Hindawi
International Journal of Zoology
Volume 2023, Article ID 6305614, 11 pages
https://doi.org/10.1155/2023/6305614

Research Article

Reproductive Behavior of *Bubalus arnee* (Kerr, 1792) (Mammalia: Artiodactyla: Bovidae) in Koshi Tappu Wildlife Reserve and Chitwan National Park, Nepal

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Received 31 May 2023; Revised 12 September 2023; Accepted 27 September 2023; Published 6 October 2023

Academic Editor: Zerihun Girma

The Asiatic wild water buffalo (*Bubalus arnee* Kerr), an endangered species featured on the IUCN Red List, is distributed across Nepal, India, Sri Lanka, Myanmar, Bhutan, Cambodia, and Thailand. This investigation delves into the reproductive and certain social behaviors exhibited by *Bubalus arnee* across distinct habitats and herd types within Nepal's Koshi Tappu Wildlife Reserve (KTWR) and Chitwan National Park (CNP) during the premonsoon and postmonsoon spans of 2018. The data were amassed through direct observations employing focal animal sampling, continuous sampling, and scan sampling methodologies. Eight primary behavioral activities were noted: chasing, courtship, grazing, mating, mowing, sniffing, urination, and wallowing. We carefully structured the gathered data and input it into a Microsoft Excel spreadsheet to facilitate analysis. For a more comprehensive visualization of reproductive behavior, we utilized the ggplot2 package within R-studio, enabling the creation of informative graphical depictions. During the premonsoon phase, Bull A exhibited six reproductive behaviors in KTWR. Notably, sniffing was the most frequently observed behavior, recorded six times (37.5%) daily, succeeded by fighting and urination with four (25%) and three (18.35%) daily observations, respectively. Chasing, mating, and courtship were each observed once (6.25%) daily. In contrast, during both seasons, Bulls B, M, and J were not observed partaking in mating activities. When analyzing the postmonsoon reproductive behavior of the observed *B. arnee* in KTWR and CNP, a notable distinction emerged. In KTWR, there were four more instances of sniffing behavior compared to CNP. In addition, courtship behavior was slightly more frequent in KTWR, occurring three times, whereas it was observed only twice in CNP. Similarly, urination activity was recorded twice as often in KTWR as compared to CNP. As a suggestion for future research, exploring the specific factors influencing the observed variations in reproductive behaviors and habitat preferences of *Bubalus arnee* could provide valuable insights into the conservation and management of these endangered species.

1. Introduction

The wild water buffalo (WWB), *Bubalus arnee* [1], is a large bovid species that is commonly found in the Indian subcontinent and Southeast Asia [2]. It is also referred to as the Asian buffalo, Asiatic buffalo, or wild Asian buffalo. In Nepal, it is popularly called "Arna." The natural range of *B. arnee* extends from central India to southern Nepal in the west and from Vietnam to Malaysia in the east. It is believed that some wild populations still exist in certain areas of Nepal, India, Bhutan, and Thailand [3]. However, domesticated and/or feral populations are widespread. In their

natural habitat, water buffaloes can be found in tropical and subtropical forests, as well as wet grasslands [4]. Although they are terrestrial animals, they rely heavily on water and spend a significant amount of time wallowing in rivers or mud holes [5]. This type of environment provides them with ample drinking water, abundant food, and dense cover [6, 7].

Bubalus arnee possesses a well-developed sense of smell, and chemical cues appear to play a significant role, especially during mating [8]. These animals also have keen hearing abilities [8]. While water buffaloes do not heavily rely on sight or defensive behaviors, they do exhibit a preference for countering threats [9]. Although physical aggression is infrequent, there is some tactile communication observed between mates and between mothers and their offspring [6, 7, 10]. Mating among water buffaloes typically follows a polygynous pattern. Maternal groups, which exist as large and loosely organized herds, are present throughout the year. During the mating season, adult males, either from bachelor groups or solitary, join the female groups and mate with the receptive females, who remain in estrus for a period ranging from 11 to 72 hours [11]. Following mating, the males are chased away by the females for the next 11 to 72 hours. A single male water buffalo can mate with multiple females, and competitive conflicts among males during mating can lead to severe injuries. Adult males, or bulls, detect the estrus phase by sniffing a female's urine and genitals. However, in the wild, young males have limited or no access to females. The timing of reproduction in this species displays some variation. In certain regions, breeding is dependent on the seasons, while in others, it occurs throughout the year. In seasonally dependent areas, breeding often takes place after the rainy season, and calves are born the following year near the onset of the rainy season. In seasonally independent areas, calves may be born at any time of the year.

The estrus cycle of female water buffaloes lasts for 21 days [12]. They have the longest gestation period among bovids, which ranges from 300 to 340 days (10-11 months) [13]. Typically, females give birth to a single calf, although twins are possible [14]. On average, a female produces one calf every two years [15]. Newborn calves weigh between 35 and 40 kg and have a reddish to yellow-brown coloration. Nursing continues from 6 to 9 months. If calves die prematurely due to predation or other reasons, the interval between births becomes shorter. Females reach sexual maturity around 1.5 years of age and remain in a maternal group within a larger herd [16]. Males, on the other hand, reach sexual maturity at 3 years old and usually leave the female group to join bachelor groups [7, 17–20]. Male water buffaloes only invest effort in mating and show no further parental involvement after impregnating females [21]. Females, on the other hand, exhibit all parental care for the young, including nursing from 6 to 9 months and protecting their calves, sometimes displaying aggression [22]. At the age of 3, males leave the maternal group, while females remain in their maternal group and inherit the loose territory of the larger female herd, which consists of multiple maternal groups [14].

