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DEPARTMENT OF BIOLOGICAL AND
ENVIRONMENTAL SCIENCES

DISTURBANCES EFFECT ON REPRODUCTION IN MOUNTAIN CHICKENS

Nordens Ark captive breeding program

Rebecka Olsson

Degree project for Bachelor of Science with a major in Biology

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Image: Mountain chicken frog (*Leptodactylus fallax)* (Erik Edvardsson)

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Abstract (SE)

Kycklinggrodan, *Leptodactylus fallax,* är en akut hotad amfibieart med en liten, kraftigt minskande vild population. Ex-situ uppfödningen är mycket viktig för bevarandearbetet men arten har visat sig svåruppfödd då den har ett komplext reproduktionssystem med yngelomvårdnad i terrestra skumbon och deras sociala beteenden är lite studerade. Den här studien undersöker, med hjälp av kameraövervakning och avelsjournaler, hur störningsmoment (från andra *L.fallax* individer samt från skötare) påverkar reproduktionsframgång och beteenden hos kycklinggrodorna på Nordens Arks avelsanläggning. En jämförelse mellan då skötarna vistades i avelsanläggningen en respektive två gånger per dygn gjordes och resultaten tydde på bättre reproduktionsframgång (fler skumbon och ungar samt en ökning av skumbonas varaktighet) då skötarna vistades mindre i anläggningen. Då kycklinggrodorna är revirhävdande undersöktes även ifall läten från andra individer kunde störa honor med skumbon och därmed påverka reproduktionsframgången negativt. Mediantiden honorna var ifrån sina bon påverkades inte signifikant av mängden ljud från andra individer, varken innan honan lämnade boet eller under tiden hon var borta. Däremot fanns en signifikant ökning i antal gånger honorna lämnade bona efter läten från andra individer jämfört med efter tystnad. Honornas beteenden i samband med att de lämnade och återvände till bona ändrades även märkbart vid läten från andra individer. Innan de lämnade bona spenderade de mer tid nära men bortvända ifrån bona, och när de återvände var de närmare bona men visade färre tendenser att ropa till dem. Studien visar på att för en lyckad reproduktion av kycklinggrodor ex-situ bör minimalt med störning från skötare förekomma. Honors beteenden runt sina skumbon påverkas även av läten från andra kycklinggrodor. Dock krävs fler studier för att avgöra ifall beteendeförändringen påverkar reproduktionsförmågan positivt eller negativt.

Nyckelord: Kycklinggroda, Leptodactylus fallax, ex-situ uppfödning, reproduktion, fortplantningsbeteenden, bo-vaktning

Abstract

The mountain chicken, *Leptodactylus fallax,* is a critically endangered amphibian with a small, severely decreasing wild population. Ex-situ breeding is an important part of the conservation actions, but the species has proven to be difficult to breed due to its complex reproduction with parental care of tadpoles in terrestrial foam nests and barely studied social behaviours. This study investigates, by breeding-journals and camera surveillance, how disturbance (from other *L.fallax* individuals and from keepers) affect reproductive success and behaviours in *L.fallax* at Nordens Ark's breeding facility. A comparison between times keepers was in the breeding facility once or twice a day respectively showed signs of better reproductive success (more foam nests and froglets, as well as an increase in foam nest length) when keepers spent less time in the facility. Due to *L.fallax* being a territorial species, the calls from nearby individuals were investigated as a potential disturbance to females with nests, therefore effecting the reproduction negatively. The median time females spent away from their nests wasn't affected by the amount of calls from nearby individuals, neither before nor during the nest leaving. However, the number of times females left their nests increased significantly at the presents of calls. The females' behaviours in connection with nest leaving noticeably changed at the presents of calls from other individuals. Before leaving, the females spent more time near but turned away from their nests, and after returning they were closer to their nests but called less to them. The study shows that for *L.fallax* to successfully reproduce ex-situ, minimal disturbance from keepers should occur. The females' behaviours around their nests changed at the presents of calls from nearby individuals. More studies will be needed to determine if this change in behaviour has a positive or negative effect on the reproduction.

Key words: Mountain chicken frog, Leptodactylus fallax, captive breeding, reproduction, reproductive behaviours, female nest guarding

Introduction

The loss of biodiversity is a serious problem in today's world. About 40% of all species, assessed by IUCN red list (IUCN, 2021), is threatened by extinction and biodiversity all over the world keeps declining. One of the most endangered animal groups are amphibians, where, of the over 7000 assessed species, around 41% are threatened by extinction (IUCN, 2021). Despite the severe threat against them, there is an underrepresentation of amphibians in research (Rosenthal et al., 2017; Silva et al., 2020) as well as conservation actions (Clark & May, 2002; Seddon et al., 2005; Silva et al., 2020), especially when it comes to the endangered amphibian species (Silva et al., 2020). Habitat loss and fragmentation, invasive species, overharvesting, toxins, climate change and pollution are just a few of the things that endanger amphibians all over the world (Gascon et al., 2007).

One of the most severe threats to amphibians is fungal diseases, in particular chytridiomycosis, a disease caused by the chytrid fungus *Batrachochytrium dendrobatidis* (commonly shortened Bd). Bd has been found in over 350 species of amphibians, spread across 14 families and 2 orders (OIE Working Group on Wildlife Diseases, 2019), and is responsible for the decline or extinction of at least 200 frog species (Skerratt et al., 2007), which is the largest ever recorded loss of biodiversity in vertebrates caused by disease (Skerratt et al., 2007). In 2001 the World Organization for Animal Health (OIE) added amphibian chytridiomycosis to the list of wildlife diseases of concern (OIE Working Group on Wildlife Diseases, 2001-2005) and has described Bd as a "highly infectious and potentially fatal pathogen" (OIE Working Group on Wildlife Diseases, 2019, p. 3). Bd spread very quickly over the whole world, probably aided by human transportation, and can now be found on all continents where wild amphibians can be found (Martel et al., 2018; OIE Working Group on Wildlife Diseases, 2019; Skerratt et al., 2007). It infects amphibians of all life stages (except eggs) with its mobile reproductive cells, zoospores, that gets to the amphibian via water or contact with other infected individuals. The zoospores penetrates the epidermis cells of the skin, where it grows into a thallus that'll later develop a zoosporangium to spread more zoospores (Martel et al., 2018; OIE Working Group on Wildlife Diseases, 2019). The infection breaks down the physical barrier of the epidermis cells and causes disruption of the important functions of the skin, such as rehydration and osmoregulation (Martel et al., 2018; Van Rooij et al., 2015). This will lead to reduced plasma potassium, sodium and chloride ion concentrations, which causes death to the infected amphibian by cardiac arrest (Van Rooij et al., 2015). One of the species that's threatened by chytridiomycosis is the mountain chicken frog.

The mountain chicken frog

Leptodactylus fallax (Müller, 1926) or the mountain chicken, also known as the giant ditch frog, the Dominican white-lipped frog or the giant woodland frog (Adams et al., 2014), is a member of the family Leptodactylidae and the largest amphibian native to the Lesser Antilles in the Caribbean (Adams et al., 2014). It can weigh up to 1 kg and reach a snout-vent-length of 21 cm, although it's commonly between 16-17 cm, with males being slightly smaller than females (Rosa et al., 2012). It has extremely powerful hindlegs that it can use to jump up to 3 meters (Hudson, 2016; Jameson et al., 2019) and males can be identified by a spur on their front feet, that's used to stimulate the female during mating (Adams et al., 2014). *L.fallax* is a nocturnal species that typically spend their days hiding in burrows and come out at night to hunt or seek out mates (Adams et al., 2014; IUCN SSC Amphibian Specialist Group, 2017; Rosa et al., 2012). They mostly eat invertebrates, such as crickets, millipedes, gastropods and coleopterans, but also spiders, centipedes and decapods and vertebrates such as snakes, other frogs, lizards and even bats (IUCN SSC Amphibian Specialist Group, 2017; Rosa et al., 2012). *L.fallax* is a completely terrestrial amphibian species that live in the dense secondary vegetation in forests up to 430 meters above sea level on islands in the Caribbean (IUCN SSC Amphibian Specialist Group, 2017; Rosa et al., 2012). They used to inhabit multiple islands, including Guadeloupe, Martinique, Saint Kitts and Nevis, but can now only be found in a 40 km^2 area on the west side of Dominica and on a less than 1 km^2 area in northern Montserrat (IUCN SSC Amphibian Specialist Group, 2017).

