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RESEARCH ARTICLE

Cherchez la femme – impact of ocean acidification on the egg jelly coat and attractants for sperm

Shawna A. Foo[1,*], Dione Deaker[2] and Maria Byrne[3]

ABSTRACT

The impact of ocean acidification on marine invertebrate eggs and its consequences for sperm chemotaxis are unknown. In the sea urchins *Heliocidaris tuberculata* and *Heliocidaris erythrogramma*, with small (93 µm) and large (393 µm) eggs, respectively, we documented the effect of decreased pH on the egg jelly coat, an extracellular matrix that increases target size for sperm and contains sperm-attracting molecules. In near-future conditions (pH 7.8, 7.6), the jelly coat of *H. tuberculata* decreased by 11% and 21%, reducing egg target size by 9% and 17%, respectively. In contrast, the egg jelly coat of *H. erythrogramma* was not affected. The reduction in the jelly coat has implications for sperm chemotaxis in *H. tuberculata*. In the presence of decreased pH and egg chemicals, the sperm of this species increased their velocity, motility and linearity, behaviour that was opposite to that seen for sperm exposed to egg chemicals in ambient conditions. Egg chemistry appears to cause a reduction in sperm velocity where attractants guide the sperm in the direction of the egg. Investigation of the effects of decreased pH on sperm isolated from the influence of egg chemistry does not provide an integrative assessment of the effects of ocean acidification on sperm function. Differences in the sensitivity of the jelly coat of the two species is likely associated with egg evolution in *H. erythrogramma*. We highlight important unappreciated impacts of ocean acidification on marine gamete functionality, and insights into potential winners and losers in a changing ocean, pointing to the advantage conveyed by the evolution of large eggs.

KEY WORDS: Egg extracellular matrix, Egg size, Target size, Broadcast spawning, Sperm chemotaxis, *Heliocidaris*

INTRODUCTION

As the ocean is on a trajectory of increased acidification because of increased uptake of atmospheric CO_2 (IPCC, 2014), there are major concerns for the functionality of the gametes of free-spawning species. These cells are fundamental for the propagation and persistence of marine populations that are directly exposed to environmental conditions (Pechenik, 1987), where surface ocean pH is projected to drop by 0.3 pH units by 2100 (IPCC, 2014). Thus far, investigation of the impacts of ocean acidification (OA) on sperm physiology and motility has been conducted with sperm isolated from the influence of egg chemistry (reviewed in Campbell et al., 2016). The impact of OA on the egg cell and its consequences for egg chemistry and sperm chemotaxis are unknown (Foo and Byrne, 2017).

The eggs of many marine invertebrates are surrounded by a jelly coat, including those of echinoderms, many molluscs and some polychaetes (Suzuki, 1989; Rosati, 1995; Farley and Levitan, 2001; Podolsky, 2002; Hofmann, 2013; Plickert, 2013). In sea urchins, the jelly coat is a polysaccharide–glycoprotein extracellular matrix that hydrates in contact with seawater and is known to be sensitive to low pH (Podolsky, 2002; Dale and de Felice, 2011; Vacquier, 2011), and so may be vulnerable to OA. In molluscs and polychaetes, the egg jelly coat can be quite diffuse (Anderson and Eckberg, 1983; Focarelli et al., 1991), and thus most studies of the chemical nature and function of the egg jelly coat have focused on echinoderms.

In echinoderms, the jelly coat serves many roles before and during fertilisation. Jelly coats provide mechanical support for the egg, reducing the shear stress that eggs experience when passing through the gonopore (Thomas and Bolton, 1999; Bolton et al., 2000). The jelly coat is an economical way to increase egg target size for sperm, thereby facilitating fertilisation success (Vogel et al., 1982; Farley and Levitan, 2001; Podolsky, 2002). The sialic acid and glycan content of the egg jelly coat shows interspecific and intraspecific differences in sea urchins, and this influences differences in the hydration of egg jelly after spawning (Jondeung and Czihak, 1982; Pomin, 2015).

The effect of removal of the egg jelly coat on fertilisation is not well understood, with conflicting results. Studies that report little or no effects of jelly coat removal are largely short-term experiments involving high levels of sperm and where removal of the jelly coat increased fertilisation rate by removal of a barrier (Hagström, 1959; Vacquier et al., 1978). In contrast, studies investigating fertilisation in sperm-limiting conditions show that removal of the egg jelly coat decreased fertilisation success (McLaughlin and Humphries, 1978; Styan, 1998). For *Lytechinus variegatus*, eggs with intact jelly coats accrued 2.2 more collisions with sperm compared with eggs without jelly coats, which required double the amount of sperm to achieve 50% fertilisation (Farley and Levitan, 2001).

Several studies of echinoids, asteroids and abalone have shown that the jelly coat possesses chemoattractive properties (Miller, 1985; Suphamungmee et al., 2010; Riffell et al., 2002). The egg jelly coat of sea urchins contains the short peptides speract and resact, which attract sperm, stimulate sperm metabolism and influence the orientation of the sperm, thereby increasing the probability of fertilisation (Miller, 1985; Matsumoto et al., 2003; Islam et al., 2008). Compounds in the jelly coat have been shown to promote directional swimming and altered swimming paths in sperm to maximise fertilisation success (Fitzpatrick et al., 2012; Jikeli et al., 2015). These compounds also stimulate the acrosome reaction to promote conspecific sperm–egg binding (Matsui et al., 1986). For the mussel *Mytlius galloprovincialis*, egg molecules act as a selective barrier to promote fertilisation by more compatible sperm, with the most successful male ejaculate having the lowest percentage of motile sperm (Fitzpatrick et al., 2012).

