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Comparative larval energetics of an ophiuroid and an echinoid echinoderm

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Abstract. The morphologically convergent larvae of the echinoderm classes Ophiuroidea and Echinoidea have been suggested to be functionally dissimilar when it comes to their capacities to feed, but little is known about whether these larvae are similar in terms of energetics. Here, we compare the energetics of early development of a tropical ophiuroid, *Ophiocoma alexandri*, and a temperate to tropical echinoid, *Arbacia punctulata*, two species with similarly sized eggs. Measurements of respiration and constituent analyses were performed on eggs and unfed larvae of both species. Members of both species showed an increase in oxygen consumption during morphogenesis followed by a lower, static rate once morphogenesis was complete (3 d for *O. alexandri* and 1.3 d for *A. punctulata*). Compared to the echinoid larvae, the ophiuroid larvae developed more slowly and had peak respiration rates that were 3.1× lower. Eggs of *O. alexandri* contained significantly more protein and significantly less triacylglycerol than eggs of *A. punctulata*. Energy utilization, as calculated via respiration measurements, closely matched decreases in energy content from the eggs to larvae as measured with biochemical constituent assays. Larvae of *A. punctulata* used 1.4× more energy to reach the pluteus stage than larvae of *O. alexandri*, and used 4× more energy during the first 9 d of larval life. These data suggest that echinoid larvae require more energy to develop to the feeding stage than ophiuroid larvae, and likewise have higher requirements for maintenance metabolism. Ophiuroid larvae may be more tolerant of low food levels due to their very low metabolic rates, but this advantage may be offset by their slower rate of development.

Additional key words: *Arbacia*, egg composition, *Ophiocoma*, respiration

One important focus of larval biology in recent decades has been larval energetics (Jaeckle & Manahan 1992; Marsh & Manahan 1999; Marsh et al. 1999; Lemos et al. 2003; Sewell 2005; Pace & Manahan 2007a). An understanding of how larvae acquire and use energy, coupled with knowledge of the biochemical constituents in eggs and larvae, can lead to a better understanding of both how ecological factors such as food and temperature affect larval success (Peck & Prothero-Thomas 2002; Moran & Manahan 2004; Meyer et al. 2007; Moran & Woods 2007; Pace & Manahan 2007b; Walther et al. 2010), and how evolution has shaped the metabolic processes that underlie energy utilization during larval development (Leong & Manahan 1997; Pace et al. 2006; Pace & Manahan 2007a; Meyer & Manahan 2010).

Most of the work on larval energetics has focused on a few specific taxa, including asteroids (Peck & Prothero-Thomas 2002; Pace & Manahan 2007b; Ginsburg & Manahan 2009), echinoids (Podolsky et al. 1994; Shilling & Manahan 1994; Hoegh-Guldberg & Emlet 1997; Marsh et al. 1999; Sewell 2005; Meyer et al. 2007; Moran & Allen 2007; Pace & Manahan 2007a,b), bivalves (Sprung 1984; Mann & G allager 1985; Moran & Manahan 2004; Chaparro et al. 2006; Pace et al. 2006; Meyer & Manahan 2010), gastropods (Jaeckle & Manahan 1989; Vavra & Manahan 1999; Moran & Manahan 2003; Moran & Woods 2007), and crustaceans (Logan & Epifanio 1978; Lemos & Phan 2001; Marsh et al. 2001; Lemos et al. 2003; Walther et al. 2010). Members of these taxa are amenable to large-scale culture through their relative accessibility, ease of spawning, production of large numbers of gametes, and often, their use in aquaculture. However, from an ecological and evolutionary perspective, it would be beneficial to understand how broadly the patterns seen in the species studied to date can be extrapolated to related groups.

