Title: The impact of acoustic signalling on offspring performance varies between three biparentally caring species

Authors

Taina Conrad[1], Magdalena M. Mair[2] Julia Müller[1], Peter Richter[1], Sophie Schödel[1], Ann-Kathrin Wezstein[1], Sandra Steiger[1]

Affiliations

1 Department of Evolutionary Animal Ecology, University of Bayreuth, Bayreuth, Germany

2 Statistical Ecotoxicology, University of Bayreuth, Bayreuth, Germany

Correspondance

Taina Conrad, Department of Evolutionary Animal Ecology, University of Bayreuth, Bayreuth, Germany, taina.conrad@uni-bayreuth.de

Abstract

Communication plays a fundamental role in the evolution of any form of cooperative behaviour such as parental care. However, it can be challenging to understand the specific role of certain signals and how they might have evolved into complex communication systems. To investigate what effect a lack of acoustic communication can have on brood care and offspring performance, we silenced parents of biparentally caring burying beetles with a non-invasive method and studied the effect on clutch and offspring performance. Moreover, by analysing three species with varying degrees of offspring dependency on parental care, we aimed to investigate how differing acoustic communication is related to the level of their dependency and if those two align in some way. We found strong effects of silencing parents on offspring performance in all three species. The lack of stridulations impacted offspring weight across all three species. However, our results point towards a species divergence in which development stage communication has the most substantial impact. Looking at larval weight at dispersal the effects seem to be in line with the larval dependency in the way we would have expected – with *N.*

orbicollis being the most strongly affected, *N. vespilloides* being also affected and *N. pustulatus* not being affected. However, looking more closely, we found various differences at other time points and also larval survival being strongly affected in *N. pustulatus*. To date, only a few studies have looked at the exact function of acoustic signals during brood care with most of them focusing on what type of different signals are emitted rather than what effect they have. Our study is one of the first to start disentangling the interplay of communication and offspring performance.

Keywords

Stridulations, biotremology, burying beetles, Nicrophorus, brood care

Introduction

Communication is a fundamental part of animal behaviour and an essential prerequisite to all animal interactions. Animals communicate all around us, all the time, in a diverse range of ways (Bradbury & Vehrenkamp, 2011). It has been shown that communication plays a vital role in the evolution of any form of cooperative behaviour (Bradbury & Vehrenkamp, 2011). Family life, which is thought to be the first step in the ‘major evolutionary transition’ from solitary to social life and eusociality (Kramer & Meunier, 2018; Szathmáry & Maynard Smith, 1995) includes many cooperative behaviours such as parental care or sibling cooperation. Studying family life can therefore be instrumental in understanding the evolution of complex animal societies and the emergence of social life in nature (Kramer & Meunier, 2018). In family life communication is key - animals must recognize a partner and be able to evaluate their trustworthiness and capabilities. They also need to coordinate their actual interactions with their partner as well as their offspring to optimize brood care and, through that, offspring performance (Bradbury & Vehrenkamp, 2011). All of these are accomplished by some form of communication, be it acoustic, vibrational, chemical or visual.

Although communication is a key component in family life it can be difficult to understand the specific role certain signals play. Parental care as a part of family life has been intensely studied in birds (Royle, Smiseth, & Kolliker, 2012) but, although birds are known for their complex vocal repertoires (Marler & Slabbekoorn, 2004), very few studies have looked into the role of vocal communication during parental care (Gorissen & Eens, 2005; Halkin, 1997). Additionally, almost nothing is known of the effect of acoustic communication per se rather than the effect of signal variation as it is usually difficult to completely shut down an entire communication channel.

In insects, on the other hand, numerous studies on communication in family life exist but here most of them focus on chemical communication (Nehring & Steiger, 2018; Stökl & Steiger, 2017). However, airborne sound as well as substrate-borne vibrations are used in many insects and have been found to play an important part in their complex communication systems (Bailey, 2003; Bennet-Clark, 1971; Claridge, 1985; Forrest, Lajoie, & Cusick, 2006; Gillham, 1992; Virant-Doberlet, Stritih-Peljhan, Žunič-Kosi, & Polajnar, 2023). In order to contribute to our general understanding of acoustic communication during complex social behaviours and their role in the evolution of parental care we used burying beetles to establish what effect a lack of acoustic communication can have on brood care and most importantly offspring performance. Moreover, by analysing three species with varying degrees of offspring dependency on parental care, we aimed to uncover how the importance of acoustic communication is related to the intensity of parental care.

Burying beetles (genus *Nicrophorus*) bury small vertebrates as food source for their larvae and then exhibit elaborate biparental care in the rearing of these larvae, which is rare among insects. Consequently burying beetles have emerged as model organisms in evolutionary and behavioural ecology (Creighton, Smith, Komendat, & Belk, 2015; Engel et al., 2016; Head, Hinde, Moore, & Royle, 2014; Jarrett, Schrader, Rebar, Houslay, & Kilner, 2017; Paquet & Smiseth, 2017; Parker et al., 2015; Rozen, Engelmoer, & Smiseth, 2008; Steiger, 2015; Trumbo, 2017; Vogel et al., 2017). Given their rather complex family life - which includes, among other behaviours, feeding of their young, defending the carcass and carcass manipulation - and the interactions between partners as well as their offspring, it is not surprising that sophisticated recognition and communication processes have evolved using chemical as well as acoustic signals (Steiger, 2015) with the former already having been intensively studied.