[1]Department of Global Ecology, Carnegie Institution for Science, Stanford, CA 94305, USA. [2]Department of Anatomy and Histology, School of Medical Sciences, The University of Sydney, Sydney, NSW 2006, Australia. [3]Department of Anatomy and Histology, School of Medical Sciences, and School of Life and Environmental Sciences, The University of Sydney, Sydney, NSW 2006, Australia.

*Author for correspondence (sfoo@carnegiescience.edu)

S.A.F., 0000-0002-7083-2377

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Accessory structures that surround the egg cell affect fertilisation by increasing egg target size, and so are considered to be under strong selection (Podolsky, 2004; Crean and Marshall, 2015). The size of the egg jelly coat differs greatly among species and this is suggested to increase egg target size and chemical attraction to sperm, especially in small eggs with respect to the sperm-limited environment expected to occur in nature (Podolsky, 2002, 2004). Many life history traits (e.g. fertilisation, larval duration, larval type) in marine invertebrates are tied to egg size evolution (Marshall et al., 2012). Thus, we need to understand the impact of OA on the egg function as well as its potential effects on sperm. In addition, the egg itself produces molecules that are released into the environment, where they mediate sperm behaviour (Vacquier and Moy, 1977; Kashikar et al., 2012). In abalone, these molecules are crucial for recruitment of sperm to the egg surface (Riffell et al., 2002). These egg functions, driven by released egg chemicals, may also be altered by lower pH.

We investigated the sensitivity of the egg jelly coat to decreased pH in sympatric, congeneric sea urchins with divergent modes of development. *Heliocidaris tuberculata* spawns a small (93 µm), negatively buoyant egg and has feeding larvae, traits considered ancestral for extant echinoderms (Raff and Byrne, 2006). In contrast, *Heliocidaris erythrogramma* spawns a large (393 µm), highly buoyant, lipid-rich egg and has non-feeding larvae (Byrne et al., 1999). The egg jelly coat is a key barrier to interspecies fertilisation (Raff et al., 1999). As the size of the jelly coat is correlated with egg size (Levitan, 2006), evolution of a larger egg in *H. erythrogramma* is probably associated with jelly coat modifications that may influence its vulnerability to OA. We hypothesised that CO_2-driven acidification would reduce the size of the jelly coat and that the magnitude of change would differ between the two species. For species that have a jelly coat vulnerable to OA, we hypothesised that this would affect sperm motility. In the first study to consider the impacts of decreased pH on the dual functionality of eggs and sperm, we hypothesise that the biology of the egg is pH dependent, and that this in turn directly affects the behavioural response of sperm in low pH conditions.

MATERIALS AND METHODS

Study species, collection sites and spawning procedure

Heliocidaris tuberculata (Lamarck 1816) and *Heliocidaris erythrogramma* (Valenciennes 1846) were collected from Long Bay (33°57′54″S, 151°15′20″E) and Edwards Beach (33°49′11″S, 151°15′8″E), Sydney, NSW, Australia, under the permit NSW DPI: P00/0015-6.0. Animals were transported in ambient seawater in a cool box and transferred promptly to flow-through aquaria. All animals were used for experiments within 7 days of collection. Spawning was induced by injection of 1–2 ml of 0.5 mol l^{-1} KCl. Eggs were examined for consistency in shape and transferred to a beaker (500 ml) of fresh filtered seawater (FSW, 1 µm).

Experimental conditions

Experimental treatments consisted of three pH_T (pH on the total scale) levels for jelly coat experiments (mean±s.e.m., control 7.97±0.02, 7.78 ±0.04 and 7.56±0.03; Table 1) or two pH_T levels for sperm chemotaxis experiments (mean±s.e., control 7.99±0.02 and 7.59±0.01; Table 2). Treatments were based on model projections for end-of-century surface ocean waters in southeast Australia (IPCC, 2014). To achieve experimental treatments, FSW was bubbled with a mixture of air and CO_2, and pH adjustment was tracked using a pH meter [Wissenschaftilich-Technische Werkstatten (WTW), Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim, Germany] and probe (WTW SenTix 41 pH electrode). Probes were calibrated using National Institute of Standards and Technology (NIST) high precision buffers with pH 4.0, 7.0 and 10.0 (ProSciTech, Thuringowa Central, QLD, Australia). pH_T was determined using the spectrophotometric method with *m*-Cresol Purple indicator dye (AO321770; Acros Organics, Thermo Fisher Scientific, Waltham, MA, USA) and an Ocean Optics USB4000 spectrometer connected to a bluLoop 395–750 nm LED light source (Ocean Optics Inc., Largo, FL, USA) following the procedures outlined in standard operating procedure (SOP) 6b of Dickson et al. (2007) and the equations of Liu et al. (2011). Experiments were conducted at 21°C. The mean salinity of the treatment water was 34 psu, and dissolved oxygen remained >90%.

Samples of the water (250 ml) were collected from each experiment and fixed with 100 µl of saturated HgCl. These were used to determine total alkalinity (TA) by potentiometric titration (Metrohm 888 Titrando, Herisau, Switzerland), using certified reference standards (Dickson et al., 2007). Experimental partial pressure of CO_2 (P_{CO_2}) (Tables 1 and 2) values were determined from TA, temperature, pH_T and salinity data using CO2SYS with the dissociation constants of Mehrbach et al. (1973) as refitted by Dickson and Millero (1987).

