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Corso di laurea /First Cycle Degree (B.Sc.) in Animal Care

Social behaviour and activity levels in a group of six captive Asian smallclawed otters (*Aonyx cinereus*)

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SUMMARY

The Asian small-clawed otter is one of the most common species held in zoos and aquariums due to its high charismatic role with public, and its playfulness, docile and gregarious nature. Although the species have been highly investigated in captivity, little is known about its wild populations (*Kruuk, 2006*). According to IUCN, the species is considered Vulnerable and in decrease, mostly because of habitat loss and pet trade. It is therefore essential to develop knowledge on the behaviour of these otters in order to be able to refine their management and well-being in captivity, but also to promote educational programs that offer precious moments of empathic connection with the animal world.

To achieve the goal, this study tries to investigate the activity levels and the behavioural allocations in a family of six captive Asian small-clawed otters held at Cappeller zoo (VI, Italy). Another objective of the research is to analyse social networks in order to detect possible patterns of interactions, individual antagonisms or preferences.

The analysis has been divided in three macro-periods, from 29th May 2023 to 28th July of the same year. The first two weeks were dedicated to initial observations to recognise animal and to develop the working ethogram while the remaining 25 days were dedicated to official data recording.

The statistical analysis, which is still ongoing, confirmed a significant influence of observation time on animal visibility (p-value=3.487e-06) and behavioural allocations (p-value<0,002), thus demonstrating the relevance of this information to properly plan guided tours and animal management. On average, the otters were visible only for 50% of the total recording time and the most exhibited behaviours were rest and looking around (6.7-14% and 8.2-13.1% of the total time respectively).

Social behaviours represented only the 5-9% of the total time, with the mother (Fm) and one of the male offspring (M1) being the preferred individuals for social rest and allogrooming. On the contrary, the presumably youngest male offspring Mp, and the other unique female offspring Fn, were the most playful.

1. INTRODUCTION

1.1 The role of zoological gardens

A zoo, abbreviation of zoological garden, is a "permanent establishments where animals of wild species are kept for exhibition to the public for 7 or more days a year..." (COUNCIL DIRECTIVE 1999/22/EC of 29 March 1999). Contrary to certain popular belief, these structures have values and legal obligations that go far beyond mere entertainment and therefore invest these places of scientifical and educational importance. Dealing with its scientifical and conservational role, a zoo located in the European Union must not only guarantee the welfare of the animals it houses, but it also must be involved in research and conservation projects aimed to protect biodiversity and species' natural habitat, as well as to help developing technique to properly care and breed animals in captivity. Considering their presence in the territory and their public attractiveness, especially to children and families, zoos have also another fundamental role that is education. In particular, their focus should be put on the promotion of animals' habitats and behavioural knowledge, in addition to conservation and environmental respect and protection. Essential to fulfil the educational role is to provide to visitors not only the simple tour of the park, but also information and engaging experiences able to catch their attention and to remain impressed to stimulate their awareness and thoughts. One way proposed to achieve this result is to focus on human emotional connection with animals, through visit experiences that promotes views from different perspectives, immersion into animal's world and that highlights individual's character and specificity (Packer, 2010).

1.2 Study aim and possible applications

The aim of this study is to provide an initial knowledge on the levels of activity and social relationships in a group of six Asian small-clawed otters housed at Cappeller Zoo (VI). In a zoo it's very important to know the activity levels and daily habits of each species because in this way it's possible to monitor their well-being and to plan properly their management, in particular regarding training and enrichment programmes. Furthermore, the precise awareness of animals' routine might be useful to schedule guided tour and other visitor experience in those moments in which animal's behaviour offer more emotional engagement and hints for thoughts. Besides, the behavioural studies carried out in captive populations might also offer a starting point knowledge to understand and explore more in deep the behaviour of wild conspecifics.

1.3 Asian small-clawed otter

The Asian small-clawed otter is the smallest and least aquatic among the otter family. Due to their high adaptability, they can inhabit a wide range of habitats, from tropical wetlands to lake, canals, forests, and mountain streams. The species range runs through Himalaya, South Asia, Philippines and Indonesia (Figure 1) but the actual population spreading is decreasing. The IUCN Red List consider this species as Vulnerable (Figure 2) and the population is decreasing due to habitat destruction for human agriculture and settlement purposes (De Silva, 2015).

