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First mating report of tayra (*Eira barbara*) in the wild, in Panamá

Primer reporte de apareamiento de tayra (*Eira barbara*) en medio silvestre en Panamá

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There is little information about the mating process of *Eira barbara* (tayra or black cat, as it is known in Panamá), reported only in captivity, by Poglayen-Neuwall (1975). Two camera traps were placed, 220 m apart, within the Altos de Campana National Park, in the border area with the community of El Chileno. The sounds emitted by individuals during the event were analyzed using the PREMIERE PRO-6.0.0 and Raven Pro1.5 software. We describe stages of this process evidenced in a sequence of 8 videos of 10 seconds each, captured by one of our camera traps; we consider the event as a single mount. Both individuals make sounds during the event, which we interpret as frequent clicks 3.19 KHz dominant and 3.23 KHz dominant frequency trills. We compared this event with the one reported in captivity by Poglayen-Neuwall (1975). Both in captivity and in our report, the nuchal grasp and the occasional dragging of the female by the male are observed. We also carried out the first bioacoustics analysis of the mating process of this species.

Key words: Altos de Campana National Park; bioacoustics; camera trap; clicking calls; mating; trills.

Existe poca información acerca del proceso de apareamiento de *Eira barbara* (tayra o gato cutarro, como se le conoce en Panamá), reportado únicamente en cautiverio, por Poglayen-Neuwall (1975). Se colocaron dos cámaras trampa, separadas 220 m, dentro del Parque Nacional Altos de Campana, en la zona limítrofe con la comunidad de El Chileno. Se analizaron los sonidos emitidos por los individuos durante el evento mediante los programas PREMIERE PRO-6.0.0 y Raven Pro1.5. Describimos etapas de este proceso evidenciado en una secuencia de 8 videos de 10 segundos cada uno, captado por una de nuestras cámaras trampa; consideramos el evento como una sola monta. Ambos individuos hacen sonidos durante el evento, que interpretamos como clics con frecuencia dominante de 3.19 KHz y trinos frecuencia dominante de 3.23 KHz. Comparamos este evento con el reportado en cautiverio por Poglayen-Neuwall (1975). Tanto en cautiverio como en nuestro reporte se observa el agarre nucal y el arrastre ocasional de la hembra por parte del macho. También realizamos el primer análisis bioacústico del proceso de apareamiento de esta especie.

Palabras clave: Apareamiento; bioacústica; cámara trampa; llamados en forma de clics; Parque Nacional Altos de Campana; trinos.

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Mammals are an essential part of the ecosystems in which they inhabit since they play critical roles in these environments (Wright 2003; Morrison *et al.* 2007); whether in their role as top predators (Sunquist and Sunquist 2002), plant consumers (Fragoso 1999), seed predators (Galetti *et al.* 2015), or seed dispersers (O'Farrill *et al.* 2013), they exert a profound influence on their surroundings that goes beyond species interactions (Morrison *et al.* 2007). Altered or disturbed ecosystems often lack the large and medium-sized mammals' populations that historically inhabited them (Morrison *et al.* 2007); their absence, in turn, could trigger negative cascading effects in the food web, as many of these mammals serve as keystone species in their environment (Bond 1994; Wright 2003). Most of these species are elusive and their natural history is poorly known, so it is imperative to use tools that allow us to learn more about their life traits, population status and function within the ecological communities of the environment (Grotta-Nieto *et al.* 2020).

