Discriminating predation attempt outcomes during natural foraging using the post-buzz pause in
Japanese large-footed bat *Myotis macrodactylus*

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Running title:

Post-buzz pause as a proxy for predation success

Key words:

Echolocation, terminal buzz, acoustic indices, trawling bats, prey selection

Summary statement (15-30 words)

We investigated the natural foraging behavior of wild *Myotis macrodactylus* and found that the
length of the post-buzz pause is a useful acoustic indicator of predation success.

26 **Abstract**

27 Bats emit a series of echolocation calls with an increasing repetition rate (the terminal
 28 buzz), when attempting to capture prey. This is often used as an acoustic indicator of prey-capture
 29 attempts. However, because it is directly linked to foraging efficiency, predation success is a more
 30 useful measure than predation attempts in ecological research. The characteristics of echolocation
 31 calls that consistently signify predation success across different situations have not been identified.
 32 Due to additional influencing factors, identification of these characteristics is particularly
 33 challenging for wild bats foraging in their natural environment compared to those in flight chambers.
 34 This study documented the natural foraging behavior of wild Japanese large-footed bat *Myotis*
 35 *macrodactylus* using synchronized acoustic and video recordings. From the video recordings, we
 36 could assign 137 attacks to three outcome categories: prey captured (51.8%), prey dropped (29.2%),
 37 and failed attempt (19%). Based on previous indications from laboratory studies that the length of
 38 the silent interval following the terminal buzz (post-buzz pause) might reflect the prey capture
 39 outcome, we compared post-buzz pause durations among categories of attack outcomes. The post-
 40 buzz pause was longest in the case of successful capture, suggesting that the length of the post-buzz
 41 pause is a useful acoustic indicator of predation success during natural foraging in *M. macrodactylus*.
 42 Our finding will advance the study of bat foraging behavior using acoustic data, including
 43 estimations of foraging efficiency and analyses of feeding habitat quality.

44

45

46 **Introduction**

47 Echolocating bats obtain information about the outside world by emitting sonar signals and
 48 listening to their echoes. This information helps them to find and capture prey, avoid obstacles and
 49 navigate (Fenton, 1990; Simmons et al., 1979). During foraging, insectivorous bats emit a series of
 50 echolocation calls that vary in pulse length, frequency structure, and repetition rates, and can
 51 therefore be grouped into three phases: the search, approach, and terminal phases (Griffin et al.,
 52 1960; Kalko and Schnitzler, 1993; Schnitzler and Kalko, 2001; Simmons et al., 1979). The "terminal
 53 buzz" is a rapid increase in the repetition rate that occurs just before a capture (Fig. 1A, Fujioka et al.,
 54 2014; Schumm et al., 1991). This unique acoustic feature reflects a "fast decision response" (Geberl
 55 et al., 2015), and has been used as an important acoustical indicator of capture attempts by bats
 56 during foraging (Britton and Jones, 1999; Griffin, 1958; Jakobsen and Surlykke, 2010; Racey and
 57 Swift, 1985).

58 Bats do not always succeed in capturing prey. Visual observations, photographs, and video
 59 recordings of foraging bats have shown that they sometimes drop prey or fail to capture it (Acharya
 60 and Fenton, 1992; Britton and Jones, 1999; Kalko, 1995; Schnitzler et al., 1994). For instance,
 61 *Eptesicus nilssonii*, an aerial-hawking bat, was found to successfully capture moths during natural
 62 foraging in only 35–36% of attempts (Rydell, 1992; Rydell, 1998). The capture success of the
 63 pipistrelle bat varies according to prey size, i.e., 60–70% of small-sized insects and 30–40% of
 64 large-sized insects, such as moths, were captured successfully (Kalko, 1995). Photographs of *Myotis*
 65 *daubentonii* foraging above the water surface have shown that the bats sometimes miss their prey or
 66 mistake it for other objects, such as floating leaves (Kalko and Schnitzler, 1989). Therefore, acoustic
 67 measures of predation success are desirable. These could be used to estimate the foraging status of
 68 bats from their emitted echolocation sounds, enabling measurement of temporal changes in energy

69 intake and bat foraging efficiency. The use of acoustic parameters to determine predation outcome
70 would greatly contribute to our understanding of natural foraging behavior, with bats as a model.

