



# Investigation of Warm Water Enrichment on Drinking Behavior in Older Rhesus Macaques

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**Abstract** – Older animals in a managed setting (e.g., zoos) may require special care. Previous studies have suggested reduced water intake in rhesus macaques may result from cold environment and aging, leading to a decline in their welfare. This study aimed to assess whether providing warm drinking water influences water consumption and preference in aged rhesus macaques. The experiment was conducted on 11 older rhesus macaques at the Kyoto City Zoo, Japan. Observations were conducted for 42 days between November 21, 2024 and April 12, 2025. Containers filled with room-temperature water or warm water were placed in an indoor habitat, and the drinking behavior of the rhesus macaques was recorded for 20 min. No significant difference was found in the drinking frequency between the two water temperatures. However, the time spent drinking in each bout was significantly longer when warm water was provided than when room-temperature water was provided. Furthermore, when room-temperature and warm water were placed simultaneously, the drinking behavior of rhesus macaques was significantly biased toward the warm water container. In other words, rhesus macaques showed a preference for drinking heated water, suggesting that installing warm water may improve the drinking environment for older rhesus macaques.

**Keywords** – Animal welfare, Environmental enrichment, Elderly care, Paired choice, Primate

Zoos are home to animals of all ages that require age-appropriate care throughout their lives (Mellor et al., 2015). In particular, as animals age, their physical and social needs and the care they require change (Krebs et al., 2018). In zoos and other settings, animals may live beyond the average lifespan of their wild counterparts (e.g., Chimpanzee, *Pan troglodytes*: Che-Castaldo et al., 2021; Lion, *Panthera leo*: Okabe & Matsunaga, 2021; Rhesus macaque, *Macaca mulatta*: Inoue-Murayama, 2024; Leopard cat, *Prionailurus bengalensis*: Okabe et al., 2024). Both wild and captive studies on aged animals have suggested that older animals are more sensitive to cold environments than younger animals (Saito et al., 2023; Terrien et al., 2008; White et al., 2011). These studies suggest that aged animals may experience higher energy expenditure to maintain body temperature and an increased physical burden compared to their younger counterparts. Therefore, aged animals may require special care in cold conditions.

Wild rhesus macaques live in cold regions at altitudes over 3,000 m and are naturally adapted to the cold. The skeletal structure of the rhesus macaque has been found to exhibit greater adaptation to cold environments compared to other macaque species (Weinstein, 2011), and genetic analyses have identified the expression of genes linked to enhanced cold tolerance (Wang et al., 2025). However, the rhesus macaques drink less water in cold environments (Oddershede & Elizondo, 1980), and their reduced water drinking becomes more pronounced with age (Schroederus et al., 1999). Similarly, research on humans has shown that water intake decreases with age, making people more susceptible to dehydration (Farrell et al., 2008). Age-related water loss due to dehydration is associated with higher mortality, morbidity, and

disability in older adults (Hooper et al., 2014). Taken together, these findings suggest that reduced water intake in aged rhesus macaques might be associated with morbidity and may affect their welfare.

For zoo primates, heat sources and shelters have been suggested to improve welfare in cold environments (McCann, 2013). The welfare of zoo animals has recently been analyzed based on the "Five Domains" model (Mellor & Beausoleil, 2015), an animal welfare assessment framework that consists of four physical/functional domains: (1) Nutrition, (2) Physical Environment, (3) Health, (4) Behavioral Interactions, and (5) Mental State. Domains (1) through (4) are constructed as ultimately influencing the state of domain (5). Within this model, providing access to water is a key component of positive welfare in the Nutrition domain. Based on previous research on rhesus macaques (Oddershede & Elizondo, 1980), keeping them in cold environments in zoos may reduce the amount of water they drink.

