Question from Joth Davis: *Measurements in both the open Pacific Ocean and recently Puget Sound have indicated an increase in dissolved carbon dioxide concentrations at depth and occasionally in up-welled surface waters. This has raised considerable concern for pelagic larvae and other organisms that have either aragonite or calcite based shells during some portion of their life cycle. Using planktotrophic and lecithotrophic invertebrate larvae as examples, please discuss how changing ocean chemistry might affect the biology, behavior and physiological ecology of these organisms. Please consider how changes in temperature, salinity,carbonate cycle, pH, alkalinity and other physio-chemical parameters might impact these organisms in an environment characterized by increasingly corrosive seawater.*

The dependence of marine invertebrate larvae on environmental cues for survival in and settlement from the plankton make them particularly sensitive to macro- and microscale changes in ocean chemistry and circulation patterns. Both planktotrophic and lecithotrophic larvae are obligate plankton constituents for hours to weeks, depending on their life history. Changes to ocean pH, temperature, and salinity have the potential to significantly affect where these larvae are distributed, their rate of development, and their settlement success. Specifically, the duration of the larval stage for both planktotrophs and lecithotrophs is dependent on synchrony between the biology, behavior, and ecology of the species. From fertilization through adulthood, marine invertebrates are attuned to environmental cues, which trigger a wide range of physiological processes and lifestage changes. There is potential, however, for acclimation and at least population-specific survival in the face of broadscale environmental changes due to the inherent genotypic and phenotypic variability in the offspring of broadcast spawners.

Lecithotrophic and planktotrophic larval stages have both evolved in marine invertebrates, sometimes even within taxonomically related species, with each strategy demonstrating a different tactic for adaptation to the marine environment. The majority of marine invertebrates have planktotrophic larvae (most bivalves, many echinoderms), but a number of taxa – *Haliotis* spp., the sea urchin *Heliocidaris erythrogramma*, and the clam *Gemma gemma* among them – have evolved a shorter larval planktonic period. Typically, planktotrophic larvae are considered “feeding” larvae and lecithotrophs are “nonfeeding” since they possess a large yolk-sac that is sufficient for their time in the plankton. However, both bivalves (*Crassostrea gigas*) and gastropods (*Haliotis rufescens*) have demonstrated the ability to absorb dissolved organic material (DOM) through larval structures in contact with the environment (Manahan 1989). Planktotrophic species do have smaller eggs and greater fecundity than lecithotrophic and their larvae spend one to three weeks longer in the plankton (Strathmann 1985). The larger eggs of the lecithotroph have more organic material/nutrients overall and proportionally compared to planktotrophic eggs (Strathmann 1985). These characteristics appear to support a trade-off between the larval strategies where the greater parental investment in lecithotrophic larvae means less larval mortality. This appears to be the case for the lecithotroph *Gemma gemma* with an estimated larval mortality of 0.052 per day compared to *C. gigas* with an estimated mortality of 0.13-0.22 per day (Strathmann 1985). This fecundity-mortality trade-off may balance out any supposed advantage to one larval strategy over the other by the time juvenile and adult stages are reached (Strathmann 1985).

Larvae have evolved a number of strategies to combat a hostile ocean environment while in the plankton, including absorption of DOM in the case of nutritive stress, but some stresses, such as ocean acidification, are relatively novel and there has not yet been potential for adaptation. Puget Sound has seen a decrease in pH of 0.05-0.15 units since pre-industrial times (Feely et al. 2010), a significant drop especially for marine calcifiers. The highest concentrations of CO2 are in the near-surface waters of the ocean (Sabine et al. 2004), where larvae in the plankton tend to congregate. The upper ocean acidification in the North Pacific is proportional to the anthropogenic increase in atmospheric CO2, enforcing that the present-day pH changes are outside the range of natural variability (Byrne, R. et al. 2010). Upwellings of deeper water are also typically more acidic and bring a host of other stressors, including different temperature, salinity, nutrients, and disease. Corrosive oceanic upwellings along the west coast of North America bring up water that is both low in pH (sometimes as low as pH 7.6) and undersaturated with respect to aragonite (Feely et al. 2008). Aragonite is an important component of many larval shells and when seawater is undersaturated, this particular form of calcium carbonate is less available to calcifying organisms. Also in the North Pacific, the pH minimum zone (pH<7.3) is generally coincident with the oxygen minimum zone since both are a result of decomposition of organic matter (Byrne, R. et al. 2010). Continued acidification of waters from increased anthropogenic CO2 will only exacerbate these problems and create increasingly hostile environments for marine biota.

