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Evolution of maternal lipid provisioning strategies in echinoids with non-feeding larvae: selection for high-quality juveniles

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ABSTRACT: In marine invertebrates where larval planktotrophy is the ancestral life history, the evolutionary switch to lecithotrophy depended on modifications of oogenesis to produce energy-rich eggs that support development to the juvenile stage. In echinoderms, this involved a change from small eggs dominated by readily metabolised triacylglycerol (TAG) to large eggs containing various types of energetic storage lipids. We characterised lipid provisioning in the large (400 µm diameter) eggs of the echinometrid sea urchin *Heliocidaris erythrogramma* which has lecithotrophic larvae to compare with the small (90 µm diameter) eggs of the planktotroph *H. tuberculata*. We also investigated egg lipids in temnopleurids with contrasting modes of development: *Temnopleurus alexandri* and *Holopneustes purpurascens* (egg diameter: 125 and 600 µm, respectively). In the planktotrophs, TAG was the major energetic lipid. Egg energetic lipids in the lecithotrophs were largely diacylglycerol ether (DAGE) with TAG and wax ester also present. We used rapid juvenile development in *H. erythrogramma* to characterise lipid depletion through metamorphosis to the 14 d old juvenile. Larval development did not significantly deplete energetic lipids, with 70% of the DAGE remaining for the juvenile. TAG supported larval development with a 20–30% decrease by Day 3 with no further depletion to Day 14. DAGE levels decreased around metamorphosis, followed by a gradual depletion, but 49% of these reserves remained on Day 14. Thus, DAGE provisioning provides a significant nutritive buffer for a considerable time post settlement. Selection to produce a high-quality juvenile has driven egg evolution in echinoids with lecithotrophic development.

KEY WORDS: Eggs · Evo-devo · Planktotrophy · Lecithotrophy · Metamorphosis · *Heliocidaris* · *Holopneustes* · Echinoidea

1. INTRODUCTION

For marine invertebrates with pelagic development, the egg is the complete investment of the mother to her offspring, with differences of maternal provisioning in species with feeding and non-feeding larvae. For many phyla (e.g. echinoderms, some molluscs and polychaetes), planktotrophic development in species with small eggs is considered to be the ancestral life history from which independent evolutionary transitions to a large egg and lecithotrophic development have occurred (Strathmann 1985, Emlet et al. 1987, Haszprunar et al. 1995, Hart 1996, Duda & Palumbi 1999, McEdward & Miner 2001, Hart 2002, Byrne 2006). Evolutionary change in egg and larval traits (evolution of development, 'evo-devo') profoundly influences the biogeography and population structure of marine invertebrates, with the planktotrophic–lecithotrophic dichotomy being of major importance for marine ecology and life-history theory (O'Connor et al. 2007, Marshall et al. 2012, Barbosa et al. 2013, Puritz et al. 2017). The egg size–fecundity trade-off

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has major implications for maternal–larval fitness relationships, dispersal potential and population connectivity (Vance 1973, Levitan 2000, O'Connor et al. 2007, Moran & McAlister 2009). Egg size is also used as the target size parameter for sperm in models of fertilization kinetics, and egg size evolution is also suggested to be influenced by sperm environment (e.g. number of sperm available for fertilization), with larger eggs providing a more suitable target in low-sperm conditions (Levitan 1993, 2000, Styan et al. 2005).

For species with small eggs and planktotrophic development, maternal energetic lipids are used to build the feeding larvae (Sewell 2005, Prowse et al. 2009, 2017, Moran et al. 2013). As the mother provides only a small fraction of the energy required for the full length of larval development, the size, condition and fitness of the recruiting juveniles are determined by larval experience (e.g. stress, food supply) over the weeks to months that they spend in the plankton (Bertram & Strathmann 1998, Miller & Emlet 1999, Levitan 2000, Phillips 2004, Emlet & Sadro 2006, Pechenik 2006). Feeding larvae have to assimilate material to construct the juveniles, with the associated increased risk of mortality the longer they disperse (Lamare & Barker 1999, Byrne et al. 2008a).

In contrast to earlier notions that the extra provisions in the large eggs of species with lecithotrophic development are required to sustain development in the absence of feeding (Mortensen 1921), most of the egg energetic reserves are partitioned for the early juvenile (Emlet & Hoegh-Guldberg 1997, Prowse et al. 2009, Falkner et al. 2015). For these species, the large egg is a direct transgenerational link between mother and juvenile and can be used as a proxy for the size of the recruiting offspring (Marshall et al. 2008). Large-egg species can rapidly achieve metamorphic competence, with the potential outcome of lower planktonic mortality and higher reproductive fitness with respect to the number of juveniles produced and their success (Havenhand 1993, McEdward & Miner 2001). These juveniles start their benthic life far better provisioned than those resulting from planktotrophic development (Emlet & Hoegh-Guldberg 1997, Byrne & Cerra 2000, Villinski et al. 2002).

The ancestral-type small eggs of echinoderms with planktotrophic development contain low levels of energetic lipids, the most important of which is triacylglycerol (TAG), a readily metabolised fuel (Sewell & Manahan 2001, Sewell 2005, Prowse et al. 2009, Whitehill & Moran 2012, Peters-Didier & Sewell 2017). In contrast, the large eggs of species with lecithotrophic development are stocked with high levels of storage lipids including diacylglycerol ether (DAGE) and wax and methyl esters (Villinski et al. 2002, Prowse et al. 2009, Falkner et al. 2015). The independent evolution of a large egg and lecithotrophic development across echinoderm taxa has resulted in distinct differences in egg lipid profiles (Jaeckle 1995, Byrne et al. 1999, Villinski et al. 2002, Prowse et al. 2009, Falkner et al. 2015).

We examined the evolution of maternal investment in sea urchins by comparing egg biochemistry in closely related species with contrasting planktotrophic and lecithotrophic development. The marked difference in the egg and larval phenotypes in *Heliocidaris* species (family Echinometridae) has provided insights into marine invertebrate evo-devo (Raff & Byrne 2006). Evolution of lecithotrophy in this genus is associated with extensive modifications in gene expression, gene regulatory networks and egg biochemistry (Villinski et al. 2002, Raff & Byrne 2006, Israel et al. 2016). *H. tuberculata* (90 µm diameter egg) and *H. erythrogramma* (400 µm diameter egg) have negatively and positively buoyant eggs, respectively (Byrne et al. 1999, 2001, Raff & Byrne 2006). These species are estimated to have diverged ~5 million yr ago (Mya) (Zigler et al. 2003). We also investigated egg lipids in 2 temnopleurids with feeding and non-feeding larvae (*Temnopleurus alexandri* and *Holopneustes purpurascens*; 600 and 125 µm diameter eggs, respectively) which are estimated to have diverged >30 Mya (Smith et al. 2006, A. Kroh pers. comm.).

A previous study using plate-based thin-layer chromatography indicated that the novel lipid class in the eggs of *H. erythrogramma* and *H. purpurascens* was wax ester (WE) (Villinski et al. 2002). More recently, analyses of asteroid and ophiuroid eggs using a 1-step development hexane-based solvent system and thin layer chromatography-flame ionization detection (TLC-FID), which separates TAG and DAGE into distinct peaks, have indicated that the primary energy storage lipid in large echinoderm eggs is DAGE (Prowse et al. 2009, Falkner et al. 2015). We used TLC-FID to characterise maternal lipid provisioning in *H. erythrogramma* in comparison with that in its congener *H. tuberculata*, as well as for egg lipid provisioning in the 2 temnopleurid species.