Given these concerns about water intake in cold environments and aging, it is worth considering thermal comfort strategies related to water. For instance, the Japanese macaque (*Macaca fuscata*) regulates its body temperature in cold environments through hot spring bathing although this has mainly been documented in wild and provisioned populations (Zhang et al., 2007; Takeshita et al., 2018). More directly relevant to drinking behavior, a recent zoo study suggested that providing warm water to giraffes in cold environments may increase their water intake (Okabe et al., 2022). Therefore, warm drinking water may increase the water intake of older rhesus macaques and improve their welfare. In this study, to verify the effects of providing warm drinking water to rhesus macaques, we investigated (1) whether rhesus macaques have a preference for warm water and (2) whether providing warm drinking water increases drinking frequency and duration in rhesus macaques.

## Methods

### Ethics Statement

The research protocol for the rhesus macaques was approved by the ethics committee of the Kyoto City Zoo, Japan (KCZ-2024-042).

### Animals and Husbandry

Eleven rhesus macaques housed at the Kyoto City Zoo participated in this experiment. The sample population included one male and ten females (Table 1). The male was neutered, and one female was spayed (via ovarioectomy). All individuals were over 18 years of age (average age = 23.18 years old). Previous studies (Mattison, 2012; Schroederus et al., 1999; Uno, 1997), defined individuals aged over 16 or 20 years as belonging to the older age group, thereby classifying most of participants in this study within the geriatric range. All individuals had consumed warm water at a previous zoo event ("Monkeys' Hot Springs (Saru-Onsen)"). Additionally, we note that some of the participants may have exhibited malignant neoplasms; however, this was deemed unlikely to influence behavioral observations.

The rhesus macaque exhibit at Kyoto City Zoo comprised of an outdoor and indoor habitat. The outdoor habitat consisted of multiple raised platforms and dirt, with multiple trees planted. The macaques had free access to water from a shallow water moat and a plastic container (this container was different from the one used in the experiment: 43.5 × 29.6 × 14.0 cm) filled with tap water during the day. The indoor habitat had a three-dimensional structure consisting of logs on a concrete floor. Feeding was performed outside three times per day at: 9:00 (commercial monkey feed pellets), 11:30 or 13:30 (vegetables and fruits), and 15:30 (vegetables and fruits).

**Table 1***Information on Individual Rhesus Macaques Used in the Study*

Individual No.	Sex	Name	Age	Notes
456	Male	Tono	27	Neutered
465	Female	Chiiko	27	
475	Female	Tsubone	26	
486	Female	Tempura	25	
490	Female	Boko2	25	
551	Female	Kankan	23	Spayed
565	Female	Nano	22	
569	Female	Saeko	21	
570	Female	Agi	21	
574	Female	Koyuriko	20	
581	Female	Yoko	18	

Typically, the rhesus macaques were released into the outdoor habitat during the diurnal hours and are not permitted access to the indoor habitat. However, on days with low temperatures (generally with a maximum temperature of approximately 10°C), following cleaning of the indoor habitat, the doors were opened to permit macaques to move freely between the indoor and outdoor habitats. During these periods, the macaques had access to room temperature water from drinking containers within the indoor habitat. In instances where outdoor temperatures were cold and the indoor habitat was already accessible, the rhesus macaques were guided to the outdoor habitat to prepare for observation. During the observation period, all the participants were housed in an indoor habitat at night.

### Observation Methods

Several methods exist for determining animal preferences (Turner et al., 2025). Because all individuals were present during observations and because rhesus macaques exhibit complex social hierarchies (Fushing et al., 2011), dominant individuals may have influenced the outcomes. Thus, rather than relying on initial choice alone, we assessed preferences by recording the frequency and duration of water consumption within a specific period. All observations were performed in the indoor habitat.

