority of uptake occurs. For example, the importance of uptake may be manifested through maternal effects which Mousseau and Dingle (1991) defined as “developmental influences extended across life cycle stages in which genetic or environmental differences in the maternal generation are expressed as phenotypic differences in the offspring”. Shick (1975) demonstrated this with his study of DOM and the cnidarian, *Aurila aurita*. He showed that polyps in water with DOM produce more ephyrae and a greater percentage of healthy ephyrae relative to starved controls. In addition to latent effects and maternal effects, which are the common major transitory periods in an organisms life cycle, effects may also be expressed across both juvenile-adult and embryo-larvae boundaries of the life cycle. In this study we sought to test the hypothesis that effects of DOM transport on growth, survival, and reproduction are more likely to occur in life stages subsequent to the stage in which the majority of uptake occurred.

METHODS

To test the hypothesis that effects of DOM transport are more likely to occur in life stages following that in which uptake occurred, all known studies examining ecological effects of DOM uptake were reviewed (Table 2). For all studies, each result was placed into one of the following four categories: 1) the authors tested across life stages and an effect of DOM uptake was found, 2) the authors tested within a life stage and an effect of DOM uptake was not observed, 3) the authors tested across life stages and an effect was not found, 4) the authors tested within a life stage and an effect was found (Table 3). Studies in the first two categories support the hypothesis that the benefits of DOM uptake are more likely to be realized after the stage in which uptake occurs while studies in the last two categories do not. For example, Manahan (1982) tested the effect of DOM uptake on shell length of the oyster, *Crassostrea gigas*. Because no effect was found and DOM transport occurred during the larval phase as did the quantification of shell length the parameter was placed in category 2. The result thus supported the hypothesis. Based on these criteria, 24 separate results were assessed from 10 different journal articles. A chi-squared test was then used to determine if the ratio of results that supported the hypothesis were greater than expected by chance alone.

RESULTS AND DISCUSSION

Based on these criteria, 24 results were assessed from 10 different studies. Of these, eight were in category one, eight were in category two, one was in category three, and seven were in category four (Table 3). The chi-squared number was 4.297 with 1 degree of freedom yielding a significant p-value of 0.039. With 16 of 24 results (65%) supporting the hypothesis this analysis suggests that the ecological benefits of DOM uptake are more likely manifested in stages after the stage in which uptake occurred (Fig. 1). This is intuitive as the accumulation of a specific amount of free amino acids, monosaccharides, and fatty acids may need to occur before other changes based on that accumulation manifest. Of the eight results that did not support the hypothesis six were testing for biomass differences within the larval stage, the life stage when uptake occurred. Of these, each study found that biomass increased or remained constant in animals exposed to DOM. Because the larvae tested were non-feeding and surviving on endogenous reserves, the maintenance of biomass due to DOM uptake is an important occurrence for their survival. In conclusion, it appears that the benefits of DOM uptake are usually manifested after the stage in which uptake occurs and if benefits do occur within the stage in which uptake occurs, they are in the form of biomass increases or maintenance of endogenous reserves. Intuitively, it seems that organisms may utilize the added biomass not only as an energy source but to affect changes, which have been observed, in later life stages. For example, Johnson and Wendt (2007) demonstrated that lophophores of the ancestrulae of *Bugula neritina* juveniles were larger if the larvae had developed in seawater with DOM.

A weakness with pooling the results data from past studies was that negative results were used to draw conclusions about the hypothesis. Results in category two of this analysis were used to support this study's hypothesis despite the fact that in the original experiments no effect were found for those parameters. For example, Ferguson (1980) found that there was no effect of DOM on organ sizes of the sea star *Echinaster*. Because the organ sizes were determined in the adult life stage, the same stage in which the sea stars were exposed to DOM, the result was used to support our hypothesis. The problem with using these data is that there may be effects for a parameter within a life stage in which DOM was transported that were simply not examined. For example, adult *Echinsater* may transport DOM and use it to increase movement but if movement was not examined we would falsely assume that the data for the species supported the hypothesis of this study. Though this analysis was the best that could be done using data that had already been gathered on the subject, more relevant information about the generalized affects of DOM on marine invertebrates can be generated in the future by testing for multiple effects of DOM transport for one species. For example, a more rigorous way to test the hypothesis would be an experiment which tested for a biomass effect, multiple effects within a life stage, and multiple effects across life stages for a single species.

With the addition of the data gathered from these experiments (see CH 2 and CH3) to the information in the literature examining the ecological effects of DOM transport to *S. purpuratus*, we begin to better understand the realized energetic benefits of DOM transport to the species. In the present experiment we tested the affects of DOM on the timing of embryonic development. The time of completion of the first cellular division and the time of hatching were assessed for embryos reared in the presence and

absence of DOM. While no differences were observed for the time of first cell division, suggesting that DOM does not influence the timing of early embryonic development, there was an effect on the time of hatching, an event that occurs close to the time of transition from embryo to larvae for the species. These results and results from other studies examining the ecological effects of DOM uptake by *Strongylocentrotus purpuratus* generally fit the model suggested above. In the first experiment of this study the tested parameter was within the embryonic life stage and no effect was observed supporting the idea that the benefits of DOM uptake are not usually manifested during the stage in which most transport occurs. In the literature already published on the topic, Shilling (1995) and Miner (2004) both tested larval arm length for embryos reared in different concentrations of DOM. Their results, which found that larvae of embryos swimming in DOM-enriched conditions had significantly shorter arms, support the concept that the manifestations of DOM uptake usually occur after the stage in which the majority of uptake occurred. Lastly, Shilling and Manahan (1990) measured the biomass of larvae swimming in artificial seawater without DOM and filtered seawater with DOM and found that larvae in seawater with DOM had significantly greater dry weight. They demonstrated an effect thus supporting the idea that if an effect is seen within the life stage during which transport occurs it is usually an increase in endogenous reserves. Finally, in the second experiment of this study there was an effect on the tested parameter, time of hatching, despite the fact that the tested parameter and all DOM transport occurred in the embryonic life stage. Though this may initially seem to counter the proposed hypothesis, the time of hatching is very close to the transition point between the embryo and larval life stages of the purple sea urchin. In addition, these data may