Camera traps have been one of the most novel and reliable methods for monitoring terrestrial mammal populations (Karanth *et al.* 2004), its main advantages are: the low stress generated to the individuals photographed (McCleery *et al.* 2014; Hobbs and Brehme 2017) and that they enable monitoring elusive species or ones that occur in low densities (Moruzzi *et al.* 2002), such as the neotropical mustelids (Yensen and Tafira 2003; Hernández-Sánchez *et al.* 2017; De Roux *et al.* 2019). Although it is argued that its cost is high, it is more cost-effective in time and money when compared to the intensive field days required to obtain the same observational level (Cutler and Swan 1999; Moruzzi *et al.* 2002); this is in addition to the recent emergence of increasingly cheaper reliable technologies (Rico-Guevara and Mickley 2017; Droissart *et al.* 2021). This method allows important variables to be obtained, such as: relative abundance (Maffei *et al.* 2004), activity patterns (Ávila-Nájera *et al.* 2016), density (Karanth and Nich-

ols 1998; Silver *et al.* 2004; Sollmann *et al.* 2011), diversity (Aranda *et al.* 2012), and animal behavior (Monteza-Moreno *et al.* 2020).

In Panamá, this method has been applied in different ecosystems: tropical rainforest (Moreno and Bustamante 2009); very humid premontane forest (Loria and Méndez-Carvajal 2017); premontane tropical rainforest (González-Hernández *et al.* 2020); dry forest (Méndez-Carvajal 2013); premontane forest and cloud forest (Méndez-Carvajal 2012) to obtain information regarding population status of mammalian umbrella species such as: felids (Moreno and Bustamante 2009), canids (Hody *et al.* 2019), ungulates (Moreno and Meyer 2014; Meyer *et al.* 2016) and to assess the status of large and medium-sized terrestrial mammal communities (Meyer *et al.* 2015). Through the use of camera traps, records have been obtained that have allowed inferring the reproductive behavior of some species (Aranda *et al.* 2012; Mandujano 2019), this has been implemented in other groups such as birds, by placing cameras at nests or nest entrances (Hudson and Bird 2006).

In mammals, reproductive behavior has been inferred through indirect observations, such as the presence of pups (Aranda *et al.* 2012; Ouboter and Kadosoe 2016; Appleton *et al.* 2018; Jansen *et al.* 2020) or pairs of individuals in solitary species (Morrow and Nicole 2009; Ouboter and Kadosoe 2016; Appleton *et al.* 2018). Whereas, direct observations of reproductive behavior in the wild are rare and anecdotal (Faller-Menéndez *et al.* 2007).

Reproductive studies on tayras (*Eira barbara*; or "gato cutarro" as it is known in Panamá) have been conducted in captivity, focusing on copulatory behavior, always highlighting their tendency to vocalize during the act (Poglayen-Neuwall 1975), gestation and care of the cubs (Poglayen-Neuwall 1978; Presley 2000), and analysis of the estrous cycles of the females (Poglayen-Neuwall *et al.* 1989). There are no reports of tayras reproducing in the wild, the closest being the suggestion of reproductive behavior in the Barro Colorado Biological Station, Panamá (Kaufman and Kaufman 1965), but this does not show conclusive evidence of the act and thus remains speculation. The literature (Poglayen-Neuwall 1975, 1978; Poglayen-Neuwall and Poglayen-Neuwall 1976) establishes that *E. barbara* is a diurnal polyestrous species; males are reproductive throughout the year and reach sexual maturity at 18 months of age; females reach metabolic and reproductive maturity by 22 months of age, although adult size for the species is reached in only 6 months (Presley 2000). According to Poglayen-Neuwall *et al.* (1989), females older than 8 years have longer estrous cycles (72-163 days) compared to younger females (38-75 days). Gestation may last a little more than 2 months, and they usually have 1 to 3 offspring that remain with the mother for 200 to 300 days (Poglayen-Neuwall and Poglayen-Neuwall 1976).

The information generated in captivity can be influenced by multiple factors, mainly by captivity *per se* (without omitting changes in behavior as a result of breeding animals together, which are solitary in the wild), which can provide data and observations that do not correspond to behavior in the wild (*e.g.*, Poglayen-Neuwall 1978). Therefore, gathering information from the natural environment that complements what is observed in captivity is one of the best tools for understanding the reproductive behavior of species.

The objective of this paper is to present data on the *in situ* reproduction of a pair of *E. barbara* in Altos de Campana National Park. The event and its details are described: duration of each mating, movements and vocalizations emitted by each individual.