### Preliminary Observation

*Preliminary Observation* was conducted for 8 days from November 21 to December 1, 2024 (Table 2). Two plastic containers (66.8 × 44.2 × 14.6 cm; Gifu Plastic Industry Co., Ltd., Gifu, Japan) were installed in the indoor habitat and filled with room temperature water. When macaques entered the room, they traversed a log to reach a bench where they could access the plastic containers filled with water placed in front of the bench. The two plastic containers were positioned 80 cm apart, referred to as Eastern and Western (Figure 1). Because rhesus macaques frequently engage in drinking behaviors after foraging (Oikawa et al., 1982), 80 g of glutinous barley cereal (Weed Planning Co., Ltd., Shiga, Japan) was dispersed across the indoor habitat to encourage drinking behavior. To ensure consistent feeding conditions, the rhesus macaques were fed between 11:30 and 12:00 on the day of the observation. Afterwards, the chute door between the indoor and outdoor habitat was opened. The experiment began between 13:30 and 14:30 on each observation day. Following the opening of the indoor habitat, an observer proceeded to the visitor area and recorded the number of instances in which rhesus macaques drank water, the location of the plastic container used for drinking, and the names of individuals engaged in water consumption for 20 min.

The data collection method employed was 1–0 sampling, at intervals of 1 min. Before and after the observations, the air temperature in the indoor habitat and the water temperature in the plastic container were recorded using thermometers (CTH-27L, CUSTOM Corporation, Tokyo, Japan) and water thermometers (T101C-JP, Shanghai Daoqian Touziguangli Youxiangongsi, Fuzhou, China), respectively. In addition, the initial outdoor temperature was obtained from the Japan Meteorological Agency (Kyoto Local

Meteorological Office, 5 km from the observation site) via its official website (<https://www.data.jma.go.jp/stats/etrn/index.php>).

### Preference Observation

After *Preliminary Observation* were recorded, and the water in both containers was replaced with warm water on January 9, 2025. Warm water was prepared by heating water in a metal bucket using a pipe heater (Nagacom-DB1000L; Daiei Dennetsu Kogyo Co., Ltd., Osaka, Japan). The locations of the containers, feed distribution, observation times, and sampling methods used were identical to those employed in the *Preliminary Observation*. The *Preference Observation* was divided into three distinct periods.

The first observation period (Both warm period) was conducted for 8 days between January 18 and February 15, 2025. During this period, the water in both plastic containers was heated to a temperature range of approximately 30–35 °C, and the drinking behavior of rhesus macaques was documented. Subsequent to the observation period, a decline in water temperature was recorded, with a mean decrease of  $1.78 \pm 0.60$  °C compared to the initial temperature.