Table 1. Water conditions in jelly coat experiments for each species

pH	Measure	*Heliocidaris erythrogramma*	*Heliocidaris tuberculata*
Control	pH_T	7.96±0.02	7.98±0.02
	P_{CO_2}	513.95	489.41
	TA	2289.23	2303.17
	DIC	2071.51	1267.99
7.8	pH_T	7.78±0.04	7.78±0.03
	P_{CO_2}	940.74	817.34
	TA	2304.75	2308.68
	DIC	2179.95	1445.07
7.6	pH_T	7.53±0.03	7.59±0.03
	P_{CO_2}	1436.54	1326.72
	TA	2297.6	2300.78
	DIC	2230.82	1717.72
	Temperature	21	21
	Salinity	34	34

Values for pH_T (pH on the total scale; means±s.e.), total alkalinity (TA; µmol kg^{-1}) and dissolved inorganic carbon (DIC; µmol kg^{-1}) measured per treatment are shown. Partial pressure of CO_2 (P_{CO_2}, µatm) was calculated in CO2SYS using the data on TA, temperature and salinity (*n*=5 for *H. erythrogramma*, *n*=4 for *H. tuberculata*).

Jelly coat experiments

For each species, eight females were used. For each female, egg counts were determined in 100 µl aliquots from the egg suspension

Table 2. Water conditions for sperm chemotaxis experiments

pH	Measure	Mean±s.e.
Control	pH_T	7.99±0.02
	P_{CO_2}	485.75±13.72
	TA	2458.52±3.98
	DIC	2227.38±2.81
7.6	pH_T	7.59±0.01
	P_{CO_2}	1388.52±23.62
	TA	2456.90±3.12
	DIC	2394.24±7.35
	Temperature	18.66±1.12
	Salinity	34.87±0.26

Values for pH_T, TA (µmol kg^{-1}) and DIC (µmol kg^{-1}) measured per treatment are shown. P_{CO_2} (µatm) was calculated in CO2SYS using the data on TA, temperature and salinity (*n*=3).

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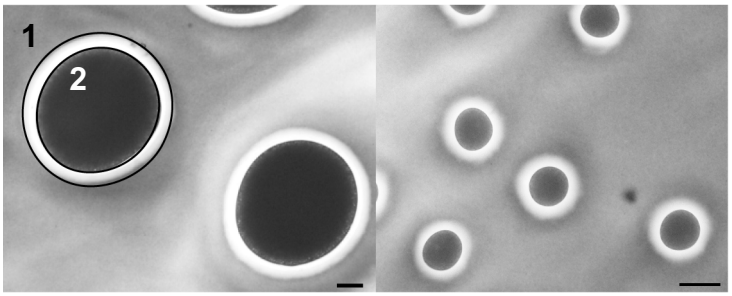

Fig. 1. Eggs of *Heliocidaris erythrogramma* (left) and *Heliocidaris tuberculata* (right) suspended in Sumi ink, which allows the extracellular jelly coat to be visualised. The total area of the egg plus jelly coat (1) minus the egg area only (2) was used to estimate jelly coat area. Scale bars: 100 µm.

and transferred to treatments within 60 s of spawning. Approximately 200 eggs were placed into containers (100 ml glass jars), one for each of the three pH treatments and one for each of the five time points (0, 5, 10, 15 and 30 min). Thus, each time point for each pH treatment had an independent jar. Jelly coats expand to approximately 80% of their maximum thickness within 2 min of contact with seawater (Podolosky, 2001), and so the time points covered the greatest changes in jelly coat size. At each time point, a sample of eggs was taken from each pH treatment and suspended in Sumi ink (Holbein, Chuo-Ku, Osaka, Japan) so that the jelly coat could be visualised microscopically (Fig. 1) using an Olympus DP73 digital camera mounted on an Olympus BX60 microscope. This method was repeated for the eggs of eight females per time point for each species.

The cross-sectional area of the egg and jelly coat, and the egg only was measured for 10 eggs per pH and time point using ImageJ (National Institutes of Health, Bethesda, MD, USA) (Fig. 1). The area of the jelly coat was calculated by subtracting the area of the egg from the whole egg and jelly coat area. The percentage change in jelly coat area at each time point compared with the start time was calculated to analyse the hydration of the jelly coat over time.

Egg and jelly coat area measurements indicated changes in egg target size (fertilisation models, *sensu* Podolsky, 2004; Fig. 1) in response to decreased pH. As the spawned eggs were not consistently spherical, the diameter (D) of each egg was determined by finding the equivalent area diameter, treating the measured area (A) of the egg as if it were that of a circle, where:

$$D = 2\sqrt{\frac{A}{\pi}}. \quad (1)$$

The equivalent diameter was then used to calculate the volume (V) of both the egg and the egg plus jelly coat, where:

$$V = \frac{4}{3}\pi\left(\frac{D}{2}\right)^3. \quad (2)$$

The relative size of the jelly coat (Bolton et al., 2000), the ratio of the diameter of the egg plus jelly coat to the diameter of the egg, allowed the size of the jelly coat to be compared across different females, which produced eggs of varying size. Target size (T) of the jelly coat was calculated using the equivalent diameter, where:

$$T = 4\pi\left(\frac{D}{2}\right)^2. \quad (3)$$

Effects of low pH on sperm chemotaxis in *H. tuberculata*

The egg seawater treatment contained eggs pooled from multiple females to incorporate potential differences in sperm and egg compatibility (Evans et al., 2012). Approximately equal numbers of eggs from each female were collected into pH 8 or pH 7.6 FSW. Egg counts were conducted immediately after spawning, and eggs were pipetted into 120 ml jars containing their respective treatment water at two concentrations to create two levels of egg chemistry in the water: 100 and 1000 eggs ml^{-1}. The jars were filled and capped to prevent gas exchange and swirled every 5 min for 30 min to mix the eggs through the water. Eggs were then removed from the FSW to create egg seawater containing dissolved egg jelly and egg molecules. The pH of the water remained unchanged after the dissolution of egg and egg jelly molecules.