L.fallax become sexually mature within their first 2 years of life (Jameson et al., 2019) and has a rather unique reproduction system for an amphibian, with the whole process, from mating to tadpole development, being completely terrestrial (Gibson & Buley, 2004). The breeding season is initiated by the rainfall season and start with a characteristic calling from the males (Davis et al., 2000).The males usually call between

February and September on Dominica and the calls will differ between individuals, between bouts of calling and even between calls within a bout (Davis et al., 2000). The bouts can vary from minutes at a time to more than 2 hours (Gibson & Buley, 2004), but if multiple males are within hearing distance from one another, their calls will not overlap (Davis et al., 2000). The males are territorial and will become aggressive and fight, by rearing against and wrestling other males, for burrows (Gibson & Buley, 2004; King et al., 2005). This aggression is thought to be initiated by a peptide called Leptodactylus aggression-stimulating peptide (LASP) that males produce in their skin secretion (King et al., 2005). Once a male has a burrow and the dry season has ended, around April on Dominica (Davis et al., 2000), the male will call out to females from the burrows. The females will call back, with a quieter and distinctively different sounding call than the males (Davis et al., 2000), before entering the burrows to start amplexus, the mating process where the male grasp the female with his front legs (Fig. 1A)(Davis et al., 2000; Gibson & Buley, 2004). The couple often initiate and end amplexus a few times (Gibson & Buley, 2004) before a foam nest with fertilized eggs is produced at the bottom of the burrow (Fig. 1B)(Davis et al., 2000; Gibson & Buley, 2004; IUCN SSC Amphibian Specialist Group, 2017). Both male and female calls quietly during amplexus (Gibson & Buley, 2004). After the nest is formed the female will stay to guard and care for it, while the male usually will stay close to, but not in, the burrow to help guard the nest (Gibson & Buley, 2004; IUCN SSC Amphibian Specialist Group, 2017).

The foam nest will develop a strong and flexible skin within the first 24 hours (Gibson & Buley, 2004) and the female will regularly renew the foam to stop the nest from drying out (Davis et al., 2000; Gibson & Buley, 2004). Once the larvae, between 26-43 per nest (Gibson & Buley, 2004), are hatched they carry no yolk sac and therefore don't survive on vitelline (Davis et al., 2000). Instead, *L.fallax* has obligatory oophagy where the female will feed the larvae every 1-7 days with unfertilized eggs (Gibson & Buley, 2004; IUCN SSC Amphibian Specialist Group, 2017), supplying larvae with up to 10 000 – 25 000 eggs during their development (Gibson & Buley, 2004). The larvae in a nest will develop at slightly different speed from one another, between 42-57 days (Davis et al., 2000; Gibson & Buley, 2004), leading to a period of 2-3 day during which the metamorphosed frogs will leave the foam nest (Gibson & Buley, 2004). Even after leaving the nest, the froglets will usually stay close to the burrows for 1-2 weeks (Davis et al., 2000; Jameson et al., 2019).

Figure 1. (A) A captive *Leptodactylus fallax* couple in amplexus (photo: G. Garcia/Durrell). (B) A *Leptodactylus fallax* female guarding a foam nest in an artificial burrow at Nordens Ark's breeding facility (photo: R. Olsson).

L.fallax faces many threats, inter alia invasive species such as rats, dogs, cats and livestock (Hudson, 2016), volcano eruptions on Montserrat (Hudson, 2016; IUCN SSC Amphibian Specialist Group, 2017), hunting by humans (the mountain chicken has been the national dish at Dominica and between 8000 and 36 000 individuals could be harvested every year)(IUCN SSC Amphibian Specialist Group, 2017; Tapley et al., 2014) and habitation loss and fragmentation due to human activities that leaves the population severely fragmented with 7-8 subpopulations, where all of the subpopulations each contain less than 30% of the total population (IUCN SSC Amphibian Specialist Group, 2017). The biggest threat, however, is the outbreak of chytridiomycosis, that plagued the islands during the last 2 decades (Hudson, 2016; IUCN SSC Amphibian Specialist Group, 2017).

L.fallax had a population that was considered stable on both islands, with the smallest on Montserrat containing around 1000 individuals and the Dominican population being considerably larger (IUCN SSC Amphibian Specialist Group, 2017), until Bd was detected on Dominica in 2002 and Montserrat in 2009, leading to one of the fastest declines of a species ever recorded (Hudson, 2016; IUCN SSC Amphibian Specialist Group, 2017). Within 18 months of the chytridiomycosis outbreak on Dominica, the population declined with over 85%, and the same decline could be seen on Montserrat only 13 weeks after the chytridiomycosis outbreak there (Adams et al., 2014; Hudson, 2016; IUCN SSC Amphibian Specialist Group, 2017). Today only 2 individuals on Montserrat and 130 individuals on Dominica is left (Hudson, 2016; IUCN SSC Amphibian Specialist Group, 2017) and the species has been deemed critically endangered (CR)(IUCN SSC Amphibian Specialist Group, 2017).

There have been many conservational efforts made to protect *L.fallax,* including protected areas on the Centre Hills of Montserrat (IUCN SSC Amphibian Specialist Group, 2017), a national captive breeding program on Dominica (Adams et al., 2014; Tapley et al., 2014) and a ban on hunting the frogs on Dominica introduced in 2003 (IUCN SSC Amphibian Specialist Group, 2017; Tapley et al., 2014). In 2008 a program called Mountain Chicken Recovery Program (MCRP) started, which takes care of the conservational actions for *L.fallax*, guided by a 20 year long-term recovery strategy plan (Adams et al., 2014). The MCRP consists of several organisations, including Durrell Wildlife Conservation Trust, Chester Zoo in England, Zoological Society of London (ZSL), Nordens Ark in Sweden, the Department of Environment (DOE) on Montserrat and the Forest, Wildlife and Park Division (FWPD) in Dominica (Mountain Chicken Recovery Programme, 2021). It handles among other things the captive breeding programme, reintroduction (first done in early 2011) of *L.fallax* in the wild and research on chytridiomycosis (Hudson, 2016). Part of the chytridiomycosis research have included an in-situ treatment of Bd on the wild population on Montserrat (Adams et al., 2014; Hudson, 2016; Hudson et al., 2016; IUCN SSC Amphibian Specialist Group, 2017). The frogs were treated with the antifungal drug itraconazole and the treatment was deemed successful as a short-term conservation method, as it increased the survival and the loss of infection among the treated frogs. According to models, the treatment could increase the extinction time of a population by 60%, but it does not give a long-term protection against Bd (Hudson, 2016; Hudson et al., 2016).

Another important conservation action is the *L.fallax* captive breeding program. When Bd was first detected on Montserrat in 2009, 50 healthy individuals of *L.fallax* were captured and transported to different zoos in Europe to be part of an ex-situ safety net population that could be used to repopulate Dominica and Montserrat in the future in case the species goes extinct in the wild (Adams et al., 2014; IUCN SSC Amphibian Specialist Group, 2017). The captured individuals were considered genetically representative of the Montserratian population (Hudson, 2016), and therefore also representative of the Dominican population, since the two populations are genetically identical (Hedges & Heinicke, 2007). Captive breeding is deemed, by the Amphibian Conservation Action Plan (ACAP)(Gascon et al., 2007), to be vital for the short-term survival of many species that face severe urgent threats, such as chytridiomycosis. However, captive breeding face many difficulties and some species are much harder to maintain as viable populations in captivity than others (Tapley et al., 2015). For a captive breeding program to be successful it is important to learn what husbandry each species requires and create an environment as close to the species natural habitat as possible, to improve the species welfare and breeding but also to keep it ready for future reintroduction. When it comes to amphibians, there is a lack of research and information on how to breed and keep many species, especially for species such as *L.fallax* with relatively new breeding programs (Tapley et al., 2015). More research, both on *L.fallax* in the wild but also on how to breed them successfully ex-situ, is needed and will be important for the conservation of the species.

Since *L.fallax* is a territorial species (Gibson & Buley, 2004; Jameson et al., 2019; King et al., 2005) and it's unknown what their optimal social structure is in captivity during breeding season (Jameson et al., 2019), it is possible that the close proximity to other *L.fallax* individuals affect the reproductive success. It has been observed that females with nests are easily disturbed and if there are too many disturbances, they can abandon their nests (Jameson et al., 2019), thus not providing the eggs and additional foam necessary for the tadpoles survival (Davis et al., 2000; Gibson & Buley, 2004; IUCN SSC Amphibian Specialist Group, 2017). On that note, it's reasonable to believe that keepers being in and around the enclosures could disturb the frogs and affect their reproductive success negatively. A female guarding a foam nest can also be disturbed by other frogs, preventing her from successfully caring for her nest, and it is recommended to keep the frogs in pairs during breeding (although other social structures have in some cases provided offspring as well)(Jameson et al., 2019). This is a question that have been raised by the personnel at Nordens Ark, whether their low breeding success is caused by their females being disturbed by nearby calling *L.fallax.*

The breeding facility at Nordens Ark keeps the largest captive population of *L.fallax* in the world at the moment (Nordens Ark, 2021). However, the breeding success has as mentioned been low, with only 1 fertile litter (containing 4 froglets) during 2016 and 3 fertile litters (together containing 15 froglets) during 2020, and the personnel is working on finding more effective breeding methods. With the total number of individuals as low as it is in this species, with only 32 individuals in the wild (IUCN SSC Amphibian Specialist Group, 2017) and 207 individuals in the EEP (EAZA Ex-situ Programme)(K. Försäter, personal communication, 2021), it is of utmost importance that the breeding facilities succeed in producing new individuals, as every new individual is important to *L.fallax* survival as a species.