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Some evolutionarily distinct lineages of invertebrates such as ophiuroids and echinoids (Phylum Echinodermata) possess larval forms that have convergently evolved striking morphological similarities (Strathmann 1988). Due to the ease of obtaining gametes from echinoids and the comparative difficulty of obtaining ripe gametes from ophiuroids, echinoid larvae have been studied in much greater detail than ophiuroid larvae (Strathmann 1987; Hart & Podolsky 2005), but some comparisons have been made between the two larval forms. Both ophiuroids and echinoids possess "pluteus" larval forms, which have long arms with ciliated bands supported by skeletal rods (Fig. 1). The pluteus larvae of many ophiuroids ("ophioplutei") have two arms that are held at a lower angle than the other arms, which can change their stability in shear (Grünbaum & Strathmann 2003). Ophioplutei have lower clearance rates (volume of water cleared of particles per unit time) than echinoid plutei ("echinoplutei") when standardized to ciliated band length, indicating that ophioplutei may be less effective feeders due to fewer and less dense cilia (Hart 1996). This observation led to the hypothesis that ophioplutei, because of their low clearance rates, might minimize their energetic needs relative to body size to keep energy use on par with feeding capacity (Hart 1996).

Egg biochemical composition has been frequently examined in echinoids. In echinoids, the small eggs of planktotrophic species contain primarily protein, structural lipids (phospholipids and sterols), and triacylglycerols, which are used as energy stores to fuel development (Moran & Manahan 2004; Byrne et al. 2008; Prowse et al. 2008, 2009). Carbohydrates generally do not comprise more than 13% of weight or energy content of echinoderm eggs (Jaeckle 1995).

As a first step toward determining whether there are fundamental differences in the energetics of larval development between members of Ophiuroidea and Echinoidea, we measured oxygen consumption and performed proximal biochemical composition analyses of embryos and early larval stages of an ophiuroid, *Ophiocoma alexandri* (Lyman 1860) (Fig. 1A), which was fortuitously obtained in ripe condition. For comparison, we performed parallel analyses with an echinoid, *Arbacia punctulata* (Lamarck 1816) (Fig. 1B). *Arbacia punctulata* and *O. alexandri* both have small, similarly sized eggs and planktotrophic development, and while their adult distributions do not overlap throughout their ranges (*A. punctulata* is an Atlantic species, and *O. alexandri* was collected in the Pacific Ocean), larvae of both species experience similar temperatures during development and can be reared at the same temperature.

Methods

Obtaining and rearing larvae

Ophiocoma alexandri. Adults of *O. alexandri* were collected from the Pearl Islands, Panama, in June 2009, and from Isla Taboguilla, Panama, in August 2010. The animals collected in 2009 began spawning after collection, probably due to a combination of a rise in water temperature and gentle shaking from transportation. Eggs from a single female were fertilized with sperm from two males. Animals collected in 2010 were induced to spawn by injection of 0.5 M KCl into the body cavity within a few hours of collection. Eggs from two females were mixed with sperm from two males to obtain zygotes. In 2010, respiration measurements were only made at four timepoints and no biochemical samples were taken. In both 2009 and 2010, embryos and larvae were reared at the Naos Laboratory of the Smithsonian Tropical Research Institute (Panama City, Panama) at 28°C in 1 μm filtered sea water without food. Final samples and data were taken 7 d after the larvae reached the pluteus stage; after this point,

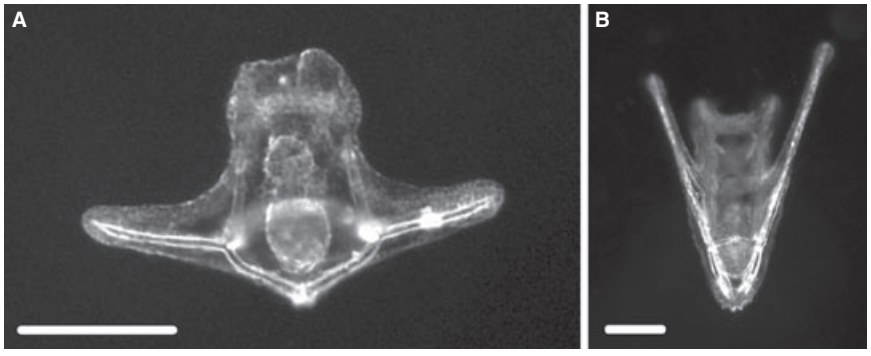

Fig. 1. Photomicrographs of plutei. **A**. Ophiopluteus of *Ophiocoma alexandri*. **B**. Echinopluteus of *Arbacia punctulata*. Scale bars=100 μm.

there were insufficient larvae remaining to take respiration measurements or biochemical samples.