Sperm were activated at a concentration of 1 µl dry sperm ml^{-1} in pH_T 8 and pH 7.6 water that had not been treated with eggs (control) and in experimental FSW that had been exposed to eggs (100 or 1000 eggs ml^{-1}), which resulted in a total of six treatments. A 2 s video was taken after 5 min at 40× magnification with an image capture rate of 28 frames s^{-1} (using an Olympus DP73 digital camera mounted on an Olympus BX60 microscope). Slides and coverslips were prepared using a 1% BSA solution, and coverslips were held ∼2 mm above the slide using Plasticine supports to let the sperm swim freely. This was replicated four times for each egg concentration and pH level across six different males.

Sperm videos were analysed using the computer-assisted sperm analysis (CASA) plugin for ImageJ. The threshold was pre-set for each video and manually adjusted to prevent misidentification of sperm by the CASA software. Swimming velocity was measured as curvilinear velocity (VCL), referring to the speed of the sperm along its whole swimming track. The percentage of moving (motile) sperm, VCL and the curvature of the sperm swimming path (linearity) were calculated for each video. Linearity is a percentage of the ratio of straight-line velocity to the average velocity of the sperm along its trajectory.

Table 3. Results of individual three-way ANOVA analyses on the effects of decreased pH on jelly coat area for two echinoid species

Source	d.f.	MS	*F*	*P*
H. erythrogramma				
Female	7	1249.7	10.484	**0.0001**
Time	3	23.169	0.10891	0.9534
pH	2	18.88	0.15488	0.8531
Female×time	21	212.73	1.7847	**0.0167**
Female×pH	14	121.9	1.0227	0.4251
Time×pH	6	53.644	0.54264	0.7679
Female×time×pH	42	98.857	0.82934	0.7720
H. tuberculata				
Female	7	1.0614×10^5	407.72	**0.0001**
Time	3	867.69	0.90256	0.4589
pH	2	55,214	32.652	**0.0001**
Female×time	21	961.36	3.6931	**0.0001**
Female×pH	14	1691	6.496	**0.0001**
Time×pH	6	490.36	1.7732	0.1231
Female×time×pH	42	276.54	1.0623	0.366

Significant results ($P \leq 0.05$) are indicated in bold.

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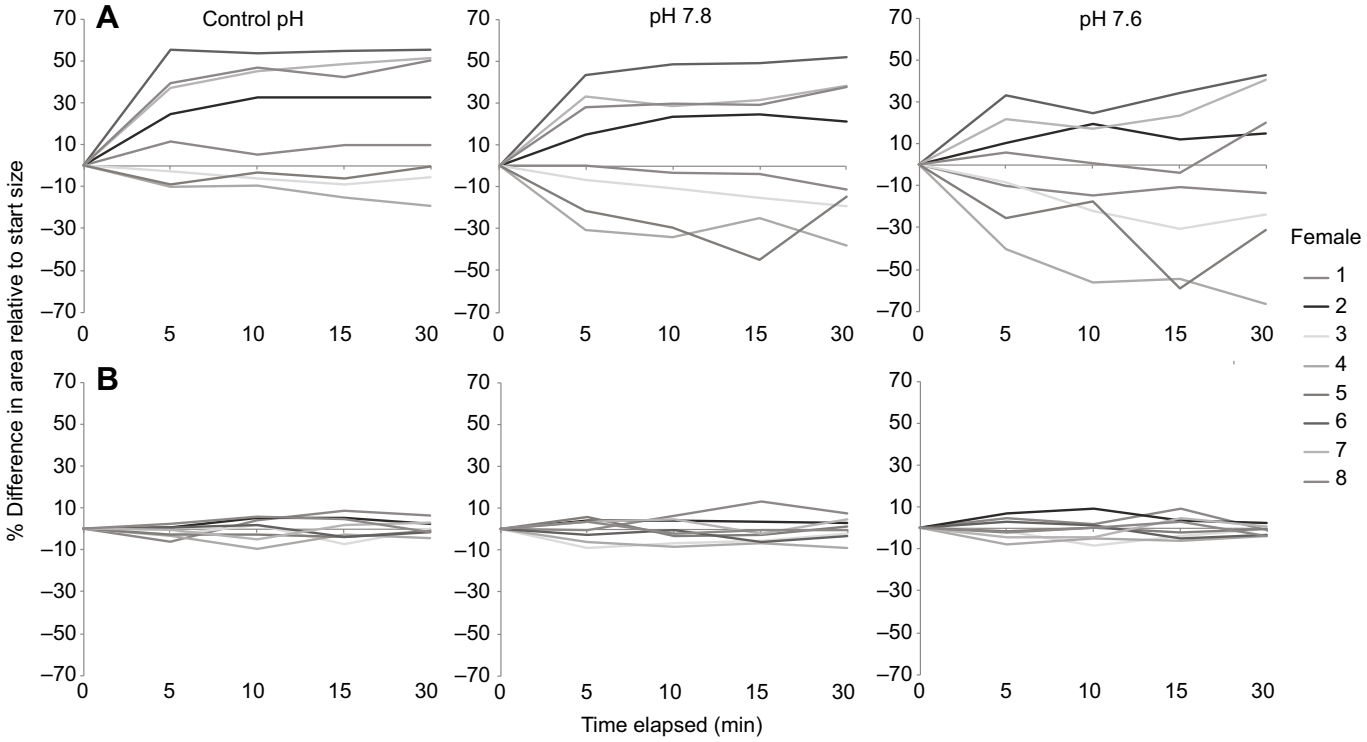

Fig. 2. Effects of decreased pH over time on the size of the jelly coat around the eggs of *Heliocidaris*. The percentage difference in jelly coat area relative to the initial size is shown for individual females across each pH level for (A) *H. tuberculata* and (B) *H. erythrogramma*.