The study area is located in the northwest end of Altos de Campana National Park, with a humid tropical climate and average annual rainfall of more than 2,500 mm. In addition, it is important to note the different forms of relief from valleys, rugged hills and steep slopes, ranging from 300 m to high points such as Cerro Campana at 1,030 m and Cerro La Cruz at 905 m, where there is a temperature oscillation between 15 to 22 °C (Ministerio de Ambiente 2021), limited to the northeast and northwest by farms with fragmented ecosystem and used for anthropogenic activities belonging to the community of El Chileno.

The cameras were installed due to interactions between cattle and jaguar reported by the inhabitants of El Chileno. Installation sites were selected using two criteria: 1) in places with defined trails (felines have been reported to use them with great frequency to patrol their action areas; Chávez *et al.* 2013), and 2) in places where there are no trails (to avoid decreasing the probability of capture of other species such as ungulates and small mammals that do not frequent trails as much; R. Moreno, pers. comm. 2019).

Two camera traps (Ancheer model RD 1006) were installed 220 m apart. Each camera (C01PNAC: 8° 42′ 21.48″ N, 80° 0′ 2.61″ W; and C02PNAC: 8° 42′ 28.97″ N, 80° 0′ 2.33″ W; Figure 1) was placed in a tree 50 cm above the ground, to obtain records of small, medium and large mammals (Noss *et al.* 2013), the undergrowth was removed with a machete just in front of the cameras, to clear the view, in an area of 15 m^2, no olfactory attractants were used. The equipment was installed on March 25, 2018 and was removed on June 15 of the same year.

Sampling effort was quantified by the number of hours (24 each full day or its respective fraction if not completed) multiplied by the number of days that each camera trap was active. For the total, both quantities were added together.

Bioacoustic analysis. The PREMIERE PRO-6.0.0.0 software (Adobe CS6; https://www.adobe.com/es/; Vbroadcast Limited 2021) was used to extract the audio from each video, followed by the Raven Pro1.5 software (Center for Conservation Bioacustic 2014). Determinant aspects for this type of analysis, mentioned below, were analyzed with frequency graphs.

For each vocalization, we analyzed the duration, interval, maximum, minimum and dominant frequencies. Spectral

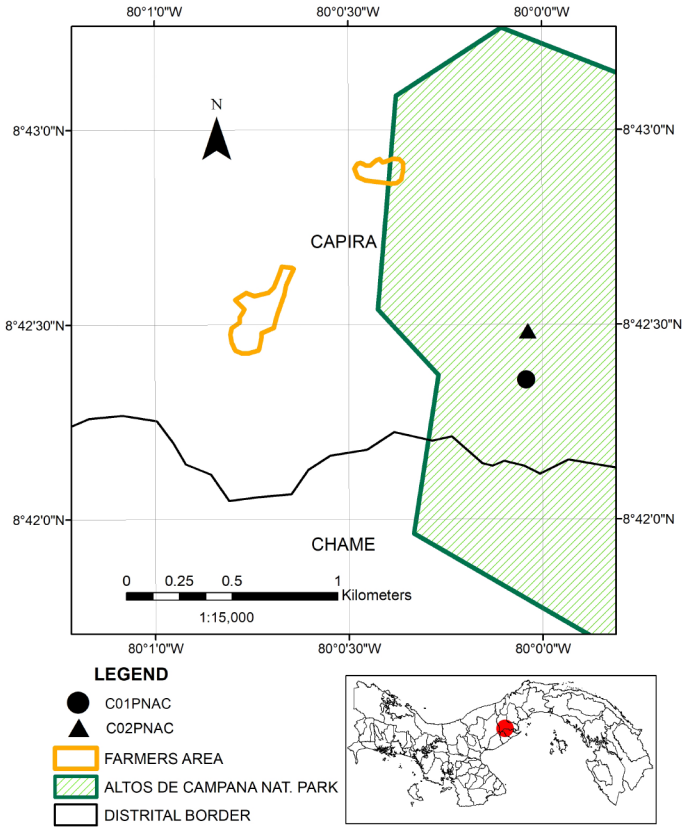

Figure 1. Camera traps location map: shows the northwest limits of the Altos de Campana National Park (green line to the right), the location of the camera traps, C01PNAC (circle) and C02PNAC (triangle) and the producer farms community of El Chileno, where the inhabitants reported incidents with jaguars (orange polygons).