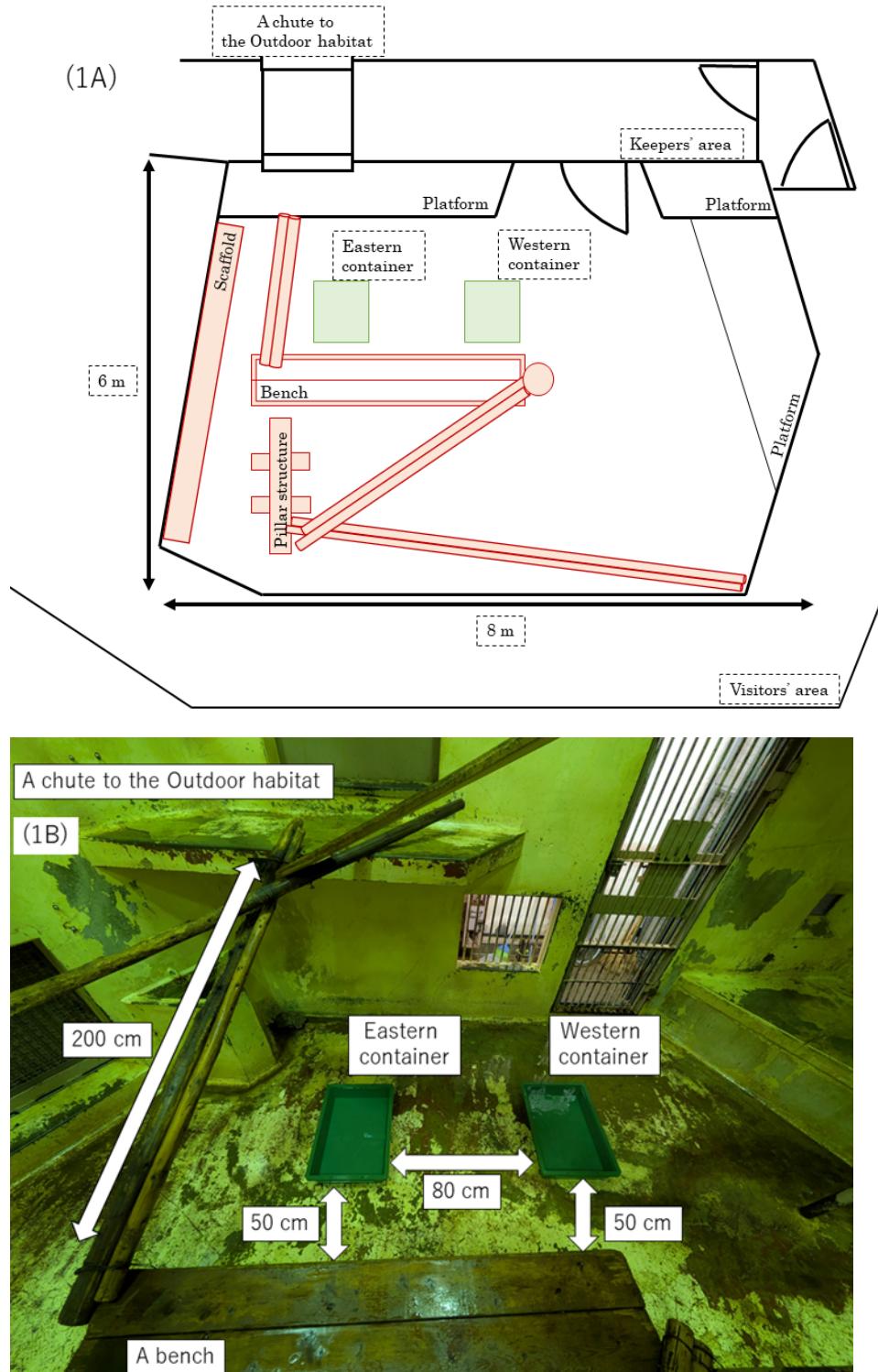
The second observation period (East warm period) was conducted for 8 days between February 25 and March 9, 2025. In this period, only the eastern container was filled with warm water, and the western container was filled with room-temperature water from the tap. Before the East warm period commenced, macaques were given a week to acclimate to the new locations of the warm and room temperature water. The third observation period (West warm period) was conducted for 8 days, from March 17 to March 28, 2025. During this period, the western container was filled with warm water, whereas the eastern container contained room-temperature tap water. Similar to the East warm period, the rhesus macaques were acclimated to the container and warm water placement for one week prior to the commencement of the West warm period.

**Table 2**

*Details of the Date of Each Period and Container Set-Up*

Experiment	Date	Container Temperature	
		East	West
Preliminary (Baseline)	Nov. 21 – Dec. 1	Room Temperature	Room temperature
Both warm	Jan. 18 - Feb. 15	Warm	Warm
East warm	Feb. 25 - Mar. 9	Room temperature	Warm
West warm	Mar. 18 - Mar. 28	Warm	Room Temperature
Bout West warm	Mar. 29 - Apr. 4	Warm	Room Temperature
Bout East warm	Apr. 8 - Apr 15	Room temperature	Warm

*Note.* Warm water was approximately 30–35 °C

**Figure 1***Experimental Setting*

*Note.* 1A shows a schematic representation of the indoor habitat for rhesus macaques, while 1B shows a photograph of the layout of the containers utilized in the experiment.

