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Exposure to predator kairomones influences egg number and size in *Littorina littorea*

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Abstract

Predator kairomones play an important role in intertidal ecosystems, but knowledge on their potential role in influencing maternal effects is lacking. The aim of this study was to test whether egg production by female *Littorina littorea* was influenced by short-term exposure to predator kairomones before egg laying. Laboratory populations were exposed to predator cues from the intertidal crab *Carcinus maenas* for nine days, and egg number, egg size, and survivorship of the offspring were measured and compared with those from reference populations. Snails exposed to predator cues produced significantly more eggs, which were also significantly smaller than those produced from mothers in control seawater, suggesting a trade-off between egg size and number. This effect was consistent over time, but egg production decreased in each treatment over the course of the study. Furthermore, eggs from the predator cue treatment had lower survivorship. These results suggest that the presence of predator kairomones in marine environments could significantly alter the reproductive investment strategies in prey species.

Keywords: maternal effects, development, fitness, inheritance, evolution.
Introduction
The recent surge of interest in parental effects and developmental plasticity is giving greater emphasis to the role that the environment plays in evolution (Jablonka & Lamb, 2002; West-Eberhard, 2003; Laland et al., 2014). Maternal effects are a direct causal link between the maternal genotype or phenotype and offspring phenotype (Rasanen & Kruuk, 2007; Wolf & Wade, 2009). Within the context of natural selection, maternal effects may be adaptive and thus increase the fitness of the mother and of her offspring.

One way that maternal effects can be manifested is if the mother experiences certain environmental conditions just before, or at the time of, oogenesis (Chambers & Legget, 1996) that cause changes in maternal behaviour, hormones, or investment of resources to her offspring. Such changes may have a substantial effect on the success and life history of the progeny. Nutrient provisioning is the most thoroughly researched maternal effect, and can influence offspring number, individual size, and development (Solemdal, 1967; Burgess & Marshall, 2011; Marshall & Keough, 2007; Allen et al. 2008; Jensen et al., 2013). The amount of maternal provisioning allocated to progeny is limited by the amount of energy the mother has obtained, and the portion that she must utilize for her own growth and maintenance (Bernardo, 1996). The remaining energy can be utilized to allocate important provisioning for the offspring, and is often positively correlated with organism size. There may also be a trade-off between energy supplied to offspring number and offspring size (Ebert, 1993; Bernardo, 1996; Marshall et al., 2008), and the nature of this trade-off will affect the fitness of both the mother and her offspring. It is generally considered that fitness increases with initial size (Messina & Fox, 2001), since greater size may provide a competitive advantage and more resources (Berg et al., 2001), however a greater size may also increase susceptibility to predation (Marshall & Uller, 2007).

In addition to nutrient provisioning, environmentally-induced maternal effects have been observed as a response to other stressors, including salinity (Solemdal, 1967; Jensen et al., 2013), pollution (Marshall & Keough, 2007; Marshall 2008), and temperature (Kaplan, 1987; Burgess & Marshall, 2011). There is less research into the effect of maternal exposure to biotic cues, such as predator kairomones. Kairomones are diffuse chemical cues released by predators that prey encounter and potentially respond to in aquatic habitats (Nordlund & Lewis, 1976). There is some evidence that detection of such predator cues can induce maternal effects in terrestrial (Fowler, 2005) and several freshwater species (Mikulski & Pijanowska, 2010; Tollrian, 1995; Resetarits & Wilbur, 1989). Predator stress can have a strong influence on maternal provisioning, as it is likely to drive maternal trade-off decisions to maximize fitness. For instance under the stress of a predator, it may be beneficial to produce larger offspring with more developed defenses (Schwab & Allen, 2014), or perhaps a greater number of offspring to optimize the chance of several escaping predation. However, few empirical studies on predator-induced maternal effects have been conducted on marine organisms to date (but see Schwab & Allen, 2014). Given the well-documented importance of predator kairomones in influencing the biology of marine
organisms (for example Agrawal \textit{et al.}, 1999; Weber & Declerck, 1997), it is likely that such effects occur in the ocean.

Here we investigated predator induced maternal effects in the common marine intertidal gastropod \textit{Littorina littorea} (Linnaeus, 1758). It is widely known that marine snails exhibit predator-induced plasticity (Appleton & Palmer, 1988; Trussel, 2000), therefore it is justifiable to hypothesize that maternal transgenerational plasticity may also be at play. The intertidal crustacean \textit{Carcinus maenas} is a common and natural predator of \textit{L. littorea}, and previous research confirms the ability of \textit{L. littorea} to detect cues released by \textit{C. maenas} (Jacobsen & Stabell, 1999). This study aimed to examine whether exposure to predator cues from \textit{C. maenas} influenced the number, size, and survivorship of \textit{L. littorea}.