Aim and Research Questions

This study will look at how potential disturbances affects breeding and behaviours in *L.fallax* in the hopes of getting a better understanding of why there have been so few fertile litters at Nordens Ark's breeding facility.

The following three hypotheses will be tested;

H_A – Fewer disturbances from the keepers will increase the foam nest length.

H_B – The number of times a female leaves her foam nest increases with the presence of other calling mountain chicken frogs.

H_C – How long a female stays away from her foam nest increases with the presence of other calling mountain chicken frogs.

Method

Nordens Ark's breeding facility

This project was done at Nordens Ark's breeding facility for the mountain chickens. The breeding facility was finished in 2015 and is a biosecure facility that's not open for visitors. It consists of 2 separate quarantine enclosures, 3 enclosures for juveniles after metamorphous and 2 enclosures for growth, as well as the breeding enclosures (Appendix 1). There are 4 separate breeding enclosures, each containing 2 parts that can be separated from each other, so the keepers can choose to either let the frogs have access to the whole enclosure or just one of the parts. Each part is 1x3x2.5 meters and have a nesting burrow, made by a PVC pipe, with 3 chambers available for the frogs to make nests in. The enclosures are made to look as close to the frog's natural habitat as possible, with mulch covered ground and Monstera sp. plants. Both the temperature and air humidity are closely regulated.

During the years 2019 to 2021 20 adult frogs were kept at Nordens Ark, 12 of them were kept in the breeding enclosures at any given time. Enclosure 1 housed one male and one female, while enclosure 2 housed two females and two males (during 2019 all four frogs had full access to the enclosure except when both females had nests, but during 2020 and 2021 the frogs were separated with one pair in enclosure 2.1 and one in 2.2) and enclosure 3 and 4 each housed one female and two males (Table 1).

During 2019, the keepers would enter the breeding facility 2 times per day to do husbandry procedures and note observations and climate measurements. However, in an attempt to minimize the disturbances of the frogs, the keepers' routines were changed and as of the 13th January 2020 they only enter once a day, between 7am and 9.30am.

The keepers recorded a number of things about the frogs and the enclosures every day, the information kept and saved in excel journals. For each enclosure temperature and air humidity were recorded every day, as well as behaviors, such as calling, aggressive behaviors, sitting out in the open and sitting by the water, and what and how much the frogs were fed. For each breeding enclosure, the keepers also noted amplexus and the presence of foam nests. Some days, the keepers also included additional notes in the journals about the frog's behaviors, special events or anything the keepers deemed worth writing down. These daily recordings (except for the additional notes) were summarized for every month, both for each enclosure separate and a summary of all the breeding enclosures together.

Table 1. *Leptodactylus fallax* held in the breeding enclosures 1-4 during the breeding seasons of 2019-2021. The frogs in enclosure 1, 3 and 4 had access to both parts of the enclosure. After 2019, enclosure 2 was divided in two parts, 2.1 and 2.2, leaving one male and one female in each part. All individuals are referred to as the last 4 digits of their identifying transponder number.

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Collecting data

Foam nest length

The length of the foam nests was determined using the data recorded in excel journals by the keepers at Nordens Ark and compiled in a separate excel file. All nest from 2019 and 2020 were looked at and for each nest it was noted which year it appeared, in which breeding enclosure, to which female it belonged, which males were present in the breeding enclosure when the nest was constructed (if there were multiple males present it wasn't possible to determine which of them sired the nest), the date the nest first appeared, the date the nest disappeared, the length of the nest (in days present), the number of froglets the nest produced and, if any, additional notes. Each foam nest was also given an individual number 1-56, in order of appearance.

The date a foam nest first appeared, the start date, was estimated by the keepers and noted with a 1 in their journals. Most of the time the nest was discovered and recorded in the journal the day it first appeared but, in some cases, an older nest was discovered and the keepers made an estimation of its start date, in which case that date was used.

In the keepers' journals there was a system for "nest control" where every seventh day after a nest first appeared the burrow would be examined to see if the nest was still present and in good health. If the nest was present a 1 would be noted in the journals, if not, a 0 would be noted. However, this system wasn't consistently used and most information about the nests could be found in the additional notes in the journals. Due to this inconsistent recording, the date a foam nest disappeared, the end date, was sometimes hard to determine. The end dates were consequently divided into 4 categories, as follows; 1: The date it was noted that the nest was removed or that there were remnants of an old abandoned/destroyed nest in the enclosure. 2: The date it was noted that there wasn't a foam nest in the enclosure anymore or that a foam nest could no longer be detected. 3: The last date a foam nest was mentioned as present in the enclosure. 4: The last date before a new foam nest appeared in the enclosure or the date the female was seen in amplexus again and therefore could be assumed to have abandoned her previous foam nest.

Dates of category 1 was seen as most secure and was used in all cases where it existed. Dates of category 2 was seen as almost as secure and used if a date of category 1 couldn't be found. Dates from category 3 and 4 were not as accurate since the true end date probably was somewhere in between the two. Therefore, in the cases where neither an end date from category 1 or 2 could be found, the duration in days of the foam nest was calculated using both end date from category 3 and 4, leaving two different lengths of the nest. If the differentiation between these two dates were more than 10 days, the nest was excluded from the data (6 nests were excluded due to this criteria). A mean of the two nest lengths was then used as the "true" length of the nest (this was used on 13 nests). If the only end date available was of category 4 (due to the nest never being mentioned again after its first discovery), the nest would also be excluded (2 nests were excluded due to this criteria). In 2020-12-21 the frogs were moved to different enclosures to form new couples for the new breeding season, and all nest present at that date was removed. All of these nests were excluded from the data (5 nests). 1 nest was also present over the turn of the year 2020-2021 and was also excluded. This left 11 foam nests from 2019 (none excluded) and of the 45 foam nests from 2020 14 was excluded, leaving 31 nests (Appendix 2).

Female nest guarding

During the breeding season of 2020 two cameras were installed in the burrows of breeding enclosure 1.1 and 2.1. In 2021 six more cameras were installed so that all the burrows in the breeding enclosures had one camera each. The cameras were placed in such a way that the whole burrow with all 3 chambers was visible, but not the tunnel out of the burrow or any other part of the enclosure. The cameras recorded with both picture and sound continuous all hours a day during the whole breeding season, and the recorded films were saved to a hard drive regularly. The cameras were not changed to daylight saving time; therefore, camera time (ct) were used when referred to the time showed on the camera. The films were viewed, using VSPlayer, allowing the frogs behaviours to be observed without disturbing them. Only the films that included amplexus or a foam nest, identified by Nordens Ark staff, were used in this study.

Due to technical issues with the sound recording on the cameras, the films from enclosure 2 and 3 (from the breeding season 2021) could only be used to find time females left and returned to their nests, time they were away from the nests and some behavioural observations.

Leaving nest

The films were fast-forwarded to find the times the female left her foam nest and then the film was observed from 2 hours before she left the nest until she came back to it. During that time a behavioural observation was done, with continuous registration of any sounds the frogs made. Due to the placing of the cameras, it wasn't possible to determine which individual the sounds came from (except if the sound was made by a frog inside the burrow). It was therefore possible that the sounds were made by the male who sired the nest, or by the female herself once she left the nest, in which case the sounds wouldn't be a disturbance to the female. However, since it couldn't be determined, all sounds not made by a frog visible inside the burrow were seen as a potential disturbance.

The data collected from the films were compiled in an excel file and for every time a female left her nest the following data would be noted: the nests individual number, the enclosure the nest was in, the female, the date the female left the nest, the time the female left (ct), came back (ct) and the total time she was away, the minutes of sound that could be heard from 2 hours before the female left until the minute she left, the minutes of sound that could be heard while the female was away as well as, occasionally, additional notes.

The time the female spent away from the nest was counted from the minute she left the burrow until she returned to the burrow. She was deemed to have left the burrow when she was completely out of sight from the camera and to have returned when she entered the view of the camera again. However, it wasn't possible to determine how far from the nest she went or what she did during her time away from the nest. It is therefore possible that she spent the time away from her nest sitting in or just outside of the tunnel leading to the burrow.

The sounds were divided in 3 categories: Calling (short calls, about one every second), barking (short yapping sounds, like a small dog barking) and drilling (a high 'krrrr' sound that start low and get higher toward the end). The exact times the different sounds could be heard, as well as the duration of the bout, were noted during the observation of the films, but summarised as total minutes (without specifying when and how long each bout was) in the excel files. The total minutes were summarized both for each sound category separate as well as for all the sounds together. A fourth sound was also noted; ticking (a short fast ticking sound). This sound was summarized separate in the same way as the others but excluded from the total summation of all the sounds because it wasn't certain this type of sound was from a frog and since it was always present at the same time as other types of sounds.