Larval provisioning for the juvenile in planktotrophic developers (Byrne et al. 2008a) and maternal provisioning for the juvenile in lecithotrophic developers (Emlet & Hoegh-Guldberg 1997) indicates strong selection to support the early juvenile in both life history modes. This stage is considered to be a particularly weak link in the biphasic life history of

marine invertebrates (Gosselin & Qian 1997). The planktonic–benthic metamorphic transition is one of the most important events in the life of marine invertebrates and involves extensive body rearrangement and functional changes. These changes are likely to incur significant energetic costs, but this has rarely been investigated, especially to the advanced juvenile stage (but see Villinski et al. 2002). We used the rapid development of *H. erythrogramma,* where the juvenile stage is reached within 5–6 d, to characterise the dynamics of lipid depletion through metamorphosis and the early benthic stage. In this species, most of the egg lipids are extruded into the embryonic blastocoel as large droplets where they are evident throughout larval development (Emlet & Hoegh-Guldberg 1997, Byrne et al. 1999, 2001). We determined what proportion and type of egg lipids are used to fuel embryonic and larval development through to metamorphosis and what provisions are reserved for the benthic juvenile.

With respect to the proportion of different lipid classes, the large eggs of echinoderms with lecithotrophic development are not simply scaled up versions of the small eggs (Prowse et al. 2009, Falkner et al. 2015). We hypothesised that the switch to lecithotrophy in echinoids involved a novel maternal lipid provisioning strategy with hypertrophic production of long-term energy storage lipid (sensu Lee et al. 2006), in particular DAGE, as predicted by Prowse et al. (2009), or alternately due to production of WE, as previously reported (Villinski et al. 2002). During development, we hypothesised that the TAG reserves would be exhausted in *H. erythrogramma* during construction of the larval body as in echinoids with planktotrophic development (Sewell 2005, Byrne et al. 2008b, Prowse et al. 2017) with other energetic lipid types reserved for the juvenile (Emlet & Hoegh Guldberg 1997, Villinski et al. 2002). The lipid condition index of the juvenile *H. erythrogramma* was compared with that determined for juvenile *H. tuberculata* (Prowse et al. 2017) to assess the comparative advantage of lecithotrophy.

2. MATERIALS AND METHODS

2.1. Spawning, egg sampling and fertilization

Heliocidaris erythrogramma, H. tuberculata and *Holopneustes purpurascens* were collected in Chowder Bay (33° 50′ 29″ S, 151° 15′ 11″ E) and *Temnopleurus alexandri* was collected from Camp Cove (33° 51′ 30″ S, 151° 14′ 00″ E) in Sydney Harbour, Australia. To obtain eggs for lipid analysis, spawning was induced by intracoelomic injection of 0.5 M KCl. The eggs were collected from the top of the urchin and transferred to 2 l beakers of filtered seawater (FSW 1.0 µm, Millipore), 1 for each female. After a rinse in FSW, the eggs were placed in a 100 ml volumetric cylinder and the concentration (number ml^{-1}) of the eggs in suspension was determined from aliquots using a Sedgewick-Rafter counting chamber. Three egg samples per female (*H. erythrogramma*, n = 6 females with 30 eggs per subsample; *H. tuberculata*, n = 3 females, 700 eggs; *H. purpurascens*, n = 2 females, 30 eggs; *T. alexandri*, n = 3 females, 700 eggs) were aliquoted into 1.5 ml tubes. Each tube was briefly centrifuged, the excess seawater removed, and the samples stored at −80°C until analysis. Twenty eggs $female^{-1}$ were photographed using an Olympus stereo microscope and measured using image J (NIH).

For *H. erythrogramma*, eggs from 3 females were pooled in 3 different combinations (Females 1, 2; Females 1, 3; Females 2, 3), and each pool of eggs was placed in a 2 l beaker filled with FSW. The eggs in each beaker were fertilized with sperm mixed equally from 2 males. This was followed by a rinse and renewal of the FSW. Each population of embryos was then split into 2 l beakers of FSW (5 embryos ml^{-1}) for rearing with daily renewal of the FSW. Samples of the eggs were collected (as above) to quantify the lipid classes present at the start of development. The cultures were maintained in a constant temperature room (20°C) with gentle bubbling from a glass pipette to maintain circulation. Water changes (90%) were conducted daily by reverse filtration. On Day 4, larvae from each beaker were transferred to 200 ml culture dishes for settlement with a small piece of geniculate coralline algae (*Amphiroa* sp.) to induce metamorphosis. The settled juveniles were maintained in these dishes until 14 d post fertilization (dpf) prior to formation of the mouth. Samples of swimming larvae (3 dpf), metamorphosing–metamorphosed larvae (6 dpf) and definitive juveniles (11 dpf, 14 dpf) were collected (as above, with n = 30–40 for each time point). As larval settlement is asynchronous in *H. erythrogramma*, the Day 6 sample was a mixture of swimming larvae, attached larvae and metamorphosing larvae.

2.2. Lipid extraction and analyses

Lipid was extracted from frozen samples as described by Prowse et al. (2008). Total lipid extracts were dissolved in a known volume of chloroform (5–30 µl) before spotting on Chromarods of an Iatroscan

Mark V^{new} TLC/FID system. Lipids were separated and quantified in *H. erythrogramma*, *H. purpurascens* and *T. alexandri* using the 1-step development of hexane–diethyl ether (96:4 v/v) which separates TAG and DAGE into distinct peaks (Phleger et al. 1997, Prowse et al. 2009). The presence of DAGE was confirmed by comparison with a lipid standard provided by P. Nichols (CSIRO) and P. Virtue (University of Tasmania), and quantification based on the TAG standard (Prowse et al. 2009). Total energetic lipid per sample was calculated as the sum of DAGE, TAG, WE and aliphatic hydrocarbon (AH). Structural lipid was determined from the sum of the structural neutral lipid cholesterol and the structural polar lipids, which include phospholipid (PL) and acetone mobile polar lipid (AMPL). Quantification of lipids in *H. tuberculata* is from Prowse et al. (2017); here we used additional egg samples to test for the presence of DAGE using the 1-step development as described above to confirm that the major energetic lipid class is TAG. Amounts of DAGE and TAG for each female were normalized to egg volume (ng nl^{-1}) using the mean egg volume for each species; as the units are weight of lipid per unit egg volume, we follow Jaeckle (1995) in referring to this as lipid density. Lipid class depletion during development of *H. erythrogramma* was also assessed using a lipid condition index based on the energetic lipid:ST ratio as applied to development in a range of taxa including *H. tuberculata* (see Prowse et al. 2017). This index was calculated for the egg, larva (3 d) and metamorphosing juveniles (6 d) to compare lecithotrophic (*H. erythrogramma*) and planktotrophic development (*H. tuberculata*).

2.3. Statistical analyses

Data on lipid classes and total lipid in the eggs of *H. erythrogramma* were analysed using a 1-way analysis of variance (ANOVA) with female as a fixed factor. Data on lipid class and total lipid utilization over development of *H. erythrogramma* were also analysed with a 1-way ANOVA with time as a fixed factor. The assumptions of ANOVA were checked through graphical analysis of residuals, and Levine's (homogeneity of variance) and Shapiro-Wilk (normality) tests were non-significant. Tukey's post hoc tests were used to assess significant tests ($\alpha = 0.05$). Lipid density for TAG and DAGE in the eggs of *H. erythrogramma* and *H. tuberculata* were compared using Student's *t*-test. All analyses were conducted in R 3.4.3 (R Core Team 2018) or in the Systat module within Sigmaplot 14.0.

