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Original Article

Maternal programming of offspring antipredator behavior in a seabird

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Predation risk is an important environmental factor for animal populations, expected to trigger maternal effects to prepare offspring for living in an environment with predators. Yet, evidence of adaptive anticipatory maternal effects in wild animals is still weak. Here, we explored this question in a wild colony of yellow-legged gulls, *Larus michahellis*. To this aim, prior to laying we exposed mothers to either mink decoys or nonpredator rabbit decoys and explored the antipredator behavior of 118 chicks at the age of 2 days. We found that chicks from second-laid eggs by predator-exposed mothers crouched faster after hearing a playback with adult alarm calls than chicks from second-laid eggs by control mothers. Besides, chicks from third-laid eggs by predator-exposed mothers were lighter than control chicks, but this was not due to differences in egg volume. Our results suggest that predator-exposed mothers modified offspring phenotype via eggs to cope with predators, although only in chicks from second-laid eggs. Maternal transference of corticosterone could underlie chick behavioral plasticity. Results support the role of maternal effects as a form of phenotype programming to forewarn offspring about environmental hazards.

Key words: **antipredator behavior, developmental programming, maternal effects, predictive adaptive response, yellow-legged gull.**

INTRODUCTION

It has been hypothesized that mothers can shape the phenotype of offspring via maternal effects to prepare them for the environment they will encounter at birth (reviewed by Uller 2008; Love et al. 2013; Sheriff and Love 2013). Mothers can transfer signals or cues for example into eggs that affect phenotypically the early development of the progeny. The potential benefit of these maternal cues, enabling the offspring to adapt to their current environment, can be termed a predictive adaptive response (Gluckman and Hanson 2004; Bateson et al. 2014). For instance, daphnids born from mothers prenatally exposed to predator chemical cues grow larger helmets and survive better than offspring from mothers in a control environment (Agrawal et al. 1999). Nevertheless, the importance of this form of adaptive developmental plasticity that spans generations in natural populations is still under debate (Uller et al. 2013; Bateson et al. 2014; Nettle and Bateson 2015).

Predation risk is one of the most significant environmental factors affecting animal populations (e.g., Krebs et al. 1995; Sheriff et al. 2010; 2015), but only a few empirical studies have investigated whether it triggers transgenerational predictive adaptive responses for predator evasion. Thus, mothers prenatally exposed to predators produce offspring with greater immobility in an insect (Storm and Lima 2010), tighter shoaling behavior in a fish (Giesing et al. 2011) and stronger avoidance to predator odor in a mammal (St-Cyr and McGowan 2015). In an environment with high predation pressure, the induction of fearfulness, anxiety, and reduced locomotor and exploratory behaviors can promote offspring survival (reviewed by Sheriff and Love 2013). Thus, maternal effects may represent a flexible mechanism of developmental "programming" for antipredator defense (Love et al. 2013).

In birds, mothers allocate several active substances into eggs and they may modify offspring morphology via eggs when exposed to predators (Saino et al. 2005; Fontaine and Martin 2006; Coslovsky and Richner 2011). Yet, there is poor evidence for maternal programming of offspring behavior in birds (e.g., competition/foraging via begging; Smiseth et al. 2011) and we lack studies analyzing maternal influences on offspring behavioral defenses via eggs. Interestingly, under harsh conditions (e.g., high predation risk), parents are expected to favor offspring with high survival

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prospects (e.g., Haig 1990; Davis et al. 1999; Parker et al. 2002). Hence, by intraclutch differential investment mother birds have the potential to favor chicks with stronger competitive advantage over their sibs, which are those hatched from eggs in the first laying positions (Schwabl et al. 1997; Gil et al. 1999).

Here, we explored whether mothers induce offspring antipredator behavior and whether they do it according to laying order in a wild colony of a long-lived bird, the yellow-legged gull, *Larus michahellis*. To this aim, we confronted mothers either to mink decoys or to nonpredator rabbit decoys before and during laying. To disentangle maternal effects via eggs from the effects of the rearing environment, prior to incubation onset we transferred whole clutches of predator and nonpredator treatments to nests of naïve (nonexposed) mothers. Gulls respond to stuffed predators in a similar way as to live predators (Kruuk 1964) and, in dense colonies inhabited by minks, they show a dramatic response to mink decoys compared to rabbit ones, including flocking over and even diving at them (Clode et al. 2000). Yellow-legged gulls typically lay 3-egg clutches and maternal investment into eggs show a systematic relationship with laying sequence, characteristic of Laridae, where egg mass decreases with laying order and results in last chicks with very low competitive advantage and survival prospects (e.g., Parsons 1970; Royle and Hamer 1998; Rubolini et al. 2005).