Mortality and reproductive success directly impact the viability of the population and are frequently reported [23]. Body condition indicators such as body mass reflect an animal's energy reserves and reproductive ability, thus being closely linked to survival and the population's establishment in a new habitat. Therefore, body condition serves as a convenient measure to assess the outcome of translocation efforts. Studying reproductive behavior offers valuable insights into the reproductive status of a species' population. Ideally, in order to sustain genetic diversity within a viable population, there should be an equal number of reproductive individuals with a 1 : 1 sex ratio [14]. Therefore, the focus of management efforts should center on preventing alterations to the habitat, controlling interbreeding with domestic buffalo, managing forest fires, addressing habitat loss due to climatic conditions and natural disasters (such as floods, droughts, and soil erosion), and minimizing human disruptions such as illegal grazing of domestic cattle and the collection of firewood. These factors have a significant impact on the survival of these animals and can ultimately lead to the species' extinction. The Asian wild buffalo population faces a substantial threat, as it is currently limited to KTWR as a remaining population and has been exposed to various levels of threats for decades. Despite the consistent implementation of existing laws and bylaws to safeguard the population of wild buffalo in the wildlife reserve area through various interventions, enforcement actions have been lacking. There is an urgent need for comprehensive and effective management of the reserve and endangered species such as wild buffaloes. In addition, there is a dearth of essential biological information regarding wild buffaloes, including available habitat for them and other species, ecological and social behavior, population growth trends over time, and distinctions in molecular characteristics among wild, feral, and domestic breeds. This underscores the crucial need to streamline fundamental information for future effective management. In this context, this study aims to propose conservation measures for the wild breed of Asian wild buffaloes in the natural habitat of KTWR. However, currently, there is insufficient data available on the reproductive and social behaviors of *B. arnee* [24]. This research seeks to gather data and information on these specific topics to assist wildlife managers in the effective preservation of the endangered *B. arnee* and the improvement of habitat management. Specifically, the study aims to evaluate the reproductive behavior of *B. arnee* in two designated areas: Koshi Tappu Wildlife Reserve (KTWR) and Chitwan National Park (CNP).

2. Materials and Methods

2.1. Study Area. This study took place in two distinct settings: Koshi Tappu Wildlife Reserve (KTWR), which serves as a natural habitat, and Chitwan National Park (CNP), where an enclosure covering 175 square kilometers and 30 hectares was created. CNP is famous for its population of single-horned rhinoceroses and hosts around 120 tigers. The park showcases diverse habitats, including floodplains and grasslands. Previous research has suggested that CNP provides a favorable environment for *Bubalus arnee* [25]. The *Bubalus arnee* population is exclusively found in KTWR, where the main challenge is interbreeding with domestic

buffalo. In an effort to address this issue, the government of Nepal translocated a total of 15 animals (13 from KTWR and 2 from a zoo) to CNP, where they are being managed in a closed enclosure.

Koshi Tappu Wildlife Reserve derives its name from Nepal's largest river, the Koshi. The reserve's headquarters are located in Koshi Rural Municipality-3, Sunsari. Geographically, it is situated between 26°33′57″ to 26°43′40″N and 86°55′15″ to 87°05′02″E. Established in 1976 with an initial coverage of 65 km^2, it was expanded in 1980 to include the floodplains of the Koshi River. The primary objective was to protect the remaining population of *B. arnee*. It was designated as a Ramsar site on December 17, 1987, and a buffer zone was established in 2004. KTWR encompasses an area of 175 km^2, with an additional 173 km^2 designated as a buffer zone. It spans the alluvial floodplain of the Koshi River, extending across parts of the Sunsari, Saptari, and Udayapur districts (Figure 1).

Similarly, Chitwan National Park is located in the southern regions of Bagmati, Madhesh, and Gandaki provinces in Central Nepal, encompassing the Chitwan, Makawanpur, Parsa, and Nawalparasi districts. Its geographical coordinates range from 27°20′19″ to 27°43′16″N and 83°44′50″ to 84°45′03″E on the floodplain of the confluence of Rapti and Narayani rivers in the lowland of central Nepal. Originally designated in 1973 with an area of 544 square kilometers, the park was later expanded to 932 km^2. In 2016, the park and its buffer zone were amended to cover a total area of 729.37 km^2, and currently, they span 952.63 km^2. The park's major boundaries are formed by rivers, hillocks, fireline, and the Nepal-India international border. Due to its exceptional biological resources of universal significance, CNP was designated a UNESCO World Heritage Site in 1984 (Figure 1).

2.2. Natural Habitat (KTWR) and Managed Site (CNP) for the Translocated Water Buffalo. The *B. arnee* have found their existing habitat in the tropical forests of Koshi Tappu Wildlife Reserve (KTWR), which are characterized by the presence of big rivers, flood plains, open patches of grassland, running water, and riparian sites. Meanwhile, in Chitwan National Park (CNP), the managed sites for the translocated wild water buffaloes are chosen by the park authority, specifically at old Padampur, which encompasses approximately 30 hectares and is enclosed with mesh wire fencing. This enclosure serves multiple purposes, providing protection from predators such as tigers and other wild animals, as well as facilitating regular monitoring. Within KTWR, the wild water buffaloes primarily inhabit the muddy waters of the tropical landscape. The river banks and beds are dominated by species such as *Senegalia catechu* and *Dalbergia sissoo* [26]. Other riverine forests consisting of deciduous species such as *Trewia nudiflora* and *Bombax ceiba* also serve as locations where the wild water buffaloes spend their time. Approximately 80% of the land area is covered by tall grass species, including Saccharum and Phragmites. The flowing water of Sapta Koshi, a river, provides an ideal habitat for wallowing, and the wild water buffaloes spend a significant portion of their daytime engaging in this behavior. The riparian trees, shrubs, grasses, and availability of water create favorable conditions for grazing and wallowing.