3. RESULTS

3.1. Egg lipid profiles

The eggs of the 6 *Heliocidaris erythrogramma* females had mean diameters ranging from 390–405 µm diameter (Table A1 in the Appendix) with a mean ± SE total lipid content of 6234 ± 440 ng egg^{-1} (Table 1). Five lipid classes were identified: DAGE (4518 ± 390 ng egg^{-1}) was the major energy storage lipid (72.5% of total lipid), with TAG and WE comprising 10.2 and 5.4% of total lipid, respectively, and the rest of the lipid being structural (Table 1, Figs. 1 & 2A). There was a significant difference between

Table 1. Egg lipid composition (ng egg^{-1}) of *Heliocidaris tuberculata* (n = 3), *Heliocidaris erythrogramma* (n = 6), *Temnopleurus alexandri* (n = 3), and *Holopneustes purpurascens* (n = 2) as mean (SE) and % of total lipid. AH: aliphatic hydrocarbon, AMPL-PL: acetone-mobile polar lipids and phospholipid, DAGE: diacylglycerol ether, ST: sterol, TAG: triacylglycerol, WE: wax ester. Data for *H. tuberculata* from Prowse et al. (2017). nd: not detected

Lipid type	*Heliocidaris tuberculata*	*Heliocidaris erythrogramma*	*Temnopleurus alexandri*	*Holopneustes purpurascens*
Energetic lipids				
DAGE	nd	4517.83 (390); 72.47%	nd	4956.67 (111.75); 46.5%
TAG	8.44 (1.74); 39%	632.83 (75); 10.15%	26.28 (2.00); 31.9%	723.71 (23.48); 6.8%
WE	nd	339.17 (57); 5.44%	1.09 (0.06); 1.3%	194.10 (12.53); 1.8%
AH	0.95 (0.17); 4.39%	nd	0.28 (0.01); 0.3%	712.96 (143.91); 7.3%
Structural lipids				
ST	1.27 (0.18); 5.86%	137.26 (10); 2.20%	11.64 (1.40); 14.1%	380.67 (6.43); 3.5%
AMPL+PL	11.0 (0.41); 50.1%	606.5 (79); 9.72%	43.02 (2.88); 52.3%	3621.56 (50.58); 34.0%
Total lipid	21.66 (3.9)	6233.66 (440)	82.30 (2.77)	10657.53 (239.19)

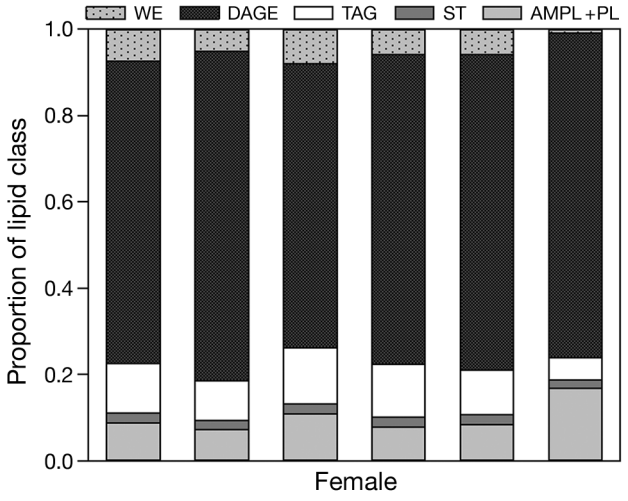

Fig. 1. Proportional contribution of lipid classes in the eggs of 6 *Heliocidaris erythrogramma*. AMPL + PL: acetone-mobile polar lipids and phospholipid, DAGE: diacylglycerol ether, ST: sterol, TAG: triacylglycerol, WE: wax ester

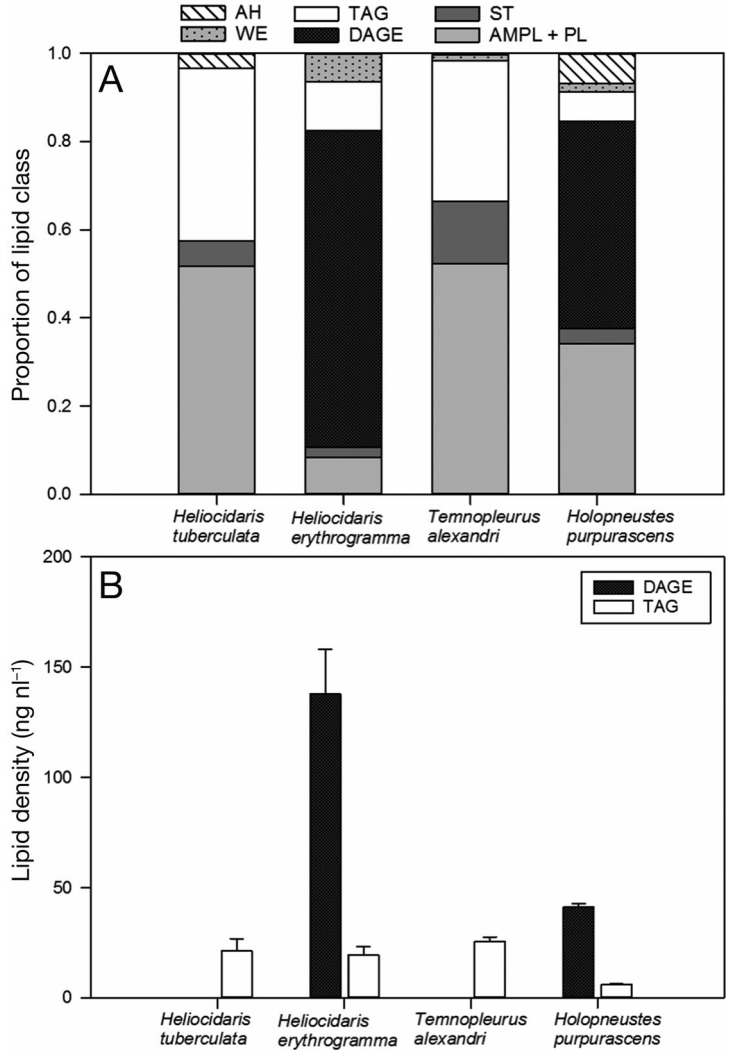

Fig. 2. (A) Proportional contribution of lipid classes to total egg lipid content in *Heliocidaris tuberculata* (n = 3; data from Prowse et al. 2017), *H. erythrogramma* (n = 6), *Holopneustes purpurascens* (n = 2) and *Temnopleurus alexandri* (n = 3). AH: aliphatic hydrocarbon; other abbreviations as in Fig. 1. (B) Lipid density for the 2 main energetic lipids, DAGE and TAG (ng nl^{-1})

females in the total lipid content of the eggs ($F_{5,12}$ = 7.83, p = 0.0017), due to between-female variation in DAGE content (ANOVA: $F_{5,12}$ = 11.55, p = 0.0003). The mean DAGE content was 4518 ± 390 ng egg^{-1} (n = 6) with a range from 3058–5450 ng egg^{-1}, as reflected in the different proportion of this lipid in the eggs of the different females (Fig. 1). The mean TAG and WE content were 633 ± 75 ng egg^{-1} (range 304–835 ng egg^{-1}, n = 6) and 339 ± 57 ng egg^{-1} (range 369–550 ng egg^{-1}, n = 6), respectively.