Starting from mating and throughout their parental care, both parents produce audible sound using their stridulatory organs. All *Nicrophorus* species possess stridulatory organs in which a plectrum (located on the ventral side of the elytra) is moved across the pars stridens (located on the fourth and fifth abdominal segment) to produce airborne sound and substrate vibrations (Darwin, 1871; Fabre, 1899; Hall, Mason, Howard, Padhi, & Smith, 2013; Niemitz, 1972; Pukowski, 1933). The innovation of stridulatory files in Nicrophorinae has been suggested to be critically linked to the origin of parental care (Cai et al., 2014) but research on this and the specific function of the stridulations during different behaviours remains rare. Hall et al. (2013) were the first to investigate defensive signals and the morphology of the stridulatory organs of 8 North American *Nicrophorus* species in a comparative study and have found significant differences in the airborne signals between species. However, whether these differences are biologically relevant remains unclear. Marking by elytral clipping was shown to significantly reduce brood size in *Nicrophorus americanus*, probably because stridulatory sound was significantly altered (Hall, Howard, Smith, & Mason, 2015). There is also evidence that larvae can use the sound produced by the parents to find the cadaver (Niemitz, 1972; Niemitz & Krampe, 1972). This highlights the importance of these stridulatory signals but there are also contrasting studies like the one by Schrader and Galanek (2017) which found no effect of the stridulations on the success of parental care. So overall previous studies have found mixed results which could have several reasons, one of them being that they examined different species and, as we know, that can make quite a difference in *Nicrophorus* (Capodeanu-Nägler et al., 2017; Capodeanu-Nägler, 2018; Otronen, 1988; Trumbo, 1992, 1994; Wilson, Knollenberg, & Fudge, 1984). Some of the studies also used invasive techniques such as elytral clipping which also meant they lacked a true control group (Hall et al., 2015; Jarrett et al., 2017). Finally, all studies looked at only a few reproductive parameters and did not account for between-individual variation in brood size, which leads - due to the strong dependency of larval mass on brood size - to high variation in larval growth between broods (Bartlett, 1988; A.-K. Eggert & Müller, 1997; A. Eggert, Reinking, & Müller, 1998; Scott & Traniello, 1990; Steiger, 2013; Steiger, Richter, Müller, & Eggert, 2007). Consequently, we still do not really understand the role of stridulatory signals in these species, nor do we know all the contexts in which these signals are produced.

Although all *Nicrophorus* species exhibit parental care, larval dependency is quite varied and ranges from facultative to obligatory parental care, depending on the species (Capodeanu-Nägler et al., 2016; Trumbo, 1992). Capodeanu-Nägler et al. (2016) were able to show that the larvae exhibit a differential dependency on parental feeding but not on pre-hatching care. In *N. orbicollis*, which is highly dependent for example, larvae do not survive in the absence of parental feeding. Further studies on other *Nicrophorus* species show that there is a continuous spectrum in larval dependency across *Nicrophorus* species (Steiger, personal communication). This pattern of differences in offspring dependency in closely related species provides us with an excellent opportunity to investigate patterns of dependency on communication signals. Presumably those species in which offspring rely completely on parental care might have also evolved to be more dependent on acoustic communication to account for the higher need for interactions (Freeberg, Dunbar, & Ord, 2012). Consequently, a disruption of their communication should have larger consequences than in species which are less dependent on parental care.

Here we provide an in-depth study of the effect of acoustic communication on successful brood care, using three species with varying degrees of dependency - *Nicrophorus pustulatus* as an example of an independent species, *Nicrophorus orbicollis* as an example of a highly dependent species and *Nicrophorus vespilloides* as an intermediately dependent species mirroring the selection of species in previous research (Capodeanu-Nägler et al., 2017; Prang, Zywucki, Körner, & Steiger, 2022). We also used a non-invasive technique including a true control to silence the parents. Since we know that *Nicrophorus* stridulates during pre- as well as post-hatching care we included clutch and egg size in our measurements as well as numerous measurements for offspring performance such as average larval weight and larval survival at different time-points, all the while controlling for variation in brood size by supplying parents with a set number of larvae. With this approach we hope to gain an insight into the role of communication during brood care and whether the acoustic communication correlates in some way with larval dependency.

Materials and Methods

Rearing and maintenance of beetles

Experimental beetles used were descendants of beetles collected from carrion-baited pitfall traps. *N. vespilloides* beetles were caught in a forest near Bayreuth, Germany (49°55’18.192’’N, 11°34’19.9488’’E), *N. orbicollis* were caught near Big Falls, Wisconsin, USA (44°36’59.0” N, 89°00’58.0” W) and *N. pustulatus* were caught near Lexington, Illinois, USA (40°39'57" N, 88°53'49" W). All beetles were maintained in temperature-controlled chambers at 20 °C on a 16:8 h light:dark cycle. Before the experiments, groups of up to 5 adults of the same sex and family of each species were kept in small plastic containers (10 × 10 cm and 6 cm high) filled with moist coconut coir. To ensure optimal outbreeding we used the program Kinshipper (www. kinshipper.com, Bayreuth, Germany) to calculate optimal mating pairs. Beetles were fed freshly cut larvae of both darkling beetles (*Zophobas morio*) or whole fly larvae (*Lucilia sericata*) ad libitum twice a week. At the time of our experiments, beetles were virgin and between 30 and 40 days of age.

Mating pairs and silencing of beetles

Mating pairs were chosen according to the program Kinshipper, their pronotum width documented with a stereo microscope equipped with a camera (Stemi 305, Zeiss, Berlin, Germany) and then assigned randomly to the silenced or control group. Beetles were then anesthetized using CO_2 and subsequently silenced by gluing a small (approx. 4mm) piece of parafilm (Bernis Inc., Neenah, Wisconsin, USA) onto the stridulatory organ using super glue (Super Glue Ultra Gel, Pattex ©, Henkel AG &Co KGaA, Düsseldorf, Germany). The control beetles were treated the same way but the parafilm was placed onto the lower part of the abdomen where it would not interfere with the stridulatory organ. After the attachment of the parafilm, beetles were kept anesthetized for approximately 10 more minutes to allow the glue to fully dry. Successful silencing was checked visually and audibly during handling throughout the experiment. Additionally, microphone recordings from different studies have proven for this method to be reliable (unpublished data).

Experimental design

To study the effect that a lack of communication has on offspring performance we compared silenced beetles (both parents without the ability to stridulate) with a control group.

Reproduction was induced by providing each mating pair with a 20 g (± 2.5 g) thawed mouse carcass (Frostfutter.de—B.A.F Group GmbH, Germany). In *N. vespilloides* mice were provided in light and beetles moved to the dark after 5 h. In the nocturnal species, *N. pustulatus* and *N. orbicollis*, mice were provided in the dark.

