Using latent effects to determine the ecological importance of dissolved organic matter to marine invertebrates

Dean E. Wendt[1] and Collin H. Johnson[2]

Biological Sciences Department and Center for Coastal Marine Sciences, Cal Poly, San Luis Obispo, CA, USA

Synopsis The uptake and utilization of dissolved organic matter (DOM) by marine invertebrates is a field that has received significant attention over the past 100 years. Although it is well established that DOM is taken up by marine invertebrates, the extent to which it contributes to an animal's survival, growth, and reproduction (that is, the ecological benefits) remains largely unknown. Previous work seeking to demonstrate the putative ecological benefits of DOM uptake have examined them within a single life stage of an animal. Moreover, most of the benefits are demonstrated through indirect approaches by examining (1) mass balance, or (2) making comparisons of oxyenthalpic conversions of transport rates to metabolic rate as judged by oxygen consumption. We suggest that directly examining delayed metamorphosis or the latent effects associated with nutritional stress of larvae is a better model for investigating the ecological importance of DOM to marine invertebrates. We also provide direct evidence that availability of DOM enhances survival and growth of the bryozoan *Bugula neritina*. That DOM offsets latent effects in *B. neritina* suggests that the underlying mechanisms are at least in part energetic.

Introduction

All organisms ranging from the simplest bacterium to the most sophisticated multicellular animal need energy for maintenance, growth, and reproduction, and a wide variety of mechanisms supplying that energy have evolved. One such system that has developed among aquatic organisms involves the cellular transport and utilization of dissolved organic matter (DOM) directly from the surrounding medium. It is well known that heterotrophic bacteria and protozoans use this abundant source of carbon and nitrogen for growth and reproduction (for example, Crawford and others 1974; Williams and others 1976; Williams 2000). Although first hypothesized over 100 years ago for metazoans such as sponges and cnidarians (see Jørgensen 1976 for a historical review), early progress in DOM research suffered from difficulties associated with measuring minute fluxes of DOM relative to large ambient concentrations. The application of more sophisticated technologies such as radiotracer studies and HPLC led to a renewed interest in the field during the past several decades and, although we have a very good understanding of the types of compounds involved and the rates of transport, the actual contribution of DOM to the nutrition, growth, reproduction, and survival of marine invertebrates remains largely unknown. The paucity of data in this area is in part due to lack of a good model systems for testing the ecological effects of DOM. In the few cases where experiments have been performed, researchers have exclusively looked for effects within a single life stage of an animal (for example, Ferguson 1980).

Over the past few decades biologists studying marine invertebrates have begun to recognize and demonstrate that events occurring in one portion of a life cycle can have dramatic and long-lasting effects at another stage in the life cycle (see Pechenik and others 1998; Pechenik 2006; this volume for recent reviews). For example, delayed metamorphosis or short-term nutritional stress of larvae can affect postmetamorphic growth, survival, or reproduction in many marine invertebrates (for example, Woollacott and others 1989; Miller 1993; Wendt 1998; Phillips 2002; Wacker and von Elert 2002). Following Pechenik (2006) we use the term "latent effects" to refer to such phenomena. We suggest that examining latent effects in embryos or larvae are a novel and direct method of determining the ecological importance of DOM transport. We also suggest that this approach provides a suitable test for the hypothesis that certain latent effects are mediated in part through depletion of larval energy reserves (for example, Pechenik and Eyster 1989; Pechenik and others 1993; Wendt 1998; Thiyagarajan and others 2002; Marshall and others

2003; reviewed by Pechenik and others 1998; Pechenik 2006).

In this paper we will provide (1) a brief review of what is known about the importance of DOM for growth, reproduction, and survival of marine invertebrates, (2) an overview of latent effects that result from delayed metamorphosis or nutritional stress, (3) a discussion of previous ways of determining the importance of DOM to marine invertebrates, (4) the results from a model system that utilized a direct examination of latent effects to determine ecological importance of DOM, and (5) suggestions for other suitable means of investigating the longstanding and as yet unanswered question—does DOM matter for marine invertebrates?