Arbacia punctulata. Adults of *A. punctulata* were obtained from Gulf Specimen Marine Lab (Panacea, FL, USA) in April 2010. Adults were spawned via intracoelomic injection of 0.5 M KCl. Eggs from 11 females were fertilized with sperm from 11 males; all gametes were mixed into a common pool of fertilized eggs. A common pool of fertilized eggs was used to ensure that there would be sufficient numbers of larvae to complete the experiment, factoring in sampling and mortality. Embryos and larvae of *A. punctulata* were reared at 27±1°C in artificial seawater (Instant Ocean) in laboratories at Clemson University (Clemson, SC, USA) without food. Final samples and measurements were taken 7 d after the larvae reached the pluteus stage, as for *O. alexandri*. In the absence of food, neither species developed beyond the pluteus stage (2-arm for *O. alexandri* and 4-arm for *A. punctulata*).

Metabolic rates

For *O. alexandri*, metabolic rates and biochemical samples were taken when embryos reached the gastrula (1 d), prism (2 d), and pluteus stages (3 d), and at 24–48 h intervals after reaching the pluteus stage. For *A. punctulata*, metabolic rates were measured, and samples were taken at the gastrula (0.5 d), prism (0.8 d), and pluteus (1.3 d) stages and at 2 and 7 d after reaching the pluteus stage. Metabolic rates were measured using the μBOD method of Marsh & Manahan (1999). Briefly, larvae were loaded into gas-tight glass vials of ~500–600 μL in volume and incubated at their respective rearing temperatures for 3–6 h. After the incubation period, the oxygen concentration in each vial was measured using a Strathkelvin 1302 Clark-type oxygen electrode (North Lanarkshire, Scotland). Oxygen consumption h^{-1} $vial^{-1}$ was regressed against the number of larvae in each vial; the slope of this line yields the amount of oxygen consumed per larva per hour, which was used as a proxy for overall metabolic rate. As in Marsh & Manahan (1999), the standard error of the regression line of consumption (per vial) against number of larvae (per vial) was used as the standard error of the respiration rate at each timepoint.

Biochemical samples

Eggs and larvae were pooled within each species and biochemical samples were taken from the pooled larvae. Multiple (6–10) samples of a known number of eggs or larvae (1500–5000, depending on age and stage) were collected in microcentrifuge tubes and frozen at –80°C. For each biochemical assay (protein, lipid, carbohydrate), the contents of three of these microcentrifuge tubes were homogenized and subsampled for analysis; each of the three microcentrifuge tubes was treated as an independent replicate. Each replicate was subsampled and assayed in triplicate, and the replicate value was calculated as the mean of the three subsamples to ensure the accuracy of our methods of analysis. The mean and standard error of the three replicates from each timepoint were used for statistical analyses.

Lipid analysis

Lipid was extracted with the 2:2:1 (v/v/v) methanol: water:chloroform solution described by Bligh & Dyer (1959) with modifications by Moran & Manahan (2003). The quantities of lipid classes in each sample were determined using an Iatroscan MK6 flame ionization detection-thin layer chromatography system, and samples and standards were prepared as described by Moran & Manahan (2003). Total lipid was calculated by adding the quantities of the separate lipid classes; this method yields results similar to other methods of total lipid quantification (Moran & McAlister 2009). Briefly, fatty alcohol (stearyl alcohol) was added to each sample prior to lipid extraction to serve as an internal standard. The extracted lipids were resuspended in a known volume of chloroform (~20 μL). The lipid extract was then loaded onto quartz Chromarods (n=3 per sample, 1–3 μL extract per rod), developed in a 60:6:0.1 (v/v/v) hexane:diethyl ether:formic acid solution for 30 min, and analyzed using the Iatroscan system (Iatron Laboratories, Inc., Tokyo, Japan) and PeakSimple v3.88 software (SRI Instruments, Menlo Park, CA, USA). The following lipids were used as standards: tripalmitin for triglyceride (TG), stearyl alcohol for fatty alcohol (ALC), cholesterol for free sterol (ST), and L-α-lecithin for phospholipid (PL). Samples of eggs were also developed using the protocol described by Prowse et al. (2009) using a 96:4 v/v hexane:diethyl ether solution; no diacylglycerol ether (DAGE) was detected, consistent with expectations for small planktotrophic eggs.