After the egg-laying period, but before larvae hatched (see Capodeanu-Nägler et al. 2016), parents and the carcass were transferred to new plastic containers filled with coconut coir. The eggs were left to hatch in the old container, which we checked every 2 h for the presence of newly hatched larvae. We weighed the larvae when they hatched (0 h), before providing each couple of beetles with a brood of 10 newly hatched larvae of mixed parentage (within either group of silenced or control beetles) to control for variation between families and individual differences in behaviour

(Rauter and Moore 1999). This set-up enabled us to control brood-size and measure larval growth in more detail than would be possible with natural broods. As females exhibit temporally-based kin discrimination in which they kill any larvae arriving on the carcass before their own eggs would have hatched but accept larvae that arrive after their own eggs have begun to hatch (Müller and Eggert 1990), we provided couples with larvae only after their own larvae had begun hatching. We established broods to attain a minimum sample size of 15 for each group within each species (n = 20 silenced and n = 18 control for *N. orbicollis*, n = 21 silenced and n = 20 control for *N. pustulatus*, n = 15 silenced and n = 19 control for *N. vespilloides*) in two repetitions of the experiment. As larval begging and parental feeding is most pronounced in the first 48 h (Capodeanu-Nägler, 2018; Per T. Smiseth, Darwell, & Moore, 2003), larvae were weighed again after 48h and at dispersal. All newly eclosed adults were counted and pronotum size was measured using ImageJ (NIH, Bethesda) and the pictures taken before.

Examining clutch and egg size

To test if the number of eggs or egg size differs between silenced and control beetles, we ran an additional experiment with the same treatment groups as described above. Here we removed the eggs after laying, counted them for each mating pair and then measured the length (i.e. longest axis) and width (i.e. widest distance perpendicular to the length axis) of 10 randomly chosen eggs from each couple using ImageJ (NIH, Bethesda) and pictures taken with a camera attached to a stereo microscope (Stemi 305, Zeiss, Berlin, Germany).

Statistics

For all analysed response variables, we fit fixed effects models with treatment (either silenced or control), size of male parent, size of female parent, carcass weight and experiment (either first or second experimental repetition) as fixed effects and an interaction term for male * female parent size:

$$response = treatment + size\ male\ parent * size\ female\ parent + carcass\ weight + experimen$$

Linear models were fit to continuous response variables (average egg length, average egg width, hatching time, average larval weight right after hatching, average larval weight after 48 hours, average weight at dispersal, average adult offspring size). Generalized linear models (GLMs) with Poisson distributed error structure and log link were fit to egg counts. GLMs with binomial error structure and logit link were fit to k out of n data (number of surviving larvae after 48 hours, number of offspring surviving to dispersion, number of offspring surviving to adulthood).

Residuals of linear models were checked visually based on standard residual plots and by plotting residuals against predictors. Residuals of GLMs were checked using DHARMa (version 0.4.6, (Hartig & Hartig, 2017)). Additional dispersion parameters were fit to GLMs where necessary. In cases of excess numbers of zeros in k out of n responses (*N. pustulatus*, number of surviving offspring at all three time points), analysis was split into two steps: first, a GLM with binomial error structure and logit link was fit to model the presence/absence of surviving offspring; second, a GLM with Poisson distribution and log link was fit to offspring numbers including only samples with surviving offspring. The contributions of different predictors to the variance in the data were tested via type II ANOVAs (linear models) and Likelihood Ratio Tests (GLMs) using the *Anova()* function (*car* package; (Fox & Weisberg, 2019)). Effect sizes were calculated using the *emmeans* package (Lenth R, 2023) and are reported as mean differences (control - silenced) for continuous responses, ratios for count data (silenced/ control) and odds ratios (odds silenced/ odds control) for binary outcomes. For all effect sizes, 95% confidence intervals (CI) are provided. All analyses were done in R version 4.3.1 (for a full list of package versions see session info in the supplemental online material). All graphs were produced using Sigma Plot 14.0 (Systat Software, Chicago, IL, USA).

Ethical Note

All methods used in this study were kept as non-invasive as possible and no animals were permanently harmed during the experiments. The parafilm falls off after a couple of weeks due to grooming leaving the animals as before.

Results

Clutch size and egg size

Silenced parents had on average more than 30 percent fewer eggs than unsilenced control parents in *N. vespilloides* (silenced/control ratio (CI): 0.69 (0.53,0.90); χ^2= 7.50, P = 0.006; Fig. 1). In contrast, this reduction in egg numbers was not observed in *N. orbicollis* (silenced/control ratio (CI): 1.13 (0.90, 1.43); χ^2_1= 1.14, P = 0.286) and *N. pustulatus* (silenced/control ratio (CI): 1.1 (0.93, 1.29); χ^2_1= 1.17, P = 0.279).

N. vespilloides eggs of silenced parents were on average shorter (control-silenced (CI): 0.09 mm (0.02, 0.16); F_1 = 6.22, P = 0.019), but not narrower (control-silenced (CI): 0.03 mm (-0.006, 0.06); F_1=2.96, P =0.096; Fig. 2) than those of control parents. There was no effect of treatment on egg length or width in either *N. orbicollis* (length control-silenced (CI): -0.01 mm (-0.09, 0.07); F_1 = 0.08, P = 0.781; width control-silenced (CI): -0.02 mm (-0.04, 0.005); F_1 = 0.03, P = 0.873) or *N. pustulatus* (length control-silenced (CI): -0.01 mm (-0.06, 0.04); F_1 = 0.16, P = 0.691; width control-silenced (CI): 0.0004 mm (-0.02, 0.02); F_1 = 0.002, P = 0.964).

Offspring performance

We found no difference in the hatching time of larvae from silenced versus control parents in any of the three species (*N. orbicollis* control-silenced (CI): -0.82 h (-11.2, 9.54), F_1 = 0.026; *N. vespilloides* control-silenced (CI): -0.66 h (-7.40, 6.07), F_1 = 0.04; *N. pustulatus* control-silenced (CI): 2.57 h (-1.05, 6.19); F_1 = 2.09; all P > 0.1; Fig. S1).