We explored behavioral responses against predators in 2-day old chicks by means of 2 standard tests, the time to crouch after hearing a playback of adult alarm calls in response to a predator (see Impekoven 1976; Noguera et al. 2017, in gulls) and the time in tonic immobility, an innate paralysis response generally considered as indication of fearfulness and reactive behavior (Erhard et al. 1999; in gulls, see Rubolini et al. 2005; Kim and Velando 2015). Rapid crouching and immobility reduce predation risk and is related to immediate survival in gull chicks (Impekoven 1976). If mothers adjust offspring behavior according to predator exposure, we predict that chicks of predator-exposed mothers would crouch faster and remain longer in tonic immobility than chicks of control mothers. According to theoretical models, if mothers in a harsh environment favor offspring with high reproductive value (Haig 1990; Davis et al. 1999; Parker et al. 2002), we expect that chicks hatched from the first 2 laid eggs show stronger behavioral responses and grow heavier compared to third chicks especially in predator-exposed mothers.

METHODS

Ethics

Methods were performed in accordance with the Guidelines for the Treatment of Animals in Behavioral Research and Teaching from the Animal Behavior Society (2012). The study was approved by Xunta de Galicia and Parque Nacional das Illas Atlánticas (permit number (380/2013) and complies with the Spanish current laws (RD53/2013).

Experimental procedure

The study was conducted in 2013 on a large breeding colony of yellow-legged gulls in Sálvora island, Parque Nacional das Illas Atlánticas (42°28′N, 09°00′W). We chose 12 flat densely populated subcolonies that were on average (mean ± SE) 84.3 ± 5.2 m wide (range: 63–126 m) and separated by a minimum distance among their central parts of (mean ± SE) 155.6 ± 22.9 m (range: 82.6–332.4 m). We assigned them randomly to either a predator-exposed treatment (n = 6 subcolonies exposed to mink decoys) or a nonpredator environment (n = 6 exposed to rabbit decoys) before and during laying. In the predator treatment, we used 3 taxidermy mounts of American minks (*Neovison vison*), an alien species that was first detected in the island in 2003 and was very abundant until 2013 (Velando et al. 2017). It exerted dramatic effects on the native fauna, but after a culling program promoted by the National Park, Sálvora island was apparently free of minks during the study year (2013; Velando et al. 2017). Thus, we can be sure that any effect of predator exposure would be the result of our experimental model, not the threat of live minks on the island. In the previous years, gulls represented on average 22% of the American mink diet in Sálvora island, which used to increase up to 75% during June and July, coinciding with the period of chick development (Romero 2007; see also Pérez et al. 2012). American mink decoys are recognized by gulls as a threat in the study colony as shown in a previous study, causing circling and hovering above the predator and sometimes diving at it (on average, 32.8 ± 0.2 gulls flocked over the decoys and 2.5 ± 0.1 attacked them directly; Serafino 2006). In a pilot study, we also confirmed that mink decoys used in our study always triggered the typical alarm behavior of adult gulls. Control subcolonies were exposed to 3 domestic rabbit (*Oryctolagus cuniculus*) decoys, which are very abundant in the island and gulls never responded to rabbit decoys in the pilot study. We cannot discard that gulls moved from one control subcolony to an adjacent predator subcolony to flock over the mink decoy or to attack it. In any case, this would weaken treatment effects.