## Bout Length Observation

After *the preference observation*, the duration of drinking per bout in rhesus macaques was investigated as an ancillary study. The placement of the containers remained unaltered from *the Preliminary and Preference Observations*. As in previous observations, cereal was scattered around the indoor habitat. However, the daytime feeding time for rhesus macaques was changed to between 13:30 and 14:30 to encourage increased water intake. Subsequently, the indoor habitat was opened within 30 min, after which continuous observation commenced. A single drinking bout was defined as a rhesus macaque's approach and subsequent departure from the container, and the duration for which it kept its face on the water surface was measured. Concurrently, the names of the individuals who imbibed the water and the location of the plastic receptacle from which they drank were documented. To record the duration of drinking, a video camera (HC-VX985M, Panasonic Corporation, Tokyo, Japan) was installed in the visitor area, and the behavior of the rhesus macaques was recorded.

From March 29 to April 4, 2025 (Bout west warm period), the western container was filled with warm water while the eastern container was filled with room-temperature water, and observations were conducted for 5 days. Subsequently, we conducted a second set of observation, in which the positions of the warm and room-temperature water were reversed. The macaques were allowed a 3-day acclimation period to the new warm water location. Following this, observations (Bout east warm period) were again conducted for 5 days from April 8 to April 15, 2025.

## Statistical Analysis

Water temperature, air temperature in the indoor habitat, and outside temperature in each period (*Preliminary Observation*, *Preference Observation*, and *Bout Length Observation*) were compared using the Steel–Dwass test. Comparisons of laterality in the total drinking frequency (based on 1–0 sampling) of rhesus macaques during *the Preference Observation* were performed using the chi-square test with Haberman's definition of adjusted standardized residuals. Furthermore, the total drinking frequency over 8 days for each rhesus macaque during the *Preliminary Observation* period and the Both warm period was compared using the Wilcoxon signed-rank sum test. The duration of each bout length when warm water and room-temperature water were used during *the Bout Length Observation* was compared using Welch's t-test. Statcel4 (OMS Publishing, Saitama, Japan) was used as the statistical processing software in this study, with the significance level for the statistical analysis set at .05.