Importance of DOM for growth, reproduction, and survival of marine invertebrates

The earliest reports of the use of DOM by invertebrates date back to the latter part of the 19th century. Formal investigation of utilization of DOM by marine invertebrates was first widely acknowledged by August Pütter (1908) who measured oxygen consumption for a marine protozoan and determined that energy gained from clearing food particles from seawater was probably not sufficient to support the organism's basal metabolic rate. Indeed, he showed that the protozoan would have to remove all the food contained in 9.4 liters of seawater in 1 h to support its metabolism. This indirect evidence led Pütter to invoke the use of DOM as a possible supplement to meet basic metabolic needs. His suggestion was further supported by additional calculations showing that the DOM necessary to support the organism's metabolic demand was contained in about 0.5 ml of seawater. Pütter's conclusions, however, are necessarily based on accurate measurement of several variables as follows: (1) metabolic rate, (2) concentrations of DOM, (3) concentration of particulate food, and (4) clearance rates. Clearly, inaccuracies in any of these parameters could substantially change the strength of his conclusions. In fact, Krogh (1931) pointed out that Pütter had greatly underestimated phytoplankton concentrations and greatly overestimated the abundance of DOM. Krogh went on to cite other investigators who called into question Pütter's measurement of metabolic rates, specifically maintaining that his estimates were probably too high. In Krogh's (1931) critical and extensive review he generally concluded "that there is no convincing evidence that any animal takes up dissolved organic substances from natural water in any significant amount…" but he also stated in his conclusions that there is "strong evidence from experiments… that tadpoles, mussels, and probably many other animals can absorb organic substances from fairly concentrated solutions and at least for a considerable period thrive and grow without particulate food." In sum, Krogh's basic conclusions were that animals at artificially high concentrations have the ability to take up and even utilize DOM, but they do not have this capacity to any appreciable extent under natural conditions. That is, the uptake and utilization of DOM, according to Krogh, is not significant at ecologically realistic concentrations.

Decades of further research following this initial debate have still not conclusively determined the extent to which invertebrates are able to utilize DOM under natural conditions. In particular, 2 questions need to be resolved to accurately assess the ecological significance of DOM: (1) Can invertebrate animals take up DOM across their integument under ambient concentrations? (2) If so, does the transported DOM contribute to maintenance, growth, reproduction, or survival?

Analytical techniques have advanced significantly since Krogh and Pütter's time. Through a series of elegant experiments, there is now ample direct evidence showing that invertebrates can take up DOM across their integument at close to ambient concentrations (for example, Manahan and others 1982; Manahan and others 1983; Davis and Stephens 1984a). However, how DOM contributes to metabolic maintenance, growth, reproduction, or survival remains a contested issue. Part of the reason this controversy has continued for so long is that the vast majority of data deal mostly with the uptake of DOM, while there remains almost no direct evidence assessing parameters such as growth, reproduction, or survival of animals in the presence and absence of DOM.

Evidence for the uptake of DOM by invertebrates

Following the critical review of Krogh (1931), research on utilization of DOM by invertebrates stalled for several decades (Jørgensen 1976). Investigations eventually revived because of major technological improvements that allowed direct measurement of the uptake of DOM. At the forefront was the classic study by Stephens and Schinske (1961), who documented uptake of DOM for a large group of soft-bodied invertebrates using colorimetric techniques. They showed that representative animals from 10 phyla removed significant quantities of glycine from solution during an observation period of 16–24 h. The only phylum tested that did not contain animals capable of removing this amino acid was Arthropoda. The researcher's findings, however, were brought into question because

the concentration of compounds being removed from solution was far in excess of normal levels in seawater (mM versus μM). To address this criticism, Stephens (1962) used C^{14}-labeled glucose to show uptake by the solitary coral *Fungia* sp. at concentrations as low as 6 μM. The results with ^{14}C-labeled compounds were extended through the use of autoradiography and it was clear that transported amino acids were being incorporated into tissues (for example, Ferguson 1967; Manahan and Crisp 1982). Concurrent with, and following, the early work of Stephens and his colleagues, several investigators showed that polychaetes, echinoderms, sponges, coelenterates, molluscs, and pogonophorans could take up and accumulate DOM in their tissues (see Jørgensen 1976 for a detailed review). Perhaps the most taxonomically diverse study after the work of Stephens and Schinske was that of Ferguson (1982). He demonstrated for 21 species in 7 phyla that DOM could *potentially* account for 1–10% of metabolism (see discussion below). In fact, he showed levels of net uptake in 2 echinoderm species that were sufficient to account for more than 20% of the organisms' metabolism. For free-swimming invertebrate larvae, the ability to utilize DOM would be especially beneficial; larvae are commonly transported away from adult habitats and must continue swimming until they encounter a site suitable for settlement and metamorphosis, a process that could last for months (see Pechenik 1990 for review). This ecological rationale was the basis for Manahan and Crisp's (1982) groundbreaking work on larval stages of mussels (see also, Manahan and others 1982; Manahan and Richardson 1983). Moreover, for nonfeeding larvae that must rely solely on endogenous reserves, use of DOM could have a clear advantage in terms of survival and dispersal ability (Jaeckle and Manahan 1989a; Jaeckle and Manahan 1989b), especially considering that detrimental latent effects arising from prolonged periods of swimming (for example, Woollacott and others 1989; Pechenik and others 1993; Wendt 1996, 1998) have been shown to have energetic underpinnings at least in part (Wendt 2000; Marshall and others 2003).