In *N. orbicollis*, no difference in larval weight right after hatching was observed between silenced and control parents (control-silenced (CI): -0.12 mg (-0.31, 0.08); F_1 = 1.47, P = 0.235; Fig. 3). Larval weight of N. orbicollis after 48 hours and larval weight at dispersal was lower in groups with silenced parents as compared to control parents (larval weight after 48 hours, control-silenced (CI): 7.95 mg (1.13, 14.8); F_1 = 5.67, P = 0.024; weight at dispersal, control-silenced (CI): 40.20 mg (0.49, 79.90); F_1 = 4.29, P = 0.047; Fig. 4 and 5). This weight difference was also apparent in adult offspring with pronotum width being smaller in offspring of silenced parents as compared to control parents (control-silenced (CI): 0.03 mm (0.006, 0.06); F1 = 6.18, P = 0.019). In *N. vespilloides*, the weight of larvae right after hatching from silenced parents was lower than in control groups, but the difference was very small (control-silenced (CI): 0.12 mg (0.02, 0.21); F_1 = 5.80, P = 0.023; Fig. 3). After 48 hours and at dispersal, the difference was not significant any more (larval weight after 48 hours, control-silenced (CI): 6.88 mg (-0.42, 14.2); F_1 = 3.74, P = 0.064; at dispersal control-silenced (CI): 13.3 mg (-1.26, 27.8); F_1 = 3.51, P = 0.072; pronotum width of adult offspring, control-silenced (CI): 0.08 mm (-0.03, 0.20); F_1 = 2.30, P = 0.142; Fig. 4

and 5). In *N. pustulatus*, larval weight after hatching did not differ between treatments (control-silenced (CI): 0.008 mg (-0.05, 0.06); $F_1 = 0.10$, $P = 0.754$; Fig. 3). After 48 hours, larval weight was lower in silenced as compared to control group parents (control-silenced (CI): 5.32 mg (0.05, 10.6); $F_1 = 4.33$, $P = 0.048$; Fig. 4), but differences disappeared again at dispersal (control-silenced (CI): -5.86 mg (-33.6, 21.9); $F_1 = 0.19$, $P = 0.667$; Fig. 5) and in adult offspring (pronotum width, control-silenced (CI): -0.10 mm (-0.27, 0.07); $F_1 = 1.54$, $P = 0.226$).

Offspring survival did not differ between silenced and control parents in *N. orbicollis* (survival after 48 hours, silenced/control odds ratio (CI): 1.01 (0.72, 1.4); $\chi^2_1 = 0.001$, $P = 0.975$; survival to dispersal, silenced/control odds ratio (CI): 0.96 (0.69, 1.36); $\chi^2_1 = 0.04$, $P = 0.835$; survival to adulthood, silenced/control odds ratio (CI): 0.87 (0.66, 1.16); $\chi^2_1 = 0.85$, $P = 0.356$; Fig. 6). In *N. vespilloides*, offspring survival was reduced in offspring of silenced parents as compared to control parents (48 hours, silenced/control odds ratio (CI): 0.77 (0.68, 0.89); $\chi^2_1 = 13.70$, $P = 0.0002$; dispersal, silenced/control odds ratio (CI): 0.70 (0.57, 0.86); $\chi^2_1 = 11.53$, $P = 0.0007$; adulthood, silenced/control odds ratio (CI): 0.58 (0.45, 0.76); $\chi^2_1 = 15.71$, $P = 7.4$ e-05). In seven of the 21 silenced *N. pustulatus* parents, none of the offspring larvae survived for 48 hours, while this did not happen in any of the 18 control parents (presence-absence of surviving larvae after 48 hours/at dispersal/to adulthood, silenced/control odds ratio (CI): 0.08 (0.006, 1.02); $\chi^2_1 = 5.27$, $P = 0.022$). Among the parents with surviving offspring, the number of surviving offspring was significantly smaller in silenced parents at dispersal (silenced/control odds ratio (CI): 1.15 (1.02, 1.29); $\chi^2_1 = 5.46$, $P = 0.019$), but not after 48 hours (silenced/control odds ratio (CI): 1.07 (1.00, 1.15); $\chi^2_1 = 3.83$, $P = 0.050$) or to adulthood (silenced/control odds ratio (CI): 1.14 (0.998, 1.3); $\chi^2_1 = 3.70$, $P = 0.054$).

Discussion

Our results clearly show that there are effects of silencing parents on offspring performance in multiple *Nicrophorus* species. However, the details of the effects differ from species to species.

One surprising result was that *Nicrophorus vespilloides* is the only species already affected pre-hatching with silenced parents laying fewer and smaller eggs which leads to already smaller larvae at hatching. We did not expect to find an effect already during pre-hatching that so strongly affects the offspring. There are various studies showing that numerous parameters can have an effect on clutch size or egg size such as body size (Steiger, 2013), nutritional state (Steiger, Peschke, Francke, & Müller, 2007), carcass size (Müller, 1987) or even the female's social environment (Paquet & Smiseth, 2017; Richardson, Stephens, & Smiseth, 2020). Females are therefore capable of regulating the amount of resources they allocate into their egg laying depending on their circumstances (Sheldon, 2000). It is possible that a lack of acoustic communication from their partner signals a low-quality male in this species, which leads to the female saving resources in favour of future reproductive opportunities. Additionally, larval survival was also affected with far fewer larvae surviving till dispersal and consequently fewer new adults emerging. This can be explained at least in part by the poor-quality eggs they emerged from. However, we believe this is also due to an important role of the stridulations in feeding behaviour, which leads to feeding being impaired in the beginning and consequently some larvae dying within the first 48 hours. Females have been observed to stridulate on top of the carcass seemingly calling the larvae for feeding (Prang, Streller, personal communication). If this proves to be true, feeding is probably affected by a lack of stridulations. After the initial 48h the remaining larvae then have more than enough resource to feed from and less competition which enables them to make up for their initial disadvantage (Bartlett, 1988; A.-K. Eggert & Müller, 1997; Scott & Traniello, 1990).

At first glance this result seems surprising as it appears to be in contrast to the results of Schrader and Galanek (2022) who found no effect of silencing the parents in *N. vespilloides*. However, Schrader and Galanek used natural brood sizes instead of our standardized ones, which probably led to far more variation which in turn would have obscured the differences. Additionally, it is possible that our method of using glue and parafilm is superior to elytral clipping in detecting any effects as it allowed us to have an actual control group that was treated in the same way which the authors admit was lacking in their study (Schrader & Galanek, 2022). Finally, they focused on only a few reproductive parameters and as mentioned did not account for between-individual variation in brood size, which leads - due to the strong dependency of larval mass on brood size - to high variation in larval growth between broods (Bartlett, 1988; A. Eggert et al., 1998; A.-K. Eggert & Müller, 1997; Scott & Traniello, 1990; Steiger, 2013; Steiger, Richter, et al., 2007).