In mid-April and once a day until the last egg was laid (mean ± SE: 11.50 ± 0.15 days), a mink decoy (randomly selected each day for each predator-exposed subcolony) was covered with an opaque cloth and fixed on the ground with metal tent pegs in the center of the subcolony. A 70 m string was attached to the cloth to uncover the mink manually from a hidden position outside the subcolony. Ten minutes after placing the mink decoy (the typical length of time needed until gulls were quiet after our visit), it was uncovered for 15 min, after which it was removed from the subcolony. Similarly, every day until the last egg was laid (10.67 ± 0.18 days) a rabbit decoy (randomly selected each day for each predator-exposed subcolony) was presented uncovered in the center of each control subcolony. In the pilot study, we realized that the action of uncovering the rabbit decoy provoked per se certain degree of alert and fright on gulls, and thus it was not adequate for the control treatment. Hence, we decided to present the rabbit uncovered all the time. Therefore, in our experiment, the response of gulls to mink decoys included both effects, the decoy and the action of uncovering it. Rabbit decoys were exposed for 25 min to match the time that mink decoys were present in their respective subcolonies, with the difference that minks were covered for the first 10 min and then uncovered for the last 15. Human disturbance was similar in the presentation of mink and rabbit decoys. Time of presentation of mink and rabbit decoys was daily changed to prevent habituation (i.e., each day we inverted the path followed to reach all the subcolonies; besides, in some days, decoy presentation started in the morning and other days in the afternoon). Treatments did not differ in the number of days of exposure, laying date or hatching date (all $P > 0.62$).

In each subcolony, we selected the first 4 nests (n = 48 nests) with a complete clutch of 3 eggs that we found in a random search close to the place where the decoys were exposed (±15 m). We avoided nests that laid the first egg within 3 days after the first decoy presentation, since some maternal effects, including hormones, are transferred to eggs at least 3 days before laying (Henriksen et al. 2011).

On the day of laying, the eggs were measured with a digital caliper (accuracy 0.01 mm) and their volume was calculated with the formula: length × width^2 × 0.52, as previously described (Hoyt 1979). On the day of clutch completion, whole clutches were exchanged with 3-egg clutches with similar laying date (±1 day) from one nonexperimental area located at a minimum distance of 200 m from the nearest subcolony. This was done to disentangle maternal effects via eggs from the effects of the rearing environment.

We visited foster nests daily, beginning 2 days before the estimated hatching date. During pipping, chicks were individually marked on the tip of the bill with nontoxic acrylic markers (ArtCreation, Royal Talens, The Netherlands). At hatching, chicks were marked with a leg Velcro band. On the day of hatching (day 0), chicks were blood sampled in the brachial vein for sex identification and samples were kept in absolute ethanol. Sex was identified as previously described (Fridolfsson and Ellegren 1999). At day 2 of age, chicks were weighed and their tarsus length was measured, except one chick that was not measured. During incubation, 4 clutches were abandoned (3 in the predator treatment and 1 in the control treatment) and also 1 egg in the predator treatment. In addition, 8 chicks from control mothers and 5 from predator-exposed mothers died before day 2 of age. Hatching success (91%) and nestling survival probability from 0–2 days of age (90%) were similar to or above those reported in previous studies in the study colony (e.g., Kim et al. 2011; Velando et al. 2013; Diaz-Real et al. 2016).

Chick antipredator response

The day of each behavioral test, we transported various focal chicks at a time from their foster nests in individual cloth bags to the observation site, located outside the breeding colony and free of nests to avoid disturbance by gull noise. Since all chicks were raised in foster nests randomly located in a nonexperimental subcolony (the same for all chicks), chicks in both treatments were at the same distance from the testing location. When 2 siblings had hatched on the same day, which occurred in 18 nests, they were transported one by one to the observation site to minimize nest desertion. In these cases, the order of collection of siblings was randomized (first chicks were tested first in 10 nests while second chicks in 8). The chicks waiting to be tested were placed in the shadow far enough to prevent that they could hear the playback. The approximate range of time elapsed from collection to the start of the test was 15 min for the first chick to be tested to 50–60 min for the last one. Testing order of all focal chicks was randomized. Behavioral tests were performed inside an enclosure (34 × 88 × 110 cm, LWH) with opaque walls that prevented external visual stimuli but allowed similar light conditions to the outside environment. All behavioral tests were performed individually (in the absence of real sibling competition) and were observed by the same person (A.L.). The observer knew the foster nest but not the original nest and thus was blind to treatment.