Latent (carryover effects) in marine invertebrates

The idea that embryonic and for marine invertebrates larval experience can have dramatic and even long-lasting effects on postmetamorphic growth, reproduction, and survival has received significant attention over the past decade (see Pechenik and others 1998; Pechenik 2006 for recent reviews). Recent work has demonstrated that environmental stresses as embryos or larvae such as low nutrition (for example, Phillips 2002, 2004), delayed metamorphosis (for example, Woollacott and others 1989; Pechenik 1993; Wendt 1998), exposure to pollution (for example, Ng and Keough 2003), or salinity stress (Pechenik and others 2001) can produce latent effects; that is, the effects of experiences during early development are not manifest until after metamorphosis. For example, a prolonged larval stage in bryozoans has been shown to affect postmetamorphic size (Wendt 1996), growth (Woollacott and others 1989; Wendt 1998), and reproduction (Wendt 1998). Likewise, Maldonado and Young (1999) demonstrated reduced postmetamorphic size and lower rates of colony development after prolonged swimming by larvae of the sponge *Tedina* sp. In addition to prolonged swimming, nutritional stress can also affect juvenile performance. For instance, Phillips (2002, 2004) demonstrated for the mussel *Mytilus galloprovincialis* that nutritional stressing of larvae affected growth and survival of juveniles. In a different study reduced growth rates were documented for juveniles of the slipper-shell snail *Crepidula fornicata* following limitation of food during early larval life (Pechenik and others 2002). Interestingly, in *C. fornicata* juveniles exhibited reduced growth rates even when larvae were returned to normal food concentrations and had resumed normal growth rates prior to metamorphosis. Thus, despite the fact that larvae had apparently recovered from the nutritional stress as juveniles they still exhibited latent effects in the form of reduced growth rates. For nonfeeding larvae, higher rates or longer duration of energy expenditure can be viewed as a form of nutritional stress. Marshall and colleagues (2003) showed that increased swimming activity by larvae of the ascidian *Diplosoma listerianum* resulted in slower colony development. Many species of nonfeeding larvae tend to show latent effects after prolonged larval swimming (for example, Nielson 1981; Woollacott and others 1989; Orellana and Cancino 1991; Pechenik and others 1993; Wendt 1996, 1998; Maldonado and Young 1999).

It has often been suggested that the latent effects of prolonged larval swimming are mediated through depletion of energy stores. Energy used in swimming is at the expense of that otherwise available for metamorphosis and postmetamorphic growth, resulting in smaller and slower-growing individuals (for example, Pechenik and Eyster 1989; Pechenik and others 1993; Wendt 1998; Thiyagarajan and others 2002; Marshall and others 2003; reviewed by Pechenik and others 1998; Pechenik 2006). In addition to energetic underpinnings, changes in transcription or translation at key times during development could shift or slow normal developmental processes as suggested by Pechenik and

colleagues (1998). We suggest that in cases where DOM has the ability to offset latent effects arising from nutritional stress or protracted larval swimming the underlying mechanisms must be at least in part due to depletion of energy stores. For example, our results with the nonfeeding larvae of the bryozoan *Bugula neritina* show that access to DOM significantly affects the ability of individuals to complete metamorphosis as well as their postmetamorphic size (C. H. Johnson and D. E. Wendt, unpublished data). Results such as these clearly support the energy-limitation hypothesis, although they do not discount other mechanisms of action.

Indirect determination of the ecological importance of DOM

The ability to take up DOM has been convincingly established for certain marine invertebrates, although it remains to be ascertained whether DOM actually contributes to growth, reproduction, or survival. The basic hypothesis concerning utilization of DOM stems from the rationale that marine invertebrates, because of their limited or variable food supply, need an additional energy source to meet their metabolic requirements. In light of the fact that DOM is very abundant in marine waters, the use of DOM in this way seems plausible. Despite this clear rationale, it is surprising that after almost a century of formal investigation the actual contribution of DOM to the nutritional reserves is still not understood for any animal. In part, this lack of understanding stems from deflection of attention over the past 40 years toward determining whether animals were the main agents of transport of DOM, and whether they could carry out transport at naturally occurring concentrations. Through a series of rigorous and detailed experiments in several laboratories (for example, Grover Stephens, Donal Manahan) it has been satisfactorily demonstrated that many marine invertebrates can and do transport DOM. Most of the data, however, do not address by *direct* methods the ecological benefit of the uptake of DOM. More explicitly, the following question has yet to be answered: How much does the utilization of DOM contribute to the energy budget of an animal and to its growth, reproduction, and survival? These ecologically based questions have been the underlying driving force of research in this area since its formal inception by Pütter in the early 1900s, but they still remain unexplored.

The 2 most common indirect methods for estimating the utilization of DOM by marine invertebrates are (1) comparisons of metabolic rate and energy gain from the assumed catabolism of transported DOM (for example, Jaeckle and Manahan 1989a; Jaeckle and Manahan 1989c; Jaeckle 1994; Ben-David-Zaslow and Benayahu 2000), and (2) using mass balance to compare the energy required to that available from endogenous sources (for example, Jaeckle and Manahan 1989b; Shilling and others 1996; Wendt 2000). For example, comparisons of metabolic rate and energy gain from the assumed catabolism of transported DOM use the following protocol: The metabolic rate of an animal is measured via oxygen consumption per unit time (that is, mol of O_2 consumed per individual per hour). In a separate experiment the transport rate of a dissolved compound (for example, glucose or an amino acid) is determined (mol transported per individual per hour). By then converting the transported compound into oxygen equivalents (that is, the amount of O_2 required to catabolize the transported compound) it can be estimated how much DOM is contributing to an animal's metabolism. For instance, the catabolism of 1 mol of alanine requires 3 mol of O_2. Thus, for every mol of dissolved alanine transported, 3 mol of O_2 are respired by the animal, assuming the animal is indeed using alanine as a metabolic substrate. Therefore, by dividing the oxygen equivalents of the transported DOM by the total O_2 consumption of the animal, one can estimate the percent contribution of DOM to the animal's energy requirements. Some authors, and likely many readers, have often concluded that these types of calculations demonstrate the percentage of metabolic demand that is met through the use of DOM.