For *N. pustulatus*, we found that larval weight at hatching was the same for larvae from control parents as larvae from silenced parents. After 48 hours though, there was a significant difference in the weight of surviving offspring of silenced parents and larvae of control parents. Since the first 48 hours are most important for parental feeding (Rauter & Moore, 2002; Per T. Smiseth, Lennox, & Moore, 2007; P. T. Smiseth & Moore, 2002) we believe that parental feeding is probably impaired during this time leading to lower weights in larvae with silenced parents. However, since *N. pustulatus* larvae are a comparatively independent species (Capodeanu-Nägler et al., 2016) they are able to make up for this disadvantage during the remaining time until dispersal leading to the effect of the treatment vanishing. More important than these weight effects is, however, the large proportion of couples having lost their brood entirely in the silenced treatment (seven out of 21 with no surviving offspring). We believe that since larvae of this species can survive on their own the high number of broods without any surviving larvae stem from infanticide by the parents. In *Nicrophorus,* mothers are able to identify their own larvae through timing - how long after laying their eggs should larvae arrive – and any larvae arriving too early are killed (Bartlett, 1987). Since fathers do not know exactly when the eggs were laid, it is possible that females use stridulatory signals to communicate if larvae should or should not be killed. If both parents are silenced, this communication might not be possible, and “mishaps” could happen in which the father accidentally kills the brood. It is also possible that the female deems the brood or mating partner unsuitable and decides to invest in future reproductive opportunities instead (Richardson & Smiseth, 2021; Sahm, Prang, & Steiger, 2022).

N. orbicollis reacted similar to *N. pustulatus* with a difference in larval weight at 48 hours. However, they did not manage to make up for this difference in the remaining brood care time and still showed a difference in larval weight at dispersal with larvae of silenced parents being significantly lighter in weight. In consequence, adult offspring from silenced couples were smaller than in control groups. Just like in the other species we believe that the parents’ feeding behaviour is disrupted (Conrad, unpublished data) and because *N. orbicollis* larvae are highly dependent on parental care (Capodeanu-Nägler et al., 2016) they cannot feed themselves sufficiently. Again this result differs from that of Schrader and Galanek (Schrader & Galanek, 2022) who found no effect of silencing in *N. orbicollis*. Apart from the reasons mentioned earlier for *N. vespilloides*, they also only looked at total brood mass and breeding success at dispersal, which might have been insufficient to detect differences.

Overall, our manipulation revealed that the lack of acoustic communication impacted offspring weight across all three species under study. However, our results point towards a species divergence at which development stage communication plays an important role - with *N. vespilloides* being the only one of the three where the effects already influence pre-hatching care. Looking only at larval weight at dispersal one could argue that the effects on offspring performance seem to be in line with larval dependency in the way we would have expected – with *N. orbicollis* being the most strongly affected and *N. pustulatus* not being affected. However, looking closely at the results, all three species are affected at different times, and we believe more research is needed to fully understand these dynamics. Moreover, the fact that some *N. pustulatus* parents lose their entire brood is, after all, a drastic effect. An important next step would involve examining both the behaviour and the specific signals produced, assessing their complexity and quantity.

Consequently, our study was a very important first step in showing that acoustic communication is indeed vital during brood care in these species, and it will be interesting to discover what exactly is communicated and how brood care is coordinated. To date only a few studies have looked at the exact function of acoustic signals during brood care with most of them focusing on what type of different signals are emitted rather than what effect they have (Charrier, Mathevon, Jouventin, & Aubin, 2001; Kavelaars, Lens, & Müller, 2019; Moss, Tumulty, & Fischer, 2023; Vergne, Pritz, & Mathevon, 2009). We know, for example, that in *Umbonia crassicornis* females exchange vibrational signals with their offspring when they are under attack from a predator (Hamel & Cocroft, 2019). In the subsocial shield bug *Parastrachia japonensis* mothers use a “provisioning call” directed towards their offspring and a new study on poison frogs (*Ranitomeya imitator*) found that signal evolution of their acoustic signals is likely associated with their cooperative parental behaviour (Moss et al., 2023). Another example comes from zebra finches where parents seem to coordinate their tasks through vocalizations (Boucaud, Perez, Ramos, Griffith, & Vignal, 2017). We believe task allocation as well as parent-offspring communication is likely to play a key role in *Nicrophorus* and further studies will reveal the interplay of communication, task allocation and larval dependency.

Data Availability

All data and code will be made available upon publication.

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Figure Legends:

Figure 1: Number of eggs laid by silenced or control parents of the three species (*N. orbicollis, N. vespilloides and N. pustulatus*). The numbers within the box represent the number of couples per group (n). The medians, quartiles and outliers (circles) are shown. Significant differences are marked by stars (*GLM, $P < 0.05$).