Statistical analyses

To determine the effect of decreased pH on jelly coat area, the data were analysed using a three-way ANOVA in GMAV, with time and pH as fixed factors, and individual female as a random factor.

Linear regression analyses were performed to assess the relationship between mean egg volume and mean jelly coat volume. To avoid the confounding influence of absolute egg area and jelly coat size, jelly coat volume only (with egg volume subtracted) was used to normalise the data (Levitan and Irvine, 2001; Podolosky, 2001). In addition to the eight females used in this study, data from eight other females of each species from Foo (2015) were used for this analysis.

For sperm chemotaxis experiments, VCL, percentage motile sperm and linearity were analysed using a three-way ANOVA in GMAV, with pH and egg seawater concentration as fixed factors, and individual male as a random factor. For both data sets, the assumptions of homogeneity of variance were confirmed using Cochran's test. Where there were significant effects, Student–Newman–Keuls (SNK) tests were used for *post hoc* analyses ($P<0.05$).

RESULTS

Egg traits in control conditions

For *H. tuberculata*, the mean±s.e.m. egg diameter was 93.2±1.2 µm and the mean±s.e.m. egg plus jelly coat thickness was 148.1±4.8 µm (n=160, 10 eggs per female, 16 females). For *H. erythrogramma*, the mean±s.e.m. egg diameter was 393.2±4.5 µm and the mean±s.e.m. egg plus jelly coat thickness was 509±5.8 µm (n=160, 10 eggs per female, 16 females). Thus, the jelly coat provided an increased egg target size of 153% for *H. tuberculata* and 68% for *H. erythrogramma*. The relative size of the jelly coat was 1.3 for *H. erythrogramma* and 1.6 for *H. tuberculata*. Thus, the jelly coat

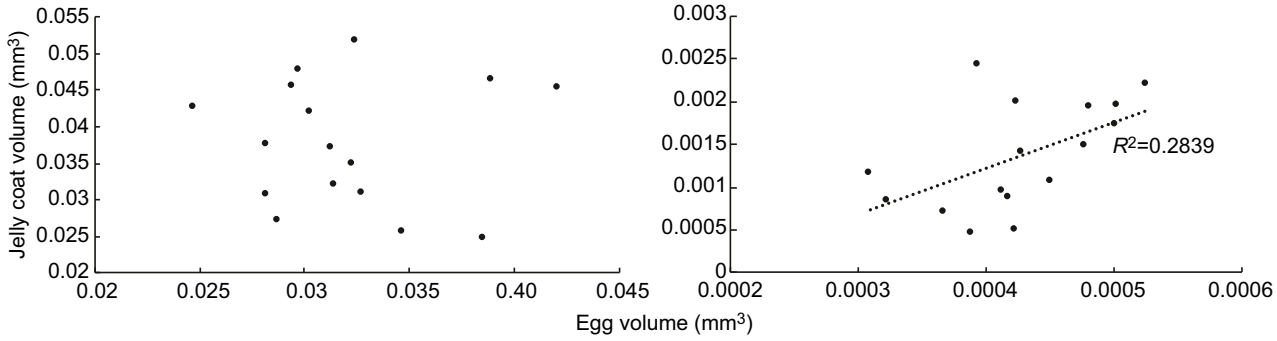

Fig. 3. Relationship between mean egg volume and mean jelly coat volume across 16 females of *H. erythrogramma* (left) and *H. tuberculata* (right). A positive relationship was evident only for *H. tuberculata*, where larger eggs also had a larger jelly coat (n=16, $P<0.05$).

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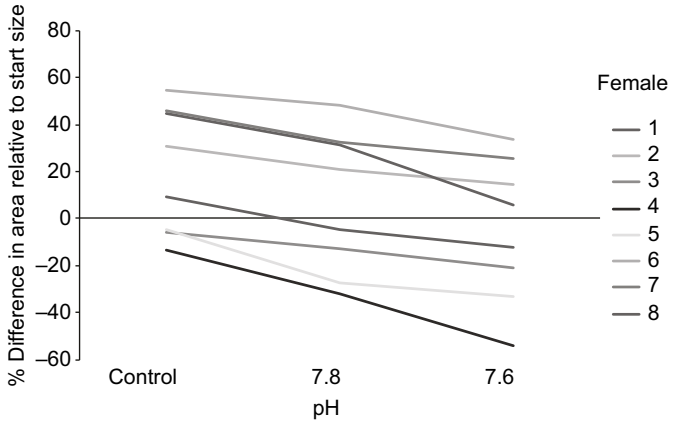

Fig. 4. Effects of decreased pH on *H. tuberculata* jelly coat area, shown for each female across pH levels. The percentage difference in jelly coat area relative to initial size (line from 0) pooled over the four time points is shown for eight females for each pH treatment.

contributes substantially more to egg target size in the small egg species, *H. tuberculata*.

For both species, there was variation in jelly coat size depending on egg source. Significant effects of female and female×time for both species indicated that the size of the jelly coat in control conditions differed across females, and that this varied over time (Table 3, Fig. 2A,B). For *H. tuberculata*, four females showed a slight increase in jelly coat thickness over time, whereas two females showed a slight decrease (Table 3, Fig. 2A). There was a significant positive correlation between the egg volume and jelly coat volume. Thus, bigger eggs had larger jelly coats (Fig. 3).