and temporal parameters (frequency) were analyzed, and power spectra were calculated in Raven Pro1.5 (Window: Blackman, Discrete Fourier transform DFT): 2,048 samples, 3 dB filter bandwidth: 0.16 KHz; grid spacing 0.021 KHz; overlap 70.1 % (Charif *et al.* 2004).

Both cameras were active for 82 days 15 hours each, resulting in 3,942 effective camera hours of sampling. On June 15, 2018, at 8:34 am in the C01PNAC camera was recorded the first of 8 videos that captured the act of copulation between a pair of tayras (*E. barbara*), each video has a duration of 10 seconds. The sun rose at 5:59 am and the temperature recorded showed a variation of 1 °C (from 21 to 22 °C) in the 43 minutes that the event lasted (Table 1; Figure 2).

Acoustic properties of the reproductive event. During the reproductive act both individuals emitted tonal rhythmic sounds in the form of short clicking calls (clicking calls; Poglayen-Neuwall 1975) and trills (short, rhythmic series of noisy sounds; Poglayen-Neuwall 1975; Peters 1984). The female emitted a click before the trill at a dominant frequency of 3.19 KHz (fundamental frequency 2.44 KHz) with a duration of 0.06 s, immediately followed by a guttural trill at a dominant frequency of 3.23 KHz (fundamental frequency of 2.34 KHz) and a duration of 0.54 s; a second series of clicks and trills were recorded at dominant frequencies of 3.19 KHz (fundamental frequency of 2.39 KHz) and 2.44 KHz (fundamental frequency of 1.73 KHz), respectively. The interval between this series of clicks and trills was 3.1 s and 1.2 s between two measured cycles, and in one of the cases the click was separated from the trill by 0.57 s. The male emitted a trill at a dominant frequency of 2.79 KHz with a duration of 0.48 s, alternating between female calls (Figure 3).

The individuals present uniform coloration patterns (without spots) in the fur, on the head a dark brown hood up to the neck and the rest of the body black, only in the female, and only in the second video, it can be appreciated the typical throat patch used to identify individuals of *E. barbara* (Villafañe-Trujillo *et al.* 2018). The recorded event lasted 43 minutes; however, at the beginning of the first video the male was already mounting the female, so the exact duration of coitus is unknown, nor was courtship behavior recorded prior to copulation, it is possible that it occurred near the photo-trapping station but outside the camera's range. The whole event is considered as a single mount, since the male does not release the female from the first capture, it is possible that, at some point, while they are getting adjusted, the penetration was interrupted; however, it is a detail that is not perceptible in the videos. The number of mounts in captivity is variable with up to 13 mounts per day (Poglayen-Neuwall 1975) and copulation both in captivity and in the wild occurs crouching, standing or lying on its side. It should be noted that in captivity the presence of lordosis on the female's back was not observed or mentioned (Poglayen-Neuwall 1975; Alves and Borstlemann 2010), a behavior that is visible in the videos filmed.

Breeding events reported in captivity (Poglayen-Neuwall 1975) record average durations between 22 to 45 minutes, sometimes events where the male was unsuccessful (no penetration), a specific case of 45 minutes where the male mounted the female 10 times during this period, and a case of long duration copulation (86 minutes) where the male lost intravaginal contact once (Poglayen-Neuwall 1975), so the duration of our event is within the range reported by Poglayen-Neuwall (1975) in captivity.

The sexual act occurs in a similar way in both scenarios (captivity and wild); however, in the wild this type of events are recorded by chance, being the one reported in this work the first with videographic evidence captured by camera trap, which allowed us to perform the first analysis of bioacoustics of the species during the sexual act in the wild. The video can be found on the youtube channel of Fundación Los Naturalistas (https://youtu.be/v_kXBJEyMkc).