The impacts of ocean acidification and other forms of climate change on marine larvae begin even before fertilization. Stressful environmental conditions for adults can result in catabolism of carbohydrate and protein stores, as seen in the eastern oyster, *Crassostrea virginica*, which could mean there are fewer resources available for parental investment (the yolk-sac) in the larvae (Thompson et al. in *The Eastern Oyster*). Dissolved CO2 in the marine environment could also enter gametes and lower intracellular pH, preventing fertilization and development (Kurihara 2008). Low pH has inconsistent effects on fertilization success across taxa of broadcast spawners. Decreased fertilization has been documented in the urchin *Heliocidaris erythrogramma*, the coral, *Acropora digitifera*, and sea cucumber *Holothuria* spp., but not in the Pacific oyster *Crassostrea gigas* (Havenhand et al. 2008; Morita et al. 2009; Havenhand & Schlegel 2009). In a review of fertilization success in urchins under acidified conditions, the only significantly negative effect was seen in *H. erythrogramma* (Dupont et al. 2010b).

The pace of larval development is important for development of normal morphology and maintaining synchrony with resources. In a variety of laboratory studies across a range of marine taxa, larvae exposed to low pH exhibit slower development than larvae in control conditions. Sub- and supraoptimal temperatures and salinities evoke similar developmental responses (Zimmerman & Pechenik 1991; Calabrese 1969). Concurrently, these slower developing larvae also exhibit more morphological abnormalities and it is difficult to dissect apart the interaction between developmental rate and physical abnormalities, but they do seem to co-occur. In two species of planktotrophic urchin, *Hemicentrotus pelcherrimus* and *Echinometra mathaei*, larvae exposed to acidified conditions (pH 7.77 and below) demonstrated less early cleavage and a deformed pluteus stage (Kurihara & Shirayama 2004). Similarly in the planktotrophic brittlestar *Ophiothrix fragilis*, larvae exposed to pH of 7.9 and 7.7 had higher mortality, arrested development before the 8-arm pluteus stage, and a greater proportion of abnormal morphologies than controls (Dupont et al. 2008). *Crassostrea gigas* also exhibited a lag in developmental timing at a pH of 7.4, paired with smaller size, abnormal shape, and lack of calcification (Kurihara et al. 2007). Even if ocean acidification causes only sublethal effects, it could weaken a larva’s defenses when faced with another stressor. The red abalone, *Haliotis rufescens*, showed significantly decreased survival during a brief temperature stress when larvae were reared at a pH of 7.9 compared to control pH (Zippay & Hofmann 2010). Similarly, two barnacle species, *Elminius modestus* and *Semibalanus balanoides*, experienced sublethal effects of low pH that only turned lethal upon exposure to warmer temperature (Findlay et al. 2009). Elevated CO2 also affects the physiological stress response in the urchin *Strongylocentrotus franciscanus*. At high CO2 equivalent to pH of 7.87 and 7.98, the urchin larvae were less able to upregulate the gene *hsp70* under thermal stress (O’Donnell et al. 2009). Heat shock protein 70 (Hsp70) is a key component of the physiological stress response to a variety of environmental stressors. If the preliminary stress of ocean acidification inhibits larval response to other stressors, then the confluence of environmental stress from global climate change could easily overwhelm some marine invertebrates.

