

Electronic Supplementary Material for:

Parental and embryonic experiences with predation risk affect prey offspring behavior and performance

Sarah C. Donelan¹ and Geoffrey C. Trussell

Proceedings of the Royal Society B: Biological Sciences

doi: 10.1098/rspb.2018.0034

¹sarah.donelan@gmail.com

Description of buoyant weighing technique to convert measured shell mass into actual shell mass

We destructively sampled *Nucella lapillus* offspring from each parental risk experience x embryonic risk experience treatment combination in order to derive a relationship between actual shell mass and approximate shell mass as measured by a non-destructive technique (buoyant weighing, [1, 2]). Buoyant weighing produces a nondestructive measurement of approximate shell and tissue weights of individual *Nucella*, but a destructive regression is required to describe the relationship between these approximations and actual shell and tissue weights. We first weighed *Nucella* that were submerged in room temperature seawater to obtain approximate shell mass, and then weighed *Nucella* that had been allowed to dry in air to obtain approximate total mass (shell + tissue). Each snail was then placed individually in an aluminum foil square, dried in a drying oven (60°C) for one week, and cracked open with a hammer. All shell pieces were transferred to an aluminum weigh dish and weighed to the nearest milligram. All masses are in milligrams (mg) and n = 25 for each regression. Methods of predation risk exposure (waterborne risk cues from the green crab, *Carcinus maenas*) are described in the Methods section.

Risk-experienced parents:

Embryos exposed to risk:

$$\text{Shell mass} = (1.5858 * \text{Submerged mass}) - 10.286; R^2 = 0.993$$

Embryos not exposed to risk:

$$\text{Shell mass} = (1.6033 * \text{Submerged mass}) - 19.525; R^2 = 0.997$$

Risk-naïve parents:

Embryos exposed to risk:

$$\text{Shell mass} = (1.5668 * \text{Submerged mass}) + 3.528; R^2 = 0.999$$

Embryos not exposed to risk:

$$\text{Shell mass} = (1.6667 * \text{Submerged mass}) - 28.371; R^2 = 0.995$$

ANOVA and likelihood ratio results

Table 1. Summary of results from three-way ANOVAs that examined the effects of parental experience with risk, embryonic experience with risk, and current experience with risk on the refuge use (a) and foraging activity (b) of offspring *Nucella lapillus*. Analyses were performed on replicate averages. $n = 8$ for all treatment combinations except: - parental risk / - embryonic risk / + current risk and + parental risk / + embryonic risk / + current risk.

Effect	a) refuge use		b) foraging activity	
	F _{1,54}	p-value	F _{1,54}	p-value
Parental experience with risk (P)	4.57	0.03	1.43	0.24
Embryonic experience with risk (E)	1.11	0.30	0.13	0.72
Current experience with risk (C)	55.7	< 0.0001	466.7	< 0.0001
P x E	0.07	0.80	0.53	0.47
P x C	7.88	0.007	0.009	0.93
E x C	2.38	0.13	0.005	0.94
P x E x C	0.070	0.80	2.22	0.14

Table 2. Summary of results from three-way ANOVAs that examined the effects of parental experience with risk, embryonic experience with risk, and current experience with risk on the growth (a) and growth efficiency (b) of individual offspring *Nucella lapillus*. Parent pair (i.e., family) was also included as a random effect nested within the parental experience with risk treatment to account for potential differences in risk responses between families. Replicate was also included as a random effect nested within the parental, embryonic, and current risk treatments because there were multiple *Nucella* offspring in each replicate. Embryonic bucket ID was included as a random block effect. $n = 8$ for all treatment combinations except: - parental risk / - embryonic risk / + current risk and + parental risk / + embryonic risk / + current risk.