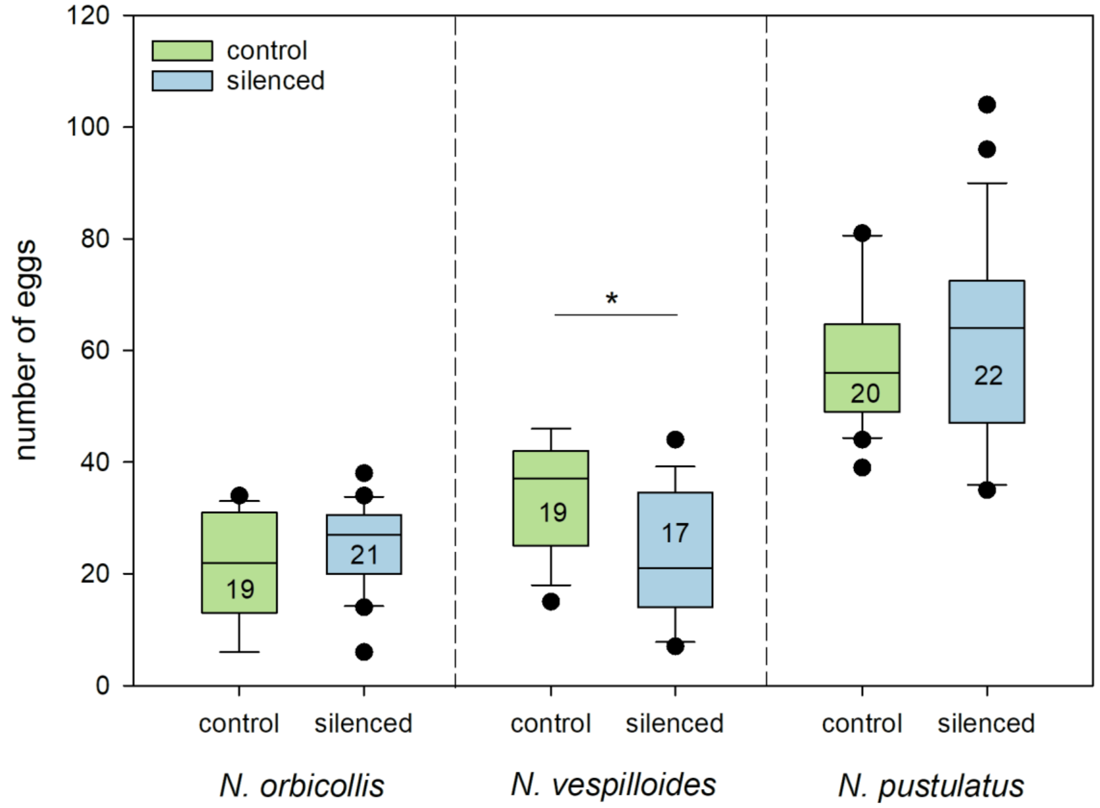
Figure 2: Comparison of egg length and egg width between silenced and control parents of *N. vespilloides*. The numbers within the box represent the number of couples per group (n). The medians, quartiles and outliers (circles) are shown. Significant differences are marked by stars (*GLM, $P < 0.05$).

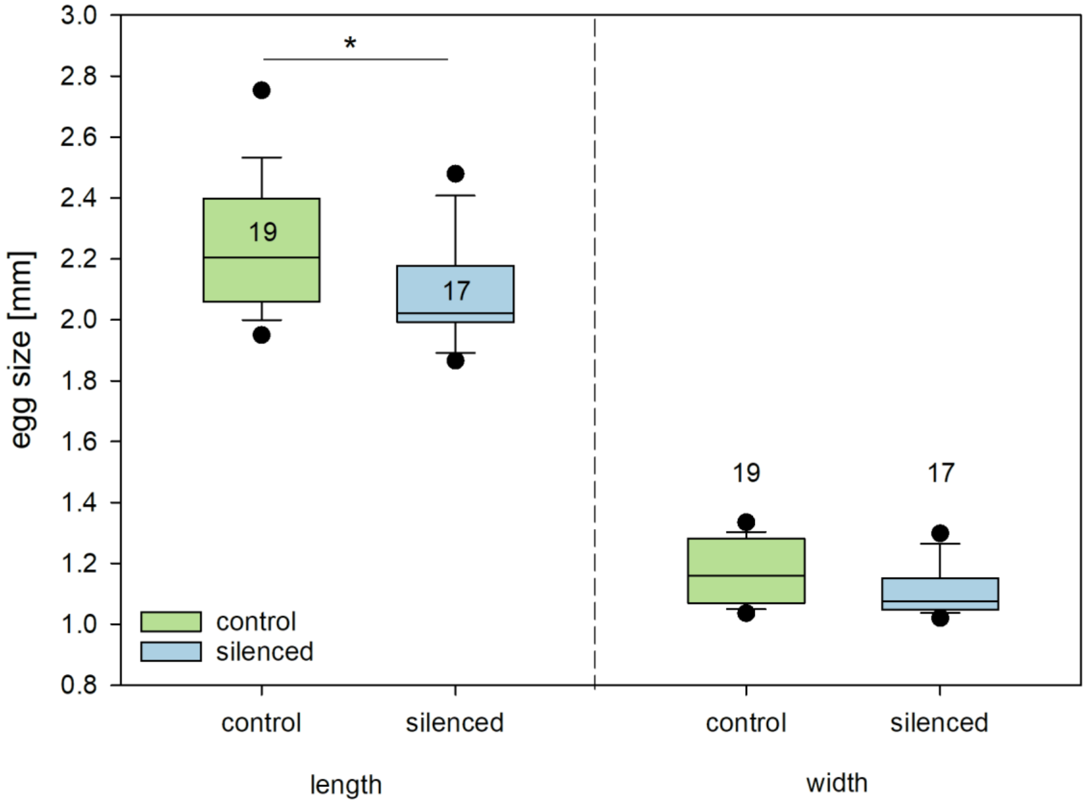
Figure 3: Comparison of larval weight at hatching of silenced or control parents of the three species (*N. orbicollis, N. vespilloides and N. pustulatus*). The numbers within the box represent the number of couples per group (n). The medians, quartiles and outliers (circles) are shown. Significant differences are marked by stars (GLM, *$P < 0.05$).