Acidification is obviously instigating a number of developmental abnormalities across taxa, which could be due to a variety of organism-environment interactions. The most striking is the disruption of the formation of calcified structures in these larvae. As pH changes due to addition of CO2, the carbonate chemistry of the water also changes. Increasing dissolved CO2 causes an increase in dissolved inorganic carbon in the water. As dissolved CO2 increases in the ocean, it makes carbonate ions (CO32-) less available to calcifying organisms, causing undersaturation with respect to calcite and/or aragonite. Anthropogenic CO2 increases do not directly affect the total alkalinity (TA) of water, which is also involved in carbonate chemistry. Changes to TA occur with changes to the charge balance of seawater since TA depends on a variety of carbonate and other ions (Schulz et al. 2009). Both changes in dissolved inorganic carbon and TA can influence concentrations of HCO3-, CO32-, and H+, which are all significant ions in ocean acidification and calcification. Dissolved CO2 can also cross biological membranes and decrease extracellular and intracellular pH of marine organisms. Not much work has been done on this front in larvae since they are too small and mobile for most of the procedures available, but adult marine invertebrates vary taxonomically in their ability to regulate intracellular pH in the face of ocean acidification (Lannig et al. 2010; Thomsen & Melzner 2010). One of the main strategies of juveniles and adults is to dissolve CaCO3 structures so that bicarbonate (HCO3-) is in the hemolymph and available for buffering of pH changes (Marchant et al. 2010). Currently, the consensus is that larvae without shells and with abnormal appendages are victims of a corrosive environment, although there is a small chance that the larvae are using HCO3- for internal buffering instead of shell building.

The timing of larval development is intimately linked to the number of hours, days, or weeks a larva spends in the water column, thus impacting larval synchrony with resources and exposure to certain environmental conditions. Increased planktonic duration could easily instigate nutritive stress for both lecithotrophic and planktotrophic larvae. Lecithotrophs depend on a yolk-sac for their nutritional needs during their short planktonic existence. If development and settlement – during which the larvae become competent to feed – are delayed, the larvae could run out of food. Planktotrophic larvae are dependent on food availability from the environment during their extended planktonic phase. Spawning and fertilization occur according to specific environmental cues, which in part could be to optimize conditions for larval development (Thompson et al. in *TEO*). As the environment changes from ocean acidification, increased upwellings, and warming, these cues could become asynchronous with resource availability. Planktotrophs are more sensitive to this asynchrony at certain points during development. *C. gigas* larvae that were deprived of food for a few days at different points in development recovered better from the stress at later developmental stages, but showed greatest decreased metamorphosis when starved at 6 days post fertilization (Kheder et al. 2010).

Aside from nutritive stress, increased planktonic duration also increases the probability that a larva will encounter adverse conditions. On a short time scale these adverse conditions could come in the form of upwelled water that is more acidic, has higher salinity, lower temperature, and is sometimes associated with prevalence of disease. Upwelled waters have been linked to *C. gigas* larval mortalities in the eastern Pacific (Elston et al 2008). Other stochastic changes in the water column could prove equally as detrimental. Normal development and growth rate in the coot clam, *Mulinia lateralis,* were significantly less at salinities and temperatures both lower and higher than their optimum physiological range (Calabrese 1969).

The last important component to larval timing in the water column is advection by currents. Planktotrophic larvae necessarily travel farther than lecithotrophic since they are in the water longer. The trade-off in the case of larval advection is frequency of environmental turnover. Planktotrophic larvae have a better chance of finding suitable habitat when there is a higher variability between sites. If sites are consistently good or bad, more larvae are exported from good sites than are returned (Strathmann 1985). However, if the larvae are in the water column for a longer amount of time, they could be swept away to habitat that is beyond their physiological tolerances.

Larval distribution in the water column is a key component of survival and successful settlement. Oyster larvae actively remain above the halocline and will change their position in the water column depending on current speed (Kennedy in *TEO*). Echinoderms (urchins and sea stars) and crabs off of northern California showed taxon-specific but consistent associations with water masses (Wing et al. 1995). Crab settlement was triggered by increases in temperature as upwelling relaxed and urchin settlement, although more episodic, was linked to the rate of change in salinity during upwelling relaxation (Wing et al. 1995). Bivalve larvae off the East coast also show preferences for certain water masses. Two clam species, *Spisula solidissima* and *Ensis directus*, maintained their position below the thermocline during up- and downwelling events, while *Tellina* spp. and *Mulinia lateralis* stayed near the shore throughout (Shanks & Brink 2005). The consistent nearshore residence of the latter two bivalves is probably due to their ability to maintain their vertical position in the water column, which would cause entrapment in flow fields during both upwelling and downwelling (Shanks & Brink 2005). Global climate change could change the position of clines and currents and the frequency of upwelling events. The Pacific Decadal Oscillation (PDO) provides a good case study for the effects of climate change on a relatively small scale. The PDO instigates changes in ocean currents, which affect the distribution of phyto- and zooplankton in the ocean (Francis et al. 2003). The PDO fluctuates, whereas the anticipated global climate change will most likely bring broader scale and more permanent changes. Changes in circulation patterns and the halocline may affect settlement success rate in some larvae. Also, abnormal larvae may not have the mechanisms necessary to detect cues and become competent to settle. There is potential for adaptation in at least some marine invertebrates. After the initial bottleneck of early larval mortalities at heightened temperature, the lecithotrophic urchin *H. erythrogramma* settled more quickly than larvae raised at ambient temperatures (Byrne et al. 2011). With adequate genetic diversity or phenotypic plasticity, at least some larvae may be able to overcome the obstacles posed by climate change.