71 Laboratory studies investigating the acoustic characteristics of predation success have
72 found that the length of the silent period at the end of a terminal buzz (post-buzz pause) is longer
73 when predation is successful compared to when it is unsuccessful (Acharya and Fenton, 1992;
74 Britton and Jones, 1999; Surlykke et al., 2003; Übernickel et al., 2013). It is assumed that the length
75 of the post-buzz pause reflects the time required for a bat to bend its head towards its tail membrane
76 pouch and grasp the prey (Acharya and Fenton, 1992; Kalko and Schnitzler, 1989). Furthermore, if a
77 predation attempt fails, the next search is expected to start earlier, leading to a shorter post-buzz
78 pause. However, this relationship has not been confirmed in field experiments (Britton and Jones,
79 1999). Thus, to the best of our knowledge, the echolocation call characteristics that enable
80 discrimination between successful and unsuccessful predation in naturally foraging bats have not yet
81 been identified.

82 The purpose of this study was to investigate whether the length of the post-buzz pause
83 could be used as an acoustic indicator of successful predation in bats during natural foraging. We
84 hypothesized that the length of the post-buzz pause would depend on the attack outcome. In
85 particular, if a bat successfully captured its prey, we expected to find a longer post-buzz pause, with
86 a shorter pause reflecting a failed attempted, as demonstrated in a previous laboratory experiment
87 with *Myotis daubentonii* (Britton and Jones 1999a). Furthermore, if a bat dropped its prey, we
88 expected the post-buzz pause to have an intermediate length.

89

90 **Materials and Methods**

91 *Study species and setting*

92 The target species was Japanese large-footed bat *Myotis macrodactylus*, a member of the
93 family Vespertilionidae. This species emit a frequency-modulated (FM) pulse with a fundamental
94 frequency falling from approximately 90 to 40 kHz, and a second harmonic (Fig. 1B, Fukui et al.,
95 2004; Luo et al., 2012). In Japan, these bats feed mainly on prey from the orders of Diptera,
96 Trichoptera, and Lepidoptera (Funakoshi and Takeda, 1998), and typically trawl for prey by flying
97 above the water surface (Luo et al., 2012). Bat species that predominantly use trawling for prey
98 capture are particularly suitable for detailed observations of natural foraging behavior via acoustic
99 and video recordings because the feeding sites can be easily identified.

100 Our study site was a 20 m diameter pond (Fig. 2A) in Tomakomai Experimental Forest
101 (42°43'N, 141°36'E), a research facility of Hokkaido University in Tomakomai, Hokkaido, Japan.
102 The pond is part of a Horonai stream (about 3 m wide) that enters the pond from one side and exits it
103 on the other side (Fig. 2A). Bats regularly use the open space above this pond for foraging after
104 sunset during early summer and fall. In most of the cases that we observed, the bats appeared
105 upstream or downstream of the stream, foraged above the pond for a certain period of time, and
106 exited via the downstream side of the stream. The average temperature, average humidity, weather
107 conditions, and sunset times during the recording period are shown in Table 1.

108 Although this site is designated as a wildlife reserve by the local government, no
109 permission was required for this survey because it did not include any endangered or protected
110 species (Fukui et al., 2019), and we did not engage in any animal capture or habitat disturbance.

111 *Microphone array recordings*

112 Bat echolocation calls were recorded using four T-shaped microphone array units, which
113 comprised four omnidirectional electret condenser microphones (1/8-inch condenser microphones:
114 models FG-23329-C05 and FG-23629-P16; Knowles Electronics, Itasca, Italy) (Fig. 2B, Fujioka et

115 al., 2011). The distance between the central microphone, M1, and each of the three equally spaced
116 outer microphones was 0.9 m (Fig. 2B). The four units in the microphone arrays (arrays 1–4) were
117 arranged such that echolocation calls emitted from anywhere around the pond area could be recorded.