DAGE was not detected in the eggs of *H. tuberculata* (88.5–92.3 µm diameter, Table A1). These eggs have a total lipid content of 21.7 ng egg^{-1} with the energetic lipid being TAG (39% of total lipid) and with trace levels of WE (not shown, see Prowse et al. 2017) (Table 1, Fig. 2A).

The eggs of *Holopneustes purpurascens* (595–624 µm diameter, Table A1) had a mean total lipid content of 10 657 ± 239.19 ng egg^{-1} (Table 1). DAGE (mean 4956.67 ± 111.75 ng egg^{-1}) was the major energy storage lipid (46.5% of total), with AH, TAG and WE being 7.3, 6.8 and 1.8% of total lipid, respectively (Fig. 2A, Table 1).

The eggs of the planktotrophic developer *Temnopleurus alexandri* (125 µm diameter) had a low total lipid content (82.3 ng egg^{-1}). TAG was the major energy storage lipid (31.9% of total), with small amounts of WE (1.3% of total) (Fig. 2A, Table 1). DAGE was not detected in the 1-step development.

The lipid density of the 2 main energetic lipids illustrated in Fig. 2B shows that the density of TAG was not significantly different in the 2 *Heliocidaris* species (t = –0.482, df = 7, p = 0.644), so that the TAG amount remained proportionally the same per unit volume in the lecithotrophic egg. DAGE density in the eggs of *H. erythrogramma* was much higher (Fig. 2B). Despite their much larger size, the eggs of *H. purpurascens* had a significantly lower DAGE density compared with those of *H. erythrogramma* (t = 4.496, df = 6, p = 0.004). For the 2 temno-

Table 2. One-way ANOVA of data on maternal lipids over 5 time points (Time 0 [D0, egg], D3 [larva], D6 [metamorphosis], D11 and D14 [juvenile]) in development from the egg to the 14 d old juvenile of *Heliocidaris erythrogramma*. Lipid abbreviations as in Table 1

Lipid class	Time $F_{4,10}$	p	Tukey's post hoc
DAGE	5.38	0.0142	D0 = D3 > D6 = D11 = D14
TAG	8.06	0.0036	D0 > D3 = D6 = D11 = D14
WE	12.94	0.0006	D0 > D3 > D6; D3 > D11 > D14; D6 > D11 > D14; D0 = D11 = D14
ST	2.47	0.113	
AMPL + PL	8.24	0.0033	D0 = D3 = D6 = D11; D0 > D14; D3 > D14; D6 > D14
Total energetic lipids	9.31	0.002	D0 = D3; D0 > D6, D0 > D11, D0 > D14; D6 = D11 = D14
Total structural lipids	6.28	0.009	D0 = D11 = D14; D3 = D11; D3 > D14; D6 = D11, D6 > D14
Total lipids	5.11	0.0167	D0 = D3 = D6; D0 > D11; D0 > D14; D3 > D14; D6 = D11 = D14

pleurids, the density of TAG was higher in *T. alexandri* (t = −7.778, df = 3, p = 0.00442), indicating that as the egg gets bigger, the TAG amount does not increase at the same rate as egg size in the lecithotrophic *H. purpurascens*.

3.2. Lipid dynamics in development of *H. erythrogramma*

There was a significant change in all lipid classes, except ST, through development in *H. erythrogramma* from the egg (Time 0) through the swimming larva (Day 3) to metamorphosis-settlement (Day 6) and juvenile stages (Days 11 and 14) (Table 2, Fig. 3). The largest lipid class, DAGE, was not used to fuel larval development (to Day 3), and only decreased on Day 6 in association with metamorphosis, to 70% of the level in the egg (Table 2, Fig. 3). By Day 14, the juveniles still had 49% of their initial DAGE levels. TAG was used to construct the larvae (~190 ng TAG used) on Day 3, with 71% of this lipid remaining with no further depletion to the 14 d old juvenile stage (Table 2, Fig. 3). Overall, the WE levels remained relatively unchanged (~100 ng used), but Tukey's post hoc results were difficult to interpret (Table 2).

The lipid condition index (total energetic lipid:ST ratio) of *H. erythrogramma* changed from 38.1 for the egg to 34.8 for the larva and to 20.2 for the metamorphic period on Day 6, with the latter two considerably higher than that for *H. tuberculata* at metamorphosis (Table 3).

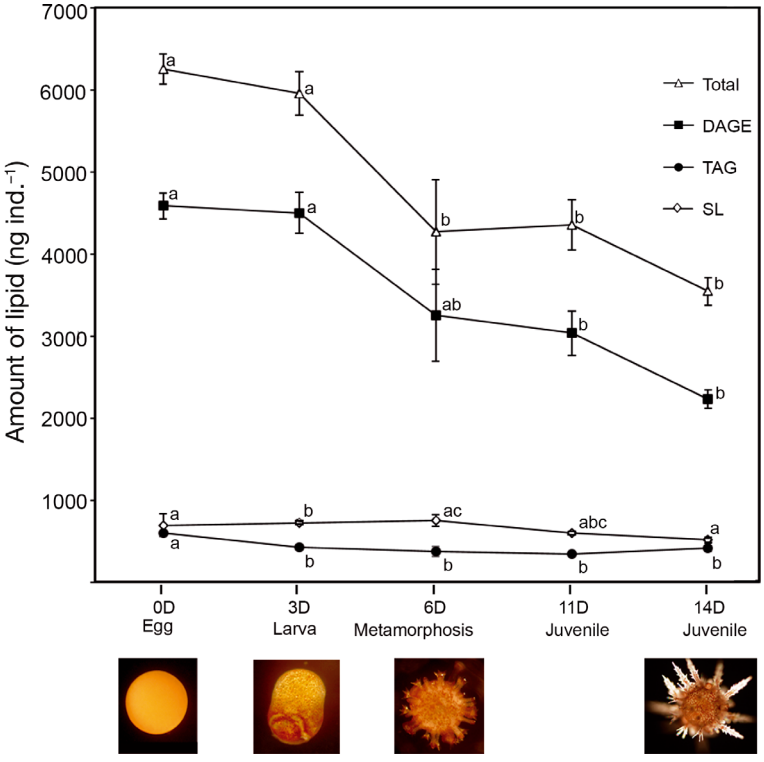

Fig. 3. Lipid depletion (mean ± SE) in development of *Heliocidaris erythrogramma* from Day 0 (0D, egg) to the larva (Day 3), metamorphosis (Day 6) and the juvenile (Days 11 and 14). Total: total lipid, DAGE: diacylglycerol ether; SL: total structural lipid (sterol, acetone-mobile polar lipids and phospholipid); TAG: triacylglycerol. The letters indicate results of Tukey's post hoc test; time points that have the same letter did not differ

Table 3. Comparison of the lipid condition index (total energetic lipid:sterol ratio) of eggs, larvae and newly metamorphosed juveniles of the planktotrophic developer *Heliocidaris tuberculata* and the lecithotrophic developer *H. erythrogramma*. Data for *H. tuberculata* from Prowse et al. (2017)