For *H. erythrogramma*, *post hoc* analyses showed that the jelly coat of one of the eight females increased in thickness between 10 and 15 min, whereas another female showed a slight decrease (Fig. 2B). The jelly coat of the other six females remained similar in size across all time points. There was no correlation between the egg volume and jelly coat volume in *H. erythrogramma* (Fig. 3).

Table 4. Results of three-way ANOVA analyses on the effects of decreased pH and egg seawater on sperm velocity, motility and linearity

Source	d.f.	MS	*F*	*P*
Velocity				
pH	1	4522.885	12.534	**0.017**
Egg concentration	2	2195.036	7.146	**0.012**
Male	5	10,738.490	20.003	**0.000**
pH×egg concentration	2	281.196	2.141	0.168
pH×male	5	360.964	2.748	0.082
Egg concentration×male	10	307.331	2.339	0.098
pH×egg concentration×male	10	131.375	3.067	**0.002**
Motility				
pH	1	0.002	0.427	0.542
Egg concentration	2	0.047	3.622	0.066
Male	5	0.050	4.224	0.073
pH×egg concentration	2	0.007	10.32	0.391
pH×male	5	0.006	0.822	0.561
Egg concentration×male	10	0.013	1.843	0.175
pH×egg concentration×male	10	0.007	2.798	**0.004**
Linearity				
pH	1	0.229	17.371	**0.009**
Egg concentration	2	0.073	19.537	**0.000**
Male	5	0.029	1.894	0.223
pH×egg concentration	2	0.001	0.887	0.442
pH×male	5	0.013	7.828	**0.003**
Egg concentration×male	10	0.004	2.228	0.111
pH×egg concentration×male	10	0.002	1.401	0.189

Significant results ($P \leq 0.05$) are indicated in bold.

Effects of OA on the jelly coat

For *H. tuberculata*, there were significant effects of pH as well as a significant interaction between female and pH (Table 3) on the egg jelly coat area. Exposure to pH 7.8 and 7.6 reduced the area of the jelly coat by 11% and 21%, respectively (Figs 2A and 4). The negative effect of pH varied with female ($P<0.0001$), with the egg jelly coats of some females being more susceptible to decreased pH (Fig. 4). *Post hoc* analyses showed that the egg jelly coats of two females were unaffected by exposure to pH 7.8; however, the jelly coats of all females decreased when exposed to pH 7.6 (Fig. 4). Calculations of target size showed that the mean±s.e.m. size of control eggs was 75,292±5129 μm^2. This was reduced by 9% in pH 7.8 and by 17% in pH 7.6.

For *H. erythrogramma*, there was no effect of decreased pH on the jelly coat area, and hydration level also did not differ (Table 3, Fig. 2B). Thus, decreased pH did not affect egg jelly coat size in *H. erythrogramma*.

Influence of egg chemistry and OA on sperm chemotaxis in *H. tuberculata*

In control seawater, the presence of egg chemicals caused a decrease in the velocity of the sperm of *H. tuberculata*. Exposure to low pH, however, increased the velocity, with a similar increase in velocity seen at both egg chemistry conditions (Table 4, Fig. 5). With regards to motility, the number of motile sperm decreased at control pH for both egg chemistry conditions (Table 4, Fig. 6). At low pH, the decrease in sperm motility was eliminated in the lower egg chemistry treatment, but retained in the higher egg chemistry treatment (Table 4, Fig. 6). At control pH, swimming paths of the sperm became more circular under both egg chemistry conditions (Table 4, Fig. 7B). In contrast, at low pH, swimming paths were more linear for all but one male (Table 4, Fig. 7A). There were no interactive effects between pH and egg concentration for sperm swimming path (Table 4). Overall, virtually all sperm traits seen in the presence of egg chemistry at control pH were altered at low pH.

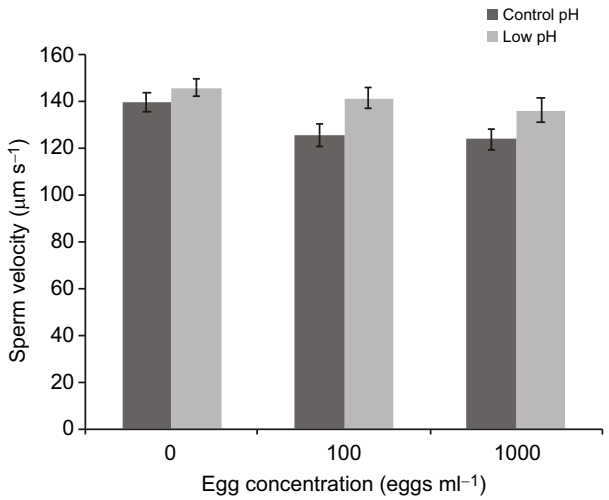

Fig. 5. Effects of decreased pH and egg concentration on sperm velocity (μm s^{-1}) in *H. tuberculata*. The mean±s.e.m. velocity for the sperm from six males are shown.

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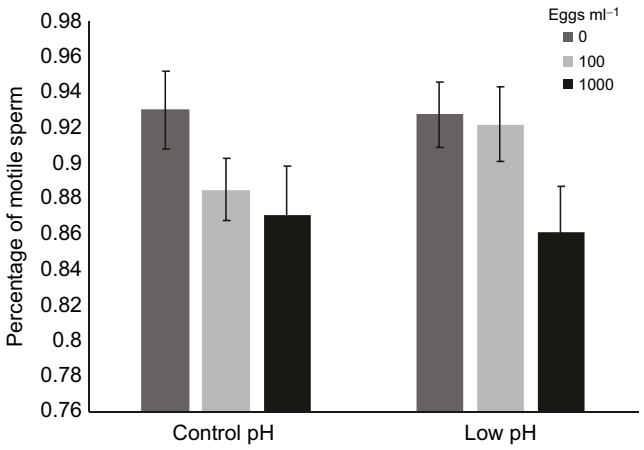

Fig. 6. Effects of decreased pH and egg concentration on the percentage of motile sperm in *H. tuberculata*. Mean±s.e.m. percentages are shown for the sperm from six males.