118 The echolocation signals recorded by the microphones were amplified and band-pass
119 filtered (10~250 kHz) using a custom-designed electronic circuit, and then digitized with 16-bit
120 precision at a sampling rate of 500 kHz using a high-speed data acquisition card (PXIe-6358;
121 National Instruments, Austin, TX, USA). The frequency response of the microphones was flat (± 3
122 dB), and ranged between 10 and 250 kHz. The output signals were synchronously stored using a
123 personal computer via a custom program created using LabVIEW 2011 (National Instruments).
124 Recordings were saved as files every 10 minutes, and recording was stopped when the batteries ran
125 out.

126 Sound data from the central microphone in each of the four microphone arrays (ch 1, 5, 9,
127 13) were analyzed. We used Cool Edit 2000 (Syntrillium Software Corporation, Phoenix, AZ, USA)
128 to display the spectrograms of the sounds (128-point FFT, Han window with overlap for N-1) and
129 extracted the terminal buzz signals with the clearest spectrograms for analysis. When the signal-to-
130 noise ratio of a pulse was poor and extraction of the sound data was difficult, the data from the other
131 channels were checked to determine the presence or absence of a terminal buzz. The post-buzz pause
132 was calculated as the time between the last sound in the terminal buzz and the start of the next search
133 pulse, as measured from the spectrogram images (Fig. 1A).

134 *Video recording and analysis*

135 The foraging behavior of the bats at the pond was recorded using high-speed cameras (LT
136 Recorder Pro (ver. 1.04; DITECT, Tokyo, Japan), in synchronization with the above-described
137 sound recordings. The observation area was illuminated by infrared floodlights (LIR-CS88; IR LAB,

Shenzhen, China) and the frame rate of the camera was set to 60 fps. An analog on/off control signal generated by a custom-made electrical circuit triggered video recordings, so that the video and sound data could be synchronously recorded and stored on the PC. The video recording was stopped when the hard drive of the computer was full (after approximately 30 minutes per measurement day).

Video images were analyzed visually using Dipp-Image Viewer (version 1.22; DITECT). In the first step, we identified scenes that showed a bat attacking prey, i.e., cases in which the tail membrane and hindfeet of a bat touched the water surface. These scenes were classified as “catch” or “failed”, and the “catch” group was further classified as “captured” (successful predation) or “dropped” (Fig. 3). Scenes were classified as "captured" when the bat caught the prey near the water surface with its feet or tail membrane and carried it away. Scenes where a bat caught prey but then dropped it were classified as "dropped". The "failed" category contained scenes in which the presence of the prey on the water surface was confirmed after the bat had attacked.

Statistical analysis

All statistical analyses were performed in the R environment for statistical computing (R Core Team 2020) and its extended packages. To test our hypothesis, generalized linear mixed models (GLMM) were built using the Template Model Builder package (glmmTMB_1.0.2.1; Brooks et al., 2017). Because several post-buzz pauses were measured in the same echolocation call sequence, and therefore from the same bat, we included “bat-ID” as a random effect in all models. Also, to account for any differences between recording events, we included the date of the recording as a random effect in all models. Hereby, the effect of bat-ID was nested within the date factor. The post-buzz pause was modeled as a function of the "attack outcome" factor (three levels; captured,

160 dropped and failed). This variable was measured in milliseconds, and comprised integer values that
161 could not have a value of 0. Therefore, we assumed a 0-truncated-Poisson distribution for all models.

162 To test for the potential influence of weather (three levels: sunny, cloudy and rainy) and
163 nightly temperature, we included each variable in a separate model. We used the Akaike information
164 criterion (AIC), corrected for small sample sizes, to select the best model (function model.sel,
165 package MuMIn_1.43.17; Barton, 2020). We examined the quality of the model fit graphically using
166 the functions in the DHARMA package (version 0.3.3.0; Hartig, 2020). We determined the overall
167 model significance by using an χ^2 test, comparing the best parsimonious model to its null model
168 containing only the random effects (function anova, package stats_4.0.3, R Core Team, 2020). A
169 $\chi^2_{\text{type-II-Wald}}$ test (function Anova, package car_3.0.10; Fox and Weisberg, 2018) was used to identify
170 significant factors within the model, and Bonferroni correction was applied to all pairwise post-hoc
171 comparisons between the levels of relevant factors (function lsmeans, package emmeans_1.5.3;
172 Lenth, 2019).