On the other hand, the site where the translocated wild buffaloes are kept in CNP is characterized by the dominance of tall riverine grasses such as Saccharum spp., Imperata spp., and Phragmites spp. These grasses serve as a primary food source for the *B. arnee*. Most of the observed *B. arnee* are seen spending their time in the grassland areas and among the tall grasses. Ponds have been constructed in this area specifically for wallowing, with water collected from drainage systems. In cases of water shortage, alternative sources such as borehole water may be used. The selected site in CNP consists of a riparian habitat along the tropical river, encompassing the banks and a combination of trees, shrubs, and grasses with ample water availability. In both protected areas, the activities of the bulls were documented during two different seasons. In Koshi Tappu Wildlife Reserve (KTWR), the behaviors of "Bull A" and "Bull B" were recorded, while in Chitwan National Park (CNP), the activities of "Bull M" and "Bull J" were documented. The selection of these bulls was based on criteria such as their large body structure, significant horn size, and their established dominance within the designated areas (Table 1).

2.3. Data Collection. Examining how a species apportions its time among various activities constitutes an integral aspect of its lifestyle and behavior [27]. Animals adapt to shifts in environments in order to optimize the use of resources for survival and reproduction [28]. The fundamental factors that influence animal behavior and activity patterns [29–31] encompass spatial distribution, food resource quality and seasonality, dietary requirements [32, 33], reproductive strategies [34], seasonal variations, weather conditions, human-induced pressures, additional mineral supplementation, and more. Just as with other mammalian species, male and female wild buffaloes exhibit dissimilar energetic needs, often associated with variations in body size, a phenomenon prevalent across most herbivorous species [35, 36]. Reports indicate disparities in feeding and resting times among sexually dimorphic species; notably, males tend to consume less and rest more than their female counterparts in such species [34], which aligns with general observations of wild buffalo behavior. An alternative explanation might stem from the fact that lactating and pregnant females allocate a greater proportion of their time to feeding, compensating for the additional energy demands [37].

The research focused on studying the behavior of *B. arnee* under management at KTWR and CNP. Behavioral observations were conducted during two specific periods: the premonsoon and postmonsoon seasons of the year 2018. In KTWR, the premonsoon season spanned from June 23 to July 9, 2018, while in CNP, it took place from July 13 to July 23, 2018. The period after the monsoon was monitored in Chitwan National Park (CNP) from August 31 to September 11, 2018, and in Koshi Tappu Wildlife Reserve (KTWR) from September 13 to September 28, 2018. These monitoring sessions occurred every seven days. The choice of

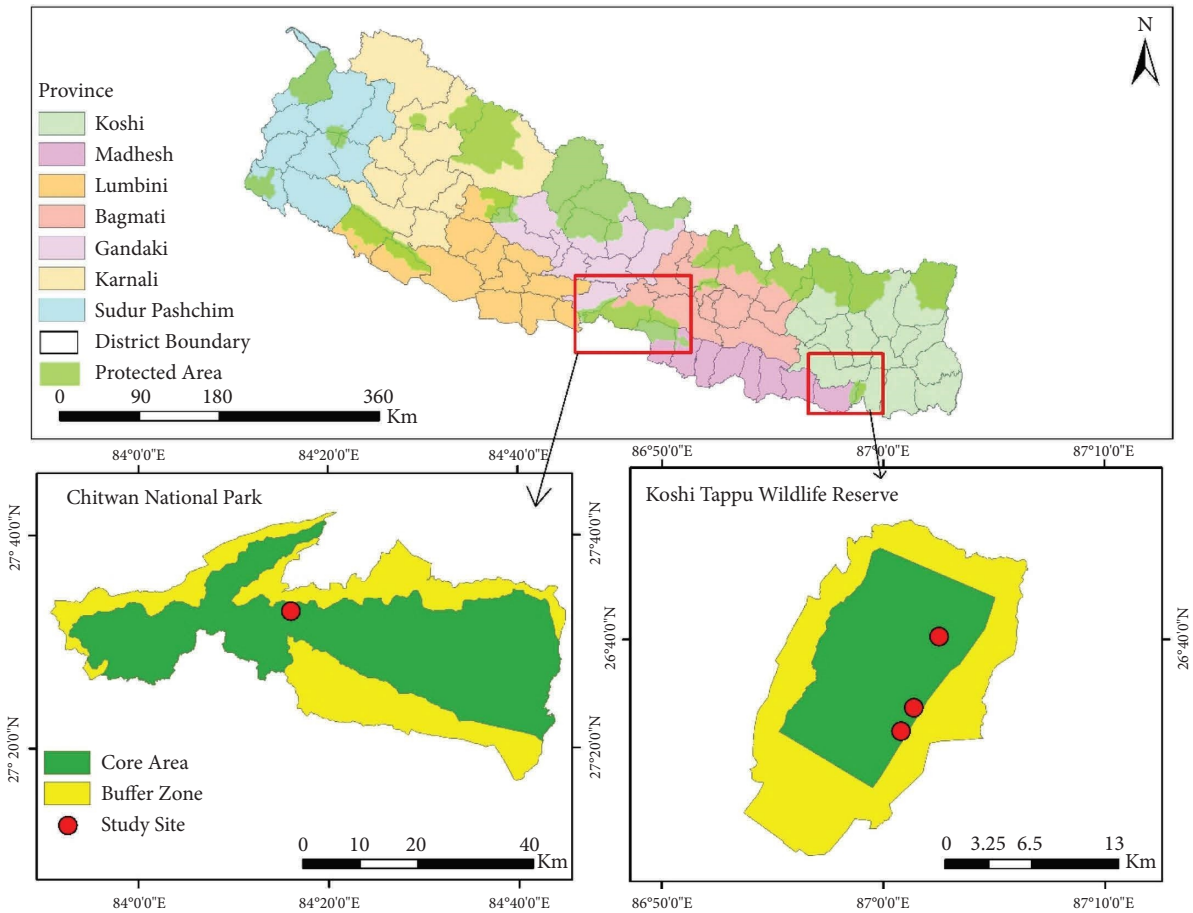

Figure 1: Map of the study area.