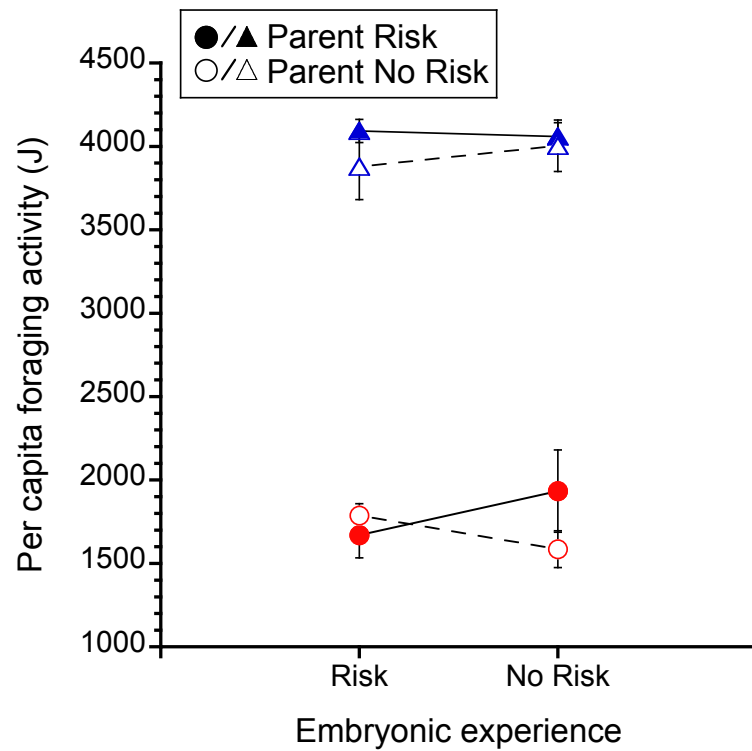
Effect	c) tissue growth		d) growth efficiency	
	F (df _{num} , df _{den})	p-value	F (df _{num} , df _{den})	p-value
Parental experience with risk (P)	1.77 (1,9.9)	0.22	1.25 (1,8.9)	0.29
Embryonic experience with risk (E)	0.23 (1,3.6)	0.66	0.05 (1,1.3)	0.85
Current experience with risk (C)	1.65 (1,3.6)	0.28	1.18 (1,1.3)	0.44
P x E	1042.6 (1,53.0)	< 0.0001	312.9 (1,53.2)	< 0.0001
P x C	4.14 (1,53.0)	0.047	8.83 (1,53.2)	0.004
E x C	2.99 (1,53.3)	0.090	4.18 (1,53.0)	0.046
P x E x C	4.31 (1,53.3)	0.043	0.59 (1,53.0)	0.44

Table 3. Summary of the results of likelihood ratio tests to determine significance of the random effects of replicate (nested within parental experience with risk, embryonic experience with risk, and current risk), parent pair (nested within parental experience with risk), and embryonic bucket (random block effect) on the growth (a) and growth efficiency (b) of offspring *Nucella lapillus*. Methods are described in [3]. Briefly, we compared a generalized least squares (gls) model with no random effects to an lme model with each random effect as appropriate. P-values were corrected (divided by 2) because likelihood ratios do not follow a X^2 distribution when testing on the boundary. Likelihood ratios were produced using the nlme package [4] and models were compared using the anova function in R [5].

	a) tissue growth		b) growth efficiency	
Effect (random)	X^2	p-value	X^2	p-value
Replicate	3.67×10^{-7}	0.50	2.48×10^{-8}	0.50
Parent pair	1.65	0.22	1.33	0.26
Embryonic bucket ID	0.81	0.18	0.77	0.19

Supplementary Figure: *Nucella* offspring foraging activity

Figure 1S. Mean (\pm SE) per capita foraging activity (J) of offspring *Nucella lapillus* in the presence (red circles) and absence (blue triangles) of current predation risk from the green crab *Carcinus maenas*. Offspring experienced the presence and absence of green crab predation risk as embryos and were produced by parents that experienced the presence (filled symbols) and absence (open symbols) of green crab predation risk. $n = 8$ for all treatment combinations except: - parental risk / - embryonic risk / + current risk and + parental risk / + embryonic risk / + current risk.



References in Supplementary Materials

1. Palmer, A.R. 1982 Growth in marine gastropods: a non-destructive technique for independently measuring shell and body weight. *Malacologia* **23**, 63-74.
2. Trussell G.C., Smith L.D. 2000 Induced defences in response to an invading crab predator: an explanation of historical and geographic phenotypic change. *Proc Natl Acad Sci* **97**, 2123-2127. (doi:10.1073/pnas.040423397).
3. Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. M. Smith. 2009. *Mixed effects models and extensions in ecology with R*. New York, New York, USA, Springer.
4. Pinheiro, J. D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2017. *nlme: linear and nonlinear mixed effects models*. R package version 3.1-131. <https://CRAN.R-project.org/package=nlme>.
5. R Core Team. 2017. R: A language and environment for statistical computing. Vienna, Austria, R Foundation for Statistical Computing.