Protein and carbohydrate analysis

Protein and carbohydrate were extracted using the TCA method of Holland & Gabbott (1971) as modified by Moran & Manahan (2003). Protein content was measured with a Pearce Micro BCA protein assay kit (Fisher #PI-23235), which utilizes a modified Lowry assay, with bovine serum albumin

as a standard. Carbohydrate content was determined using the methods described by Holland & Gabbott (1971) with modifications by Moran & Manahan (2003), using glucose as a standard.

Energy content and utilization

To calculate the total oxygen consumed by larvae during development, a curve was fitted to the respiration data and the area under that curve was calculated using the area macro function in Sigma Plot 11 software (Systat Software, Inc., Chicago, IL, USA). To determine whether the energy theoretically available from catabolism of egg biochemical constituents matched larval metabolic demand, we then compared energy use via oxygen consumption with the differences in energy content of the biochemical constituents over development. Because constituent analyses suggested that metabolism was fueled by different substrates at different stages and in the two species, we used stage-specific oxyenthalpic equivalents before and after larvae reached the pluteus stage for both taxa. Larvae of both species used lipid as a substrate prior to the pluteus stage (1.3 d for *A. punctulata* and 3 d for *O. alexandri*; see Results), so oxygen consumption through the pluteus stage was converted into joules with the oxyenthalpic equivalent of lipid, 441 kJ mol^{-1} O_2 (Gnaiger 1983). After the pluteus stage was reached, oxygen consumption was converted into energy equivalents by multiplying the moles of oxygen consumed by an oxyenthalpic equivalent based on the proportion of each biochemical constituent consumed during that time (constituent equivalent for protein, 527 kJ mol^{-1} O_2; for carbohydrate, 473 kJ mol^{-1} O_2 (Gnaiger 1983). A value of 475 kJ mol^{-1} O_2 (59% lipid, 39% protein, and 2% carbohydrate) was used for pluteus larvae of *O. alexandri*, and a value of 493 kJ mol^{-1} O_2 (37% lipid, 59% protein, and 3% carbohydrate) for pluteus larvae of *A. punctulata*. The difference of each constituent type between the egg and pluteus stages was used to determine the percent composition of the energy used during morphogenesis. Individual energy equivalents were used to determine the energy available from catabolism of biochemical reserves in the egg that were depleted during development: 24.0 kJ g^{-1} for protein, 39.5 kJ $g^{-1}$for lipid, and 17.5 kJ g^{-1} for carbohydrate (Gnaiger 1983).

Statistics

Statistical comparisons of changes in biochemical content over time were made using linear regression analyses (α=0.05). For the *O. alexandri* protein and TG lipid data, because the rate of utilization appeared to change over time, regressions were performed for data points collected during morphogenesis up to the pluteus stage (3 d), and a separate regression was performed for measurements taken after the pluteus stage was reached. For *A. punctulata* protein and TG lipid data, regressions were performed only on data collected during morphogenesis (to 1.3 d) because there were no multiple data points taken after morphogenesis was complete. All other regression analyses were performed on all timepoints combined because changes in carbohydrate, sterol, and phospholipid appeared linear. Statistical analyses were performed using JMP 9 software (SAS Institute, Inc., Cary, NC, USA).

Results

The egg diameters of *Ophiocoma alexandri* and *Arbacia punctulata* were 71.0±0.4 μm (±SE) and 73.8±0.6 μm, respectively.

Developmental and metabolic rate

Metabolic rates for both species increased during morphogenesis to the pluteus stage and then subsequently decreased and remained low for the remainder of the study. The echinoid larvae reached each developmental stage (gastrula, pluteus, etc.) 2–4× faster than the ophiuroid larvae (Table 1). This more rapid developmental rate was accompanied by metabolic rates that were, at each stage, 1.9–3.1× higher for *A. punctulata* (Fig. 2). The peak metabolic rate for larvae of *A. punctulata* was 3.1× greater than the peak metabolic rate for *O. alexandri*. When metabolic rates were normalized to protein content (protein-specific metabolic rate: Fig. 3), metabolic rates of echinoid larvae were 3.4× higher at the metabolic peak.