observation dates was guided by management expertise to guarantee a thorough study of *B. arnee* behaviors. Whenever possible, days characterized by sunny and clear weather conditions were selected for the observations. Each day, observations were made three times altogether 11 hours per day: morning (06:00–11:00 am), day (12:00–04:00 pm), and evening (05:00–7:00 pm). To facilitate the observations and record behaviors, binoculars, a camera, and a GPS instrument were utilized. The data collection involved direct observation using different sampling techniques, including focal animal sampling, continuous sampling, and scan sampling [38, 39].

2.4. Focal Animal Sampling. Wild buffaloes exhibit passive movement patterns and are typically territorial animals engaged primarily in behavioral activities during foraging, including feeding, resting, moving, wallowing, mating, and social interactions within their designated territories. They form groups of varying sizes, although some adult males may choose to remain solitary. Notably, despite the broader geographical distribution of wild buffaloes on both sides of the main course of the Koshi river, our focus was primarily directed towards the eastern block of the wildlife reserve. This emphasis stems from the fact that the primary herds are commonly found in the eastern region of Koshi Tappu [38, 39]. The focal animal sampling technique, outlined by Altmann [40] and also followed by Chalise [41, 42], was employed to document the social and ecological behavior of the wild buffaloes. The selection of focal animals was randomized for each observation session, which lasted approximately 6 hours per sampling session day. Each month, a total of 12 days were allocated, with half a session completed on one day and the remaining portion on the subsequent day, resulting in a complete full-day session.

To assess the reproductive behavior of *B. arnee* in KTWR and CNP, the focal animal sampling method was employed, with a preference for adult animals as focal subjects due to their potential influence on others within the group. This approach involved selecting specific animals (e.g., bull A, B, M, and J) and observing them at five-minute intervals using the focal animal sampling technique [40]. During these observation periods, all activities displayed by the selected animal were meticulously documented. Observations were conducted over the course of a year, encompassing both premonsoon and postmonsoon periods of 2018. On average, four animals were chosen as focal subjects each day over the course of a year. These focal animals consisted of solitary subadult to adult males, or a subadult to adult male from a male band group, or a subadult to adult male or female from the primary herds. To ensure the accuracy of data collection,

Table 1: Description of bulls in the study area.

Name/ID of bull	Descriptions and role of bull	Location
A	The dominant bull in the study area possesses a substantial horn and body size, serving as the primary mating bull, with a slight cut on the upper side of its right ear	Koshi Tappu Wildlife Reserve (KTWR)
B	The bull, identified as B, exhibits smaller horn and body dimensions compared to bull A. Bull A and bull B are frequently observed together, and bull B holds the position of the second most dominant bull in the study area	Koshi Tappu Wildlife Reserve (KTWR)
M	In the study area, only two bulls, namely M and J, were identified. Bull M served as the primary mating bull and held the title of the most dominant bull in the study area	Chitwan National Park (CNP)
J	The subadult bull is not intended for actual reproduction or mating purposes	Chitwan National Park (CNP)

* *Note*. The primary or sole recorded bull activities at KTWR included those of bulls A and B. Conversely, in CNP, only two bulls, namely, M and J, were identified.

only one animal (e.g., bull A) was observed at a given time, with the activities of other individuals within the group not being recorded or observed. After the designated observation time for a specific individual (e.g., bull A) was completed, attention shifted to bull B, followed by M and J. This sequential process continued until all animals within the group had been observed for the designated timeframe. These observations were then utilized to calculate seasonal variations in time allocation to different activities, as previously elucidated [25, 39, 43].

2.5. Continuous Monitoring. Continuous sampling involves recording the complete duration of behaviors displayed by an individual. While this approach offers a comprehensive account of behavior patterns, this sampling technique is resource-intensive and is often limited to a singular animal or a specific behavior of interest. In many research endeavors, a combination of interval and all-occurrence sampling methods proves to be adequate, enabling the recording of a broader range of behaviors across multiple focal individuals. Interval sampling necessitates the recording of behaviors at predetermined time intervals (for guidance on selecting these intervals, refer to "choosing the best sampling methods"). Our employed methodology encompassed regular observations of animal behavior. Throughout these monitoring sessions, we meticulously documented all activities demonstrated by the animals. For instance, behaviors such as chasing, fighting, rutting, mating, feeding, and nursing were recorded continuously throughout the entirety of our observations [25, 38, 39].

2.6. Scan Sampling (Group Sampling). In the group scan sampling method, we tally the number of animals engaged in a specific behavior. To understand the utilization of habitats and behavioral patterns of wild animals, we employed scan sampling. This approach involved documenting the behavior of all individuals within a group at predetermined time intervals. For instance, we observed a group of animals and recorded the behavior of each individual every 5 minutes over a twelve-hour period, conducted across 27 days during two distinct seasons. The initial season spanned from June to July, while the second season occurred in September [25, 39, 43]. In addition, we collected secondary data from various governmental bodies, including the Department of National Parks and Wildlife Conservation, the administration of Koshi Tappu Wildlife Reserve, Chitwan National Park, as well as multiple international and nongovernmental organizations (INGOs and NGOs).