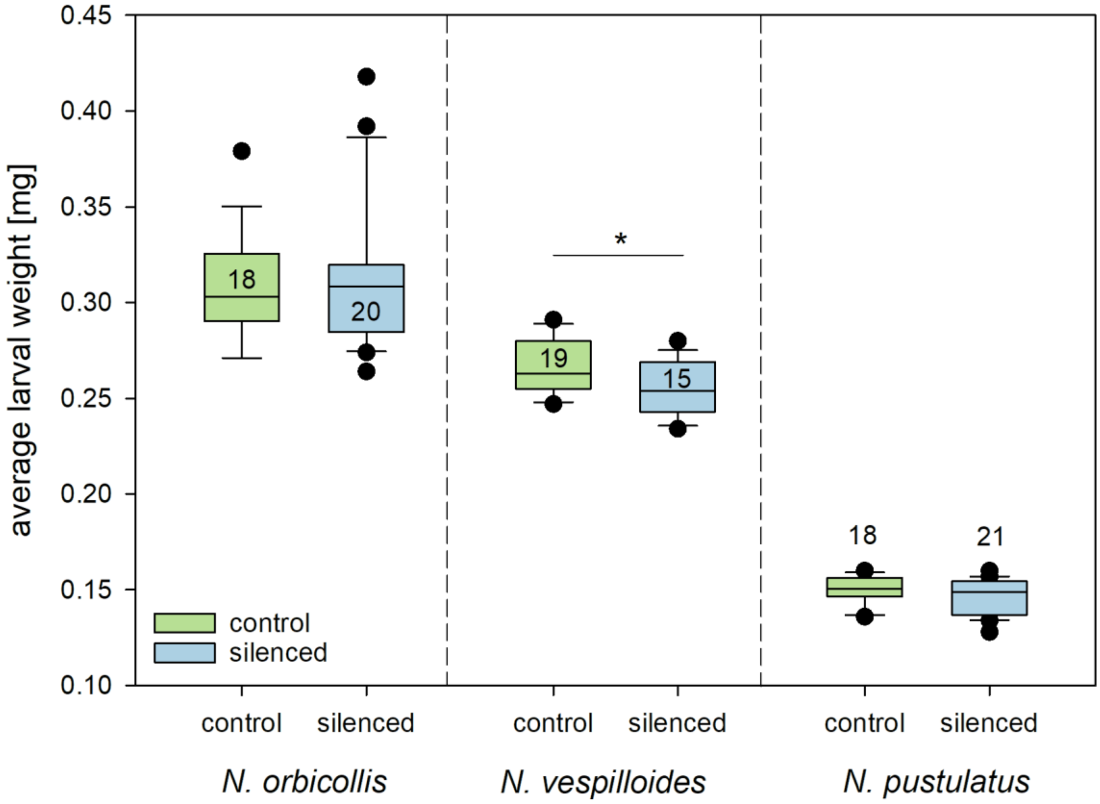
Figure 4: Comparison of larval weight 48h after hatching of silenced or control parents of the three species (*N. orbicollis, N. vespilloides and N. pustulatus*). The numbers within the box represent the number of couples per group (n). The medians, quartiles and outliers (circles) are shown. Significant differences are marked by stars (GLM, *$P < 0.05$).

Figure 5: Comparison of larval weight at dispersal of silenced or control parents of the three species (*N. orbicollis, N. vespilloides and N. pustulatus*). The numbers within the box represent the number of couples per group (n). The medians, quartiles and outliers (circles) are shown. Significant differences are marked by stars (GLM, *$P < 0.05$).

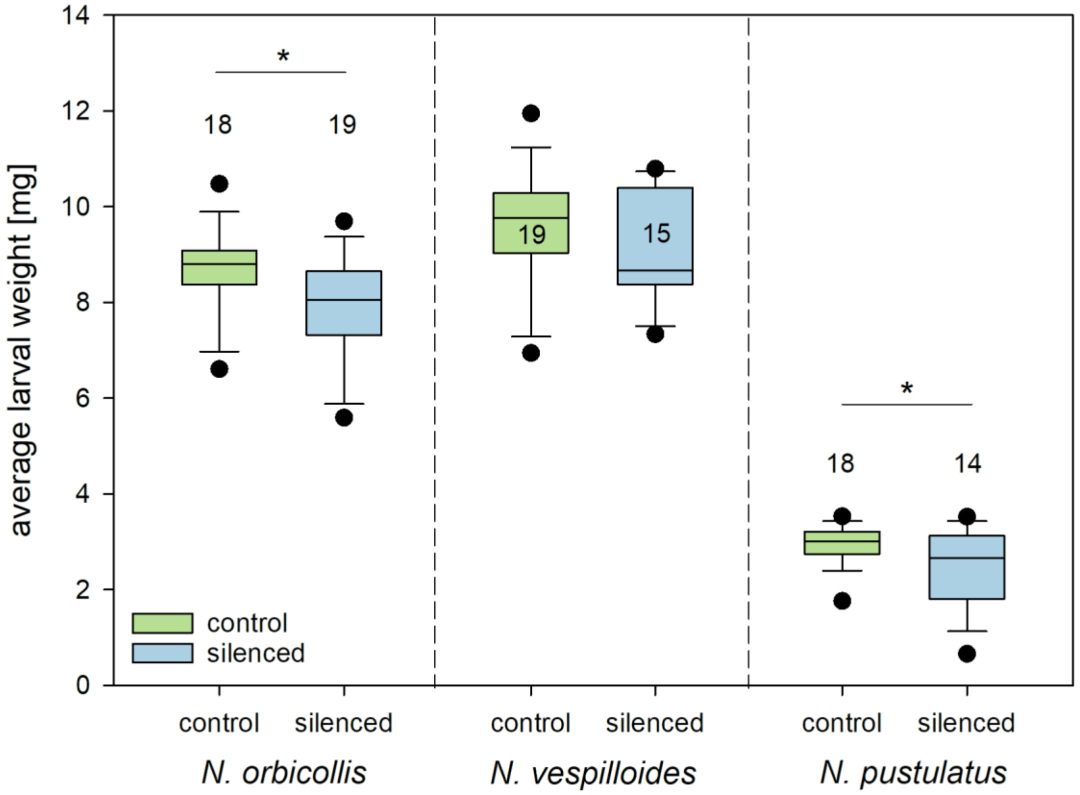
Figure 6: Comparison of larval survival at dispersal of silenced or control parents of the three species (*N. orbicollis, N. vespilloides and N. pustulatus*). The numbers within the box represent the number of couples per group (n). The medians, quartiles and outliers (circles) are shown. Significant differences are marked by stars (GLM, *$P < 0.05$).


number of eggs
120
100
80
60
40
20
0
control
silenced
*
19
21
19
17
20
22
control
silenced
control
silenced
control
silenced
N. orbicollis
N. vespilloides
N. pustulatus