If the female made any sounds while inside the burrow, it was noted in the additional notes which type of sound it was, the duration and time of the sound and whether or not she was facing the nest or another direction.

Behaviours

The behaviours of the female were observed 10 minutes before she left the nest and 10 minutes after she came back with a continuous focal observation. All her behaviours were observed, with special focus on where in the burrow she was, which way she was facing and if she made any sounds (Table 2). If a behaviour was observed it was registered as a 1, if not as a 0. The background sounds from other frogs were also noted during these observations and was divided in categories of sound present and sound not present.

Table 2. Ethogram of the observed behaviours of female *Leptodactylus fallax* at Nordens Ark's breeding facility. The behaviours are separated in 3 categories; behaviours marked [1] were included in the observation both before a female left the nest and after she came back, those marked [2] were only included before the female left the nest and those marked [3] were only included after the female came back.

Behaviour	Description
Call to nest[1]	The female faces the nest, her sides move, and she make a low calling sound
Potential call[1]	The female faces the nest, her sides move but no sound can be heard
Call away[1]	The female faces away from the nest, her sides move, and she makes a sound
Face nest[2]	The female is facing the chambre with the nest
Face tunnel[2]	The female is facing the tunnel out of the burrow
Face chambre/wall[2]	The female is facing an empty chambre or the burrow wall
Sitting near nest[2]	The female is sitting just outside or close to the chambre with the nest
Sitting in middle[2]	The female is sitting in the middle of the burrow or closer to the tunnel or an empty chambre than to the chambre with the nest
Sitting in tunnel[2]	The female is sitting in the opening to the tunnel, only part of her is visible
Straight to nest[3]	The female moves straight from the tunnel to the chambre with the nest, stopping with head by chambre opening or inside chambre
Move to middle[3]	The female moves straight from the tunnel to the middle of the burrow or stays in the tunnel opening
Move closer to nest[3]	After "move to middle", the female moves closer to the nest but stops before she reaches the opening of the chambre with the nest
Move to nest[3]	After "move to middle" or "move closer to nest", the female moves to the chambre with the nest, stopping with head by chambre opening or inside chambre
Head to nest[3]	The female has her head close to the nest, touching it or almost touching it
Head in chambre[3]	The female has her head in the opening of the chambre with the nest, but hasn't moved her head all the way to the nest

Cover nest[3]	The female is in the nest chambre, facing into the chambre, and is partly or completely covering the nest with her body
Guard nest[3]	The female is sitting in, in the opening of or just outside of the chambre with the nest, facing the tunnel

Statistics

The collected data was compiled in an excel file and the statistics in this study was made using the program SPSS-Statistics or made in excel.

Foam nest length

The foam nest length was analyzed for normal distribution with boxplots, histograms with normal distribution curves and a Shapiro-Wilk test of normality. Samples from 2019 and 2020 were analyzed both separate and together, but neither showed normal distribution. However, after a log10 transformation, the samples together and from 2020 showed normal distribution while the samples from 2019 didn't. This might be due to the much smaller sample size from 2019. A test of homogeneity of variance was made, which showed that the two sample groups had equal variances.

Due to this a Mann-Whitney U-test was preformed to test the H_A hypothesis and a bar graph with mean foam nest length for 2019 (when the frogs were disturbed by keepers twice a day) and 2020 (when the frogs were disturbed by keepers once a day) was made to visualize the possible differences between the two years.

Female nest guarding

Leaving nest

Each time a female left her nest was counted as a replication, though it should be noted that many of them were pseudoreplications due to them belonging to the same nest and female (29 occasions of nest leaving were recorded, spread over 9 foam nests and 5 females). All 29 cases were used in estimations of mean and median time females spent away from their nests, at what time and how many times a day the females left their nests. For statistics based around sound occurrence, 13 cases had to be excluded due to technical issues with the cameras sound recording, leaving 16 cases (spread over 6 foam nests and 3 females).

To test H_B and find if females left the nest more times when other frogs called, a χ^2 goodness of fit test was made, comparing number of times the females left when any sound had been present during the 2 hours before they left to number of times they left after 2 hours of silence. Since the amount of calling varied during the 2 hours, a scatter plot was made to find any correlation between minuets of calling before leaving (0-120 minutes) and time spent away from the nest (in minutes). Two Spearman correlation tests were made, one where the females were analyzed separately and one with all the females together.

To test H_C another scatterplot was made, showing the correlation between time spent away from nest (in minuets) and how many precent of that time sound had been present. To detect any correlation, two Spearman correlation tests were made, one where the females were analyzed separately and one with all the females together.

Behaviours

To get an overview of the females' behaviour in connection with nest leaving, each time a female left her nest was counted as a replication, even if there were pseudoreplications as mentioned before. There were 23 occasions in this observation (spread over 9 foam nests and 5 females) but 6 occasions (spread over 3 nests and 2 females) lacked sound due to technical issues.

The behaviours before nest leaving were grouped in 2 categories; face direction ("face tunnel", "face nest" and "face chambre/wall") and place in burrow ("sitting in tunnel", "sitting near nest" and "sitting in middle"), as were the behaviours after returning; movement ("straight to nest", "move to middle", "move closer to nest" and "move to nest") and behaviour ("head in chambre", "head to nest", "cover nest" and "guard nest"). For each category 3 separate pie charts were made showing the distribution of behaviours for all cases, the cases where sound from other frogs were observed and the cases were no sound was observed, respectively. Only the charts with all cases included the 6 cases where the videos lacked sound.

For the category "movement", a bar-of-pie chart was made where the pie showed percentage of cases where females showed "straight to nest" or "move to middle" (the two were incompatible and females always showed one of them). The bar part of the chart showed the percentage of cases where females, after "move to middle", either showed "move to nest", "move closer to nest" or stayed in middle (if "move to middle" was the only observed behaviour).

For the other 3 categories multiple of the behaviours could be observed on a single occasion, due to the one-zero sampling. The pie charts therefore represent percentages of the total amount of behaviours shown (in each category) instead of percentage of the cases.

Due to the small sample size the conditions for a chi^2-test weren't met and Fisher's exact test was used instead. Separate tests were made for each behaviour, comparing the presence or absence of the behaviour during observations with or without sounds from other frogs. The behaviours "call to nest" and "potential call" were, for the purpose of this test, counted as the same behaviour. The behaviours in the category "movement" couldn't occur at the same time and was therefore tested as one behaviour with 4 variants; "straight to nest", "stay in middle", "move to nest" or "move closer to nest".

Result

Foam nest length

When comparing the foam nest from 2019 and 2020 there were a clear but non-significant increase in mean number of days the nests were present (Fig. 2). There were also more nests present in 2020 than 2019, 45 compared to 11, and the nests from 2020 together produced 15 froglets spread over 3 nests, while no froglets were produced during 2019.

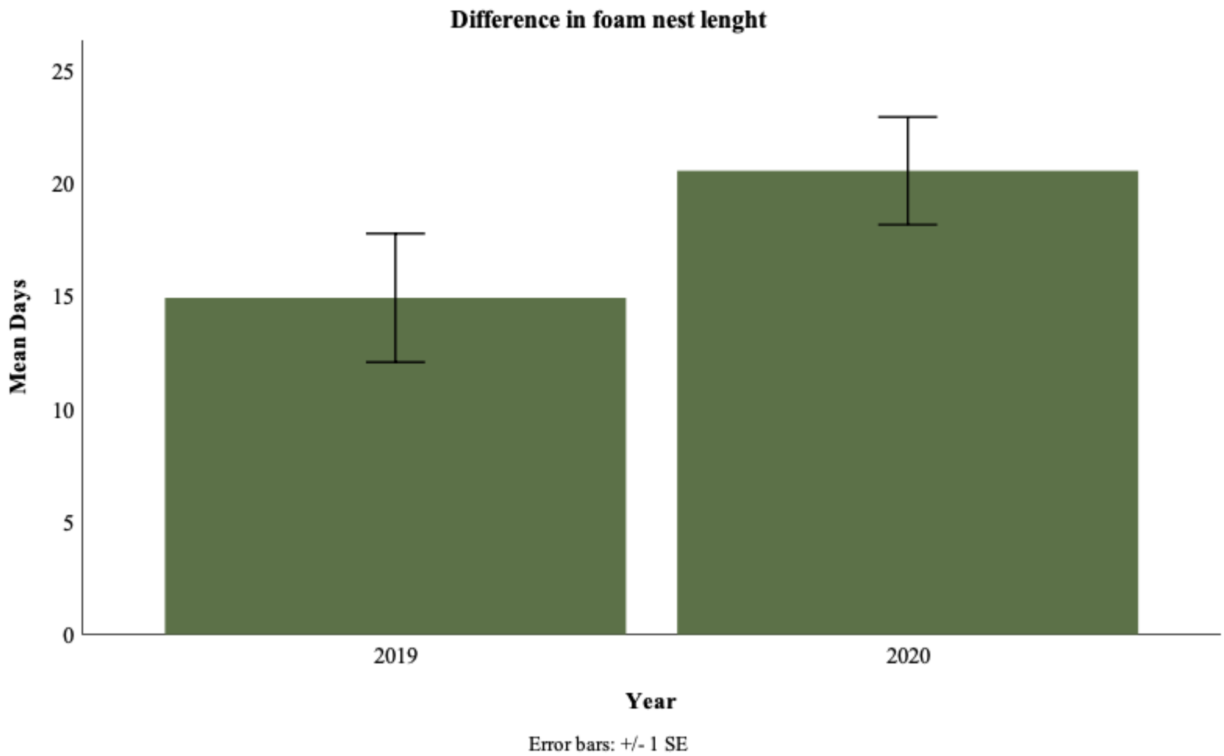

Figure 2. The mean length of foam nests from *Leptodactylus fallax*, in number of days, with a mean of 14.9 days for 2019 (n = 11) and 20.5 days for 2020 (n = 31)(p = 0.165).