2.7. Data Analysis. The data that had been gathered were meticulously arranged and inputted into a Microsoft Excel spreadsheet for the purpose of conducting an analysis. To gain a deeper insight into the reproductive behavior, we utilized the ggplot2 package within R-Studio, which enabled us to generate informative visualizations. This approach facilitated the creation of graphical representations that offered a more comprehensive understanding of the reproductive patterns.

3. Results

During the premonsoon morning phase, we noted a reduced count of reproductive activities compared to daytime. In the morning period, we observed urination in all seven (43.75%) instances, while sniffing, courtship, and chasing were witnessed in five (31.25%), one (6.25%), and three (18.75%) observations, respectively (refer to Figure 2). Notably, urination displayed a high occurrence, closely followed by sniffing and chasing, whereas courtship was only observed once during the morning. Throughout the premonsoon timeframe, in the case of "bull A" observed in KTWR across six sessions, we registered at least one reproductive activity in each instance. The most prevalent behavior was sniffing, observed in all six (37.5%) instances. Fighting was ranked second, observed in four (25%) instances, followed by urination, which manifested thrice (18.35%). Conversely, chasing, mating, and courtship each occurred once (6.25% for each activity) (see Figure 3).

We recorded various activities including fighting (4%), sniffing (24%), courtship (12%), urination (24%), and mating (32%) during the postmonsoon days. Mating emerged as the most frequently observed behavior, trailed by sniffing and urination. In contrast, courtship was observed thrice (12%) and fighting was observed just twice (4%) in the morning. Particularly for "bull A" at KTWR, the morning stood out as the preferred time for mating during the postmonsoon period. Sniffing and urination occurred six times (24%) (see Figure 4).

Throughout the daytime, the most prevalent behavior was sniffing, recorded six times (50%), trailed by courtship and mating, each occurring twice (16.66% for each activity). Urination and chasing were the least observed, each noted once (8.33% for each activity). During the postmonsoon evenings at KTWR, "bull A" exclusively engaged in courtship. During nighttime, "bull A" was observed mating and urinating once each, while sniffing occurred twice. In contrast, at CNP during the premonsoon mornings, "bull M" displayed only two reproductive activities, chasing and urination, each occurring once (refer to Figure 5).

For "bull M" at CNP during the postmonsoon mornings, urination, sniffing, and courtship were each witnessed twice (40%), while courtship occurred only once (20%). Likewise, during the same timeframe, "bull J" exhibited courtship and urination, each observed only once (50% for each activity) (refer to Figures 6 and 7).

A total of seven to nine distinct observations were conducted for the bachelor herd. Among these, grazing was the most frequently (33.33%) recorded activity during the premonsoon period, followed by standing (25.80%) and fighting (22.5%). Similarly, during the postmonsoon phase, standing (27.77%), fighting (25%), and water drinking (8.33%) were frequently observed. False mating occurred in both seasons, and urination was less commonly witnessed (refer to Figures 8 and 9).

3.1. Reproductive Behavioral Observations of Both Male and Female. All wild water buffalo exhibit a behavior known as the counter-check, which involves them reacting as if there is a potential threat or enemy nearby. They engage in chasing and running for some distance, pause momentarily to look

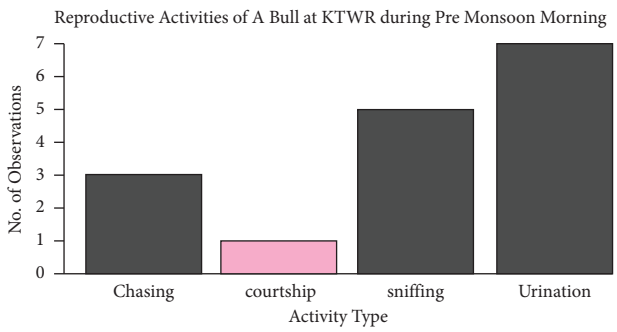

Figure 2: Reproductive activities of bull A at KTWR during the premonsoon morning.

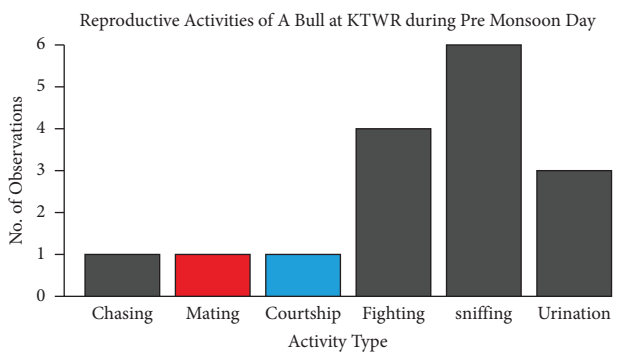

Figure 3: Reproductive activities of bull A at KTWR during the premonsoon day.

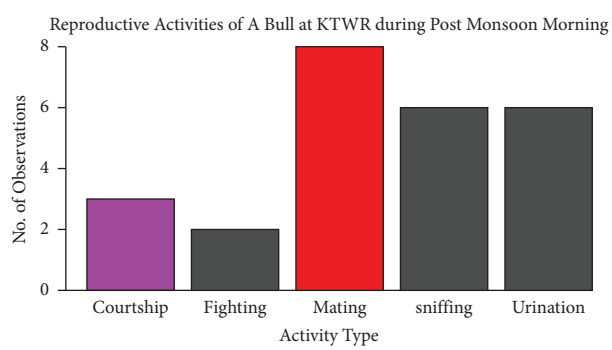

Figure 4: Reproductive activities of bull A at KTWR during the postmonsoon morning.