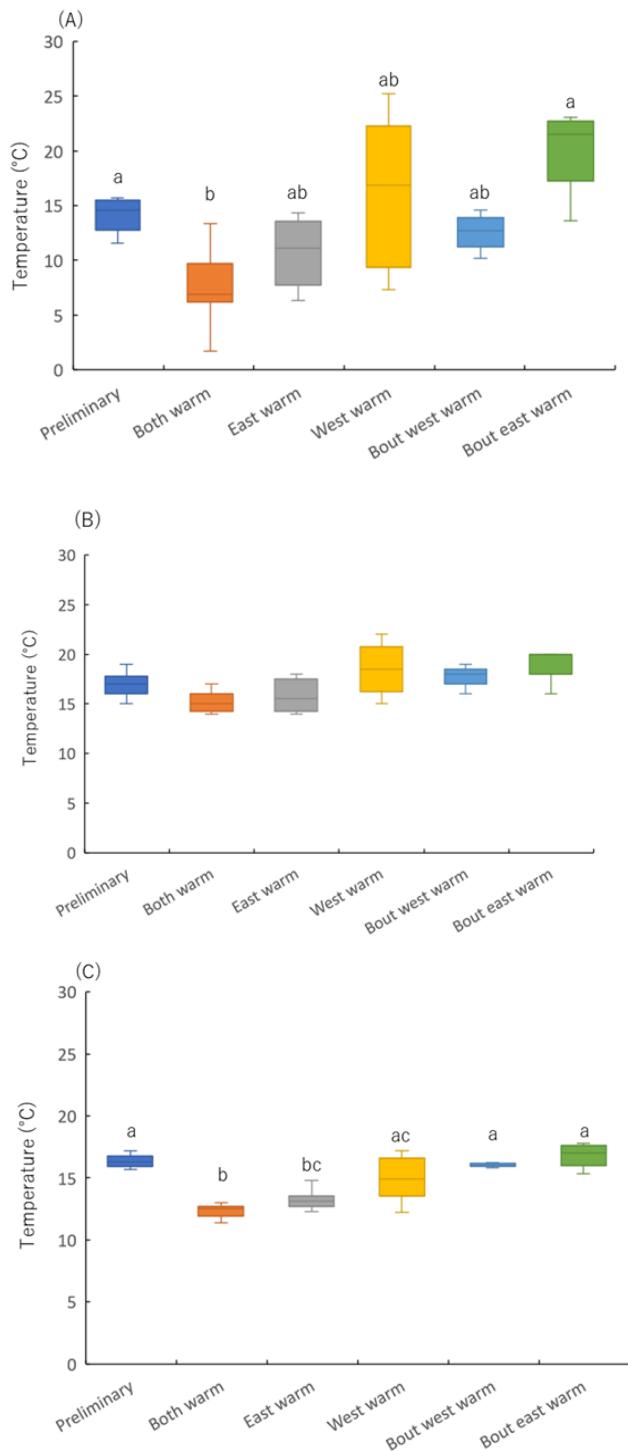
## Results

### Water Temperature, Indoor Air Temperature, and Outside Temperature Across Observation Periods

Figure 2 shows the results of comparing the outside air, room, and room water temperatures during all observation periods. The outside temperature (Figure 2A) was significantly lower during the Both warm period than during the Preliminary Observation period and the Bout east warm period (Preliminary-Both warm:  $p = .030$ , Both warm–Bout east warm:  $p = .038$ ). No significant differences were observed in the air temperature in the indoor habitat during any period (Figure 2B). The room-temperature water was significantly colder during the Both warm period than during all other periods, except for the East warm period (Preliminary-Both warm:  $p < .001$ , Both warm–West warm:  $p = .018$ , Both warm–Bout west warm:  $p = .015$ , Both warm–Bout east warm:  $p = .015$ ) (Figure 2C). The temperature of the room temperature water during the East warm period was also significantly lower than during the Preliminary Observation and the Bout Length Observation (East warm–Preliminary:  $p < .001$ , East warm–Bout west warm:  $p = .040$ , East warm–Bout east warm:  $p = .040$ ).

**Figure 2**

*Comparison of Air, Room, and Room Temperature Water Temperatures for Observation Period*



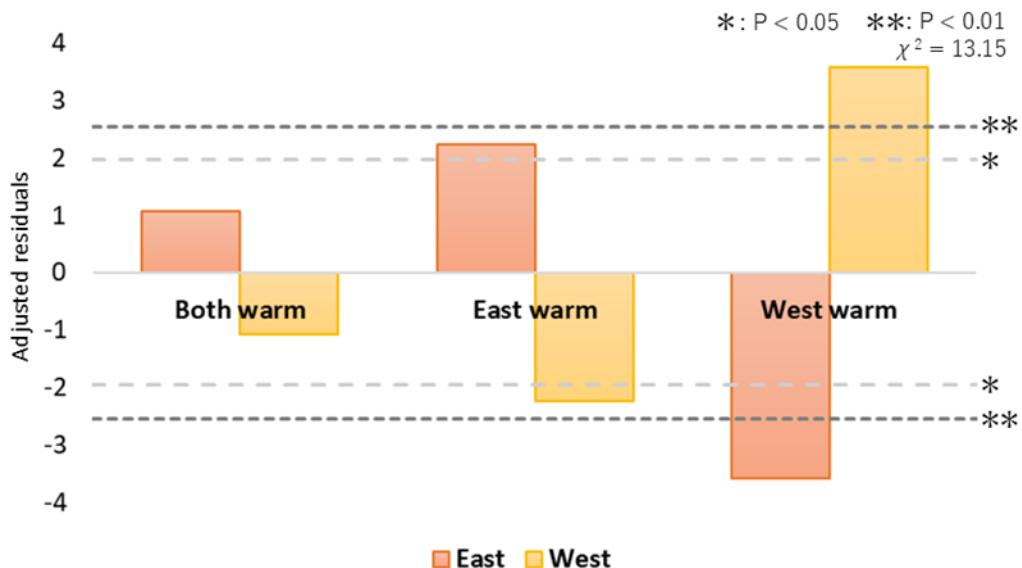
*Note.* Outside temperature (A), air temperature in the indoor habitat (B), and temperature of room temperature water (C) for each period. The letters a, b, and c indicate significant differences at  $p < .05$ . Bars that share the same letter (e.g., a and ab, or bc and ac) show no significant differences.