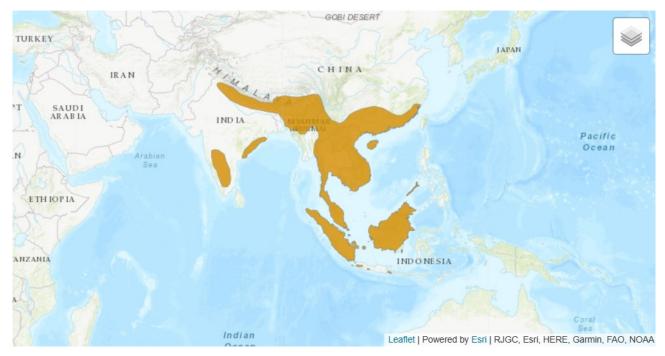


Figure 1) The map shows the distribution of Asian small-clawed otter in the World (De Silva, 2015)



Figure 2) Classification of Asian small-clawed otter according to IUCN Red List

Unlike other otter species, the Asian small-clawed otter is more gregarious and playful, and therefore it's very common in zoos exhibits. It is a species that has been much investigated from a biological, medical and behavioural point of view in captivity, while very little is known about its ecology and social structure in the wild. They usually live in family groups from four to eight individuals composed of a dominant breeding pair and many generation litters that help in predator detections and in young rising. However, they are known to be solitary hunters (*Kruuk, 2006*). According to EAZA guidelines, in captivity its accepted to house these animals in adult couples, in couples with offsprings or even in single sex groups even though it's not advisable to keep more than two adult females in the same group (*Heap, 2008*).

The aim of the present study were two:

- 1. To investigate the activity levels and the distribution of varies behaviour during day hours
- 2. To investigate the social relationship within a family of six captive Asian small-clawed otters, focusing on the group's dynamics.

2. MATERIALS AND METHODS

2.1 The history of Parco Faunistico Cappeller and the Asian small-clawed otter's exhibit

This study has been carried out at Parco Faunistico Cappeller, a zoological garden located in Cartigliano (VI). Originally a private park, the zoo has been open to public in 1998 and it owns a very peculiar collection of plants and trees. At Parco Faunistico Cappeller is also possible to find a wide variety of animal species housed in enclosures that try to mimic the species' natural habitats. As a member of UIZA (Unione Italiana dei Giardini Zoologici e Acquari), the zoo is committed to being a place for education, scientific research and for the protection of biodiversity.

This study has been carried out in the Asian small-clawed otter enclosure, which is a permanent exhibit at the Cappeller zoo (Figure 3).



Figure 3) Map of Parco Faunistico Cappeller showing the position of different species' enclosure and the suggested visitor pathway. The blue and yellow circle highlights the position of the Asian small-clawed otter's enclosure.

The Asian small-clawed otter's enclosure is developed in length, and it can be functionally divided in four major areas: the pool, the raised area, the ground-level area, and the keeper entrance. The otters are free to move between these different parts even though to reach the keeper entrance they have to climb a little fence. The first three areas are completely visible to public while the keeper entrance it's not. The enclosure also presents three different dens, two in the raised area and one on the ground-level area. These dens are very frequently used by the animals, however from the outside it's possible to see only their entrance. Next to the pool, there is a little flat rock partially hidden from visitors' eyes which can be reached by the animals only diving. The remaining part of the enclosure is enriched by little stones and wood pieces that frequently becomes part of otters' play moment, while the background is completely covered by fake rock installation which can be partially climbed by the animals. The barriers are made by glass and mesh fences with electric wires on the top.

The group of Asian small-clawed otter is fed two times per day, at 8:00 and at 16:00, and their diet is based on small fresh trouts. A general quick cleaning of the exhibit it's performed daily, while the complete change of straw in the dens and the pool's emptying occurs two or three times per week, according to season and weather conditions.