Lipid

TG content of larvae of *O. alexandri* remained high until shortly before the pluteus stage, and

Table 1. Timing of development of *Ophiocoma alexandri* and *Arbacia punctulata*.

Developmental Stage	*Ophiocoma alexandri*	*Arbacia punctulata*
Gastrula	25 h	12.5 h
Prism	71 h	18 h
Pluteus	90 h	31 h

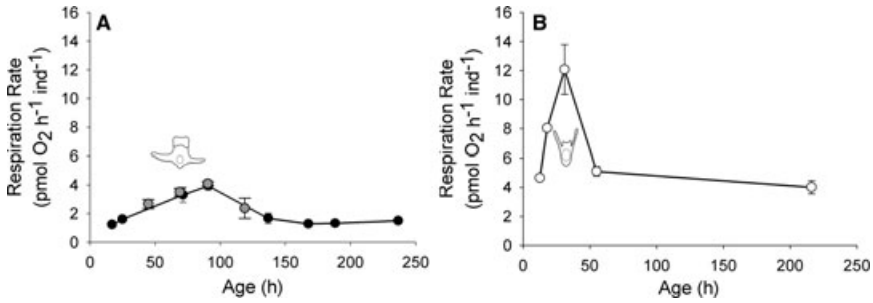

Fig. 2. **A**. Respiration rates of larvae of *Ophiocoma alexandri*. Black points are data from 2009 and gray points are data from 2010. **B**. Respiration rates of larvae of *Arbacia punctulata*. The larvae indicate when the pluteus stage was reached. Error bars are the SE of the regression slope used to calculate the respiration rate.

detectable levels of TG remained until 2 d after developing into a fully formed pluteus (137 h after fertilization: Fig. 4). TG was utilized by larvae of *O. alexandri* at a rate of 0.05 ng h^{-1} during this time (linear regression: r^2=0.873, p=0.020). ST and PL did not change over time (linear regression: p=0.606 and p=0.146, respectively). In contrast to *O. alexandri*, TG reserves of larvae of *A. punctulata* were entirely depleted shortly after reaching the pluteus stage (55 h after fertilization: Fig. 5). TG was utilized at a rate of 0.10 ng h^{-1} (linear regression: r^2=0.928, p=0.008). Over the course of the experiment, ST content of larvae did not change significantly (linear regression: p=0.388) nor did PL content (p=0.060).

Protein

Protein content did not change significantly during morphogenesis (Fig. 6; linear regression: p=0.059) or after morphogenesis (p=0.987) for larvae of *O. alexandri*. Protein content did decrease significantly during morphogenesis for larvae of *A. punctulata* (Fig. 6; r^2=0.905, p=0.013), at a rate of 0.39 ng h^{-1}. Upon reaching the pluteus stage, ophiuroid larvae contained 72.5% of the protein in the egg and the echinoid larvae contained 55.8% of the protein in the egg.

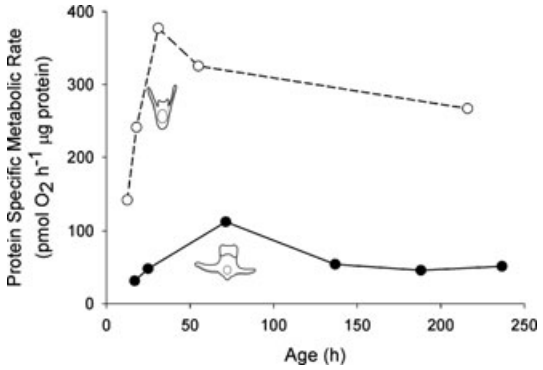

Fig. 3. Protein-specific metabolic rates of larvae of *Ophiocoma alexandri* (black) and *Arbacia punctulata* (white). The larvae indicate when the pluteus stage was reached.

Carbohydrate

The carbohydrate content of the embryos and larvae of *O. alexandri* did not change significantly over development (Fig. 7; linear regression: p=0.903). For embryos and larvae of *A. punctulata*, carbohydrate content also did not change over development (p=0.319: Fig. 7).