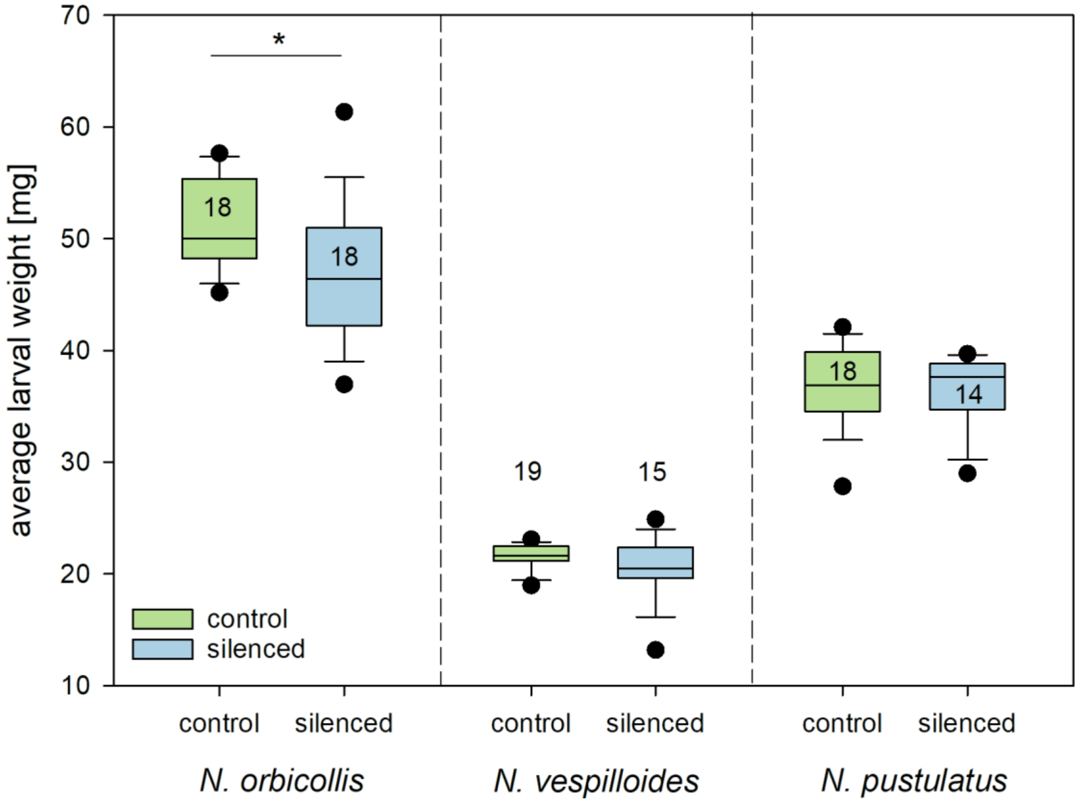

egg size [mm]
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
*
19
17
19
17
control
silenced
control
silenced
control
silenced
length
width

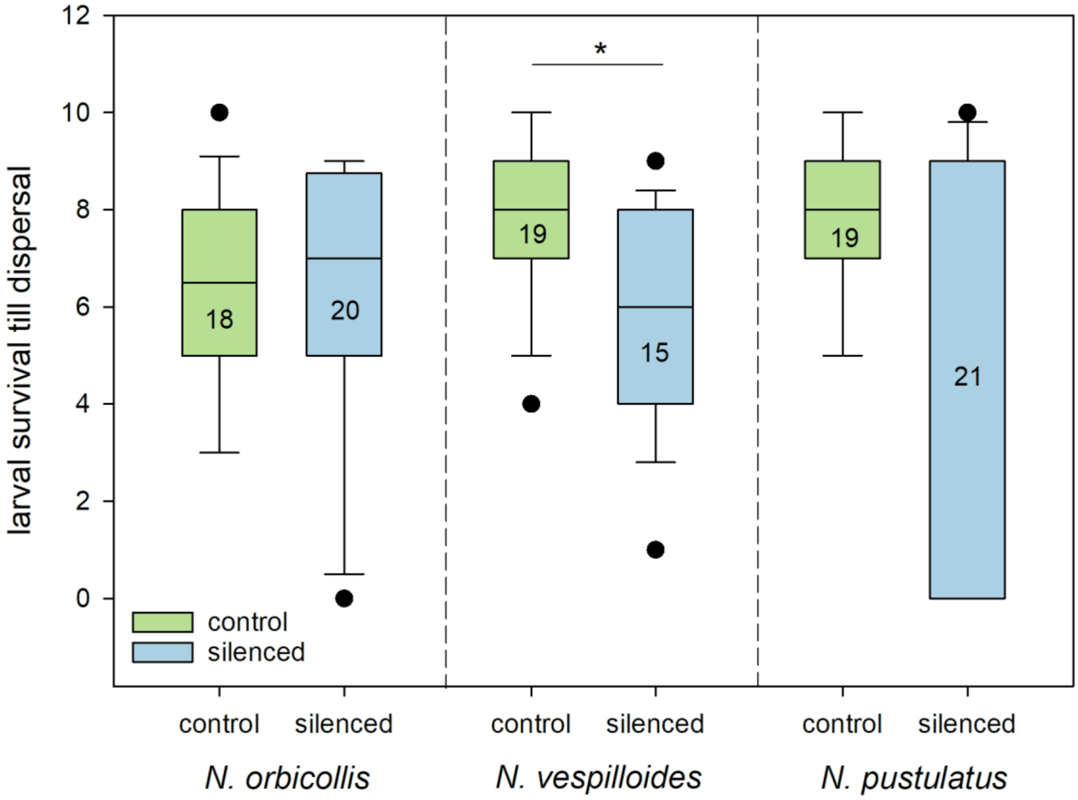

0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
average larval weight [mg]
18
20
*
19
15
18
21
control
silenced
control
silenced
control
silenced
control
silenced
N. orbicollis
N. vespilloides
N. pustulatus

735


14
12
10
8
6
4
2
0
average larval weight [mg]
*
18
19
19
15
*
18
14
control
silenced
control
silenced
control
silenced
control
silenced
N. orbicollis
N. vespilloides
N. pustulatus

736


70
60
50
40
30
20
10
average larval weight [mg]
*
18
18
19
15
18
14
control
silenced
control
silenced
control
silenced
control
silenced
N. orbicollis
N. vespilloides
N. pustulatus


12
10
8
6
4
2
0
larval survival till dispersal
*
18
20
19
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19
21
control
silenced
control
silenced
control
silenced
control
silenced
N. orbicollis
N. vespilloides
N. pustulatus

Supplementary Figures:

Figure S1: Hatching time in hours of eggs laid by silenced or control parents of the three species (*N. orbicollis, N. vespilloides and N. pustulatus*). The numbers within the box represent the number of couples per group (n). The medians, quartiles and outliers (circles) are shown. There were no significant differences (GLM, P > 0.05).