The event occurred during the month of June; captive reports (Poglayen-Neuwall 1975; Alves and Borstlemann 2010) suggest that the activity is possible throughout the year. Both in captivity and in our report, nuchal grasping and occasional dragging of the female by the male is observed. One aspect that was not possible to observe is whether the female maintained any rejection behavior towards the male during courtship, such as has been shown in captivity (Poglayen-Neuwall 1975; Alves and Borstlemann 2010) and which consists of agonistic vocalizations against the male.

Table 1. Description of the mating event of *Eira barbara* in chronological order.

Video/Hour	Description	Figure 2
1) 8:34 am	The male bites the nape of the female while riding her, arching his body to press his pelvic area against the female, keeping his forelimbs in the middle of the female's body, a process known as copulation (Granda-Serrano and Romo-Graniel 2004), the female remains in a lordosis position (Granda-Serrano and Romo-Graniel 2004), after this she gets up with the male on top and moves some steps, a process known as walk (Granda-Serrano and Romo-Graniel 2004), they fall on their left sides and the male continues with the copulation. During this first video the couple emits tenuous vocalizations (Granda-Serrano and Romo-Graniel 2004).	A, B, C
2) 8:39 am	They change position and face the camera, this time the male continues with the mount. However, he tries to accommodate himself and holds the female in the groin area and continues with the copulation, continuing to bite the nape of the female, which partially maintains the lordosis position and moves with difficulty at the time that the male tries to accommodate. The vocalization produced by both increases, but is more noticeable in the male.	A
3) 8:44 am	The couple keeps copulating lying on their left side, the male performs very fast pelvic movements (his back seems to tremble) for six seconds, then he lowers the intensity or frequency of the movement and they resume the initial riding position, finally both are placed in vertical position but the female with the body totally crouched. In this video no one vocalizes.	A, C
4) 8:50 am	Both are observed in the same position and activity as at 8:34 am. However, after three seconds the female shows a remarkable resistance to the male making a 180 degree turn from left to right; however, the mating is not interrupted, the male for his part makes pressure and after six seconds he manages to knock her down on his left side and copulation continues, during this process the male places his leg on the female's thigh and knee and maintains a strong pelvic hug (groin area), on this occasion we can clearly appreciate the male genitalia and penetration. The male vocalizes with greater intensity than the female who only emits a low growl.	A, B, C, D
5) 8:55 am	They rise from their right side where copulation is appreciated, they remain completely crouched and the male generates pressure on the female for eight seconds, then they take a few steps and fall on their left side. Only the female vocalizes very faint.	A, C
6) 9:00 am	They maintain the mount, the male accommodates himself twice, lifting and bending his back with that of the female, each time he bends down again the copulation is appreciated, generating pressure on the female, and finally forcing her to fall on her left side. The hug is pelvic and vocalizes a single individual, a single note apparently.	B, C
7) 9:05 am	The mount is kept with its back to the camera for three seconds, the male makes pelvic pressure on the female once and manages to lay her down on her left side and continues the copulation until the end of the video. They do not vocalize.	D
8) 9:11 am	Initially they are observed crouched in their entirety after two seconds both get up and settle down until they fall on their left side and the male, after the strong hug with the forelimbs, begins again. Both individuals vocalize, producing a quite loud sound.	A, C
9) 9:17 am	The male is seen leaving the place, in the opposite direction to the camera, the female had already left the scene.	-

Although, resistance from the female towards the male was observed and consisted of attempts to break free from the pelvic grasp, in captivity this behavior was accompanied by the release of musk from the female's anal glands (Poglayen-Neuwall 1975), the latter could not be confirmed due to the method employed for the observations (photo-trapping).