While this indirect approach is certainly intriguing, it has associated with it a number of weaknesses, and caution should be used when relying on these calculations to interpret claims of DOM's nutritional benefit. For example, in most cases where this approach is used it is not determined if the carbon from the transported compound is in fact respired as CO_2 by the animal. Thus, there is no direct way to interpret if the transported compound was used for respiration. In cases where radiotracers are used in the absence of HPLC, it is not clear if there is any associated efflux of the transported compound, thereby weakening basic conclusions. Furthermore, the conclusions necessarily rely on accurate measurement of respiration rates. One particularly troubling aspect with many studies that compare oxygen equivalents of transport rates to actual O_2 consumption is that measured respiration rates are almost always obtained in water that has not been enriched with DOM. The assumption is that oxygen consumption of animals does not increase in the presence of DOM. Jaeckle and Manahan (1992), however, showed that oxygen consumption of larvae of the oyster *Crassostrea gigas* increased by as much as 53% in the presence of 1 μM dissolved glucose, and calculated

that the increase in the average metabolic rate of these larvae exceeded the energy gained by the uptake of glucose. They found similar results for echinoderm larvae in the presence of dissolved amino acids. This phenomenon of elevated respiration in the presence of DOM has also been demonstrated for adult invertebrate animals (for example, Shick 1975). Clearly, an increase in measured respiration rate necessarily decreases the calculated contribution of DOM. Another issue emerging over the past few years is that continuous measurement of respiration using Clarke-type polarographic oxygen electrodes could be greatly underestimating respiration rates under certain conditions (Hoegh-Guldberg and Manahan 1995; Marsh and Manahan 1999). If this is the case, the indirect calculations outlined above may grossly overestimate the potential contribution of DOM to metabolic demand. The extent of the latter problem needs to be fully explored.

In the case of mass balance approaches, the calculated energy used from O_2 consumption is compared with the energy contained in catabolized biochemical substrates such as lipids, proteins, or carbohydrates. By this method, metabolic rate is also estimated by determining oxygen consumption. The amount of O_2 consumed is then converted to energy equivalents (that is, calories or joules). For instance, if 1 mol of lipid is catabolized through aerobic respiration, 441 kJ of energy will be released. Similarly, the values are 527 kJ/mol of O_2 for protein, and 473 kJ/mol of O_2 for carbohydrate (based on Gnaiger 1983). Thus, from the total amount of O_2 consumed one can readily calculate the energy released by oxidative processes, provided the relative composition of substrates used for respiration is known. The calculated energetic value (for example, mJ) based on the amount of O_2 consumed over time is then compared with energy contained in an equivalent mass of biochemical substrate depleted during that same period of time. For example, the combustion enthalpy for 1 g of lipid is 39.5 kJ (Gnaiger 1983). Thus, if it were found that lipid content of an invertebrate larva decreased by 1 ng (that is, 1.00×10^{-9} g) over a 5 day period, then $\sim$3.95 mJ of energy would have been liberated from the catabolism of that substrate. Similar calculations can be made for protein and carbohydrates. These values are then summed to provide an estimate of the total amount of energy liberated by catabolism of the substrates. The comparison is between energy used based on O_2 consumption and the energy liberated based on decrease in mass of the biochemical substrates. If the amount of energy used based on O_2 consumption is larger than the amount of energy available from catabolism of biochemical substrates, some additional substrate must be available. Indeed, DOM is often suggested as that additional substrate. As an indirect method, however, it suffers the same shortcomings as Pütter's work in the early 1900s, namely, inaccuracies in any of the measurements or assumptions related to the conversion factors can drastically alter final conclusions. Much of this uncertainly is commonly "hidden" from readers amidst conclusions that these types of calculations definitively demonstrate the *realized* contribution of DOM to the energy budget of an animal.