	H. tuberculata	*H. erythrogramma*
Egg	47	38.1
Swimming larva	0.07	34.8
Metamorphosing larva/juvenile	0.001	20.2

4. DISCUSSION

Evolution of lecithotrophic development in echinoderms was associated with the emergence of new strategies of maternal investment in larger eggs across the echinoderm classes (Strathmann 1985, McEdward & Miner 2001, Prowse et al. 2008, 2009, Falkner et al. 2015). Our hypothesis that evolution of large eggs in echinoids involved hypertrophic production of the long-term energy storage lipid DAGE is supported by the data for *Heliocidaris erythrogramma* and *Holopneustes purpurascens*. The eggs of these species did not have extensive stores of WE, as previously suggested (Villinski et al. 2002), although this lipid was present in low levels in the eggs of both species. For *Heliocidaris tuberculata* and *Temnopleurus alexandri*, TAG is the major energetic lipid, as reported for 9 species of echinoids with small eggs and planktotrophic development (Table 4). The dominance of TAG as the major energetic lipid across 5 echinoid families as well as in the small eggs of asteroids, ophiuroids and a holothuroid with planktotrophic development (Falkner et al. 2015, Peters-Didier & Sewell 2017, Prowse et al. 2017) provides a strong indication that TAG was the ancestral type of maternal energetic lipid provisioning in the Echinodermata.

The hypothesis that production of a large egg in *H. erythrogramma* involved hypertrophic elaboration of the ancestral lipogenic program is supported because small amounts of DAGE have been detected in ancestral-type development in *H. tuberculata* using mass spectrometry-based lipidomics (Davidson et al. 2019), although we did not detect DAGE in these eggs with TLC-FID. TAG density scaled with egg size similarly in the 2 *Heliocidaris* species, indicating that *H. erythrogramma* has conserved provisioning of this lipid class. TAG is used for larval building in this species, as it is in *H. tuberculata* (Prowse et al. 2017). The density of DAGE in the eggs of *H. erythrogramma* is much higher, showing that these eggs are not just scaled up with respect to ancestral-type egg energetic provisioning. The massive increase in egg energy in *H. erythrogramma* is due to hypertrophic elaboration of an existing program of DAGE synthesis, similar to that described for asteroids (Prowse et al. 2009) and for several ophiuroids with lecithotrophic development (Falkner et al. 2015). *H. purpurascens* has a much larger egg than *H. erythrogramma*, but less DAGE per nl volume, although levels of this lipid are still substantial. The TAG density was lower in the eggs of *H. purpurascens* compared with those of the planktotroph *T. alexandri*. To understand egg evolution in the temnopleurids, we need data on how egg lipids in *H. purpurascens* are used during development. The temnopleurid model would be promising to pursue with regard to evo-devo, as *H. purpurascens* forms a juvenile within a week of fertilization (Morris & Byrne 2005).

Table 4. Egg energetic lipid data for echinoids with development through planktotrophic (P) and lecithotrophic (L) larvae, with details for lipid classes where available. Lipid abbreviations as in Table 1

Family, species	Larva	Egg diameter (µm)	Egg total lipid (ng)	Egg energetic lipid (ng)	Reference
Echinidae					
Sterechinus neumayeri	P	179	117	75 (TAG)	Moore & Manahan (2007)
Echinometridae					
Evechinus chloroticus	P	87	34	15 (TAG)	Sewell (2005)
Echinometra lucunter	P	82	15.1	7.5 (TAG)	Emlet et al. (1987), McAlister & Moran (2013)
Echinometra vanbrunti	P	70	8.3	3.4 (TAG)	Emlet et al. (1987), McAlister & Moran (2013)
Echinometra viridis	P	91	17.5	8.3(TAG)	Emlet et al. (1987), McAlister & Moran (2013)
Heliocidaris tuberculata	P	90	29	10 (TAG)	Prowse et al. 2017
Heliocidaris erythrogramma	L	400	6234	5500 (DAGE, TAG, WE)	This study
Toxopneustidae					
Tripneustes gratilla	P	85	31	17 (TAG)	Byrne et al. (2008a,b)
Temnopleuridae					
Temnopleurus alexandri	P	125	82	26.2 (TAG)	This study
Holopneustes purpurascens	L	610	10435	5792 (DAGE, AH, TAG, WE)	This study
Strongylocentrotidae					
Strongylocentrotus purpuratus	P	80	15.67	9.28 (TAG)	Emlet et al. (1987), Matson et al. (2012)

Intraspecific variation in egg size in echinoderms is well known (e.g. Turner & Lawrence 1979), including for *H. erythrogramma* (Deaker et al. 2019), but few studies have examined variation in egg lipid chemistry between conspecific females (e.g. George 1990, George et al. 1990, Moore & Manahan 2007) as seen here across the eggs of individual *H. erythrogramma* from the same location. These females would have experienced similar abiotic conditions, and so the differences may be due to maternal trophic state and age, among other possible factors, as well as the potential for in-built phenotypic plasticity in egg provisioning (Jong-Westman et al. 1995, Marshall et al. 2008, Moran & McAlister 2009, González-Ortegón et al. 2018). Differences in the level of DAGE in the eggs among females would be expected to carry over to their offspring. Some of these mothers may provision their juveniles for a longer period than others. Larvae derived from more energy-rich eggs may also start their benthic life at a larger size, as partial removal of the lipids from *H. erythrogramma* embryos reduces juvenile size and their survival (Emlet & Hoegh-Guldberg 1997). The larvae may also have greater flexibility to delay metamorphosis and be more selective with respect to favourable settlement habitat (Pechenik 2006). The larvae of *H erythrogramma* can swim for over a month in laboratory culture, without an obvious change in size, before undergoing settlement and metamorphosis (M. Byrne pers. obs.).

Intraspecific variation in maternal provisioning, as indicated by egg size in lecithotrophs (e.g. McEdward & Carson 1987), would be of interest to follow as there is a direct link between the egg (oogenic program), juvenile condition and size, and the potential that this variation may represent a bet-hedging strategy (Marshall et al. 2008). Although egg size may not fully reflect nutritive content, there is a good relationship between these two for echinoderms (Moran et al. 2013). For *H. erythrogramma*, and other species with lecithotrophic development, phenotypic plasticity in egg size could be used to explore the trade-offs between maternal fitness to maintain nutritive condition to ensure future reproduction and the imperative to invest in progeny. Selection to produce a high-quality juvenile in *H. erythrogramma* is likely to have been an important evolutionary, as well as contemporary, feedback mechanism on oogenic processes.

We tracked lipid use over development in *H. erythrogramma*, with a focus on energetic lipids. In early development, some depletion of TAG occurred during larval building in *H. erythrogramma*, similar to that for *H. tuberculata* (Prowse et al. 2017). However, there is a big difference in lipid use. For *H. tuberculata*, ~12 ng of TAG (~100% of TAG reserves and 33% of total lipid) are used to construct the echinopluteus (Prowse et al. 2017). In *H. erythrogramma*, ~190 ng of TAG (~30% of TAG reserves and ~3% of total lipid) are used to construct the simple larva of this species, with ~440 ng remaining to support juvenile development and which may also be used to extend the larval period.