We first performed a tonic immobility test (Erhard et al. 1999; Rubolini et al. 2005; Kim and Velando 2015) on 118 2-day-old chicks. Tonic immobility is a natural state of paralysis which animals enter and is regarded as an antipredator reaction (Gallup 1977). The focal chick was placed on its back in the center of the enclosure and covered with a concave container for 10 s to gently restrain it physically in order to induce tonic immobility (see Erhard et al. 1999). After 10 s, the container was removed and the time elapsed until the chick righted itself was measured up to 120 s (hereafter, "time in tonic immobility"). If the chick had not righted itself within the time of the trial, we scored 120 s. If the chick righted itself before 15 s (then it did not reach tonic immobility) the test was repeated up to 3 times. If in the 3 attempts, the chick righted itself before 15 s, the maximum time in immobility was used. The test was performed on average 2.4 ± 0.1 times per individual.

After the tonic immobility test, we assessed the antipredator behavior of 118 chicks by registering the latency to become immobile (hereafter "time to crouch"; see Noguera et al. 2017), after hearing a playback with adult alarm calls. Prior to each antipredator behavioral test, the focal chick was released in the enclosure for 2 min to allow it to explore the new environment and reduce neophobia. During the exploration phase, most chicks (80%) moved from their original position to other sections of the enclosure and almost all chicks (92%) remained some time in a vigilant attitude, which we interpreted as a sign of receptiveness to environmental stimuli (e.g., parental alarm calls). The focal chick was then covered under a container in the central part of the enclosure to keep it calm and to start all tests in the same position, and was uncovered after 5 min. After 10 s, alarm calls were played for 40 s with an audio player (Desire A8181, HTC Corporation, Taiwan) connected to speakers (QS-631, CDS Technology Limited, China) and placed on the ground outside the enclosure. Alarm calls had been previously recorded for 10 s in the colony with a microphone (HTC RC E195). The sound was then edited with Raven Lite 1.0 (Cornell Lab of Ornithology, Ithaca, USA) to repeat 4 times the original sound to obtain a playback of 40 s (adults usually perform bursts of 3–6 calls; Tinbergen 1953). We selected a volume as similar as possible to that the chicks can normally hear in the colony and used the same playback and volume setting in all experimental subjects. The time to crouch was registered up to 110 s by the same observer. The maximum duration of the trial was decided prior to collecting data just to place a limit that allowed us to perform both behavioral tests quickly, without retaining the chicks for too long outside their nest. After each trial, the focal chicks were placed back in their nest.

Statistical analyses

We ran linear mixed models in SAS 9.4 (SAS Inst., Cary, NC, USA) to test the effect of maternal exposure to predators on egg volume, chick body mass, (log) time to crouch, and (log) time in tonic immobility. All models included as predictor variables: treatment (predator/control), chick sex, laying order (1, 2, or 3, as categorical factor), treatment × chick sex, treatment × laying order. Tarsus length was included as a covariate in the model of chick body mass. The original subcolony (nested within treatment) and nest identity (nested within subcolony) were included as random variables. The interactions were removed from full models when nonsignificant ($\alpha = 0.05$). When an interaction was significant, we obtained differences in LS-means with the DIFF option. The estimation method was restricted maximum likelihood. We additionally explored whether sex allocation in the laying sequence was affected by treatment. Thus, we performed a GLIMMIX procedure with binomial distribution and treatment, laying order and treatment x laying order as predictor variables (the random structure was the same as above).

RESULTS

Offspring antipredator behavior

The interaction between treatment and laying order significantly affected time in tonic immobility (Table 1). Chicks from second-laid eggs by predator-exposed mothers tended nonsignificantly to

Table 1

Full linear mixed models with the effect of maternal predator exposure on egg volume (cm^3), chick body mass (g), chick (log) time to crouch (s) after hearing a playback with adult alarm calls and (log) time in tonic immobility (s)