The group of Asian small-clawed otters analysed during this study is composed of six related individuals, namely the mother, four males and one female offsprings. The female and one male belong to the same last litter occurred in 2022, whereas the other three males were born in different years, and thus in 2017, 2018 and 2020.

2.2 Individuals' recognition and preliminary observations

The period of observation at Parco Faunistico Cappeller began on 29th May 2023 and finished on 28th July of the same year, for an overall duration of 8 weeks. I carried out the research during my working days at the zoo, therefore from Mondays to Fridays within 7:00 and 17:00. Due to high levels of animals' similarities, the first two weeks of the study have been dedicated completely to individuals' recognition. To identify the differences among individuals the process has been divided in two main steps. In the first days, I carried out the observations only in person, taking notes of any advisable differences among animals, starting from a generic overview to get to a more detailed analysis of body and fur details. After this first step, the analysis has been carried out by comparing carefully different pictures taken with a Nikon D3200 camera (*Appendix, pp. 28*). This phase was particularly essential to verify the reliability of the little elements noticed in-situ, taking into consideration the variation of colour due to weather and time. The pictures have also been useful to memorize the points of individual distinction in order to speed up the consequent phases of the research. The key factors that have been used to distinguish the otters were body sizes, eyes' shape and fur patches on face and neck areas. A general rule for a broad distinction it's that male otters are larger and with thicker tails than females. Looking in details, M1 and M2 were the biggest among the whole group and M1 had a

very clear and wide brown spot with a "D" shape on his right cheek. M3 instead had a clear and complete dark brown circle all-around the right eye while the other males had only a partial dark brown line just underneath the lower eyelid. Another easy recognisable otter was Fn, the unique female offspring, because of her slender body shape, thin and long neck and, in addition, because of her little light brown spot just above the right eye.

After animals' recognition, I proceed with preliminary observations aimed to let the animals adapt to my presence and to the recording instruments. This phase has also been crucial to develop the working ethogram.

The analysis has been carried out with Ad libitum sampling, both in person and with video recordings took in different moments of the day. During preliminary observations I used a Go Pro Hero 9 attached frontally to my backpack with a specific support. In a second moment, due to the sun reflex on exhibit's glass, the camera has been equipped with a polarizing filter.

2.3 Working ethogram

The Asian small-clawed otter's working ethogram (Table 1) used in this study has been developed thanks to the information acquired during preliminary observations. However, it has been expanded and compered with the ones used in other similar research on the species, in particular for the category of social behaviours (*Ross, 2002; Azevedo et al., 2015; Rossi et al. 2020*)

CATEGORY	BEHAVIOUR	DEFINITION		
INACTIVITY/ RESTING	Looking around	While resting on the ground or		
		standing in the four-leg		
		position, the otter moves the		
		head and the gaze slowly in		
		different directions, scanning		
		the surrounding environments		
		without dwelling on any		
		particular element. During the		
		whole action the body is		
		relaxed.		
	Rest	Lay on the ground without		
		doing anything. Eyes can be		

		both open or closed and the body is relaxed.
IN ATTENTION	In attention	The muscles suddenly stiffen
		and the whole body tenses in
		the direction of the stimulus.
		Head and gaze are fixed
		towards the stimulus (located
		inside or outside of the
		enclosure).
ACTIVE BEHAVIOURS	Climb/jump	Load the hindlimbs as springs
		to propel the body up in the
		vertical plane
	Ground exploration	When walking or lying on the
		ground, the animal sniff the
		ground moving the head side to
		side
	Locomotion	Walk or run to reach a different
		point within the enclosure
	Rubbing	Press and friction the body or
		the head towards enclosure's
		ground or furniture
	Rolling	The animal lies on the ground
		on his side and, by keeping the
		back in contact with the
		ground, he turns the body to lie
		on the opposite side
	Swimming	Set of limbs and tail
		movements which allows to
		propel the body on water's
		surface
	Diving	Set of limbs and tail
		movements that allows to
		propel the body underwater