For *H. tuberculata*, once the larva is formed there is no improvement in nutritional condition, even after 6 wk of planktotrophic feeding with virtually no energetic lipid reserves at metamorphosis, leading to the suggestion that these larvae use phospholipids to fuel metamorphosis (Prowse et al. 2017). In contrast, for *H. erythrogramma*, despite the energetic cost of metamorphosis, with 30% of energetic lipid stores depleted to fuel this process, the early juveniles still had massive maternal energetic lipid stores remaining (~3000 ng DAGE juvenile^{-1}). That the DAGE reserves are sequestered for the juvenile and so are physiologically inert through development is also supported by larval energetics (Hoegh-Guldberg & Emlet 1997). The lipid condition index of *H. erythrogramma* at metamorphosis (42.2) far exceeded that determined for *H. tuberculata* (0.001; Prowse et al. 2017). This is similar to the case for asterinid sea stars where the juveniles of a species with planktotrophic development, *Patiriella regularis*, had a lipid nutrition index of 1.4, whereas the indices of juveniles of the lecithotrophic developers *Meridiastra calcar* and *Parvulastra exigua* were 25.4 and 33.3, respectively (Prowse 2009, Prowse et al. 2017).

Ecological factors such as predation pressure, dispersal time, starvation and environmental abiotic stressors are likely to have influenced the evolutionary imperative to evolve a larger larva independent of an exogenous food source as well as a fitter high-quality juvenile at the start of benthic life (Vance 1973, Morgan 1995, Moran & McAlister 2009). Lecithotrophic larvae are less prone to predation by a suite of benthic predators (e.g. tunicates, mussels, anemones) than their planktotrophic counterparts (Mercier et al. 2013). For the newly formed juvenile, the nutritional buffer from a large egg, as seen in *H. erythrogramma*, allows them to focus their energy on growing their skeletal test and spines, structures that serve in protection and defence against predators (Strathmann 1981). Juvenile *H. erythrogramma* do not need to find food for some time, potentially a month or more post settlement (M. Byrne pers. obs.). The 14 d old juveniles still had 56% of maternal provisions remaining.

These reserves undoubtedly contribute to the hardy nature of these juveniles and their resilience to stress (Wolfe et al. 2013). While there are many advantages of lecithotrophy, the great diversity of species with planktotrophic propagules shows that this life history strategy has also been selected for, potentially in association with high dispersal potential, although the benefits of a larval stage and life history modes are mixed and have long been debated (Vance 1973, Havenhand 1993, Pechenik 1999, Moran & McAlister 2009, Marshall et al. 2012).

Although egg size evolution is theorised to have been influenced by the 'sperm environment' with respect to the target size that eggs present to sperm (Levitan 1993, Moran & McAlister 2009), egg target size in echinoids is also strongly influenced by the jelly coat, a feature not involved in larval nutrition (Deaker et al. 2019). The egg jelly coat increases the cross-sectional area of the egg several fold and is inexpensive to produce (Podolsky 2002). In the sand dollar *Dendraster excentricus* and sea urchin *Arbacia punctulata*, the jelly coat is estimated to cost only 2 and 7% of the energy required to produce the egg, respectively (Bolton et al. 2000, Podolsky 2001). This extracellular coat releases sperm attractants, which further increase egg target size by creating a chemical halo around the egg (Podolsky 2002, Inamdar et al. 2007, Deaker et al. 2019). Investment in the egg jelly coat differs across echinoid species, thereby differentially influencing egg target size for sperm, although the contribution of the jelly coat to egg target size is not incorporated into models of fertilization kinetics (Deaker et al. 2019). The relative importance of the different evolutionary drivers to increase egg size (offspring condition, egg target size for sperm) and the mechanisms involved (increased maternal provisioning, expansion of extracellular layers/chemicals) are difficult to tease out (Moran & McAlister 2009, Deaker et al. 2019). For the *Heliocidaris* species, the egg jelly provides a larger contribution to the increase in surface area of the egg of *H. tuberculata* than in *H. erythrogramma* (Foo et al. 2018).

Evolution of lecithotrophic development in echinoderms was associated with the emergence of new strategies of maternal investment, which also drove changes in the egg size–fecundity trade-off, likely in response to selection to improve the nutritional status of the early juvenile (Strathmann 1985, Emlet & Hoegh-Guldberg 1997, McEdward & Miner 2001, Prowse et al. 2009, Falkner et al. 2015). For the lipid-rich, positively buoyant eggs of echinoderms with lecithotrophic development, common themes in maternal provisioning with increasing egg size are emerging based on data for asteroids, ophiuroids and echinoids (Prowse et al. 2008, 2009, Falkner et al. 2015, this study). The occurrence of large DAGE-rich eggs in non-feeding larvae indicates convergent use of this lipid class to provision large eggs. However, this can vary, as seen in ophiocomid ophiuroids where methyl esters and WE (not DAGE) are the major energetic lipids in the eggs of some lecithotrophic developers (Falkner et al. 2015). Thus, in parallel with multiple and independent evolution of diverse lecithotrophic larvae in the Echinodermata, multiple patterns of maternal lipid provisioning have also evolved, but with an overall trend in the switch from dominance of TAG to other storage lipids, with DAGE being prominent.

The weight of evidence suggests that readily metabolised egg energetic lipid reserves such as TAG are most suitable for planktotrophic development (Table 4), while long-term energy stores are more appropriate fuels for lecithotrophic development. However, as seen here for *H. erythrogramma*, the eggs have a similar density of TAG as the eggs of *H. tuberculata*. Use of TAG to construct the larval body is also a feature of development in this species, albeit a very small proportion and for a much simpler larval morphology. We need more comparative data on closely related species with divergent modes of development to confirm that TAG use for larval building is a conserved feature of lecithotrophic development. To more fully understand evo-devo in the Echinodermata, we also need more data on egg biochemistry and lipid dynamics during development for holothuroids, for which just one study is available (Peters-Didier & Sewell 2017), and for crinoids for which there are no studies to date. The latter is a particularly important gap to address, as crinoids are the basal echinoderm group. Interestingly, crinoids lost the feeding larval stage millions of years ago, in association with major extinction events. Quantification of crinoid egg lipids would provide key insights into ancient (100s Mya) evolutionary change in maternal provisioning to compare with the more recent changes, as for the *Heliocidaris* species (~5 Mya) (Zigler et al. 2003).

Determination of the nutritive profile in the eggs of echinoderms with facultative planktotrophy is likely to be particularly informative (Allen & Pernet 2007). Thus far, only one study has determined total lipid levels in the eggs of a species with facultative planktotrophy, i.e. *Clypeaster rosaceus* (280 µm diameter egg, 2025 ng total lipid per egg) (Reitzel & Miner 2007). However, there are no data on the energetic lipid classes within this egg. In particular, the

DAGE content of these eggs would be insightful to determine if change in this lipid class was a key innovation associated with increased egg size to allow facultative feeding. The current paradigm is that a gradual increase in egg size reduced larval dependence on exogenous food, with the eventual loss of feeding structures, morphological simplification of larvae and finally full dependence on maternal provisions (Wray 1996, Hart 2002). This change is also likely to have been associated with oogenic and developmental plasticity. A parallel gradual increase in DAGE as egg size evolved may have been a convergent feature underlying the initial stages involved in the switch to lecithotrophy in echinoderms.