### Comparisons of Laterality Differences in the Total Drinking Frequency During the Preference Observation

Results of the residual analysis of the Preference Observation are shown in Figure 3. The drinking behavior of all the rhesus macaques was recorded during the Both warm and East warm period and for ten individuals during the West warm period. During the Both warm period, the total number of drinking events was 67 in the western container and 43 in the eastern container. In the East warm period, macaques drank 68 times from the western container and 51 times from the eastern container. In the West warm period, they drank 70 times from the western container and 17 times from the eastern container. Chi-square test and residual analysis showed no significant bias in the Both warm period, a significant bias in the drinking water from the eastern container in the East warm period ( $p = .025$ ), and a significant bias in the drinking water from the western container in the West warm period ( $\chi^2 = 13.15, p < .01$ ).

**Figure 3**

*Bias of the Container from which the Rhesus Macaques Drank During the Preference Observation*



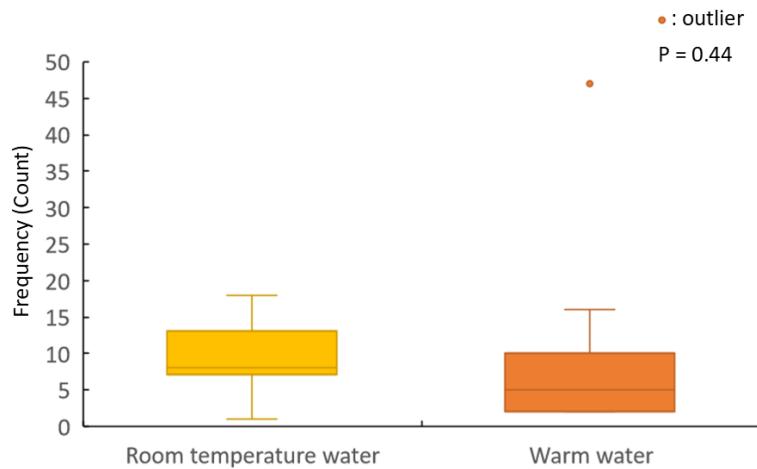
*Note.* A significant bias is observed when the values shown in the bar graph exceed the gray dashed line (\*  $p < .05$ ) or the black dashed line (\*\*  $p < .01$ ), respectively.

### The Drinking Frequency of All Animals Per Day Between the Preliminary Observation and Both Warm Period

The total drinking frequency over 8 days for each rhesus macaque during the *Preliminary Observation* period and the Both warm period is shown in Figure 4. The median number of times the macaques drank room-temperature water during the *Preliminary Observation* and warm water during the Both warm period was eight and five, respectively. No significant difference was observed in the number of drinks between the two periods ( $Z = -0.77, p = .44$ ).

**Figure 4**

*Comparison of Total Water During Frequency for Each Individual During the Preliminary Observation and the Both Warm Period*



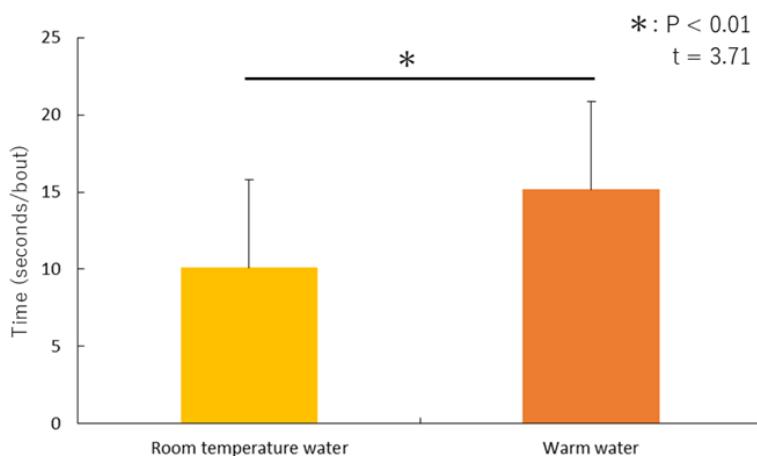
*Note.* There is one outlier for warm water.