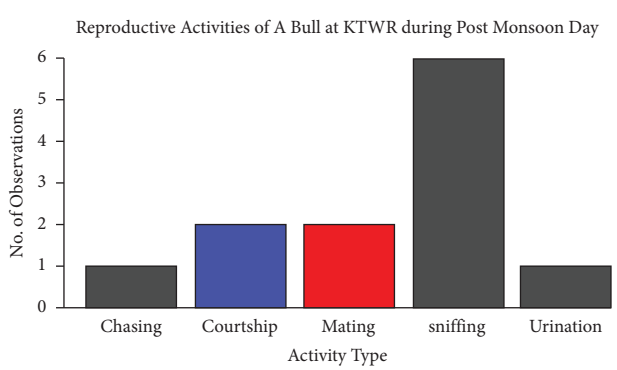

Figure 5: Reproductive activities of bull A at KTWR during the postmonsoon day.

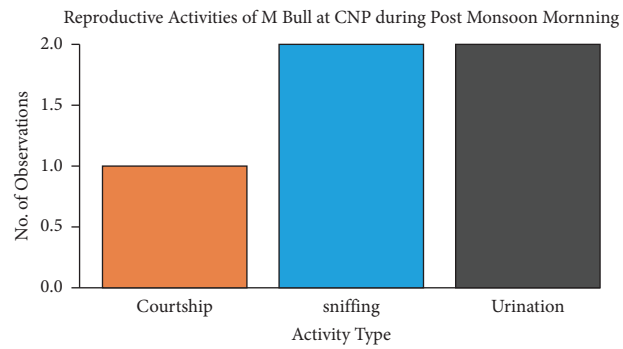

Figure 6: Reproductive activities of bull M at CNP during the postmonsoon morning.

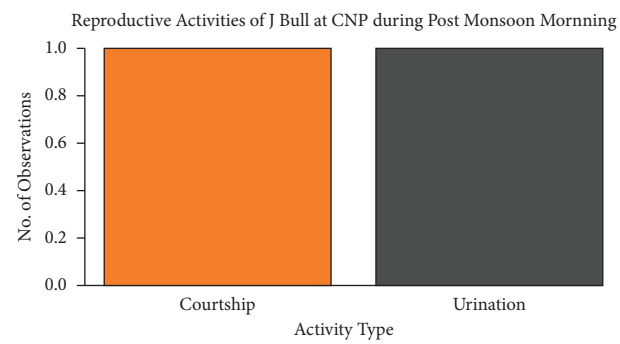

Figure 7: Reproductive activities of bull J at CNP during the postmonsoon morning.

backward and then continue the chase. The reproductive behavior of wild water buffalo, similar to many other wild animals, is influenced by seasonal changes in environmental conditions and resource availability (Figure 10). During the breeding season, male bulls compete for mates, engaging in aggressive interactions. This includes dominant males vying for mating rights over a group of females. Bulls often employ vocalizations such as loud roars and bellows to assert dominance and attract receptive females. They establish their territory and communicate their presence to females through scent marking, using a small amount of urine and feces. Dominant bulls may also display behaviors like wallowing in mud, rubbing against tree trunks, and making themselves appear more imposing to attract females. Once a bull establishes dominance, he approaches a receptive female and engages in courtship behaviors, which may include sniffing, licking the female's genital organ, and gentle rubbing. Actual mating typically occurs within one to two days.

Female wild water buffalo display signs of estrus or being in heat when they are ready for mating. These signs include increased restlessness, mounting by other females, and allowing males to approach and mate. In some cases, female wild water buffalo may synchronize their estrous cycles, leading to multiple females coming into heat simultaneously. This intensifies competition among males for mates. Females may actively choose their mates, preferring dominant and physically superior bulls, which is crucial for successful reproduction. After mating, female wild water buffalo have a gestation period lasting approximately from 9 to 11 months. They are generally

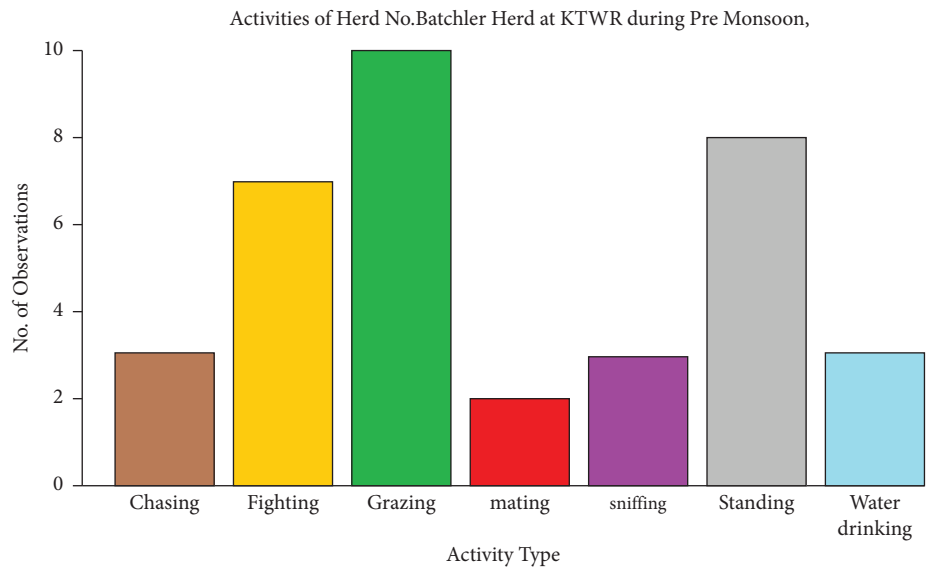

Figure 8: Activities of bachelor herd at KTWR during the premonsoon.