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LITERATURE CITED

Allen JD, Pernet B (2007) Intermediate modes of larval development: bridging the gap between planktotrophy and lecithotrophy. Evol Dev 9:643–653

Barbosa SS, Klanten OS, Puritz JB, Toonen RJ, Byrne M (2013) Very fine-scale population genetic structure of sympatric asterinid sea stars with benthic and pelagic larvae: influence of mating system and dispersal potential. Biol J Linn Soc 108:821–833

Bertram DF, Strathmann RR (1998) Effects of maternal and larval nutrition on growth and form of planktotrophic larvae. Ecology 79:315–327

Bolton TF, Thomas FIM, Leonard CN (2000) Maternal energy investment in eggs and jelly coats surrounding eggs of the echinoid *Arbacia punctulata.* Biol Bull (Woods Hole) 199:1–5

Byrne M (2006) Life history evolution in the Asterinidae. Integr Comp Biol 46:243–254

Byrne M, Cerra A (2000) Lipid dynamics in the embryos of *Patiriella* species (Asteroidea) with divergent modes of development. Dev Growth Differ 42:79–86

Byrne M, Villinski J, Popodi E, Cisternas P, Raff RR (1999) Maternal factors and the evolution of developmental mode: evolution of oogenesis in *Heliocidaris erythrogramma.* Dev Genes Evol 209:275–283

Byrne M, Emlet R, Cerra A (2001) Ciliated band structure in planktotrophic and lecithotrophic larvae of *Heliocidaris* species (Echinodermata: Echinoidea): conservation and change. Acta Zool 82:189–199

Byrne M, Prowse TAA, Sewell MA, Dworjanyn S, Williamson JE, Vaïtilingon D (2008a) Maternal provisioning for larvae and larval provisioning for juveniles in the sea urchin *Tripneustes gratilla.* Mar Biol 155:473–482

Byrne M, Sewell MA, Prowse TAA (2008b) Nutritional ecology of sea urchin larvae: influence of endogenous and exogenous nutrition on echinopluteal growth and phenotypic plasticity in *Tripneustes gratilla.* Funct Ecol 22: 643–648

Davidson PL, Thompson JW, Foster MW, Mosely MA, Byrne M, Wray GA (2019) A comparative analysis of egg provisioning using mass spectrometry during rapid life history evolution in sea urchins. Evol Dev (in press), doi:10.111/ede.12289

Deaker DJ, Foo SA, Byrne M (2019) Variability in egg and jelly-coat size and their contribution to target size for spermatozoa: a review for the Echinodermata. Mar Freshw Res 70: (in press)

Duda TF, Palumbi SR (1999) Developmental shifts and species selection in gastropods. Proc Natl Acad Sci USA 96: 10272–10277

Emlet RB, Hoegh-Guldberg O (1997) Effects of egg size on postlarval performance: experimental evidence from a sea urchin. Evolution 51:141–152

Emlet RB, Sadro SS (2006) Linking stages of life history: how larval quality translates into juvenile performance for an intertidal barnacle (*Balanus glandula*). Integr Comp Biol 46:334–346

Emlet RB, McEdward LR, Strathmann RR (1987) Echinoderm larval ecology viewed from the egg. In: Jangoux M, Lawrence JM (eds) Echinoderm studies 2. Balkema, Rotterdam, p 55–136

Falkner I, Sewell MA, Byrne M (2015) Evolution of maternal provisioning in ophiuroid echinoderms: characterisation of egg composition in planktotrophic and lecithotrophic developers. Mar Ecol Prog Ser 525:1–13

Foo SA, Deaker D, Byrne M (2018) Cherchez la femme — impact of ocean acidification on the egg jelly coat and attractants for sperm. J Exp Biol 221:jeb177188

George SB (1990) Echinoderm egg and larval quality as a function of adult nutritional state. Oceanol Acta 19:297–308

George SB, Cellario C, Fenaux L (1990) Population differences in egg quality of *Arbacia lixula* (Echinodermata: Echinoidea): proximate composition of eggs and larval development. J Exp Mar Biol Ecol 141:107–118

González-Ortegón E, Le Vay L, Mackay Walton LE, Giménez L (2018) Maternal trophic status and offspring phenotype in a marine invertebrate. Sci Rep 8:9618

Gosselin LA, Qian PY (1997) Juvenile mortality in benthic marine invertebrates. Mar Ecol Prog Ser 146:265–282

Hart MW (1996) Evolutionary loss of larval feeding: development, form, and function in a facultatively feeding larva, *Brisaster latifrons.* Evolution 50:174–187

Hart MW (2002) Life history evolution and comparative developmental biology of echinoderms. Evol Dev 4:62–71

Haszprunar G, von Salvini-Plawen L, Rieger RM (1995) Larval planktotrophy — a primitive trait in the Bilateria. Acta Zool (Stockh) 76:141–154

Havenhand JN (1993) Egg to juvenile period, generation time, and the evolution of larval type in marine invertebrates. Mar Ecol Prog Ser 97:247–260

Hoegh-Guldberg O, Emlet RB (1997) Energy use during the development of a lecithotrophic and a planktotrophic echinoid. Biol Bull (Woods Hole) 192:27–40

Inamdar MV, Kim T, Chung YK, Was AM and others (2007) Assessment of sperm chemokinesis with exposure to jelly coats of sea urchin eggs and resact: a microfluidic experiment and numerical study. J Exp Biol 210: 3805–3820

Israel JW, Martik ML, Byrne M, Raff EC, Raff RA, McClay DR, Wray GA (2016) Comparative developmental transcriptomics reveals rewiring of a highly conserved gene regulatory network during a major life history switch in the sea urchin genus *Heliocidaris.* PLOS Biol 14: e1002391

Jaeckle WB (1995) Variation in the size, energy content, and biochemical composition of invertebrate eggs: correlates to the mode of larval development. In: McEdward L (ed) Ecology of marine invertebrate larvae. CRC Press, Boca Raton, FL, p 49–77

Jong-Westman M, Qian PY, March EE, Carefoot TH (1995) Artificial diets in sea urchin culture: effects of dietary protein level and other additives on egg quality, larval morphometrics, and larval survival in the green sea urchin, *Strongylocentrotus droebachiensis.* Can J Zool 73:2080–2090

Lamare MD, Barker MF (1999) *In situ* estimates of larval development and mortality in the New Zealand sea urchin *Evechinus chloroticus* (Echinodermata: Echinoidea). Mar Ecol Prog Ser 180:197–211

Lee RF, Hagen W, Kattner G (2006) Lipid storage in marine zooplankton. Mar Ecol Prog Ser 307:273–306

Levitan DR (1993) The importance of sperm limitation to the evolution of egg size in marine invertebrates. Am Nat 141:517–536

Levitan DR (2000) Optimal egg size in marine invertebrates: theory and phylogenetic analysis of the critical relationship between egg size and development time in echinoids. Am Nat 156:175–192

Marshall DJ, Allen RM, Crean AJ (2008) The ecological and evolutionary importance of maternal effects in the sea. Oceanogr Mar Biol Annu Rev 46:203–250

Marshall DJ, Krug PJ, Kupriyanova E, Byrne M, Emlet RB (2012) The biogeography of marine invertebrate life histories. Annu Rev Ecol Evol Syst 43:97–114

Matson PG, Yu PC, Sewell MA, Hofmann GE (2012) Development under elevated pCO_2 conditions does not affect lipid utilization and protein content in early life-history stages of the purple sea urchin, *Strongylocentrotus purpuratus.* Biol Bull (Woods Hole) 223:312–327

McAlister JS, Moran AL (2013) Effects of variation in egg energy and exogenous food on larval development in congeneric sea urchins. Mar Ecol Prog Ser 490:155–167