A more direct approach: comparisons of performance in the presence and absence of DOM

In contrast to indirect approaches, direct approaches determining the ecological significance for marine invertebrates are almost wholly lacking. The most definitive answers regarding the nutritional or ecological role of DOM will come from evidence based on comparisons between animals in the presence versus the absence of DOM. Additionally, the strength of indirect methods such as mass-balance approaches will increase significantly if one demonstrates differences in energy budgets between animals that have access to DOM and those that do not. Indeed, since direct analysis will use comparisons to determine relative differences between groups and not rely on absolute values, errors due to incorrect assumptions when using conversion factors or errors associated with techniques (for example, underestimation of respiration rates) would not weaken basic conclusions. Desirable direct methods are those that could compare, for instance, decrease in energy content of tissues between animals with and without access to DOM. Moreover, to fully characterize the ecological importance of DOM, the final measure of benefit must be determined for the whole organism in terms of the animal's growth, reproduction, and survival. However, experiments investigating organismal-level parameters or comparisons of energy budgets in the presence versus absence of DOM are almost nonexistent. The very few studies that have employed direct methods thus far provide contradictory evidence. One study demonstrated in a jellyfish access to DOM increased the rate of adult asexual reproduction compared with that of animals growing without DOM (Shick 1975). Gustafson (1980) showed increased survival and greater biochemical reserves for larvae of the soft-shell clam *Mya arenaria* when exposed to relatively high concentrations of amino acids (5.1–7.3 μM) compared to starved controls. In each case, however, clam larvae fed phytoplankton had higher survivorship and biochemical reserves than did

those only having access to dissolved amino acids. Jaeckle and Manahan (1992) found an increase in dry organic weight 2 days after fertilization for the nonfeeding larvae of the red abalone *Haliotis rufescens*. Unfortunately, the dry organic weight of animals was not measured beyond 2 days, so the effect of DOM over longer periods was not determined. On the other hand, the larvae of the oyster *C. gigas* failed to show any significant increase in shell length over starved controls with access to dissolved glycine, alanine, or glucose (Manahan 1982, as cited in Manahan and Crisp 1982). Similarly, Ferguson (1980) maintained adults of the sea star *Echinaster* sp. for 160 days under the following 2 conditions: (1) seawater depleted of organic nutrients, and (2) seawater enriched with a variety of amino acids previously shown to be taken up by these animals. He found no pathological effects in adults living in nutrient-depleted water in terms of behavior, size, or chemistry of body parts, or in appearance of the epidermis. Likewise, re-enrichment of the seawater produced no detectable benefits, and negative growth occurred in all conditions despite having significant quantities of DOM. This result may seem surprising given that Ferguson (1967) previously showed net uptake and incorporation (via autoradiography) of DOM for this species. It is interesting to note that all previous published work using direct approaches have examined response variables within a given stage of a life cycle. We suggest that this approach has limited the ability to determine the full importance of DOM to marine invertebrates, especially the larval stages. Designing experiments that utilize direct examination of the ability of DOM to offset latent effects associated with larval experience may be a better methodology, and utilizing this approach may uncover the hidden ecological benefits of utilization of DOM by marine invertebrates.

Using latent effects: results from a model system

As Stephens (1988) pointed out, the most convincing demonstration that marine invertebrates are indeed the agent of uptake of DOM from seawater comes from a series of studies utilizing larvae (for example, Manahan and others 1983; Davis and Stephens 1984a; Davis and Stephens 1984b). We suggest that model systems using larvae are also best suited for determining the degree to which DOM supplements energy reserves, contributes to growth (and perhaps adult reproduction), and increases chances of survival. Larvae are ideal model systems for this line of research because: (1) they are easily handled under laboratory conditions in relatively small volumes of seawater, (2) hundreds to thousands of individuals can be procured on a daily basis for experimental replication, (3) previous work with larvae of many species has demonstrated uptake of DOM, and (4) experiments examining latent effects such as energetic content of tissues, growth (premetamorphic and postmetamorphic), and survival are readily executed.

Perhaps the best organism for addressing these questions is the bryozoan *B. neritina*, a common member of tropical and subtropical fouling communities in coastal habitats worldwide. In addition to the reasons outlined above, this species is ideal for determining the ecological benefit of DOM because: (1) the length of the larval phase can readily be prolonged using bright light or it can be curtailed by using excess exogenous KCl (Wendt and Woollacott 1995, 1999), (2) the larvae are nonfeeding and have been shown to transport DOM from seawater at quantities calculated to account for up to 72% of their basal metabolism (Jaeckle 1994), (3) previous work has demonstrated that the latent effects associated with protracted larval swimming (Wendt 1996, 1998) have energetic underpinnings at least in part (Wendt 2000), and (4) growth and reproduction are relatively easy to quantify by nondestructive means, allowing repeated assessment of these parameters on the same individuals over time (Wendt 1998). Furthermore, the energetics of larval swimming and of metamorphosis are well understood, as are the costs associated with a protracted period of larval swimming (for example, Jaeckle 1994; Hunter and Fusetani 1996; Wendt 1996, 1998, 2000; Hunter and others 1999). For example, our previous research has shown that larvae lose the ability to complete metamorphosis over time, and that by 28 h after release from a parent colony only about 20% of larvae can complete metamorphosis (Wendt 1996). For those individuals that do complete metamorphosis after 28 h of larval swimming, the first juvenile (ancestrula) lophophore was 25% smaller in height, and had 40% less surface area and 50% less volume than in individuals that were allowed to metamorphose immediately after their release from the parental colony (Wendt 1996). In a subsequent study it was shown that short-term costs of protracted larval swimming significantly compromise postmetamorphic growth and reproduction (Wendt 1998). We have recently extended this work to include a detailed examination of the ability of DOM to offset the latent effects mentioned above. In this series of experiments we removed naturally occurring DOM from seawater through UV-irradiation (Beattie and others 1961; Armstrong and others 1966; Armstrong and Tibbitts 1968). Importantly, metabolic activity of larvae of species from the phyla Arthropoda (*Artemia* sp.), Echinodermata