For the foam nests that didn't produce froglets (n = 39) the mean length was 18 days, the shortest being 4.5 days and the longest 34 days. For the nests that produced froglets (n = 3) the mean foam nest length was 55, ranging between 53 and 57 days.

Female nest guarding

Leaving nest

The mean time a female spent away from her nest was 192 minutes at a time (29 times a female left, spread over 9 nests and 5 females). However, one nest is to be viewed as an outlier since the female left it for over 12 hours every time. Because of this is the median time females spent away from their nests, 112 minutes, a better estimate.

The females left the nests more times after sound had been present during the 2 hours before they left (n = 12) than after 2 hours of silence (n = 4) (p = 0.046). The amount of sound from other frogs during the 2 hours varied a lot, but the time spent away from the nest showed no correlation to the amount of sound before the female left (Fig. 3). Separate correlation tests were made for the different females, but none showed significance (female "…0976", n = 5, p = 0.391; female "…3303", n = 10, p = 0.236; other females excluded from separate tests due to small sample sizes).

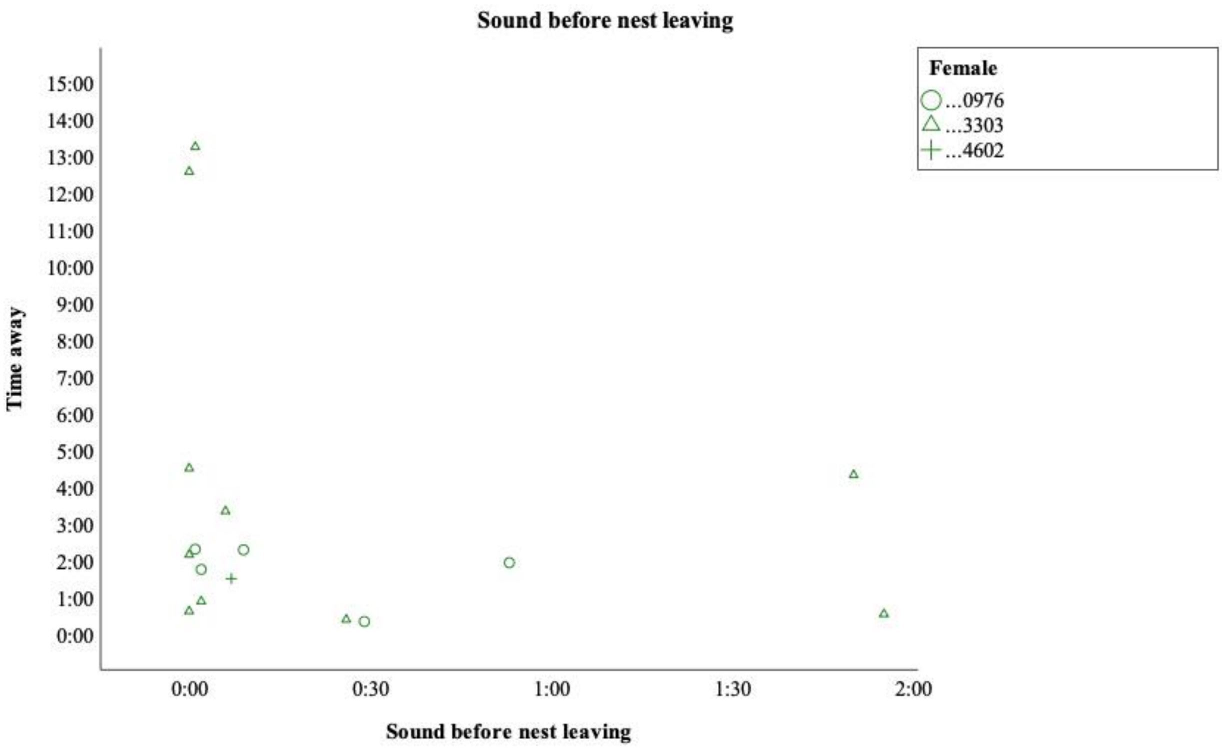

Figure 3. The total amount of sound from other frogs during 2 hours before the females left on the x-axis and the time the females spent away from the nests in hours on the y-axis. Different shapes symbolize the different *Leptodactylus fallax* females from Nordens Ark´s breeding facility. A Spearman correlation test showed no significant correlation (n = 16, p = 0.103).

Since the time away from the nest varied, a percentage was used to compare the amount of sound from other frogs during the females' time away from the nests. No correlation could be found between the two on an individual level (female "…0976", n = 5, p = 0.391; female "…3303", n = 10, p = 0.067; other females excluded from separate tests due to small sample size) but a significant negative correlation was found for the females as a whole (Fig. 4). However, when the outlier nest was excluded, no correlation could be found (n = 14, p = 0.246).

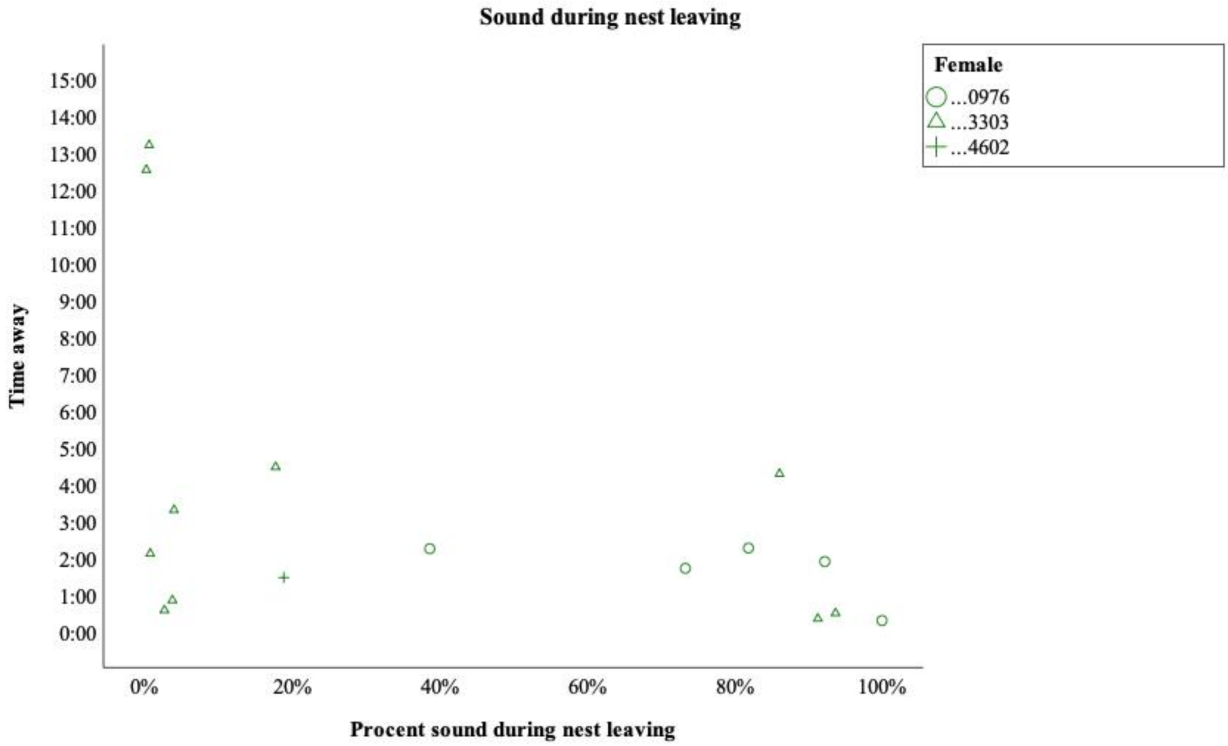

Figure 4. The time females spent away from the nests in hours on the y-axis and the percentage of sound from other frogs during that time on the x-axis. Different shapes symbolize the different *Leptodactylus fallax* females from Nordens Ark´s breeding facility. A Spearman correlation test showed a significant negative correlation ($n = 16$, $p = 0.027$).