suggest that the realized energetic effects of DOM transport are not inherently manifested because of a change in life stage, but rather simply that a certain amount of time must pass in order for enough DOM transport to occur before changes are manifested. To further elucidate the importance of DOM to marine invertebrates, future studies should test for multiple effects within life stages, multiple effects across lifestages, and biomass effects.

Figure 1. A) Percentage of results that support the hypothesis that the effects of DOM transport primarily occur in the life stage after that in which the majority of transport occurred. B) Of the results that do not support the hypothesis all but one demonstrates an effect on biomass within a life stage.

Table 1. List of all marine invertebrate phyla. X's indicate if species within the phyla have been tested for DOM transport, if transport was found, if an ecological effect of transport has been tested for, and if an ecological effect was found.

MARINE INVERTEBRATE PHYLA	Tested for DOM uptake	DOM uptake Found	Ecological Effect Tested	Ecological Effect Found
Porifera	x	x		
Placozoa				
Rhombzoans				
Orthonectida				
Cnidaria	x	x	x	x
Ctenophora				
Platyhelminthes				
Nemertea	x	x		
Rotifera				
Gastrotricha				
Kinorhynchs				
Nematoda				
Nematomorpha				
Entoprocta				
Gnathostomulida				
Priapula				
Loricifera				
Cycliophora				
Sipuncula	x	x		
Echiura	x	x		
Annelida	x	x		
Tardigrada				
Arthropoda	x			
Mollusca	x	x	x	x
Phoronida				
Ectoprocta	x	x	x	x
Brachiopoda				
Echinodermata	x	x	x	x
Chaetognatha	x	x		
Hemichordata	x	x		
Chordata	x	x		

Table 2. For all studies in the literature examining the effects of DOM transport on marine invertebrates the following are listed: the phyla of the species used in the study, the lifestage of the species during the study, the effect that the study was testing for, whether the tested was within a lifestage or between lifestages, if an effect was observed, if the result supported the hypothesis, and the citation for the experiment.

PHYLUM	STAGE STUDIED	TESTED PARAMETER	LIFE STAGE	EFFECT SEEN	HYPOTHESIS SUPPORTED	LITERATURE CITED
Cnidaria	adult/juvenile	less strobilation	among	yes	yes	Shick, 1975
Cnidaria	adult/juvenile	strobilation timing	among	yes	yes	Shick, 1976
Cnidaria	adult/juvenile	fewer ephyrae	among	yes	yes	Shick, 1977
Cnidaria	adult/juvenile	fewer healthy ephyrae	among	yes	yes	Shick, 1978
Cnidaria	adult	survival	within	no	yes	Shick, 1979
Cnidaria	adult	buds produced	within	no	yes	Shick, 1980
Mollusca	larval	increased survival	within	yes	no	Gustafson, 1980
Mollusca	larval	greater reserves	within	yes	no (biomass)	Gustafson, 1980
Mollusca	larval	biomass	within	yes	no (biomass)	Jaekle and Manahan, 1992
Mollusca	larval	shell length	within	no	yes	Manahan, 1882
Ectoprocta	larval/juvenile	lophophore size	among	yes	yes	Johnson and Wendt, 2006
Ectoprocta	larval/juvenile	metamorphic completion	among	yes	yes	Johnson and Wendt, 2007
Ectoprocta	larval/juvenile	metamorphic initiation	among	no	no	Johnson and Wendt, 2008
Echinodermata	adult	behavior	within	no	yes	Ferguson, 1980
Echinodermata	adult	mucous production	within	no	yes	Ferguson, 1980
Echinodermata	adult	organ size indices	within	no	yes	Ferguson, 1980
Echinodermata	adult	biomass	within	no	yes	Ferguson, 1980
Echinodermata	embryonic/larval	arm length	among	yes	yes	Shilling, 1995
Echinodermata	embryonic/larval	biomass	within	yes	no (biomass)	Shilling and Manahan, 1990
Echinodermata	embryonic/larval	arm length	among	yes	yes	Shilling, 1995; Miner 2005
Echinodermata	embryonic	biomass	within	yes	no (biomass)	Shilling and Bosch, 1994
Echinodermata	embryonic	biomass	within	yes	no (biomass)	Shilling and Bosch, 1995
Echinodermata	embryonic	biomass	within	yes	no (biomass)	Shilling and Bosch, 1996
Echinodermata	embryonic	cleavage/ hatching	within	no	yes	Hodges, 2009

Table 3. To test the hypothesis that the manifestation of DOM transport is most likely to occur in the lifestage after that in which uptake occurs each result from the literature was placed into one of four scenarios (A). The two-by-two table (B) used for testing the hypothesis (B) and the chi-squared results (C).

A.	ACROSS	WITHIN
OBSERVED	Sc. 1	Sc. 4
NOT OBSERVED	Sc. 3	Sc. 2

C. Chi-Squared Value	4.279
DF	1
P-Value	0.039

B.	ACROSS	WITHIN
OBSERVED	8	7
NOT OBSERVED	1	8

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