	Egg volume	Chick body mass	(log) Time to crouch	(log) Time in tonic immobility
Intercept	*coef* = 81.51 ± 1.74	*coef* = −45.66 ± 12.87	*coef* = 1.08 ± 0.14	*coef* = 1.24 ± 0.18
Predator treatment	*coef* = −1.92 ± 2.11 $F_{1,10.2}$ = 0.83 *P* = 0.38	*coef* = 6.34 ± 2.50 $F_{1,9.5}$ = 1.41 *P* = 0.26	*coef* = −0.13 ± 0.17 $F_{1,38.5}$ = 1.24 *P* = 0.27	*coef* = 0.28 ± 0.22 $F_{1,9.05}$ = 0.01 *P* = 0.93
Chick sex	*coef* = 1.03 ± 1.06 $F_{1,100}$ = 0.94 *P* = 0.34	*coef* = −0.11 ± 1.28 $F_{1,108}$ = 0.01 *P* = 0.93	*coef* = −0.32 ± 0.10 $F_{1,107}$ = 11.41 ***P* = 0.001**	*coef* = −0.24 ± 0.12 $F_{1,111}$ = 4.16 ***P* = 0.044**
Laying order	*coef (1)* = 3.30 ± 1.05 *coef (2)* = 3.58 ± 1.06 $F_{2,76.7}$ = 6.87 ***P* = 0.002**	*coef (1)* = 4.25 ± 2.08 *coef (2)* = 7.23 ± 2.13 $F_{2,79.2}$ = 2.17 *P* = 0.12	*coef (1)* = 0.05 ± 0.16 *coef (2)* = −0.33 ± 0.17 $F_{2,74.6}$ = 1.27 *P* = 0.29	*coef (1)* = −0.09 ± 0.19 *coef (2)* = 0.44 ± 0.19 $F_{2,75.8}$ = 2.47 *P* = 0.091
P. treatment x laying order		*coef* = −4.55 ± 2.77 $F_{2,75.8}$ = 4.07 ***P* = 0.021**	*coef* = 0.16 ± 0.23 $F_{2,74}$ = 3.38 ***P* = 0.039**	*coef* = −0.18 ± 0.26 $F_{2,75.2}$ = 3.90 ***P* = 0.024**
Tarsus length		*coef* = 3.80 ± 0.47 $F_{1,98}$ = 66.32 ***P* < 0.001**		

Coefficients (SE) are shown for maternal control treatment, female chick sex and first (1) and second (2) positions in the laying order.
Significant effects ($P < 0.05$) are shown in bold.

remain 3 s longer in tonic immobility than chicks from second-laid eggs by control mothers ($t_{30.4}$ = 2.02, P = 0.052). Chicks from first and third-laid eggs did not significantly differ between treatments (both $P > 0.21$). Significant differences were found within broods of predator-exposed mothers; second chicks remained more time immobile than their sibs in the first and third positions (Figure 1a; respectively, $t_{71.9}$ = 3.02, P = 0.004 and $t_{77.6}$ = 2.31, P = 0.024). We did not find differences within broods of control females (Figure 1a; all $P > 0.13$). Female chicks remained longer in tonic immobility than male chicks (Table 1). The number of times the test had to be repeated per focal chick when tonic immobility was not reached did not differ between treatments ($F_{1,7.6}$ = 1.84, P = 0.21).

All chicks except 4 crouched before 110 s (the maximum duration of the trial). We found that the interaction between treatment and laying order significantly affected chick time to crouch (Table 1). Chicks from second-laid eggs by predator-exposed mothers crouched on average 3 s earlier than chicks from second-laid eggs in the control environment (Figure 1b; t_{111} = 2.73, P = 0.007; LSmeans test), while chicks from first- and third-laid eggs did not significantly differ between treatments (Figure 1b; both $P > 0.44$). Also, chicks from second-laid eggs in the predator treatment crouched earlier than their first sibs (P = 0.018), the difference with second chicks being nearly significant (P = 0.053). Siblings in the control treatment did not differ among them in time to crouch (all $P > 0.13$). Female chicks crouched on average 2 s earlier than male chicks, the difference being highly significant (Table 1).

Offspring morphology and sex

Predator exposure treatment did not affect egg volume (Table 1), suggesting that mothers in both treatments were of similar quality. However, the interaction between predator treatment and laying order affected chick body mass at day 2 (Table 1). Chicks from third eggs laid by predator-exposed mothers were on average 6 g lighter than chicks from third eggs laid by control mothers (Figure 2; $t_{33.4}$ = −2.53, P = 0.016; LSmeans test). Chicks from first and second-laid eggs did not significantly differ between treatments (both $P > 0.44$). Also, chicks from third-laid eggs in the predator treatment were lighter than their sibs in the first and the second positions (P = 0.044 and 0.001, respectively), while siblings in the control treatment did not differ among them in body mass (all $P > 0.7$). Finally, sex allocation in the laying sequence was not affected by predator exposure (treatment × laying order: $F_{2,117}$ = 0.50, P = 0.61).