Energetics

Based on our measurements of oxygen consumption, development from the egg to the pluteus larval stage was energetically less costly for the ophiuroid larvae (0.02 mJ d^{-1} $larva^{-1}$, or 0.10 mJ total) compared with the echinoid larvae (0.12 mJ d^{-1} $larva^{-1}$, or 0.15 mJ total) by ~30%. Development from the egg through the end of the experiment was also energetically less costly for larvae of *O. alexandri* (0.02 mJ d^{-1} $larva^{-1}$, or 0.25 mJ total) compared with larvae of *A. punctulata* (0.08 mJ d^{-1} $larva^{-1}$, or 0.64 mJ total) by ~60%. The TG in the eggs of *O. alexandri* (0.15 mJ) could theoretically provide 150% of the total energy needed to fuel morphogenesis to the feeding pluteus stage; the TG in the eggs of *A. punctulata* (0.20 mJ) contained 133% of the energy needed.

For both species, energy costs measured via oxygen consumption were comparable to the amount of energy theoretically available from the measured depletion of lipid, protein, and carbohydrate during development. The larvae of *O. alexandri* experienced a 0.10 mJ reduction in energetic content between

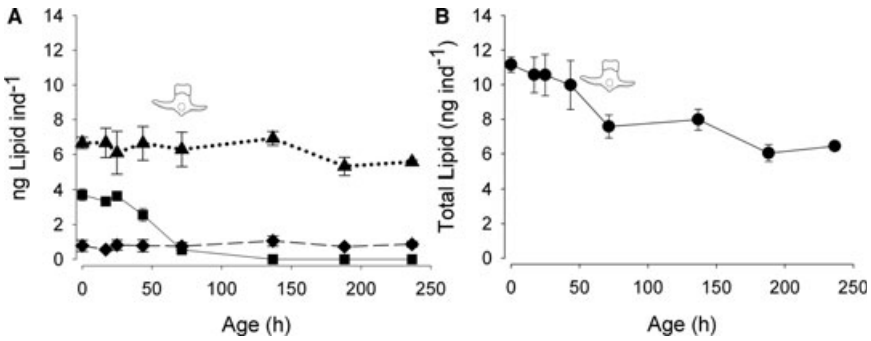

Fig. 4. Lipid content of eggs and larvae of *Ophiocoma alexandri* over development. **A**. Quantities of phospholipid (triangles, dotted line), triacylglycerol (squares, solid line), and sterol (diamonds, dashed line). **B**. Quantities of total lipid. The larvae indicate when the pluteus stage was reached. Error bars=SE (n=3).

the egg and the pluteus stage (3 d), compared to a 0.09 mJ use of energy as determined by energy loss via oxygen consumption. These larvae experienced 0.25 mJ loss in the energy content of the larvae 7 d after reaching the pluteus stage relative to the egg, with a 0.23 mJ use of energy. Relative to the energy in the egg, the energy content of newly formed plutei of *A. punctulata* was reduced by 0.15 mJ, and over this period, the larvae utilized 0.15 mJ via oxygen consumption. The energy content of larvae 7 d after reaching the pluteus stage was 0.64 mJ less than the energy in the egg with a corresponding 0.69 mJ energy use via metabolism. The echinoid larvae used 1.5× more energy (as measured via metabolic rate) to reach the pluteus stage than the ophiuroid larvae. During the 7 d after reaching the pluteus stage (after which no further visible development occurred), echinoid larvae consumed 4.2× more energy than ophiuroid larvae.

Discussion

To our knowledge, this is the first description of the energetics of ophiuroid larvae. The metabolic rates we saw in ophiuroid larvae were substantially lower than similarly sized echinoid larvae reared at similar temperatures. The low metabolic rate of these ophioplutei is consistent with their comparatively slow development to the pluteus stage. Relative to the energy in the egg, the amount of egg material consumed by the larvae of *Ophiocoma alexandri* during development to the pluteus stage was similar to *Arbacia punctulata*; the ophiuroid larvae consumed 8.7% of the energy in the egg during development to the pluteus stage, and the echinoid larvae used 10.4% of the energy in the egg. After morphogenesis was complete, the metabolic rates of larvae of *O. alexandri* were 3.3× lower than the metabolic rates of larvae of *A. punctulata*. These data highlight the importance of studying the developmental energetics of taxa whose larvae are not easily cultured in the laboratory, because larvae from such taxa may have very different energetics than better-known groups.