173

174 **Results**

175 We recorded echolocation sounds of the bat for a total of 421 minutes on six separate
176 nights (June 18 and 19 in 2018, and June 23, 25, 26, and 27 in 2019). From this time period, 220
177 minutes of synchronized video and audio recording were collected and analyzed.

178 We classified a total of 137 attacks into the three categories (captured / dropped / failed)
179 from the video recordings (Fig. 3, Movie 1). We found that bats kept hold of their prey in 51.8 %
180 (“captured”, $n = 71$) of the attacks, and dropped their prey or failed to capture it in 29.2 % ($n = 40$)
181 and 19 % ($n = 26$) of the attacks, respectively. From the 137 attacks confirmed via video, we
182 identified 135 terminal buzzes. From these buzzes, 87 post-buzz pauses could be clearly measured

183 from the spectrograms with a good signal-to-noise ratio (capture: n = 37, drop: n = 33, failed: n = 17).
 184 Based on the minimum AIC value, the model containing only the attack outcome factor was the best
 185 (Table 2). After graphically examining the model residuals, the fit was determined to be satisfactory.
 186 The best model explained significantly more variance than the null model ($\chi^2 = 162.14$, degrees of
 187 freedom [df] = 2, $P < 0.001$), and the attack outcome factor was significant ($\chi^2_{\text{type-II-Wald}} = 158.1$, df =
 188 2, $P < 0.001$). The post-buzz pause was longest in cases of successful capture, with a mean $\pm$ SE
 189 value of 200 ± 11.14 ms ($\chi^2_{\text{type-II-Wald}}$ test: capture vs. failed ratio = 0.573 ± 0.03 , df = 82, $P < 0.001$,
 190 capture vs. drop ratio = 0.767 ± 0.03 , df = 82, $P < 0.001$, Fig. 4). The post-buzz pause was shortest in
 191 cases of failed capture, with a mean value of 114 ± 7.05 ms ($\chi^2_{\text{type-II-Wald}}$ test: drop vs. failed ratio =
 192 1.340 ± 0.05 , df = 82, $P < 0.001$), while the mean post-buzz pause in cases of dropped prey had an
 193 intermediate value of 153 ± 8.64 ms.

194 We observed additional interesting behaviors in the video recordings. For example, the bat
 195 attacked small branches ("Attack branch" in Movie 2) or the same prey again after initially failing to
 196 capture it ("Repeated attack" in Movie 2). In addition, in cases of dropped prey, the bats usually
 197 dropped the item immediately after capture. However, there were a few cases where the bats
 198 dropped objects after holding them for a longer period ("Throw away" in Movie 2).

199 Discussion

200 The terminal buzz emitted by bats has traditionally been treated as an acoustic indicator of
 201 an attack on a prey item (Britton and Jones, 1999; Griffin, 1958; Jakobsen and Surlykke, 2010;
 202 Racey and Swift, 1985). However, the presence of a terminal buzz is not sufficient to determine
 203 whether predation was successful. Various potentially indicative acoustic features have been
 204 examined, such as the lengths of terminal buzz I and II, the length of the post-buzz pause, and the
 205 average interpulse interval (IPI) after the terminal buzz (Acharya and Fenton, 1992; Britton and

206 Jones, 1999; Surlykke et al., 2003; Übernicket et al., 2013). Laboratory experiments on foraging
207 behavior have indicated that the presence or absence of predation affects the post-buzz pause
208 (Acharya and Fenton, 1992; Britton and Jones, 1999; Surlykke et al., 2003; Übernicket et al., 2013).
209 In contrast, no clear evidence for such a relationship could be derived from observations of wild bats
210 during natural foraging (Britton and Jones, 1999; Surlykke et al., 2003). This was due to poor-
211 quality acoustic data and the tendency for post-buzz pauses to be relatively short as a result of
212 adaptation by the bats to the complex natural environment. To the best of our knowledge, this study
213 is the first to show that the length of the post-buzz pause can be used to measure successful predation
214 by wild bats during natural foraging.