**Materials and Methods**

**Animal collection**
Individual \textit{L. littorea} (spire height >20mm) were collected on 27\textsuperscript{th} June, 2013 from the intertidal at Mount Batten, Devon, UK (50° 35’ 75” N; 4° 12’ 65” W). In the laboratory, snails were sexed by observing the right side of the body for the presence or absence of a penis. Four males and ten females were then placed into each of ten aerated 3L aquaria containing 2.5L of 35 ppt seawater maintained at 16°C, under a 12:12 hour light/dark regime. Snails were fed \textit{ad libitum} on \textit{Ulva lactuca} and an acclimation period of six days was allowed before experiments.

**Experimental procedure**
At the start of the experiment the water in each tank was changed. Five tanks were filled with 2.5L of seawater and five with predator kairomone water, which was made up from 2.0L of seawater and 0.5L of predator kairomone water from a tank containing a single \textit{Carcinus maenas} (carapace width c60mm) in 8L of seawater. This crab had been in this tank for 1h and had been taken from a stock tank population maintained in seawater at 16°C, and fed fish once a week.

Eggs were collected from each tank after the first 24h and subsequently every 24h for nine days (at which time the snails ceased to produce eggs) by passing the full volume of water from each tank through a fine mesh filter (<0.1mm\textsuperscript{2}) and transferring them to a petri dish.

**Enumerating egg size and number**
All eggs collected were counted under a binocular microscope (60x) noting the number of occurrences of double and triple eggs (two or three eggs in the same capsule, respectively). On days 4 and 8 measurements were also made of egg capsule width. On day 4, five single egg capsules and eight double egg capsules (Fig. 1) were isolated from each treatment and photographed at x75 magnification using a high power lens and camera (IMAGINGSOURCE, PIEM zoom x75, and Image Capture software). The size of each egg was quantified using the analyzing software ImageJ. This procedure was repeated on Day 9...
using nine single egg capsules from each treatment and no double egg capsules (due to the scarcity of this type of capsule).

![Figure 1: Single (left pane) and double (right pane) egg capsules of *Littorina littorea*.](image)

**Survivorship**
At the end of the study, eggs were isolated to observe them on a daily basis for survivorship. On the 8th day of exposure to control or predator cue seawater, 10 of each capsule type (single egg and double egg) produced from each treatment were selected at random (a total of 30 egg capsules) and isolated individually in 12x8 well plates of control seawater. The control seawater in each well was replaced daily using a fine syringe. Eggs were observed daily using the high power microscope and camera, IMAGINGSOURCE, PIEM zoom x75. Upon egg morality, the number of days that the egg had survived since egg release was recorded. The observation period lasted for 7 days (after this time all animals had either hatched or died).

**Data analysis**
Egg count data were analyzed using a Two-Way ANOVA to assess the effects of time, treatment (control or cue), and their interaction. To achieve homogeneity and normalize the data, a square root transformation was used. Differences in size between the treatments and capsule type (single egg or double egg) were analyzed using a Two-Way ANOVA.

**Results**

**Egg number**
There were significant effects of time (ANOVA; $F_{7,16}=2.502$, $p=0.025$) and predator kairomones (ANOVA; $F_{1,8}=5.644$, $p=0.021$) on egg number but no significant interactive effect these two factors (Fig. 2). The number of eggs released in the predator cue treatment was significantly higher than that in the control on 5 out of 9 days. Furthermore the number of eggs produced decreased over the course of the study in each treatment (Fig. 2). It seems likely that this could be related to the possible accumulation of *L. littorea*.
stress as a result of being maintained in laboratory conditions. The cumulative number of eggs produced was 30951 in the presence of kairomones and 15484 in control conditions (Fig. 3).

Figure 2: The mean (±95% C.I.) number of eggs produced through time by *Littorina littorea* after exposure to seawater or predator kairomones from *Carcinus maenas*.

![Figure 2](image)

Figure 3: The cumulative number of eggs produced by *Littorina littorea* (n=5), after exposure to seawater predator kairomones from *Carcinus maenas*.

![Figure 3](image)

**Egg size**

For single egg capsules sampled at day 4, the mean diameter of singular eggs was significantly larger in the control treatment (ANOVA, $F_{1,5} = 5.292$, $p=0.04$; Fig. 4) at 0.162mm ±0.004, compared to 0.150mm ±0.005 for singular eggs from the kairomone treatment. There was also a significant difference in the size of eggs in twin capsules (control = 0.164mm ±0.003; kairomone= 0.156mm ±0.005; ANOVA, $F_{1,8} = 29.853$, $p<0.001$; Fig. 4). However the interaction between treatment and capsule type (single or double egg) was not significant.
Significantly larger eggs were also produced in the control treatment at 9 days, with an average egg diameter for control of 0.168mm ±0.009 compared with 0.159mm ±0.004 in the kairomone treatment (Fig. 4) (ANOVA, F$_{1,9}$ =5.162, p=0.037).

**Figure 4**: The mean (±95%C.I.) diameter of *Littorina littorea* eggs in single and double capsules, after 4 days and 9 days exposure to predator kairomones (cue) or seawater (control).

**Survivorship**

There was a lower percentage of survivorship for eggs from the predator cue treatment. Seven days after being laid only 9 eggs (60%) from the predator cue treatment had survived, compared with 13 eggs (87%) of those from the control treatment (Fig. 5).