The biochemical composition of eggs of *O. alexandri* was similar to the eggs of other planktotrophic ophiuroids (Falkner et al. 2006; Prowse et al. 2009), and the composition of the eggs of *A. punctulata*

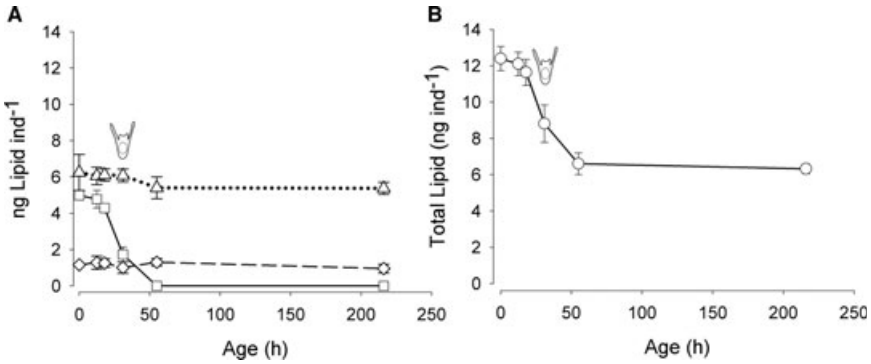

Fig. 5. Lipid content of eggs and larvae of *Arbacia punctulata* over development. **A**. Quantities of phospholipid (triangles, dotted line), triacylglycerol (squares, solid line), and sterol (diamonds, dashed line). **B**. Quantities of total lipid. The larvae indicate when the pluteus stage was reached. Error bars=SE (n=3).

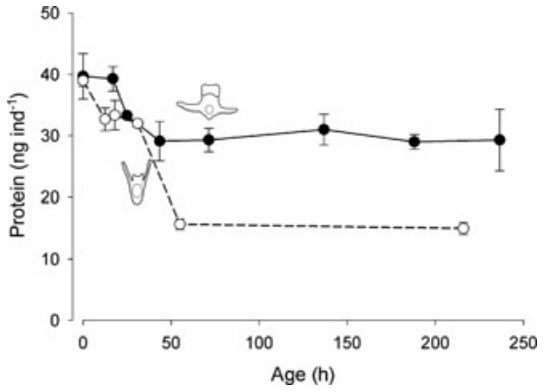

Fig. 6. Protein content of eggs and larvae of *Ophiocoma alexandri* (black dots) and *Arbacia punctulata* (white dots) over development. The larvae indicate when the pluteus stage was reached. Error bars=SE (n=3).

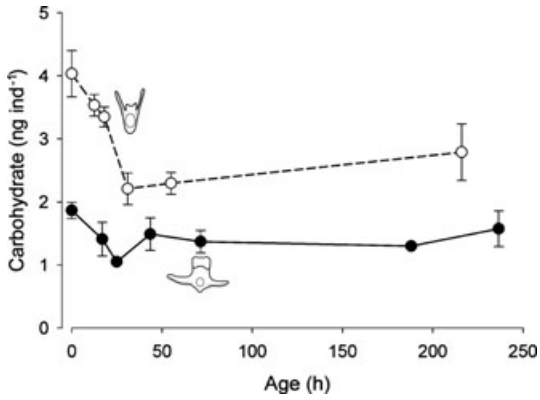

Fig. 7. Carbohydrate content of eggs and larvae of *Ophiocoma alexandri* (black dots) and *Arbacia punctulata* (white dots) over development. The larvae indicate when the pluteus stage was reached. Error bars=SE (n=3).