215

216 *Prey selection during natural foraging in trawling bats*

217 In the present study, *M. macrodactylus* bats dropped their prey during 30% of all recorded
218 attacks (40 drops out of 137 attacks). Therefore, these bats do not appear highly capable of
219 discriminating their prey, but rather make their prey selection after capture. This may explain the
220 intermediate values of the post-buzz pause observed in this study.

221 Previous studies on prey selection in bats that predominantly hunt via trawling, like *M.*
222 *macrodactylus*, have also suggested that bats have relatively weak target discrimination ability.
223 Indeed, bats have been observed to sometimes attack objects instead of prey (Barclay and Brigham,
224 1994; Kalko and Schnitzler, 1989). For example, *Myotis lufigusus* and *M. yumanensis* did not appear
225 to discriminate among targets, and attacked inedible targets (beetles and leaves) as well as edible
226 prey of the same size (moths) during natural foraging (Barclay and Brigham, 1994). Other trawling
227 *Myotis* species, such as *M. dasycneme*, *M. daubentonii*, and *M. capaccinii*, repeatedly attempted to
228 capture inedible dummy targets placed on artificial surfaces that mimicked the reverberatory

229 properties of water (Siemers et al., 2001). Therefore, this might represent a general prey selection
230 behavior in trawling bats.

231 On the other hand, bats might drop not only inedible targets, but also edible prey
232 unintentionally. For instance, in the “repeated attack” shown in Movie 2 the bat most likely dropped
233 an edible prey item because it recaptured that item after dropping it. However, since this type of
234 behavior was captured rarely by our cameras, there is a limitation to discriminate between these two
235 types of behaviors in this study. In the future, knowing what the bats have caught would help
236 distinguishing between drops of inedible or edible targets.

237 The post-buzz pause was also shown to be affected by prey size in a laboratory study with
238 *Pipistrellus pygmaeus* (Surlykke et al., 2003). However, the authors reported that the prey size did
239 not have a significant effect on the post-buzz pause in wild bats, according to acoustic recordings
240 taken during natural foraging.

241 In summary, we found a clear relationship between the post-buzz pause and predation
242 success in naturally foraging *M. macrodactylus*. However, further investigation regarding this
243 relationship, including influencing factors such as prey type and size, will be needed to develop a
244 reliable acoustic indicator of predation success in wild bats.

245 *Post-buzz pause applied: example of temporal changes in individual-specific predation* 246 *success*

247 To elucidate what insights could be gained by investigating post-buzz pauses during
248 natural foraging, we recorded the foraging behavior of individual *M. macrodactylus* bats using four
249 microphone arrays surrounding the pond, in the same experimental setting, for about 100 minutes
250 starting at 20:04 on June 15, 2016. The recorded post-buzz pauses were analyzed based on their
251 duration (represented by colors in Fig. 5) rather than whether predation was successful or not.

252 During this recording period, a total of 70 bats visited the pond, 54 of which produced feeding
253 buzzes (attacks) (note that the same bat might have entered the feeding area on multiple occasions).
254 Bats that arrived during the first 30 minutes of the recording, which was the busiest period (20:30–
255 21:00), seemed to have shorter post-buzz pauses than those that arrived during the second 30-minute
256 period. If predation success is related to the length of the post-buzz pause, then this observation
257 suggests that predation success greatly varies depending on the time of foraging, and potentially also
258 on the individual. This example shows that by using the post-buzz pause length as an acoustic
259 indicator of predation success, we may be able to investigate detailed temporal aspects of foraging
260 behavior in bats in feeding patches. Specifically, both the number of attacks and the length of the
261 post-buzz pause can be used as indicators of predation efficiency during natural foraging.

262

263 **Conclusions**

264 In this study, we conducted synchronized acoustic and video recordings of foraging
265 behavior in wild Japanese large-footed bat *M. macrodactylus*. Our data showed that the bats either
266 kept hold of their prey, dropped it, or failed to capture it after an attack. Overall, predation was
267 successful in 51.8 % of attacks. Furthermore, the post-buzz pause was significantly longer in cases
268 of successful predation than in the other two cases. This study is the first to identify differences in
269 acoustic features between successful and unsuccessful predation in naturally foraging bats. Using the
270 post-buzz pause as an indicator of successful predation will enable detailed investigations of wild bat
271 foraging behavior, including foraging efficiency and, potentially, temporal changes in energy intake
272 within feeding areas.