DISCUSSION

The contrasting vulnerability of the egg jelly coat of the two *Heliocidaris* species to near-future OA conditions has important implications for the differential resilience of species in a changing ocean, even closely related ones. Our findings provide an important model for how sperm sense the egg (Fig. 8). The jelly coat was partially stripped off of the eggs of *H. tuberculata* in relevant, near-future OA conditions, resulting in a decrease in egg target size for sperm and a likely decrease in sperm attractants. In contrast, the egg jelly coat of *H. erythrogramma* was not reduced in OA conditions. The opposite results for the two species provide an explanation as to why fertilisation success in *H. tuberculata* is reduced at low pH, whereas fertilisation in *H. erythrogramma* is resilient (Byrne et al., 2010a,b).

The eggs of *H. tuberculata* were not equally impacted by pH 7.8, with the jelly coat around the eggs of some females resilient to this level of acidification. At pH 7.6, however, the jelly coat of all eggs was reduced. OA might select against the more susceptible jelly coat phenotypes (Foo et al., 2012, 2014; Schlegel and Havenhand, 2012), as has been found for frog eggs in response to lake acidification (Shu et al., 2015). For the sea urchin *Arbacia lixula*, the egg jelly coat from females inhabiting the low pH vent sites of Ischia (mean pH 7.8) is resilient to low pH while *A. lixula* from ambient sites possess egg jelly coats which greatly decrease in size when exposed to low pH (Foo et al., 2018). Thus, the variation in the egg jelly coat response to low pH seen for *H. tuberculata* has potential as an adaptive trait if OA selects for the resilient phenotypes.

The egg jelly coat of *H. erythrogramma* was not affected by decreased pH, a feature that may contribute to the resilience of fertilisation in this species to OA, even at low sperm concentrations (Byrne et al., 2010a,b). The different sensitivities of the eggs of the *Heliocidaris* species to decreased pH may be due to differences in the chemical constituents of egg jelly. As glycan content of the egg jelly influences hydration levels, and this is known to differ between sea urchins (Jondeung and Czihak, 1982; Pomin, 2015), interspecific and intraspecific variation in glycosylation in the egg jelly could be responsible for the differences in sensitivity of the egg jelly coat to acidification. This would have flow-on effects on fertilisation success, thereby contributing to the variable outcomes noted in OA investigations with echinoderm gametes and fertilisation (Byrne, 2011; Foo and Byrne, 2017; Byrne, 2012).

In addition to the difference in the size of the egg itself, the difference in the contribution of the jelly coat to the egg target size, the relationship between egg size and jelly coat size, and sensitivity of the jelly coat to OA add to the contrasting traits of the eggs of the *Heliocidaris* species. These findings have implications for egg evolution in other marine invertebrates with egg jelly coats and potential differences in sensitivity of gametes to pH (Farley and Levitan, 2001; Hofmann, 2013; Plickert, 2013). Whether the resilience of the egg jelly coat to decreased pH in *H. erythrogramma* is also a feature of other echinoids with large eggs warrants investigation.

For *H. tuberculata*, we showed that egg chemistry significantly affected sperm behaviour, decreasing sperm velocity, motility and modifying the swimming pattern. Exposure to egg seawater increased the curvature of the sperm swimming paths, a behaviour shown to increase sperm collision rates (Jikeli et al., 2015). Sperm velocity was significantly decreased by the presence of egg chemicals, but this behaviour was completely diminished under low pH. Previous research with *H. erythrogramma* showed decreased motility and velocity of sperm isolated from egg chemistry under pH levels of 7.8 and 7.6 (Havenhand et al., 2008; Schlegel et al., 2012). In the present study, we found that a reduction in sperm motility in the presence of egg chemicals is not necessarily a negative effect, as seen for *M. galloprovincialis*, where a lower percentage of motile sperm led to increased developmental success (Fitzpatrick et al., 2012; Jikeli et al., 2015). Additionally, much OA research on sperm behaviour isolated from eggs has concluded that the decrease in sperm velocity at low pH is another negative impact of OA (Campbell et al., 2016). In a more realistic setting in the presence of egg chemicals, we see that this is a normal process and is probably essential in sperm sensing the egg.

Our results provide a model for how sperm detect the egg (Fig. 8). In the presence of dissolved egg and jelly coat molecules, the sperm

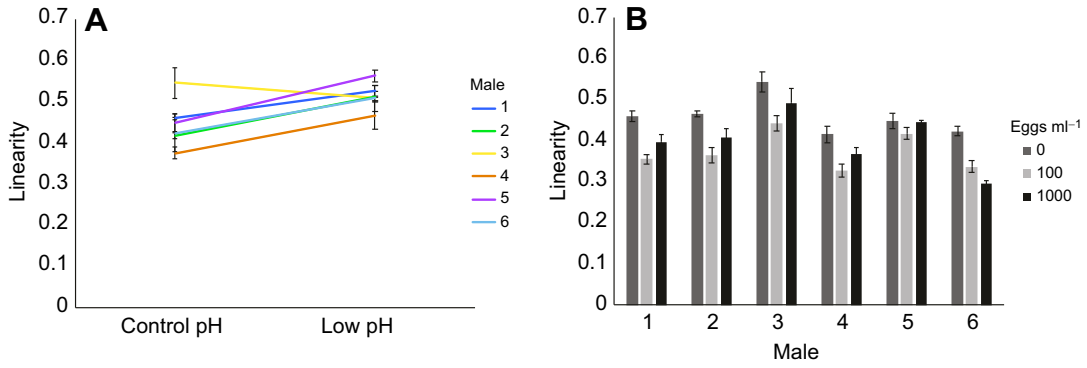

Fig. 7. Effects of decreased pH and egg concentration on the linearity of sperm swimming in *H. tuberculata*. (A) Changes in sperm swimming linearity across pH in individual males (*n*=6). (B) Changes across different egg concentrations for individual males.