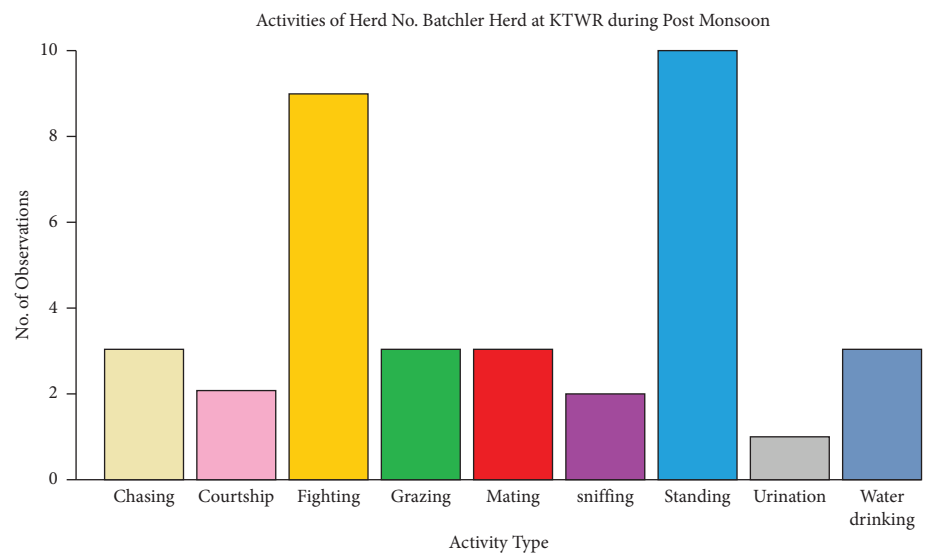

Figure 9: Activities of bachelor herd at KTWR during the postmonsoon.

protective and nurturing mothers, providing care and protection to their calves to ensure their survival.

When analyzing the postmonsoon reproductive behavior of the observed *B. arnee* in KTWR and CNP, a notable disparity was observed. Specifically, KTWR exhibited a significantly higher frequency of sniffing behavior, with four more instances than CNP. In addition, courtship behavior was slightly more prevalent in KTWR, occurring three times, compared to CNP where it occurred twice. Similarly, urination activity was observed twice as frequently in KTWR compared to CNP.

4. Discussion

The decision to translocate 15 *B. arnee* from KTWR to CNP was made by the Department of National Parks and Wildlife Conservation. The WWBs were placed in a 30-hectare enclosure within CNP, where the park authorities have implemented various measures to maintain a habitat similar to KTWR. These measures include fencing to protect the buffaloes from threats, grass cutting, water management from the Chure area, the construction of wallowing ponds, and the establishment of a view tower for better monitoring. However, the success of these measures has not been 100 percent [44]. According to Chaudhary [45], the typical habitat for WWBs is strongly associated with grasslands and water bodies, preferably in low-lying areas. The *B. arnee* tends to inhabit tall grassy areas near swamps, streams, river banks, open forests, and dry thorn scrub, particularly in floodplains. KTWR, established in 1976 and designated as Nepal's first "Ramsar site" in 1987, contains extensive wetlands that provide crucial wintering grounds for international waterfowl. The natural habitat of *B. arnee* in

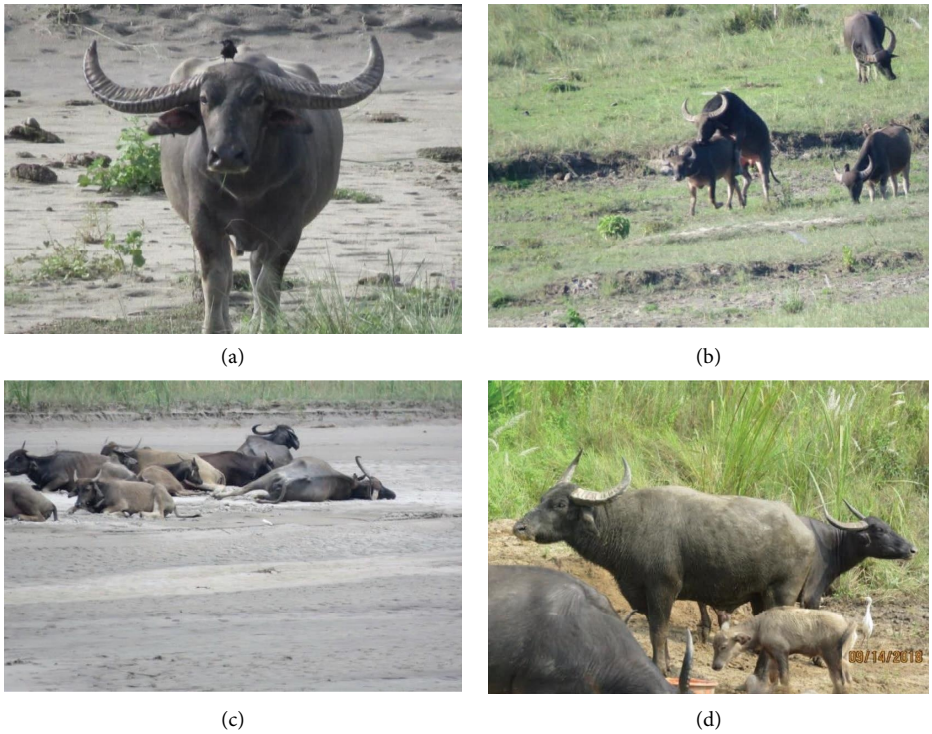

Figure 10: *B. arnee* in KTWR and CNP. (a) Bull A in KTWR. (b) Mating of *B. arnee* in KTWR. (c) *B. arnee* is resting in a sand bank at KTWR. (d) *B. arnee* in enclosure CNP.