**Figure 5**: Percentage of isolated *Littorina littorea* eggs (n=15) produced after 8 days exposure to predator kairomones or seawater that survived over the course of a 7-day observation period in control seawater.
Discussion
The main aim of this study was to investigate whether maternal exposure to predator kairomones influenced offspring number, size, and survivorship in *L. littorea*. Females exposed to kairomones from *Carcinus maenas* produced a significantly larger number of eggs than those in the control treatment. Furthermore, eggs from the predator cue treatment were significantly smaller and had lower survivorship.

In the context of maternal effects, when a parent organism is exposed to some degree of stress, it might be predicted that fecundity would be affected by changes in parental energy allocation. For instance the parent organism may respond by allocating more energy into producing more offspring, to increase fitness should its own mortality occur. Equally, the parent may respond by investing more energy into its own defence or maintenance, and therefore fecundity could decrease. Previous experimental investigations of the effects of predation stress on egg production have been equivocal. Schwab & Allen (2014) observed no maternal effect on the offspring number of the intertidal mud snail *Ilyanassa obsoleta* after exposure to *C. maenas* predator cues; a lack of response that may be related to the different natural history of *I. obsoleta* eggs (laid on surfaces rather than in the water column). Similarly, no maternal effect on fecundity was also reported in the water flea *Daphnia galeata*, when exposed to predator kairomones from two different predators, despite a maternal effect on size (Weber & Declerck, 1997). In contrast, Tollrian (1995) observed increases in both fecundity and body size in another water flea, *D. pulex*, after exposure to predator kairomones – a result more in-line with our findings. From these studies, we might conclude that the energy allocation response of organisms to stress is variable and perhaps species specific. However it is imperative to look at other effects of maternal exposure, and to look for evidence of energy trade-offs, to gain the full picture of maternal effects.

Research into energy partitioning has concluded that total maternal energy devoted to reproduction is divided into offspring size and number (Berg *et al.*, 2001), and therefore a trade-off between these two maternal effects seems likely. In our study, maternal exposure to predator cues triggered a decrease in offspring size in addition to an increase in offspring number, suggesting a trade-off between these traits. Given that smaller eggs are presumably less susceptible to predation (Marshall & Uller, 2007) perhaps this was the driving factor behind the resource allocation decisions made by *L. littorea* in this study. However maternal effects are not always adaptive (Bernardo, 1996), as seen in the literature. Tollrian (1995) reported that whilst fecundity of *D. pulex* increased after maternal exposure to predator kairomones, offspring size also increased and thus a trade-off between egg number and size was not indicated. However Tollrian (1995) also observed effects on growth rate and time to maturity, therefore there were other components to energy partitioning in his study.

Other research has reported that a stressful maternal environment correlated with the production of larger offspring to enhance survivorship (Segers & Taborsky, 2011), opposing the findings of the current study. The trade-off
between offspring size and number is a significant determinant of offspring life history (Fleming & Gross, 1990; Glazier, 1992; Mousseau & Fox, 1998a), and it seems likely that there is an ecological component to the parental organism’s energy partitioning decisions. For instance, variation in per-offspring investment can influence individual performance, whilst variation in offspring number will influence the competitive environment of the offspring (Beckerman et al 2005). Given the predator stress introduced in our study, it seems fitting that fecundity increased as size decreased, however the true reasons underpinning this trade-off can only be theorized.

Another life history trait that can be influenced by parental energy partitioning is survivorship. This study is the first to report that exposure of maternal organisms to predator kairomones results in the production of offspring with a lower survivorship, and ultimately lower fitness. In fact, there is a large gap in the literature concerning the effect of predator kairomones on offspring survivorship. This maternal effect may be associated with egg size, as smaller eggs have less resource provisioning, which is likely detrimental to proper development, and thus to offspring survival. Moran & Emlet (2001) reported that increased size correlated with increased survivorship in hatchlings of the marine gastropod Nucella ostrina. Our study reports this same finding, and this is unsurprising when considering the importance of resource provisioning in developing embryos. This correlation is well known and accepted in the literature (Fox, 1994; Chambers & Leggett, 1996), however connections between maternal experience, the consequential energy partitioning, and the potential influence on survivorship have not been made. Our study demonstrates how survivorship, in conjunction with other offspring traits, can be used to understand parental energy trade-offs in maternal effects.

**Conclusions**

We have demonstrated how maternal exposure to waterborne predator kairomones can have a considerable influence on the number, size, and survivorship of offspring. This study has illustrated the way in which environmental fluctuations, or the spatial distribution of predator and prey organisms at the reproductive time of one generation can affect the life history of proceeding generations; an intriguing concept that requires further understanding. The fitness implications of this phenomenon are of great significance to evolutionary ecologists, since the potential role of maternal effects in evolution is still undetermined. In the future, long-term, multi-generational observations will be pivotal in studying the heredity and microevolution of specific traits, and this knowledge is paramount for a true evolutionary understanding.

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