On seven occasions, the pair was observed lying on their left sides instead of the right side (once) to perform the behavior described as copulation (Granda-Serrano and Romo-Graniel 2004), where the male performs rapid pelvic movements, the male contracted his thigh muscles so fast that it gave the visual impression of vibration, a procedure that lasts between 5 and 8 seconds per repetition and can be seen in all the videos. This behavior was also observed in captivity, although only once (Poglayen-Neuwall 1975), in neither case was it an obstacle to maintaining copulation.

The female showed resistance to the male on three occasions; however, the fact of biting the nape of the female's neck during the whole event allows the male to face this resistance and to have control to some extent during the act. In the fourth video (8:50 am) we can see the greater resistance of the female; however, the male takes advantage of the above mentioned and manages to lie down next to his partner to continue with the copulation.

We note that the male tends to accommodate when they are partially crouched vertically just before lying on their sides and this is done by bending his pelvic area to the maximum and pressing it against the female forcefully, while hugging her at groin level with his forelimbs also with force, it is visually noticeable changes in the force exerted by the male against the female during the different stages of the event, the latter being where the use of greater force by the male is easily appreciated. The mating pattern and sequence reported in this work is very similar to that reported in captivity in terms of male nuchal grasping and dragging the female to accommodate him, while the female vocalizes and tries to resist, until she is fully receptive and remains silent (Poglayen-Neuwall 1975, 1978). Female vocalizations in captivity seem to occur before and at the end of copulation (Poglayen-Neuwall 1975), while the male vocalizes throughout the act. Whether in captivity (Poglayen-Neuwall 1975) or in the wild, both specimens tend to increase the intensity of the sounds at the end of the act.

In contrast to the above, cases have been reported in which the female takes the initiative by mounting the male without biting the nape of his neck and pushing his flanks

Figure 2. Photographic evidence extracted from the videos: the selected images represent *Eira barbara*'s repetitive behaviors observed in the videos from 8:34 am (first video) to 9:11 am (eighth video). A) The male being on top of the female bites her neck ("Monta"), in a pelvic hug he puts pressure on the female ("Copula"). B) Both the male and the female acquire a position of lordosis, displacement, only a few steps, of the female with the male on top. C) Both individuals lying on their left sides, continue to mount and copulate. D) The testicles of the male are appreciated at the moment that he exerts pressure on the female (copulation) during the mating.

with rhythmic movements of her waist, in addition to excessive preening by both prior to the act (Poglayen-Neuwall 1978). Although this difference could be due to the fact that the specimens were in captivity.

Tayras are diurnal mustelids (Delgado-V. *et al.* 2011; González-Maya *et al.* 2015; Braga *et al.* 2020; Villafañe-Trujillo *et al.* 2021) and this breeding event was recorded during the day contradicting the "nocturnal mating" data suggested by Kaufman and Kaufman (1965) and reinforcing that both captivity and breeding solitary animals together can generate behaviors that are not an accurate reflection of what occurs in free-living individuals.

Most studies on mustelid reproduction have been conducted in captivity in zoos and on northern hemisphere species; for example: *Neovison vison*, *Mustela lutreola*, *Martes martes*, *Mustela sibirica* (Amstislavsky and Ternovskaya

2000). As a group they possess a wide variety of reproductive strategies from which tayras differ in the absence of seasonality (Amstislavsky and Ternovskaya 2000; Presley 2000). Therefore, studies of this type (in the tropics and in the wild) become even more relevant, considering the rapid loss of habitat and diversity in the tropics (Meyer *et al.* 2020).

Field observations (Barro Colorado Island, Panamá) suggest the existence of competitive agonistic behavior between males to copulate with the female (Kaufmann and Kaufmann 1965). Although our records did not capture pre-copulation activity, there is a possibility that the males have to chase the female in the wild until she is subdued before proceeding with the reproductive act.