Females often left their nests no more than once a day, in 22 out of 29 cases they left before midday (01:00-13:00 ct) and in 14 of those 22 cases the female left during the time the keepers could be in the facility (between 6:00 and 8:30 ct) or within 30 minuets after they left (until 9:00 ct).

Only 3 times did females leave multiple times in a day. The first was female "…0976" who left her nest 2 times during the 10th of May 2020, first at 07:42 ct when she was away for 138 minutes (81.9% of that time sound could be heard) and then at 13:02 ct when she was gone for 119 minutes. The second was female "…7144" who left her nest 2 times during the 6th of May 2021, first at 03:46 ct for 4 minutes (sound unknown due to technical issues) and then at 08:36 ct for 163 minutes. The last was female "…3303" who left her nest 3 times during the 11th of May 2021, the first time at 09:31 ct for 23 minutes (of which 91.3% sound could be heard), the second at 11:06 ct for 32 minutes (93.7% with sound) and the third at 13:30 ct for 259 minutes (of which 86.1% sound could be heard). The last 2 times female "…3303" left the nest, less than 2 hours had passed since she came back to the nest until she left again and there was almost constant calling from other frogs.

Behaviours

Before leaving

Females showed a changed behaviour if sound from other *L.fallax* were present during the 10 minutes before they left their foam nests. Without any sound present the females faced all directions and mostly sat in the middle of the burrow, although both "sitting in tunnel" and "sitting near nest" occurred. When sound was present no females faced their burrows and all of them sat near their nests, although "sitting in tunnel" was observed once (Fig. 5). The behaviour "sitting near nest" increased ($p = 0.002$) and "sitting in middle" decreased ($p = 0.002$) when sound from other *L.fallax* were present compared to when it was not, while the other behaviours showed no significant difference ("face nest", $p = 0.237$; "face chambre/wall", $p = 0.304$; "face tunnel", $p = 0.584$; "sitting in tunnel", $p = 1.000$).

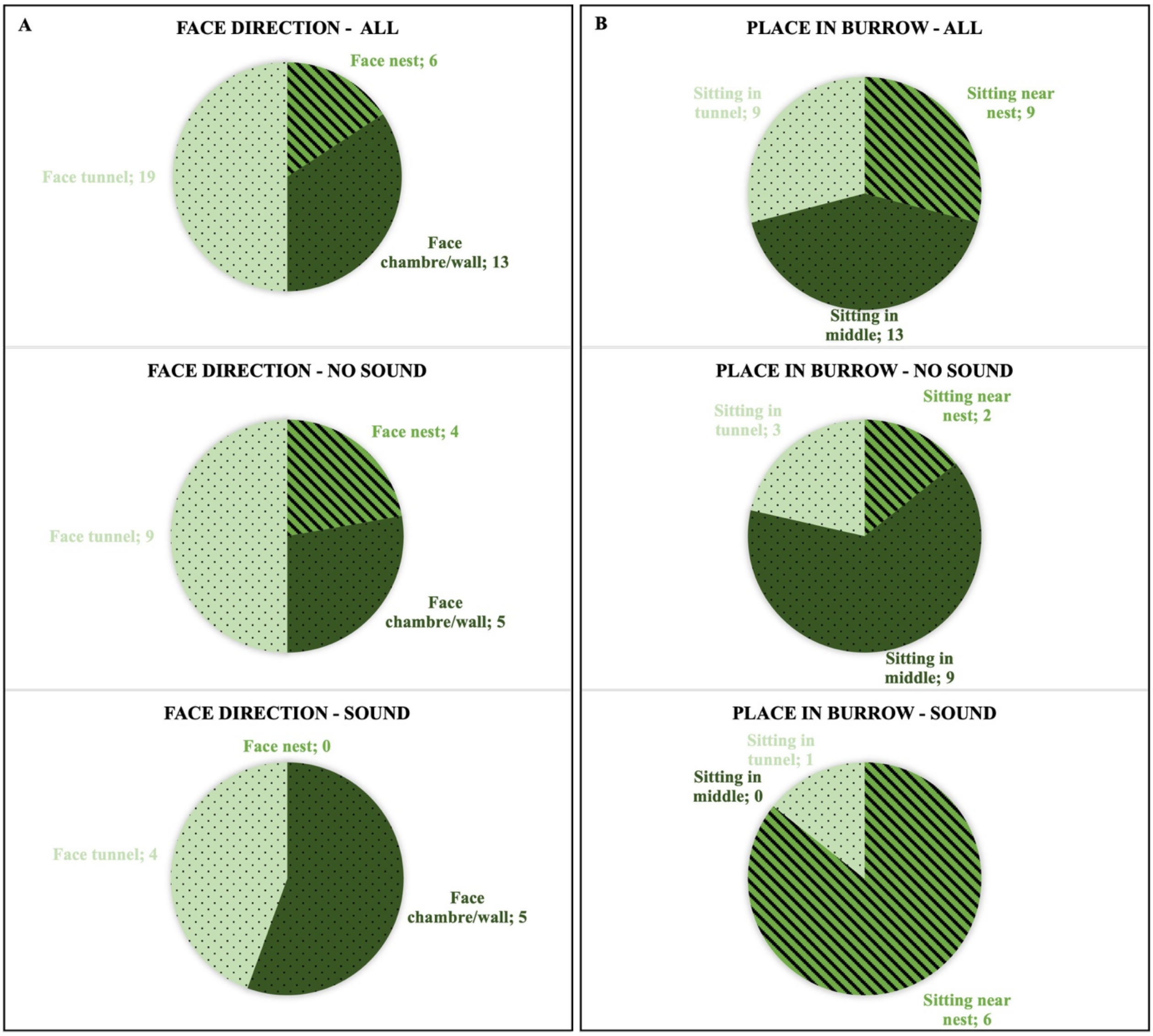

Figure 5. The distribution of observed behaviours in Nordens Ark's *Leptodactylus fallax* females during the 10 minutes before they left their foam nests. The top charts represent all cases females left (n = 23), the middle represent cases when no sound from other frogs could be heard (n = 11) and the bottom represent cases with sound from other frogs (n = 6). (A) The behaviour category "face direction". (B) The behaviour category "place in burrow".

2 potential calls to the nest could be observed before the females left, both occurring during silence, as well as one call away from the nest toward the tunnel that occurred when other *L.fallax* individuals could be heard (n = 23, of which 6 was excluded due to technical issues). The potential calls to the nest during silence weren't significantly different from during sound (p = 0.515).

After returning

In the 10 minutes after females returned to their nests their behaviours changed if sound were present. Of the behaviours from the category "movement", "straight to nest" was most common without sound present while "move to nest" was most common when sound was present (Fig. 6A). When no sound was present "head in chambre" was the most common behaviour, "head to nest" and "cover nest" were only observed once each and "guard nest" wasn't observed at all. However, while sound was present, all four behaviours were observed (Fig. 6B). None of the behaviours showed any significant difference ("movement", p = 0.332; "head to nest", p = 0.294; "head in chambre", p = 1.000; "cover nest", p = 0.131; "guard nest", p = 0.471).