DISCUSSION

We report that prenatal maternal exposure to predators induces antipredator behavior in gull chicks, although only evident in those hatched from second-laid eggs. Eggs were incubated and chicks raised by nonexposed females, so our results indicate that mothers modified egg substances to prepare some of their offspring for living in an environment with predators.

Chicks hatched from second-laid eggs of predator-exposed mothers responded faster to a potential predator attack than second chicks of control mothers. Given the survival advantage of rapid crouching behavior in semiprecocial gull chicks when predators are present in the colony (Impekoven 1976), our results suggest an important adaptive value of maternal effects via eggs on second chicks in the predator treatment. Additionally, second chicks of predator-exposed mothers remained more time in tonic immobility than their sibs, while we did not find significant differences within broods of control mothers. Our results may suggest that mothers in an environment with high predation risk performed intraclutch differential investment to optimize the cost-benefit tradeoff of current reproduction (see Sheriff and Love 2013). Mothers thus "programmed" the phenotype of certain offspring (second chicks) with high survival prospects to cope with predators, which partly agrees with our prediction. However, a passive mechanism of intraclutch allocation may be possible as well, according to which predator exposure may have caused a transitory stress response in mothers by regulating the hypothalamic-pituitary-adrenal axis, which influenced egg composition. Under this scenario, third chicks were not

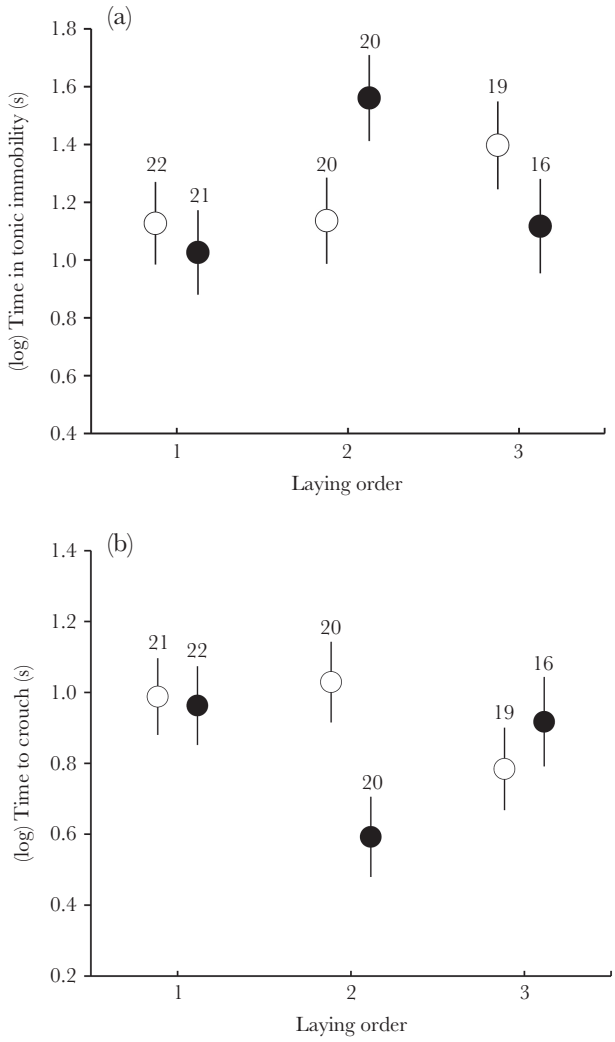

Figure 1
Effect of maternal predator exposure and laying order on (a) chick (log) time in tonic immobility (s), and (b) chick (log) time to crouch (s) after hearing a playback with adult alarm calls. Values are least-square means ± SE. White and black dots are chicks from control mothers and predator-exposed mothers, respectively. Sample sizes are specified above bars.