(*Strongylocentrotus purpuratus*), Mollusca (*H. rufescens*), and Bryozoa (*B. neritina*) was not affected by treated water as compared to untreated controls (C. H. Johnson and D. E. Wendt, unpublished data). We then added known types and quantities of DOM, and measured performance between animals in the presence and absence of DOM, focusing on latent effects such as the ability to complete metamorphosis and on the size of the lophophore of postmetamorphic individuals.

Availability of DOM was found to significantly offset the latent effect of reduced metamorphic completion associated with a protracted larval period. Following 24 h of larval swimming, animals in the DOM-enriched conditions experienced a significantly greater rate of metamorphic completion, compared to animals swimming in the nonenriched conditions. On average, animals swimming in the nonenriched conditions for 24 h experienced a reduction in completion of metamorphosis of ~70%, whereas animals swimming in the enriched conditions experienced an average reduction of only 50%. The availability of DOM was not sufficient to completely offset latent effects; an expected result given that previous studies examining DOM transport have found that uptake of DOM only accounts for a portion of an animal's total metabolic demand (for example, Jaeckle and Manahan 1989a; Jaeckle and Manahan 1989b; Jaeckle 1994). In addition to increased survivability, the presence of DOM was also sufficient to offset the latent effect of reduced lophophore size in newly metamorphosed ancestrulae. Animals swimming for 24 h in seawater enriched with 1 μM each of alanine, aspartic acid, glycine, and glucose had a 31% larger lophophore volume and 23% larger lophophore surface area, compared to animals swimming in the UV-irradiated seawater (C. H. Johnson and D. E. Wendt, unpublished data). Animals swimming under these conditions for only 1 h did not have significantly different lophophore sizes, demonstrating that these size differences did not exist at the onset of the experiment. Additionally, the respiration rates of animals swimming under these conditions did not differ significantly, demonstrating that the increase in performance associated with animals swimming and metamorphosing in the presence of DOM was due to the energy acquired from uptake, rather than due to a shift in the animal's basal metabolic activity. Overall, animals capable of supplementing their endogenous reserves by uptake of DOM during swimming and metamorphosis not only had better survivability, but also an increased postmetamorphic size, previously shown to enhance rates of growth and reproduction (Wendt 1998).

The realized benefit of DOM to growth, reproduction, and survival of marine invertebrates sill remains an open question after almost a century of focused research. We believe model systems that examine latent effects associated with nutritional stress or delayed metamorphosis (in the context of DOM availability) hold tremendous promise for determining the ecological importance of DOM to marine invertebrates. We expect the most clear benefits will be observed for species that have nonfeeding larvae, although feeding larvae undergo food limitation under natural conditions and may also likely benefit from DOM uptake during times of starvation. Future work should utilize the approach presented here to ascertain whether our findings on bryozoans have more general application to marine invertebrates (C. H. Johnson and D. E. Wendt, unpublished data).

Acknowledgments

We thank the organizers of the symposium Amy Moran and Robert Podolsky for the opportunity to present and to contribute a manuscript to the SICB symposium, Integrating Function over Marine Lifecycles. We also thank the other participants of the symposium for sharing their collective insights and knowledge about life cycles of marine organisms. An anonymous reviewer provided helpful comments and criticisms that improved the manuscript.

Conflict of interest: None declared.

References

Armstrong FAJ, Tibbitts S. 1968. Photochemical combustion of organic matter in sea water, for nitrogen, phosphorous and carbon determination. J Mar Biolog Assoc UK 48:143–52.

Armstrong FAJ, Williams PM, Strickland JDH. 1966. Photo-oxidation of organic matter in sea water by ultra-violet radiation, analytical and other applications. Nature 211:481–3.

Beattie J, Bricker C, Garvin D. 1961. Photolytic determination of trace amounts of organic material in water. Anal Chem 33:1890–2.

Ben-David-Zaslow R, Benayahu Y. 2000. Biochemical composition, metabolism, and amino acid transport in planua-larvae of the soft coral *Heteroxenia fuscescens*. J Exp Zool 287:401–12.

Crawford CC, Hobbie JE, Webb KL. 1974. The utilization of dissolved amino acids by estuarine microorganisms. Ecology 55:551–63.

Davis JP, Stephens GC. 1984a. Uptake of free amino acids by bacteria-free larvae of the sand dollar *Dendraster excentricus*. Am J Physiol 247:R733–9.