There is great variability in marine invertebrate response to the environmental factors of climate change, but within that variability lies the potential for future survival and adaptation. When a species’ range includes population-specific tolerances to environmental change or clines of local adaptation, then there may exist natural variability that will make it robust to future change (e.g. Bradshaw & Holzapfel 2001). Even if future climate changes are not within the immediate range of what the species experiences, selection for phenotypic plasticity could provide the physiological groundwork necessary to acclimatize. Lecithotrophic larvae appear to be more robust than planktotrophic when exposed to future scenarios of ocean acidification and warming. The lecithotrophic sea star, *Crossaster papposus*, actually grew faster at a pH of 7.7 when compared to ambient pH (Dupont et al. 2010a). There were no negative effects on larval survival or skeletogenesis and even positive direct effects on metabolism during the 28 days of the experiment, which included settlement and metamorphosis (Dupont et al. 2010a). Even within the lecithotrophic strategy, however, there is variability in sensitivity to fluctuations in pH and temperature. The abalone *Haliotis coccordiata* was more sensitive to changes in pH and temperature than the urchin *H. erythrogramma*. The calcification of *H. coccordiata* was even more sensitive to decreased pH of 7.8 and 7.6 than some bivalves (Byrne, M. et al. 2010). Even thought the urchins were more robust overall, an increased temperature of +40C over ambient broke the thermal tolerance of both species (Byrne, M. et al. 2010). Lecithotrophic larvae may be more immune to harsh environments if this life-history strategy evolved to minimize time in the plankton in such an environment (Dupont et al. 2010b; Byrne, M. et al. 2010). By spending less time as vulnerable larvae in an adverse environment, lecithotrophic larvae may be able to bypass negative biological and physiological effects. Planktotrophic larvae are not able to abbreviate their time in the plankton since they need those weeks for pre-metamorphosis development. Their key to survival in a changing climate will be drawing upon variability in inter-population tolerances. The planktotrophic spider crab, *Hyanus araneus*, inhabits a cline from the North Sea to the North Atlantic and shows population/latitude-specific responses to changes in temperature and CO2 (Walther et al. 2010). Temperature-dependent development has led to a shift in phenology in the wild southern population and larvae from the northern population are more temperature-sensitive in their development. Larvae from both ends of the cline were sensitive to changes in CO2, but at different stages in development (Walther et al. 2010).

Larval stages of marine invertebrate larvae are particularly vulnerable to fluctuations in environmental parameters, but pre-adaptations through life history strategies (lecithrophy) and environmental clines in population tolerances may provide the evolutionary basis needed for future adaptation. In general, echinoderms demonstrate inter-population differences in their responses to ocean acidification (Dupont et al. 2010b). Broadcast spawning is also an important strategy that serves to increase genotypic and phenotypic diversity in populations. Since so many larvae are produced through this reproductive mechanism, there is a higher probability that some will have the phenotype necessary to survive adverse environmental conditions. Sweepstakes selection is an important component of invertebrate life history and is more significant in certain environments (Hedgecock et al. 2007; Taris et al. 2009). Ocean acidification and related changes will most likely cause significant alterations to development and increase mortality in a number of species. The evolutionary trajectories that have led to lecithotrophy, broadcast spawning, and overall variability between populations will prove to be increasingly important for adaptation potential.

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