	Stone play	Rubbing, manipulating, and	
		pressing small enclosure' s	
		elements (mainly stones and	
		straw) between paws, paws and	
		body or paws and ground	
	Digging	Repetitive movement of the	
		front limbs that allows the	
		animal to move the ground and	
		create small depressions on it	
	Other interactions with the	Any other interaction between	
	enclosure	the animal and its enclosure. It	
		includes swapping straw	
		between dens, attaching to the	
		fence, moving rocks	
	Eating	Tearing, chewing, and	
		swallowing food	
SELF-GROOMING	Scratch head	When sit, the animal twists its	
		body and rub one of its	
		hindlimbs with its ears or face	
	Self-grooming	any other actions aimed to	
		scratch other body regions or to	
		keep the fur ordered	
OTHER/OOS	Other	Any other behaviour not listed	
	Out Of Sight (OOS)	The animal is not visible to	
		observer and cameras. The	
		animal is inside the dens or in	
		keeper areas (OOS gate)	
SOCIAL BEHAVIOUR	Allogrooming	Clean or smooth the fur of	
		another otter by using mouth	
		and paws	
	Social rest	Sleeping or resting in body	

Play-fight	Chase and feign fight with small bites and tackles but without aggression and wounds		
Flinch (play)	Run away from conspecifics that try to attack in play- fighting		
Agonistic behaviour	Any other behaviour where the animal tries to attack and intimidate another otter		

Table 1) Working ethogram employed during the study of activity levels and social behaviour in a group of six captive Asian small-clawed otters. The ethogram has been realised in combination with the studies from (Ross, 2002; Azevedo et al., 2015; Rossi et al. 2020)

2.4 Behavioural observations' planning

After the preliminary observations and the development of the working ethogram, I proceed with the study's behavioural observations. This phase officially started the third week of the traineeship experience but due to instrument and recording methodology's testing, it truly began only on 20th June. The overall period of definitive observations lasted six weeks for a total amount of 25 days.

The hourly pattern of observations was divided into four main categories (Table 2), distributed across days and weeks in such a way to reduce as much as possible the confounding factors to the statistical analysis. In this way it was possible to observe and study the animals' activity within the whole working hours and working week.

CATEGORY	MORNING	AFTERNOON	EVENING	
	SESSION	SESSION	SESSION	
01	8:00	11:00	14:00	
02	9:00	12:00	15:00	
03	10:00	13:00	16:00	
04	7:00	Х	17:00	

Table 2) Observations' schedule

Each session lasted 5 min of general video recording which have then been analysed with the focal continuous sampling rule. Considering the expansion in length of the exhibit and the animals' area utilisation, I decided to use two different cameras. The first one, a Nikon D3200 was placed in a fixed position to constantly record the ground-level area while the second one, a GoPro Hero 9 was attached to myself in order to follow animals' movement across the enclosure. Both cameras were provided with polarizing UV filter to limit the sun reflex on enclosure's glass. The overall recording time was of 66 sessions of 5 min each, for a total of 5h and 30 min. On 26th July, the female offspring was transferred to another zoo, therefore in her case the days of observations counted as 22, with a total amount of 59 sessions and a recording time of 4h and 55 min.

2.5 Video review, statistical analysis and limitations

Once the definitive observations were concluded, on 28th July, the study went on with another phase: the video review and the subsequent statistical analysis.

During video analysis, the behaviours performed by each otter have been transcribed into their relative Excel file. Referring to the working ethogram, each otter had, per each session, a list of all the behaviours it performed, together with the time they started, ended and their duration.

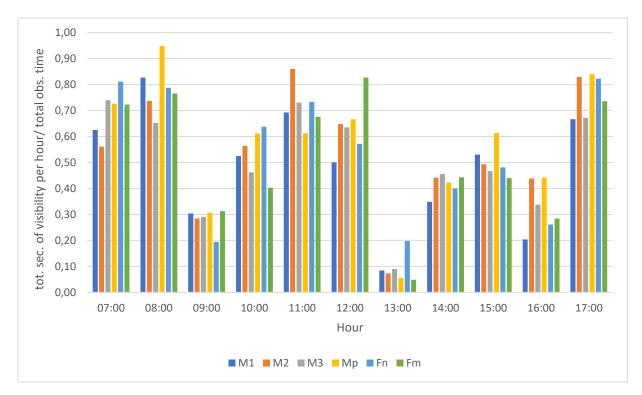
After this initial step, I made two other Excel files for each individual. The first one was a calculation of the number of occurrences, duration and frequency of each behaviour occurred within sessions, while the second one was a table of visibility. The latter considered the overall time per session in which the animal was visible and the relative percentage on the recording time (Table 3).