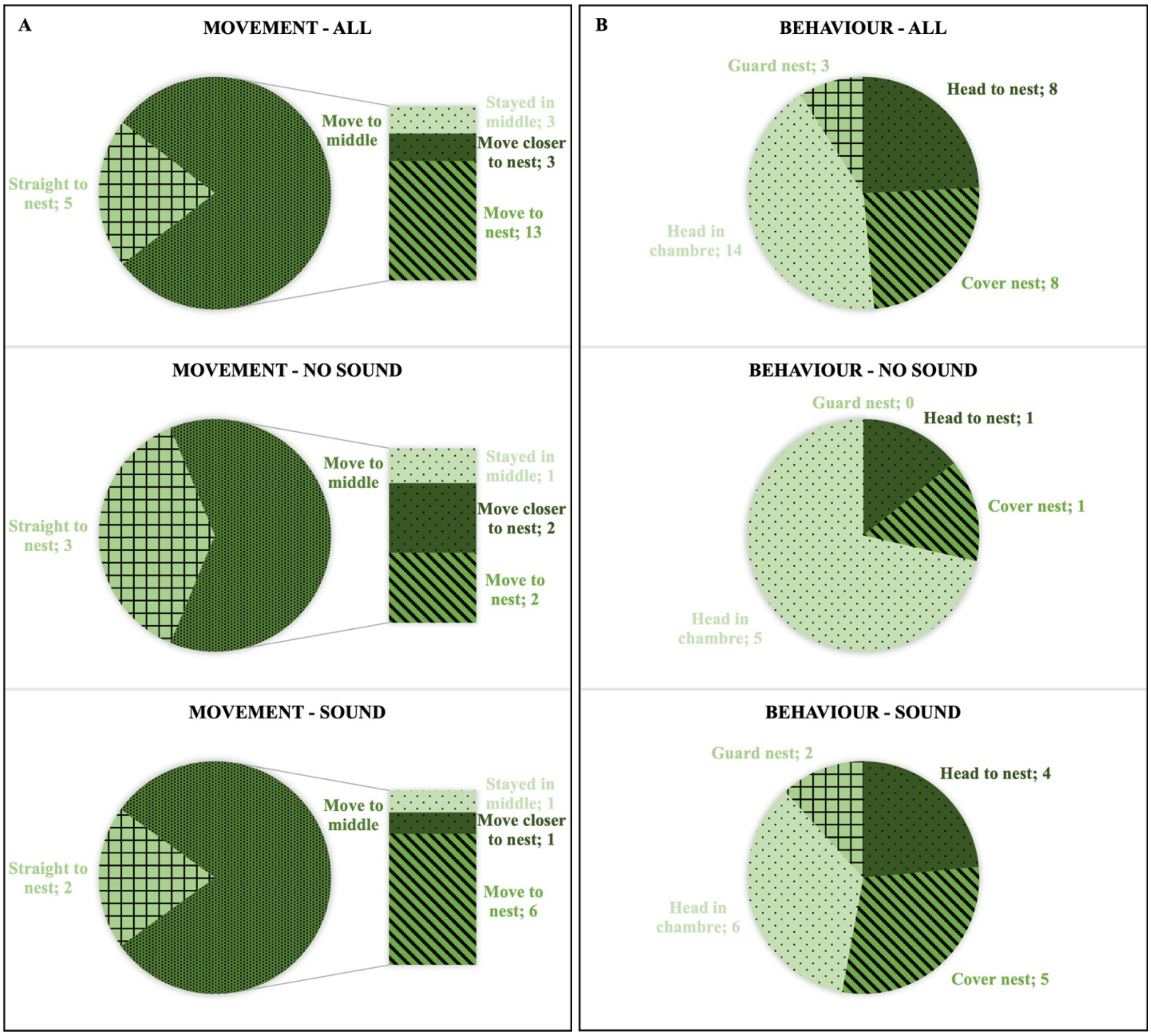

Figure 6. The distribution of observed behaviours in Nordens Ark's *Leptodactylus fallax* females during the 10 minutes after they returned to their foam nests. The top charts represent all cases females returned (n = 23), the middle represent cases when no sound from other frogs could be heard (n = 8) and the bottom represent cases with sound from other frogs (n = 9). (A) The behaviour category "movement". (B) The behaviour category "behaviour".

When the females came back to the foam nests, in 3 cases of 23 they could be observed calling with a sound almost too low to be heard and their face close to the nests. 6 of the 23 cases had to be excluded due to technical issues with the cameras sound recording, but in 4 of those 6 cases, the female could be observed making potential calls, even though no sound could be heard. In 10 of the non-excluded cases potential calls to the nest was observed. Of these 13 cases, 5 occurred while sound from other frogs were present and 8 occurred during silence. In all 4 cases where the females didn't call sound from other frogs were present. The decrease of nest calling during sound from other frogs were clear but not quite significant ($p = 0.082$).

Discussion

The increase in number of foam nest and especially froglets could be seen as signs of a greater reproduction success for the *Leptodactylus fallax* at Nordens Ark after the minimization of disturbances from the keepers between 2019 and 2020, which supports the H_A-hypothesis. Even if the increase in foam nest length wasn't significant this might simply be due to a small sample size. It might also be the case that this isn't an adequate way of measuring breeding success, as a previous study made at Nordens Ark that also used foam nest length noted (Donaldson, 2018). While it's clear that foam nests which successfully produces a surviving clutch have a longer foam nest length, giving seasons with more surviving clutches a longer mean foam nest length, a long foam nest length might not always be optimal for reproduction success. *L.fallax* females kept in captivity usually only produces one fertile clutch per breeding season, or if not the first clutch is usually extremely small (Jameson et al., 2019). If a female has a non-fertile foam nest it would be preferable for her to abandon it early so she can produce a new, hopefully fertile, clutch as quickly as possible. In these cases, a shorter foam nest length might be preferable to a longer one. Since this study didn't have a way of determining whether or not the foam nests that didn't produce froglets were fertile, it's hard to tell if the increase in foam nest length is positive for the reproduction success or not.

Another thing that could point to the keepers being a stressing factor is the fact that females seem to leave the burrows mostly during the time the keepers are in the breeding facility. They could simply leave at that particular time to feed, either because they know what time they're usually fed, have associate the keepers with feeding and therefor expect food when the keepers arrive or simply because they notice the food being placed in the enclosure. It might also be because they get disturbed by the keepers or leave the burrow to locate a potential threat and protect their nest, as *L.fallax* previously has been observed attacking humans to defend their foam nests (Gibson & Buley, 2004).

The females left more often when other frogs had been calling, which supports the H_B-hypothesis. Although, it is important to note that correlation doesn't necessarily equal causation in this case and it could be an effect of the keepers again. If other frogs are calling more because they get disturbed by keepers or because they know the keepers bring food, then that might be the reason the females left their nests. However, even if no analysis was made, there doesn't seem to be a larger amount of sound during the times keepers were in the facility compared to when they were not. The analysis didn't take into consideration the amount of sound nor how close after the sound the females left. It's entirely possible that the results might have been different if the sound was observed during a different time interval.

There were no trends that showed that the time a female spent away from her foam nest had any correlation with the amount of sounds from nearby *L.fallax,* either before she left the nest or while she was away from it. Therefore, no evidence could be found that supported the H_C-hypothesis, although there also weren't any evidence disproving it. The lack of correlation might be because of the extremely small sample size and the experiment would do well to be repeated with more females studied over a longer time period. It's also important to note that the different sounds the frogs made weren't taken into consideration due to the observers lack of knowledge about how to differentiate the sounds. It would have been interesting to see how the results differed if the different sounds were measured and tested separately. Likewise, it would have been beneficial to have an additional camera in the enclosure to detect where the female went when she left and what she did. It would've also helped the sound analysis to be able to track the frogs in the enclosure and identify if the sound heard came from the female herself or the male who sired the nest, and not from another nearby frog. As it was now, the analysis could have counted the females own calls while she was away from the nest, or the calls of the male how sired the nest. By making separate analyses with only the males' calls, a better understanding of the co-parental care of the tadpoles could be gained. Since *L.fallax* is a species where the males have been observed to guard the nest along with the females (Gibson & Buley, 2004; Jameson et al., 2019), it's possible that the female wouldn't consider the males' calls as a threat and therefore not be disturbed by them. However, calls from the male might also be what alerts the female of a potential threat, causing her to leave the burrow.

Something that further supports the hypotheses about sounds from other frogs being a disturbance to the females are the circumstances under which the females left their nests multiple times a day. While they usually only left once a day, sometimes staying in the burrow for several days in a row before leaving, on 3 occasions females were observed leaving multiple times in one day. One of the occasions, the female was only gone 4 minutes and it is very plausible that she simply was in the tunnel and didn't really leave the burrow. The other occasions, most of their time away during the first leaving had observed calls from nearby frogs (>80%). Two leavings happened closer to each other than 2 hours, and during both of them sound from other frogs could be heard almost constantly (>90%). Even if the study includes too few observations to draw any conclusions, the fact that multiple leavings only happened after a considerable amount of sound would indicate that the sound do affect the females.

Not a single foam nest that didn't produce tadpoles lasted longer than approximately a month, and most lasted far less than that. It looks like nests that survive longer than a month have a far better chance at producing surviving froglets. What's worth noting is that it's around this time, when they are approximately a month old, that the tadpoles start to go through metamorphosis and get their hindlegs (Davis et al., 2000; Gibson & Buley, 2004). The start of their metamorphosis could mean that they need less care from the female and isn't as dependent on her staying close to the nest as they are earlier in the development. Therefore, they would be less effected if the female leaves due to disturbances. However, since the female still feed the nest as often during this stage as during earlier stages (Gibson & Buley, 2004) it might be more likely that the tadpoles survive not due to them needing the female less, but due to the female leaving less at this stage. It is believed that the female is stimulated to care for the nest by some kind of cues from the tadpoles (Gibson & Buley, 2004; Jameson et al., 2019), which might also be how the female recognise her own tadpoles from others (Jameson et al., 2019), and this cue might be easier for the female to detect when the tadpoles are at this later stage in their development.

The cue might be tactile (Jameson et al., 2019) and would require the female to have direct contact with the nest, as she does when she covers it before disposing eggs and foam into it (Gibson & Buley, 2004). Behaviours when the females covered or touched the foam nest were indeed observed during this study, in the forms of "head to nest", "cover nest" and "guard nest". All 3 of these behaviours were mostly observed when sound from other frogs were present while the female returned to the burrow, although the difference was not significant (possibly due to small sample size).