Davis JP, Stephens GC. 1984b. Regulation of net amino acid exchange in sea urchin larvae. Am J Physiol 247: R1029–37.

Ferguson JC. 1967. An autoradiographic study of the utilization of free exogenous amino acids by starfishes. Biol Bull 133:317–29.

Ferguson JC. 1980. The non-dependency of a starfish on epidermal uptake of dissolved organic matter. Comp Biochem Physiol 66A:461–5.

Ferguson JC. 1982. A comparative study of the net metabolic benefits derived from the uptake and release of free amino acids by marine invertebrates. Biol Bull 162:1–17.

Gnaiger E. 1983. Calculation of energetic and biochemical equivalents of respiratory oxygen consumption. In: Ganiger E, Forstner H, editors. Polarographic oxygen sensors: aquatic and physiological applications. New York: Springer-Verlag. p 337–45.

Gustafson RG. 1980. Dissolved free amino acids in the nutrition of larvae of the soft-shell clam *Mya arenaria*. MS Thesis, Department of Zoology, University of Maine. p 77.

Hoegh-Guldberg O, Manahan DT. 1995. Coulometric measurement of oxygen consumption during development of marine invertebrate embryos and larvae. J Exp Biol 198:19–30.

Hunter E, Fusetani N. 1996. Studies on the effects of larval swimming time on settlement, metamorphosis and post-larval development of *Bugula neritina* (Bryozoa: Cheilostomatida). In: Gordon DP, Smith AM, Thompson RM, editors. Bryozoans in space and time. Wellington, New Zealand: NIWA Ltd. p 139–48.

Hunter E, Shimizu K, Fusetani N. 1999. Role of protein in larval swimming and metamorphosis of *Bugula neritina* (Bryozoa: Cheilostomatida). Mar Biol 133:701–7.

Jaeckle WB. 1994. Rates of energy consumption and acquisition by lecithotrophic larvae of *Bugula neritina* (Bryozoa: Cheliostomata). Mar Biol 119:517–23.

Jaeckle WB, Manahan DT. 1989a. Feeding by a "nonfeeding larva": uptake of dissolved amino acids from seawater by lecithotrophic larvae of the gastropod *Haliotis rufescens*. Mar Biol 103:87–9.

Jaeckle WB, Manahan DT. 1989b. Amino acid uptake and metabolism by larvae of the marine worm *Urechis caupo* (Echiura), a new species in axenic culture. Biol Bull 176:317–26.

Jaeckle WB, Manahan DT. 1989c. Growth and energy imbalance during the development of a lecithotrophic molluscan larva (*Haliotis rufescens*). Biol Bull 177:237–46.

Jaeckle WB, Manahan DT. 1992. Experimental manipulations of the organic composition of seawater: implications for studies of energy budgets in marine invertebrate larvae. J Exp Mar Biol Ecol 156:273–84.

Jørgensen B. 1976. August Pütter, August Krogh, and modern ideas on the use of dissolved organic matter in aquatic environments. Biol Rev 51:291–328.

Krogh A. 1931. Dissolved substances as food of aquatic organisms. Biol Rev 6:412–42.

Maldanado M, Young CM. 1999. Effects of the duration of larval life on postlarval stages of the demosponge *Sigmadocia caerulea*. J Exp Mar Biol Ecol 232:9–21.

Manahan DT, Crisp D. 1982. The role of dissolved organic material in the nutrition of pelagic larvae: amino acid uptake by bivalve veligers. Am Zool 22:635–46.

Manahan DT, Richardson K. 1983. Competition studies on the uptake of dissolved organic nutrients by bivalve larvae (*Mytilus edulis)* and marine bacteria. Mar Biol 75:241–7.

Manahan DT, Wright SH, Stephens GC. 1983. Simultaneous determination of net uptake of amino acids by a marine bivalve. Am J Physiol 244:R832–8.

Manahan DT, Wright SH, Stephens GC, Rice MA. 1982. Transport of dissolved amino acids by the mussel, *Mytilus edulis*: demonstration of net uptake from natural seawater. Science 215:1253–5.

Marsh AG, Manahan DT. 1999. A method for accurate measurements of the respiration rates of marine invertebrate embryos and larvae. Mar Ecol Prog Ser 184:1–10.

Marshall DJ, Pechenik JA, Keough MJ. 2003. Larval activity levels and delayed metamorphosis affect post-larval performance in the colonial ascidian *Diplosoma listerianum*. Mar Ecol Prog Ser 246:153–62.

Miller SE. 1993. Larval period and its influence on post-larval life history: comparison of lecithotrophy and facultative planktotrophy in the aeolid nudibranch *Phestilla sibogae*. Mar Biol 117:635–45.

Ng TYT, Keough MJ. 2003. Delayed effects of larval exposure to Cu in the bryozoan *Watersipora subtorquata*. Mar Ecol Prog Ser 257:77–85.

Nielsen C. 1981. On morphology and reproduction of '*Hippodiplosia*' *insculpta* and *Fenestrulina malusii* (Bryozoa, Cheilostomata). Ophelia 20:91–125.