McEdward LR, Carson SF (1987) Variation in egg organic content and its relationship with egg size in the starfish *Solaster stimpsoni.* Mar Ecol Prog Ser 37:159–169

McEdward LR, Miner BG (2001) Larval and life cycle patterns in echinoderms. Can J Zool 79:1125–1170

Mercier A, Doncaster EJ, Hamel JF (2013) Contrasting predation rates on planktotrophic and lecithotrophic propagules by marine benthic invertebrates. J Exp Mar Biol Ecol 449:100–110

Miller BA, Emlet RB (1999) Development of newly metamorphosed juvenile sea urchins (*Strongylocentrotus franciscanus* and *S. purpuratus*): morphology, the effects of temperature and larval food ration, and a method for determining age. J Exp Mar Biol Ecol 235:67–90

Moore M, Manahan DT (2007) Variation among females in egg lipid content and developmental success of echinoderms from McMurdo Sound, Antarctica. Polar Biol 30: 1245–1252

Moran AL, McAlister JS (2009) Egg size as a life history character of marine invertebrates: Is it all it's cracked up to be? Biol Bull (Woods Hole) 216:226–242

Moran AL, McAlister JS, Whitehill EAG (2013) Egg as energy: revisiting the scaling of egg size and energy content among echinoderms. Biol Bull (Woods Hole) 224: 184–191

Morgan SG (1995) Life and death in the plankton: larval mortality and adaptation. In: McEdward L (ed) Ecology of marine invertebrate larvae. CRC Press, Boca Raton, FL, 279–322

Morris VB, Byrne M (2005) Involvement of two Hox genes and *Otx* in echinoderm body-plan morphogenesis in the sea urchin *Holopneustes purpurescens* [sic]. J Exp Zool B Mol Dev Evol 304:456–467

Mortensen T (1921) Studies of the development and larval forms of echinoderms. G.E.C. Gad, Copenhagen

O'Connor MI, Bruno JF, Gaines SD, Halpern BS, Lester SE, Kinlan BP, Weiss JM (2007) Temperature control of larval dispersal and the implications for marine ecology, evolution and conservation. Proc Natl Acad Sci USA 104: 1266–1271

Pechenik JA (1999) On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. Mar Ecol Prog Ser 177:269–297

Pechenik JA (2006) Larval experiences and latent effects — metamorphosis is not a new beginning. Integr Comp Biol 46:323–333

Peters-Didier J, Sewell MA (2017) Maternal investment and nutrient utilization during early larval development of the sea cucumber *Australostichopus mollis.* Mar Biol 164: 178

Phillips NE (2004) Variable timing of larval food has consequences for early juvenile performance in a marine mussel. Ecology 85:2341–2346

Phleger CF, Nichols PD, Virtue P (1997) Lipids and buoyancy in Southern Ocean pteropods. Lipids 32:1093–1100

Podolsky RD (2001) Evolution of egg target size: an analysis of selection on correlated characters. Evolution 55: 2470–2478

Podolsky RD (2002) Fertilization ecology of egg coats: physical versus chemical contributions to fertilization success of free-spawned eggs. J Exp Biol 205:1657–1668

Prowse TAA (2009) Evolutionary ecology of maternal investment in echinoderms. PhD thesis, University of Sydney

Prowse TAA, Sewell MA, Byrne M (2008) Fuels for development: evolution of maternal provisioning in asterinid sea stars. Mar Biol 153:337–349

Prowse TAA, Falkner I, Sewell MA, Byrne M (2009) Long-term storage lipids and developmental evolution in echinoderms. Evol Ecol Res 11:1069–1083

Prowse TAA, Sewell MA, Byrne M (2017) Three-stage lipid dynamics during development of planktotrophic echinoderm larvae. Mar Ecol Prog Ser 583:149–161

Puritz JB, Keever CC, Addison JA, Barbosa SS and others (2017) Life history predicts past and present population connectivity in two sympatric sea stars. Ecol Evol 7: 3916–3930

R Core Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Raff RA, Byrne M (2006) The active evolutionary lives of echinoderm larvae. Heredity 97:244–252

Reitzel AM, Miner BG (2007) Reduced planktotrophy in the larvae of *Clypeaster rosaceus.* Mar Biol 151:1525–1534

Sewell MA (2005) Utilization of lipids during early development of the sea urchin *Evechinus chloroticus.* Mar Ecol Prog Ser 304:133–142

Sewell MA, Manahan DT (2001) Echinoderm eggs: biochemistry and larval biology. In: Barker MF (ed) Echinoderms 2000. Swets & Zeitlinger, Lisse, p 55–58

Smith AB, Pisani D, Mackenzie-Dodds JA, Stockley B, Webster BL, Littlewood TJ (2006) Testing the molecular clock: molecular and paleontological estimates of divergence times in the Echinoidea (Echinodermata). Mol Biol Evol 23:1832–1851

Strathmann RR (1981) The role of spines in preventing structural damage to echinoid tests. Paleobiology 7:400–406

Strathmann RR (1985) Feeding and nonfeeding larval development and life-history evolution in marine invertebrates. Annu Rev Ecol Syst 16:339–361

Styan CA, Byrne M, Franke E (2005) Evolution of egg size and fertilisation efficiency in sea stars: large eggs are not fertilised more readily than small eggs in the genus *Patiriella* (Echinodermata: Asteroidea). Mar Biol 147: 235–242

Turner RL, Lawrence JM (1979) Volume and composition of echinoderm eggs: implications for the use of egg size in life history models. In: Stancyk SE (ed) Reproductive ecology of marine invertebrates. University of South Carolina Press, Columbia, SC, p 25–40

Vance RR (1973) On reproductive strategies in marine benthic invertebrates. Am Nat 107:339–361

Villinski JT, Villinski JC, Byrne M, Raff RA (2002) Convergent maternal provisioning and life-history evolution in echinoderms. Evolution 56:1764–1775

Whitehill EAG, Moran AL (2012) Comparative larval energetics of an ophiuroid and an echinoid echinoderm. Invertebr Biol 131:345–354

Wolfe K, Dworjanyn S, Byrne M (2013) Effects of ocean warming and acidification on survival, growth and skeletal development in the early benthic juvenile sea urchin (*Heliocidaris erythrogramma*). Glob Change Biol 19: 2698–2707

Wray GA (1996) Parallel evolution of nonfeeding larvae in echinoids. Syst Biol 45:308–322

Zigler KS, Raff EC, Popodi E, Raff RA, Lessios HA (2003) Adaptive evolution of bindin in the genus *Heliocidaris* is correlated with the shift to direct development. Evolution 57:2293–2302

Appendix. Calculation of egg volume

Table A1. Mean (SE) egg diameters (n = 20) for each species with the number of females used (n) and egg volumes used to calculate lipid density (see 'Materials and methods')

Species	Egg diameter (µm)	Mean egg volume (nl)
Heliocidaris tuberculata (n = 3)	88.51 (0.91); 91.9 (0.84); 92.3 (0.2)	0.3937
Heliocidaris erythrogramma (n = 6)	390 (8.03); 392 (0.83); 397 (3.2); 399 (2.2); 400 (4.5); 405 (4.0)	32.8189
Holopneustes purpurascens (n = 2)	595 (11.1); 624 (7.6)	118.7563
Temnopleurus alexandri	125[a]	1.0227

[a]M. Byrne pers. obs. (egg diameters of analysed females were not available)

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