Multiple observations of females calling to the foam nest have been made during this study, which would indicate some form of acoustic cue. Acoustic signalling from tadpoles has been noted in a number of different frog species, such as *Ceratophrys cranwelli* (Salgado Costa et al., 2014), *Ceratophrys ornate* (Natale et al., 2011), *Gephyromantis azzurrae* (Reeve et al., 2011) and *Leptodactylus ocellatus* (Vaz-Ferreira & Gehrau, 1975, as cited in Reeve et al., 2011), but has yet to be confirmed in *Leptodactylus fallax*. Despite the lack of research on the subject, it is entirely possible that *L.fallax* tadpoles use acoustic signalling. The females were often observed calling to the foam nest during this study, both before leaving the nest, but mostly after returning to it. The nest calling happened mostly during silence ($p < 0.1$, assuming that all the observed potential calls were actual calls) with extremely low calls from the females and a possibility of even lower sounds from the tadpoles (although this needs to be confirmed in further studies, preferably with better sound equipment). Sound from other *L.fallax* might disturb this potential communication between female and tadpoles. It could prevent one or both of the parts to hear the other properly, which might have a negative effect on tadpole survival if the female indeed uses acoustic stimuli to determine the tadpoles' number and health. It has been observed that females can control the amount of eggs they feed to the tadpoles, depending on their number and age (Gibson & Buley, 2004), and if she due to disturbance from other frogs is unable to correctly determine the number of tadpoles, too few eggs might be dispersed to the foam nest. This could lead to death of tadpoles due to starvation.

The sound from other frogs was also observed to affect other behaviours than calling (though mostly non-significant changes, possibly due to small sample size) that might still affect the communication between female and tadpoles. If sound was present before they left, females sat closer to the nests but was always turned away from them. This could be seen as a guarding behaviour, where the female wanted to be close to and protect the nest but had all her attention out toward the calls. This behaviour, no matter what causes it, prevents the female from communicate with the nest. This might be the reason females could only be observed calling to the nest before leaving during silence. The females also moved around a lot more in the burrow if sound was present when they returned, which might be a sign of stress. As mentioned before, they also got closer to the foam nest with more behaviours like "guard nest", "cover nest" and "head to nest". These might be guarding behaviours or a way for the female to be able to hear or feel the tadpoles even with the background sound.

It might very well be the case that both acoustic and tactile stimuli from the tadpoles can alert the female of their health and numbers. Acoustic communication seems to be used mostly when it's silent around the burrow, while tactile stimuli could be used when it's too noisy for the female to be able to hear the tadpoles. Although, more studies on female-tadpole interaction, especially during different development stages, needs to be made to test this hypothesis.

In conclusion, minimizing the disturbance from keepers could be a way to increase the reproductive success in captive *Leptodactylus fallax*. Females with foam nests are affected by the calls from other nearby individuals, but it is still unknown exactly how that effects the reproduction, and more studies on the females' behaviours needs to be done to determine this.

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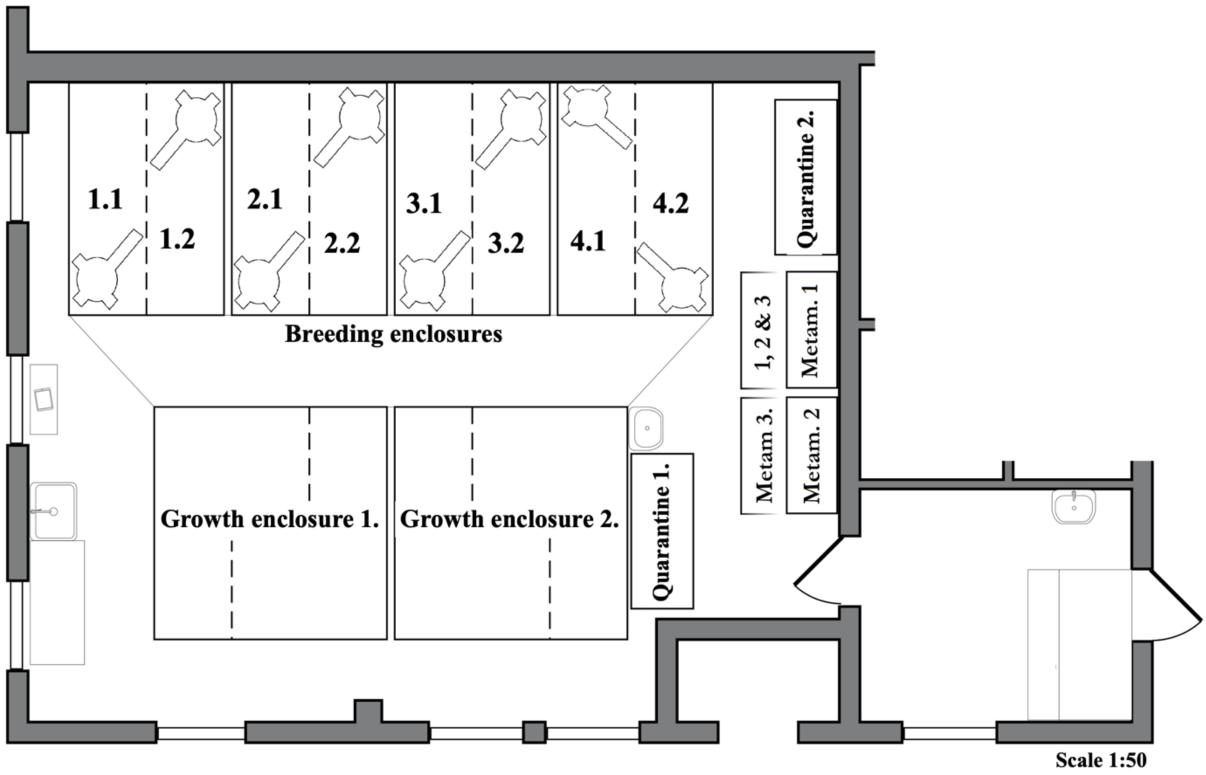
Appendix 1

The breeding facility at Nordens Ark.



Appendix 2

The foam nests used in this study, from Nordens Ark's breeding facility during 2019 and 2020. In the column "End date", dates of category 1 are black, dates of category 2 are orange and dates of category 3 are green. When the end date is of category 3, the column "Alt. end date" also have a date of category 4. The length is number of days a foam nest was present.

Year	Number	Enclosure	Start date	End date	Alt. end date	Length (days)	Froglets
2019	1	1	2019-05-17	2019-05-28	x	11	0
2019	2	1	2019-07-08	2019-07-15	x	7	0
2019	3	1	2019-08-05	2019-08-15	x	10	0
2019	4	4	2019-09-17	2019-09-24	2019-09-24	7	0
2019	5	4	2019-09-25	2019-10-02	x	7	0
2019	6	2	2019-10-04	2019-10-11	x	7	0
2019	7	4	2019-11-04	2019-11-15	x	11	0
2019	8	3	2019-11-10	2019-12-10	x	30	0
2019	9	2	2019-11-10	2019-12-06	x	26	0
2019	10	2	2019-11-11	2019-12-11	x	30	0
2019	11	4	2019-12-08	2019-12-26	x	18	0
2020	12	4	2020-03-10	2020-03-24	x	14	0
2020	13	2	2020-03-19	2020-04-12	2020-04-12	24	0
2020	14	3	2020-03-30	2020-04-24	x	25	0
2020	15	2	2020-04-13	2020-05-08	2020-05-08	25	0
2020	16	4	2020-04-25	2020-06-17	x	53	1
2020	17	1	2020-04-28	2020-06-01	x	34	0
2020	18	2	2020-05-09	2020-06-01	x	23	0
2020	19	3	2020-05-10	2020-06-01	x	22	0
2020	20	2	2020-06-02	2020-06-11	x	9	0
2020	21	2	2020-06-11	2020-06-27	x	16	0
2020	22	1	2020-06-17	2020-06-19	2020-06-24	7	0
2020	23	4	2020-06-22	2020-08-16	x	55	4
2020	24	1	2020-06-25	2020-07-15	2020-07-15	20	0
2020	25	2	2020-06-30	2020-07-14	x	14	0
2020	26	2	2020-07-14	2020-07-28	x	14	0
2020	27	3	2020-07-28	2020-08-10	x	13	0
2020	28	2	2020-07-30	2020-08-10	2020-08-19	20	0
2020	31	2	2020-08-20	2020-08-21	2020-08-30	10	0
2020	33	2	2020-09-03	2020-09-12	2020-09-15	12	0
2020	35	4	2020-09-05	2020-09-21	x	16	0
2020	36	2	2020-09-16	2020-09-23	2020-10-03	17	0
2020	37	4	2020-09-21	2020-09-30	x	9	0
2020	38	2	2020-09-28	2020-10-23	x	25	0
2020	39	4	2020-09-30	2020-11-26	x	57	10
2020	40	3	2020-10-01	2020-10-23	x	22	0
2020	41	1	2020-10-04	2020-10-23	2020-10-25	21	0

2020	42	2	2020-10-07	2020-10-31	x	24	0
2020	43	3	2020-10-24	2020-11-02	2020-11-07	14	0
2020	45	2	2020-10-31	2020-11-18	2020-11-22	22	0
2020	50	1	2020-12-03	2020-12-14	2020-12-20	17	0
2020	51	2	2020-12-06	2020-12-14	2020-12-16	10	0