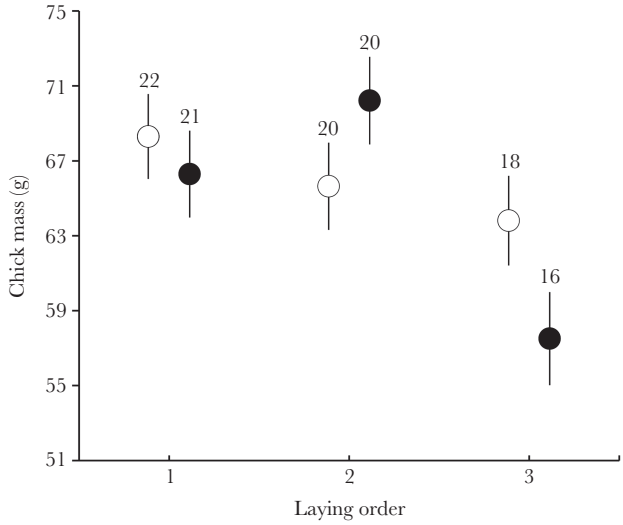

Figure 2
Effect of maternal predator exposure and laying order on chick body mass (g). Values are least-square means ± SE. White and black dots are chicks from control mothers and predator-exposed mothers, respectively. Sample sizes are specified above bars.

affected perhaps because mothers' habituated to successive predator model presentation.

Unexpectedly, first chicks were not affected by predator treatment possibly because first-laid eggs remained in the maternal genital tract on average 2 days less (mean ± SE: −2.06 ± 0.14 days) than second eggs under the influence of predator exposure. Another possibility is that all chicks received similar amounts of *in ovo* cues of maternal predator exposure but second chicks were more sensitive to them due to intrinsic differences among sibs (e.g., higher steroids contents, as occurs in other gull species; Groothuis and Schwabl 2002). Interestingly, female chicks developed faster antipredator responses than male chicks, although sex allocation in the laying sequence was not affected by predator exposure. This suggests that sex-specific mechanisms may also govern behavioral phenotypes at an early stage of life (see Ruuskanen and Laaksonen 2010; Kim and Velando 2015).

Nestlings hatched from third-laid eggs were lighter in the predator treatment than in the control environment. In other bird species, chicks of predator-exposed mothers prior to laying also grow smaller than chicks of control mothers, despite having hatched from eggs with similar size (Coslovsky and Richner 2011; 2012). As we expected, only chicks from third-laid eggs by mothers exposed to predators suffered a fitness penalty in terms of growth. Third gull chicks are typically at a disadvantage and survive worse than first and second chicks (e.g., Parsons 1970; Royle and Hamer 1998; Rubolini et al. 2005). Hence, this result corroborates that mothers perform intraclutch differential investment under high predation pressure, thereby putting last chicks with low reproductive value at a disadvantage to optimize the cost of current reproduction (Sheriff and Love 2013).

The effects of laying order and exposure to predators cannot be explained by differences in egg volume, suggesting that predator-exposed and control mothers had similar nutritional condition. Glucocorticoids, such as corticosterone, represent the main mechanism mediating adaptive behavioral responses to stressful events via eggs (reviewed in Love et al. 2013; Sheriff and Love 2013). Indeed, mother birds exposed to predators lay eggs with high corticosterone concentration (McCormick 1998; Saino et al. 2005). In lizards, experimental corticosterone injection into eggs produce offspring that stay in shelter for significantly longer time (Uller and Olsson 2006), and in birds it results in offspring with higher flight performance (Chin et al. 2009). Moreover, in our study colony, previous work showed that chicks from "stress broods" (in which 2 siblings had been implanted with corticosterone) grew more slowly and crouched on average 3 s faster than chicks of control broods after hearing adult alarm calls (Noguera et al. 2017). This was exactly the same difference in time to crouch that we found between second chicks from predator-exposed and control mothers. Therefore, egg maternal corticosterone (but also testosterone alongside corticosterone; see Guesdon et al. 2011; Coslovsky et al. 2012) could be a potential cue of high predation risk that induced stronger antipredator defenses in second offspring at the expense of lighter third chicks.

In conclusion, our results support the idea that maternal induction of defenses is a form of adaptive developmental programming

across generations that modulates predator-prey interactions (Agrawal et al. 1999). Future studies are needed to deeper explore how predation risk mediates maternal favoritism among offspring with dissimilar survival prospects and vulnerability (e.g., different laying position and different sex) and to study the long-term effects on offspring behavior.

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Data accessibility: Analyses reported in this article can be reproduced using the data provided by Morales et al. (2017).

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