### The Duration of Each Drinking Bout During Bout Length Observation

Figure 5 shows duration of the drinking per bout for room-temperature and warm water during *the Bout Length Observation*. Overall, during *the Bout Length Observation*, drinking behavior was recorded for all individuals in the container filling room-temperature water (55 bouts), while drinking behavior was recorded for nine individuals in the container filling warm water (51 bouts). The average time spent drinking room-temperature water and warm water per bout was  $10.11 \pm 5.71$  s and  $15.16 \pm 8.19$  s, respectively. There was a significant difference between the time spent drinking room-temperature water and the time spent drinking warm water per drinking bout ( $t = 3.71$ ,  $p < .01$ ).

**Figure 5**

*Comparison of Water Drinking Time Per Bout During the Bout Length*



## Discussion

Comparing the entire observation period, the outdoor air temperature and the room temperature water temperature were significantly lower in some periods than others, especially in the Both warm and East warm periods. Oddershede and Elizondo (1980) reported that exposure to a cold environment (6 °C) reduced water intake and total body water in rhesus macaques. This suggests that cold environmental conditions may have led to a reduction in the consumption of water consumed by rhesus macaques on the outdoor habitat (from a shallow water moat or a plastic case filled with tap water), especially during these two observation periods.

Drinking water preferences, particularly water temperature preferences, have not been investigated in apes or monkeys. Enrichment programs in monkey parks and zoos often include hot baths, and their effects on behavior and welfare have been explored, especially for Japanese macaques (Takeshita et al., 2018; Zhang et al., 2007). Providing warm water for drinking in such cold environments may improve welfare by increasing water consumption, even in monkeys that do not often utilize hot baths.

Regarding laterality in drinking behavior during the preference observation, a significant preference was found for the containers filled with warm water during the East warm and West warm periods. During *the Bout Length Observations*, the time spent drinking warm water was significantly longer than that to drink room temperature water. Thus, rhesus macaques may prefer warm waters.

While, when comparing the drinking frequency of the rhesus macaques, no significant difference was found between the period with room-temperature water (*Preliminary Observation* period) and warm water (Both warm period). The average time that rhesus macaques spent drinking during *the Bout Length Observation* was significantly longer when warm water was provided than when room-temperature water was provided. In other words, the change from room-temperature water to warm water may have increased the time the rhesus macaques spent consuming water, thereby increasing their water consumption. Studies on cattle have shown that the amount of water consumed per bout increases with limited drinking frequency (Williams et al., 2017). Providing warm water might increase the time rhesus macaques spend drinking water, especially in cold environments where drinking water is reduced. Therefore, providing warm drinking water to older rhesus macaques in cold environments may increase their water intake and improve their welfare.

However, this observation was conducted only for a limited period during each day, and the effects of drinking time and the amount of water consumed at other times are unknown. Notably, the provision of warm drinking water may also influence activity levels in rhesus macaques. For example, Okabe et al. (2022) reported increased feeding behavior in captive giraffes when warm water was provided, suggesting that temperature-modulated hydration could affect behavioral dynamics in other species as well. Cold-induced reductions in body temperature and elevated energy expenditure represent substantial physiological challenges for elderly animals. In Japanese macaques, the use of hot springs has been shown to stabilize body temperature (Takeshita et al., 2018; Zhang et al., 2007), indicating that warm drinking water might yield comparable benefits. Furthermore, elderly individuals generally consume less water than younger ones, posing potential welfare concerns. Given the increasing lifespan of captive animals (Che-Castaldo et al., 2021), future research should explore long-term strategies for mitigating energy loss, improving thermoregulation, and optimizing hydration to support health and welfare.

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**Data Availability Statement:** The data and materials are available from the corresponding author upon reasonable request.

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