273 **Acknowledgments**

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274 We are grateful to Fumiya Hamai, Genki Nakai, Koki Asano, Mika Fukushima, Yoshimasa Kitamura,
275 Akihiro Nakae, Yoshiki Kubota, Yuta Sakuragi, Nozomi Sonobe, and Mika Kuroda for their
276 assistance in conducting the field recordings.

277 **Competing interests**

278 The authors declare no competing interests.

279 **Funding**

280 This work was supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI
281 Grant [Numbers JP 18H03786, 16H06542 to S.H., 19K16237 to E.F., 16K00568 to D.F.] and the
282 Sasagawa Scientific Research Grant from The Japan Science Society [2020-5023, to Y.M.]. Further
283 financial support was provided by the JSPS postdoctoral fellowship and KAKENHI Grant [Numbers
284 JP 19F19090 to O.H.].

285 **Data availability**

286 The data supporting the findings of this study are available from the corresponding author upon
287 reasonable request.

288

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366

Table 1. Overview of the meta-data and weather conditions.

Day of recording	Sunset* time	Recording start	Recording duration [min]	Temperature [°C]	Humidity [%]	Weather
2018/6/18	19:14	20:00	130	13.4	79.7	rain
2018/6/19	19:15	20:15	110	14.0	79.7	rain
2019/6/23	19:16	20:27	23	15.0	77.3	sunny
2019/6/25	19:16	20:14	48	15.0	86.2	cloudy
2019/6/26	19:16	20:56	49	17.0	87.5	sunny
2019/6/27	19:16	20:29	61	17.4	83.4	cloudy

* Data were from the National Astronomical Observatory of Japan.

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381 **Table 2.** Overview of models and their comparison based on the AICc.
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Model no.	Factors			Df	Loglik	AICc	Delta	Weight
	Capture outcome	Weather	Temperature					
1	+			5	-885	1781	0	0.88
2	+	+		7	-885	1785	4	0.12
3	+		+	19	-874	1797	16	<0.001
4				3	-966	1938	158	<0.001

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Figure 1. (A) Spectrogram of an echolocation call sequence from *Myotis macrodactylus* during prey capture. The terminal buzz is emitted just before attacking the prey. The time between the attack and the next search pulse is called the post-buzz pause. (B) Example search-phase echolocation call from *M. macrodactylus*. The fundamental frequency of this frequency-modulated (FM) pulse drops from about 90 to 40 kHz, with a peak frequency of 50 kHz.

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395 Figure 2. (A) Top view of the pond. A Y-shaped microphone array system was set up at four
396 locations around the pond (total of 16 microphones) and two cameras were set up on one side of the
397 pond. (B) Schematic of a Y-shaped microphone array system consisting of four microphones, with
398 M1 at the center and three microphones (M2–M4) placed 0.9 m apart from each other.

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Figure 3. Images from video recordings of the three categories of attack outcomes. (A) “Captured”: the bat captures the prey near the water surface with its feet or tail membrane and carries it away. (B) “Dropped”: the prey is seen moving away from the bat after the bat initially caught it. (C) “Failed”: the prey can be seen on the surface of the water after the bat passed it (Movie 1).

407

408 Figure 4. Post-buzz pause length for the various attack outcomes (captured, dropped and failed).
 409 Boxplots represent the raw data for each category. Means (filled circles) and 95% confidence
 410 intervals were derived for the best model (Table 2). Asterisks indicate significant differences
 411 between factor levels based on a Bonferroni-corrected post-hoc test conducted using the emmeans-
 412 package in R. ***p < 0.001.

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 416 Figure 5. Temporal changes in the number of attacks by each bat that entered the foraging site on
 417 June 15, 2016. The time series shows the accumulation of attacks (filled circles) and the
 418 corresponding post-buzz pause lengths (color-coded). For reference, the average post-buzz pause
 419 was 200 ms for captured, 153 ms for dropped, and 114 ms for failed attempts.

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