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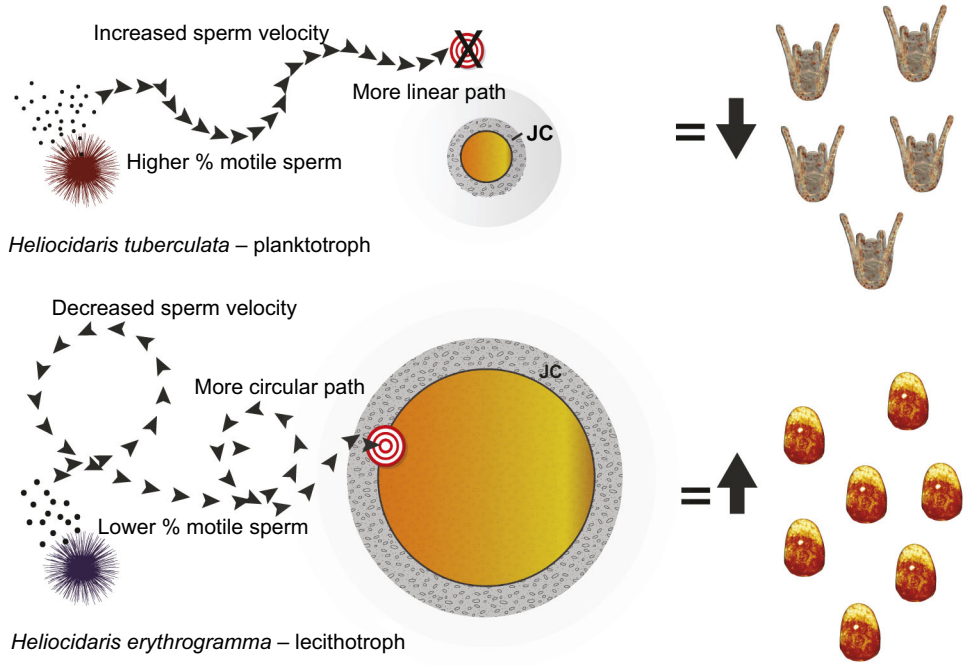

Fig. 8. A visual summary of the effects of ocean acidification (pH 7.6) on the egg jelly coat and sperm behaviour of *Heliocidaris*. For *H. tuberculata*, low pH alters the speed at which sperm swim, the percentage that are motile and the path the sperm swim along, with flow-on effects likely to contribute to a decreased number of fertilised embryos and larvae.

are steered towards the egg, are slowed down and the circularity of their swimming trajectory is increased, resulting in an increased chance of sperm meeting the egg (Fig. 8). Thus, without considering egg chemistry, the impact of stressors on sperm in isolation does not provide an integrative assessment of the impacts of acidification on their functionality.

Egg and jelly coat molecules not only mediate species-specific gamete binding but are also fine-tuned to mediate genetically compatible matings (Evans et al., 2012; Evans and Sherman, 2013). Numerous studies suggest that gametic incompatibility (the inability of sperm to fertilise eggs) can severely limit reproductive success (Kosman and Levitan, 2014) and, in some cases, lead to impaired offspring viability (Oliver and Evans, 2014; Aguirre et al., 2016). We should therefore expect selection to favour adaptations that maximise the likelihood of fusion between compatible gametes.

Our results support the suggestion that female-sourced remote regulation of sperm physiology may constitute an important evolutionary mechanism of gamete-level mate choice (Evans et al., 2012; Evans and Sherman, 2013). We found that the effects of egg attractants on the behaviour of sperm in isolation (e.g. decreased motility) are opposite to the behaviour of sperm in the presence of egg attractants exposed to low pH (e.g. increased motility). Exposure to OA eliminated or decreased the influence of the egg on sperm behaviour in *H. tuberculata*, where low pH could disrupt the chemoattraction gradient created by the egg and the ability of the sperm to sense egg cues (Fig. 8). In the abalone *Haliotis rufescens*, a natural gradient of chemicals released from the egg is necessary to promote sperm chemotaxis to the egg as well as sperm–egg interactions (Riffell et al., 2002; Krug et al., 2009). Our study highlights the potential vulnerability of these gamete recognition mechanisms to OA.

We show differing evolutionary modifications for *H. tuberculata* and *H. erythrogramma* on relative jelly coat size, with likely differences in the constituent molecules and function of the jelly coat in association with egg evolution. Recognition between the sperm and the egg is a fundamental biological event and understanding how this species-specific interaction is altered in a real-world scenario as the ocean continues to decrease in pH is crucial.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: S.A.F., M.B.; Methodology: S.A.F., D.D., M.B.; Formal analysis: S.A.F., D.D.; Investigation: S.A.F., D.D.; Writing - original draft: S.A.F., M.B.; Writing - review & editing: S.A.F., M.B.; Visualization: S.A.F.; Supervision: M.B.; Funding acquisition: S.A.F., M.B.

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