was similar to previously published data on that species (Harvey 1932; Turner & Lawrence 1979; George et al. 1997). The biochemical composition of the eggs studied here is similar to the composition reported for other planktotrophic echinoderm eggs; the energy content of the eggs contributed by protein was about 1.5× more than the energy contributed by lipid, and the eggs contained very little carbohydrate (McEdward & Carson 1987; McEdward & Chia 1991; Jaeckle 1995; McEdward & Morgan 2001; Sewell & Manahan 2001; Sewell 2005; Falkner et al. 2006; Byrne et al. 2008). Phospholipid (PL) and sterol (ST) content of the larvae of both species did not change significantly over the course of the study, which has also been noted for early development in other echinoderm species (Sewell 2005). ST and PL are largely structural and make up the major lipid fractions of the eggs of planktotrophic echinoids, and are generally not used to fuel morphogenesis (Sewell 2005; Byrne et al. 2008; Prowse et al. 2008, 2009). Consistent with previous work on other echinoderms (Podolsky et al. 1994; Sewell 2005; Meyer et al. 2007; Prowse et al. 2008) and molluscs (Moran & Manahan 2003, 2004), larvae of *A. punctulata* and *O. alexandri* utilized triacylglycerol (TG) to fuel morphogenesis; both species showed almost complete utilization of egg TG during morphogenesis to the initial feeding stage, although larvae of *O. alexandri* appeared to retain some TG past the formation of the pluteus. Because we pooled the eggs of multiple females of *A. punctulata*, we are not able to estimate the variation in biochemical content or larval respiration rate among females. However, the standard errors from our pooled samples were similar to other studies of the biochemical constituents in echinoid eggs from replicated samples of individuals or small groups of females (Moore & Manahan 2007; McAlister & Moran 2012).

While some invertebrate larvae may use protein as an energy source (Millar & Scott 1967; Lucas et al. 1979; Dawirs 1983; Whyte et al. 1987, 1992; Vavra & Manahan 1999; Moran & Manahan 2003; Prowse et al. 2008), this does not appear to be the case for echinoderms; protein was not utilized during morphogenesis by embryos and larvae of the echinoid *Strongylocentrotus purpuratus* (Stimpson 1857) (Meyer et al. 2007) or the asteroids *Patiriella regularis* (Verrill 1867) and *Meridiastra mortenseni* (O'Loughlin, Waters, & Roy 2002) (Prowse et al. 2008) when reared without food. While we did not see a significant change in the protein content of the ophiuroid larvae, we did find a significant decrease in protein content of the echinoid larvae during morphogenesis, when metabolic demand was greatest. Larvae of *O. alexandri*, because of their low metabolic demand, may be able to avoid starvation-induced catabolism of protein for longer than larvae of *A. punctulata*.

Because larval metabolic rate can be affected by water chemistry, particularly dissolved organic material (DOM) levels (Jaeckle & Manahan 1992), it is possible that some of the metabolic differences between the two taxa may have been due to rearing in water from different sources (natural tropical sea water for *O. alexandri* and Instant Ocean for *A. punctulata*). While we did not measure or manipulate DOM in our experiments, Instant Ocean has DOM levels similar to low-productivity tropical sea water (Atkinson & Bingman 1997), so this was unlikely to have an impact comparable to the large (3.1×) metabolic differences we found between taxa. Likewise, protein-specific metabolic rates of 4-armed

A. punctulata reared in Instant Ocean were very close to those of a tropical echinoid (*Echinometra vanbrunti* Agassiz 1863) reared using the same water source, temperature, and techniques as the *O. alexandri* in our study (362 vs. 377 pmol O_2 h^{-1} μg $protein^{-1}$ $larva^{-1}$, respectively). Therefore, despite differences in water chemistry, it is still clear that the larvae of *O. alexandri* utilize energy at a far lower rate than larvae of echinoids with comparable egg sizes.

Hart (1996) suggested ophioplutei might possess comparatively low metabolic rates because their low feeding cannot sustain high energy demands. Our work here supports Hart's hypothesis; larvae of *O. alexandri* have low metabolic rates compared with echinoplutei, even when metabolic rates are standardized to protein content and temperature. If other ophioplutei are similar, then ophioplutei in general may be more starvation-resistant than larvae of other echinoderm classes. The ability of larvae to persist in low food environments may be advantageous in patchy or food-poor environments (Fenaux et al. 1994). However, ophioplutei may also be unable to capitalize on concentrated patches of food if they cannot rapidly increase their metabolic rates and feeding activity when resources are plentiful.

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