Little is known about the acoustic behavior of *E. barbara* (Mumm and Knörnschild 2018), although vocalizations of a series of noisy clicks known as clicking calls have been reported (Poglayen-Neuwall 1976). There are data on the vocalizations of neonates or juveniles, in which a series of vocalizations such as squeaks, clicks, grunts, or snorts are reported depending on the state or circumstances (Poglayen-Neuwall 1978); however, during the reproductive act, we report a sequence of vocalizations never recorded before, the trills. The trills emitted during mating and copulation may more closely resemble trill songs reported for other mustelids, although the trill reported in this study appears to be faster with fewer spaces between notes than those reported for another mustelid species in another context, a free-roaming female *Mustela erminea* with her young (see Figure 3b in Peters 1984).

During mating and copulation both individuals produce short clicks and trills, the latter are emitted mainly by the male during mating, at the moment when he presses against the female; during the copulation stage when they are lying on their sides neither of the individuals produces vocalization, when the couple settles down, they produce clicks and trills with a lower intensity (volume) compared to the moment when the male presses against the female (copulation).

The recording of the vocalization of *E. barbara* during mating is the first reported for the species, as well as the recording of video in the wild through camera traps, providing important data on the reproductive biology of this species.

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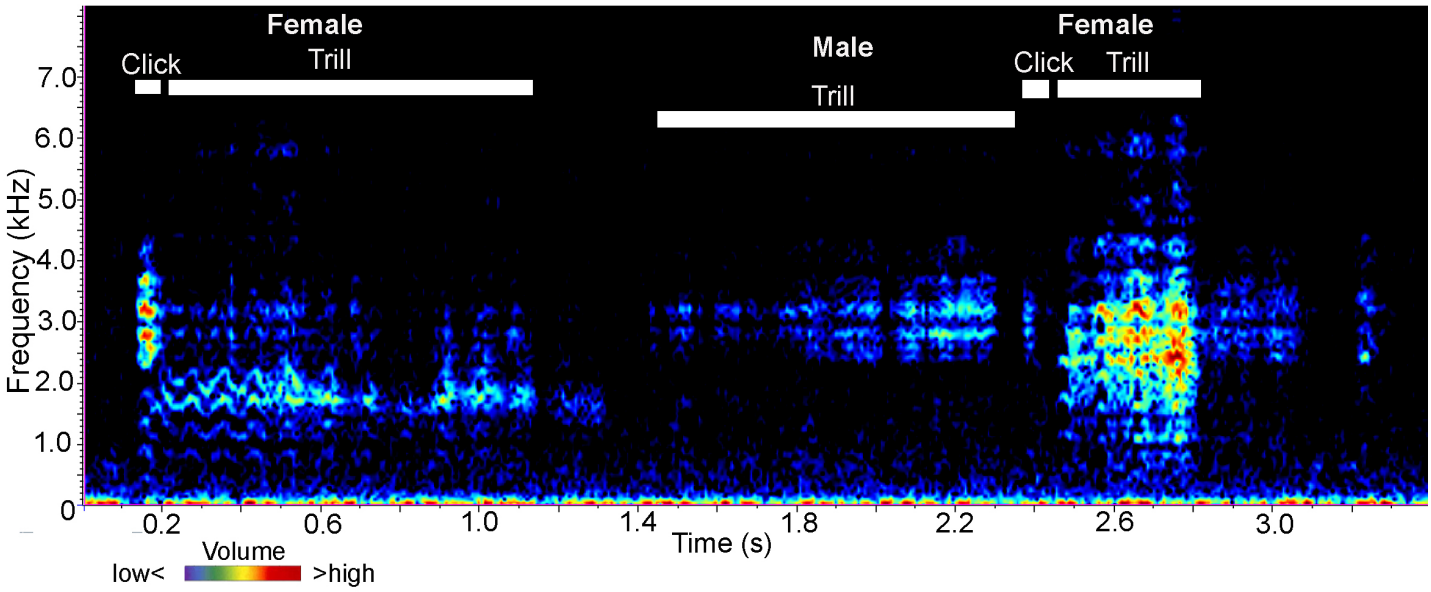

Figure 3. Sonogram of the vocalization of *Eira barbara* individuals during reproductive activity. Male and female calls obtained from the camera trap video footage are shown.

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