Orellana MC, Cancino JM. 1991. Effects of delaying larval settlement on metamorphosis and early colony growth in *Celleporella hyalina* (Bryozoa: Cheilostomata). In: Bigey FP, editor. Bryozoaires actuels et fossiles: bryozoa living and fossil. Bull Soc Sci Nate l'Ouest France, Memoirs HS 1: 309–16.

Pechenik JA. 1990. Delayed metamorphosis by larvae of benthic marine invertebrates: Does it occur? Is there a price to pay? Ophelia 32:63–94.

Pechenik JA. 2006. Larval experience and latent effects—metamorphosis is not a new beginning. Integr Comp Biol 46:323–33.

Pechenik JA, Berard R, Daniels D, Gleason TR, Champlin D. 2001. Influence of lowered salinity and elevated cadmium on the survival and metamorphosis of trochophores in *Capitella* sp. I. Invertebr Biol 120:142–8.

Pechenik JA, Eyster LS. 1989. Influence of delayed metamorphosis on the growth and metabolism of young *Crepidula fornicata* (Gastropoda) juveniles. Biol Bull 176:14–24.

Pechenik JA, Jarrett JN, Rooney J. 2002. Relationships between larval nutritional experience, larval growth rates, juvenile growth rates, and juvenile feeding rates in the prosobranch gastropod *Crepidula fornicata*. J Exp Mar Biol Ecol 280:63–78.

Pechenik JA, Rittschof D, Schmidt AR. 1993. Influence of delayed metamorphosis on survival and growth of juvenile barnacles *Balanus amphitrite*. Mar Biol 115:287–94.

Pechenik JA, Wendt DE, Jarrett JN. 1998. Metamorphosis is not a new beginning. BioScience 48:901–10.

Phillips NE. 2002. Effects of nutrition-mediated larval condition on juvenile performance in a marine mussel. Ecology 83:2562–74.

Phillips NE. 2004. Variable timing of larval food has consequences for early juvenile performance in a marine mussel. Ecology 85:2341–6.

Pütter A. 1908. Die Ernahrung der Wassertiere. Z Allg Physiol 7:283.

Shick JM. 1975. Uptake and utilization of dissolved glycine by *Aurelia aurita* scyphistomae: temperature effects on the uptake process: nutritional role of dissolved amino acids. Biol Bull 148:117–40.

Shilling FM, Hoegh-Guldberg O, Manahan DT. 1996. Sources of energy for increased metabolic demand during metamorphosis of the abalone *Haliotis rufescens* (Mollusca). Biol Bull 191:402–12.

Stephens GC. 1962. Uptake of organic material by aquatic invertebrates. I. Uptake of glucose by the solitary coral, *Fungia scutaria*. Biol Bull 123:648–59.

Stephens GC. 1988. Epidermal amino acid transport in marine invertebrates. Biochim Biophys Acta 947:113–8.

Stephens GC, Schinske RA. 1961. Uptake of amino acids by marine invertebrates. Limnol Oceanogr 6:175–81.

Thiyagarajan V, Harder T, Qian P-Y. 2002. Relationship between cyprid energy reserves and metamorphosis in the barnacle *Balanus amphitrite* Darwin (Cirripedia; Thoracica). J Mar Biol Ecol 280:79–93.

Wacker A, von Elert E. 2002. Strong influences of larval diet history on subsequent post-settlement growth in the freshwater mollusk *Dreissena polymorpha*. Proc R Soc Lond B Biol Sci 269:2113–9.

Wendt DE. 1996. Effect of larval swimming duration on success of metamorphosis and size of the ancestrular lophophore in *Bugula neritina* (Bryozoa). Biol Bull 191:224–33.

Wendt DE. 1998. Effect of larval swimming duration on success of growth and reproduction of *Bugula neritina* (Bryozoa) under field conditions. Biol Bull 195:126–35.

Wendt DE. 2000. Energetics of larval swimming and metamorphosis in four species of *Bugula* (Bryozoa). Biol Bull 198:346–56.

Wendt DE, Woollacott RM. 1995. Induction of larval settlement by KCl in three species of *Bugula* (Bryozoa). Invertebr Biol 114:345–51.

Wendt DE, Woollacott RM. 1999. Ontogenies of phototactic behavior and metamorphic competence in larvae of three species of *Bugula* (Bryozoa). Invertebr Biol 118:75–84.

Williams PJ, le B. 2000. Heterotrophic Bacteria and the ?Dynamics of Dissolved Organic Material. In: Kirchman DL, editor. Microbial ecology of the oceans. New York (NY): Wiley-Liss, Inc. p 153–200.

Williams PJ, Le B, Berman T, Holm-Hansen O. 1976. Amino acid uptake and respiration by marine heterotrophs. Mar Biol 35:41–7.

Woollacott RM, Pechenik JA, Imbalzano KM. 1989. Effects of duration of larval swimming period on early colony development in *Bugula stolonifera* (Bryozoa: Cheilostomata). Mar Biol 102:57–63.