Nepal is found within the tropical forest of KTWR, which features a complex mosaic of grassy marshes, oxbow lakes, swamp lakes, and water-retaining depressions throughout the floodplain. The open grasslands on the floodplain of the wildlife reserve serve as foraging and grazing areas for *B. arnee* and have been replicated within enclosures by creating artificial ponds and water bodies, although the availability of grassland and water bodies within the enclosures seems limited. Thus, our observations suggest that KTWR provides a suitable habitat for *B. arnee*. However, the park management in CNP, despite maintaining high standards for the natural habitat of *B. arnee*, faces challenges in providing running water for wallowing.

The results we obtained reflect the seasonal variation in the reproductive activities of *B. arnee*, specifically the bulls. Throughout the study, we observed a total of six reproductive behaviors: sniffing, fighting, urination, chasing, courtship, and mating (Figure 10). Sniffing was the most frequently observed activity, followed by fighting and urination. Chasing, mating, and courtship were observed only once. In the morning, urination was observed in all observations, while sniffing, courtship, and chasing were observed in five, one, and three observations, respectively. During the study, "bull A" at KTWR exhibited mating activities in both seasons, while "bull B" at KTWR and "bulls M and J" at CNP did not engage in mating activities during the premonsoon period (June-July). This decreased or absent mating activity during the premonsoon period may be related to the environmental conditions in the study area. Although buffaloes can adapt to hot, humid areas with muddy and swampy lands, they are also susceptible to heat stress, which directly affects their reproduction [46]. In our study site, the premonsoon period represents hot summer weather, while the postmonsoon period represents milder temperatures. It has been suggested that higher temperatures characteristic of the body core can disrupt spermatogenesis in bulls, and heat stress can impact male gametogenesis. In addition, warm summers have been associated with a lower surplus of unmated males. Vale [47] reported that the male buffalo is minimally affected by local thermoregulation of the testis due to its complex anatomical and physiological system for controlling the blood temperature in the testis. Our study's findings align somewhat with previous studies, as we found that bulls are less active during hot weather, particularly during the premonsoon period, while they remain reproductively active even at night during the postmonsoon season.

The population of WWBs has been increasing since the establishment of KTWR in 1976, with numbers rising from 63 to 441 in 2018 [25, 48, 49]. Nevertheless, despite the advancements in our understanding of the reproductive behavior of this species, a multitude of threats and challenges persists, casting a shadow over its conservation. The specter of inbreeding, exacerbated by gene pool dilution from domestic buffalo, along with the ominous shadow of diseases disseminated by domestic livestock, recurrent floods, sporadic incidents of poisoning and electrocution, and the relentless struggle for resources and space with cattle and domestic and feral buffaloes underscore the ongoing perils faced by this remarkable species [50]. The endeavor to establish an alternative population of wild water buffaloes (WWBs) through the translocation of fifteen individuals,

twelve from KTWR and three from the central zoo, encountered heartrending setbacks. Tragically, five of these translocated individuals succumbed to the fury of a devastating monsoon flood a mere four months after their relocation [51]. In addition, the burgeoning tiger population has emerged as a formidable threat to WWBs within the CNP [52]. These pressing challenges underscore the urgency of implementing swift and effective interventions to fortify the preservation of the wild buffalo population. As we confront these multifaceted challenges, resolute and immediate actions are imperative to secure a thriving future for this species within its habitat.

5. Conclusion

In conclusion, our investigation offers valuable insights into the reproductive conduct of *B. arnee* within Koshi Tappu Wildlife Reserve and Chitwan National Park throughout the pre- and postmonsoon phases. The notable prevalence of sniffing, fighting, and urination underscores the significance of these behaviors in the reproductive dynamics of *B. arnee*. Notably, a reduced frequency of reproductive activities was observed during the premonsoon morning compared to daytime. Nevertheless, during the postmonsoon period, reproductive activity was evident among bulls at both study locations. Intriguingly, while one bull participated in mating, three others did not engage in this behavior during both seasons, emphasizing the need for further investigation into the factors shaping the mating behavior of *B. arnee*. When analyzing the postmonsoon reproductive behavior of the observed *B. arnee* in KTWR and CNP, a notable distinction emerged. In KTWR, there were four more instances of sniffing behavior compared to CNP. In addition, courtship behavior was slightly more frequent in KTWR, occurring three times, whereas it was observed only twice in CNP. Furthermore, considering the critical jeopardy faced by the natural habitat of *B. arnee* within the study areas, the outcomes of our study hold substantial ramifications for conservation endeavors. A comprehensive conservation strategy that involves mobilizing buffer zone communities and enforcing robust legal measures must be devised to safeguard the *B. arnee* population within these regions. Such measures are crucial to ensuring the perpetuation of the species and its native environment for the welfare of future generations.

Data Availability

The data used to support the study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors thank Dr. Ram Chandra Kandel, Director General, Department of National Park and Wildlife Conservation, and the Hattisar team of KTWR and CNP for their help during field visits and data collection. We are also thankful to Professor Dr. Bala Ram Bhatta, Dean Faculty of Forestry at AFU, for his kind help and suggestions throughout this research. This research was financially supported by the World-Wide Fund for Nature (WWF Nepal Hario Ban Program).

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