Starting from this data, to compare animals and to understand in which moment they were generally more visible to visitors, I calculated the ratio between the seconds of visibility of each otter per hour over their total recording time (Figure 4). Then, to overview animals' time budgets as they could be seen by visitors, I calculated, over the whole observation time, the amount of seconds each animal spent performing the behaviours listed in the ethogram (Figure 5 to 8). In a second moment, the same calculation was performed on each animal's visibility time, thus allowing to have a more precise idea of their individual differences and their real time budgets, even considering its partiality. (Figure 9). Another objective of the study was to explore the temporal distribution of animal behaviours, in order to be able to define the time slots in which they are most active, in which they perform particular behaviours or any stereotypies. To do this, considering behaviours one by one, I summed all the amount of seconds it was performed considering each animal and each hour (Figure 10). The last step

of the analysis was the evaluation of social relationship. To achieve this goal, I counted the number of interactions each otter had with the others per any of the social behaviour listed in the ethogram, and with those data I realised three different sociograms (Figure 11 to 13). Thanks to this graphical representation of social interactions, it was possible to observe the social dynamics within the group. As a final step, behavioural distribution and animals' visibility have been statistically analysed with the free software R Studio. The statistical analysis was carried out with a Two-Part model in order to normalise data's distribution (*Boulton and Williford, 2018*). The variables, initially considered as binomial, have been processed with a logistic regression model, with Pearson-Fisher chi-square test and, subsequently, with a generalised mixed model and type III Wald chi-square test for the analysis of deviance table. All statistical tests were two-tailed, with the significance level set at p < 0.05. The statistical analysis of behavioural distribution is still ongoing.

It is important to consider that all the observations were carried out only in one group of otters housed in the same enclosure, and that all the recordings were done from 7:00 to 17:00, therefore only during daylight and park's opening hours. Consequently, the results of this study cannot be generalised for the species. However, these findings provide a basal knowledge of captive Asian small-clawed otter partial time budgets, activity levels, individual differences and sociality, that might be further explored and compared with previous and subsequent researches.

3. RESULTS AND DISCUSSION



3.1 Visibility and behavioural time budget

Figure 4) This chart shows animals' visibility across day hours. The ratio is calculated as total seconds of visibility per hour/ total observation time.

	M1	M2	M3	Mp	Fn	Fm
Total visibility	48%	54%	50%	57%	54%*	51%

Table 3) Percentage of visibility of each animal. The percentages are calculated as: total seconds of visibility/ total recording time x 100%. The total recording time was of 19800 sec. * Percentage calculated on a different recording time (17700 seconds).

The visibility's analysis carried out shows that, on average, all the otters were Out of Sight, and therefore not visible to visitors, for almost half of the observation time (43-52%) even if with great variability across hours. (Table 3). Indeed, considering animals and days as random effects, the statistical model confirmed the significance of hours to explain the different chances to see or not the animal across the day (Wald chisq. Test p value=3.487e-06). This finding of course provide proves

that it may be better to plan visitors' tour in the proper park opening moment to enhance the chances to truly observe this elusive species.

The percentages of total visibility however were very different from the ones obtained by *Cuculescu-Santana et al. (2017)* with two Asian small-clawed otter male siblings, held in an indoor enclosure at the Blue Reef Aquarium Tynemouth, in England. In this case, in summer, the percentage of OOS was only at 2.5%. On the contrary, the result found with this study is in compliance with the one of *Bandoli et al (2023)*, which was done with a couple of unrelated otters, a male and a female, housed in an outdoor enclosure at Giardino Zoologico di Pistoia. In this case, the OOS corresponded to 40.48% for the female and 42.70% for the male.

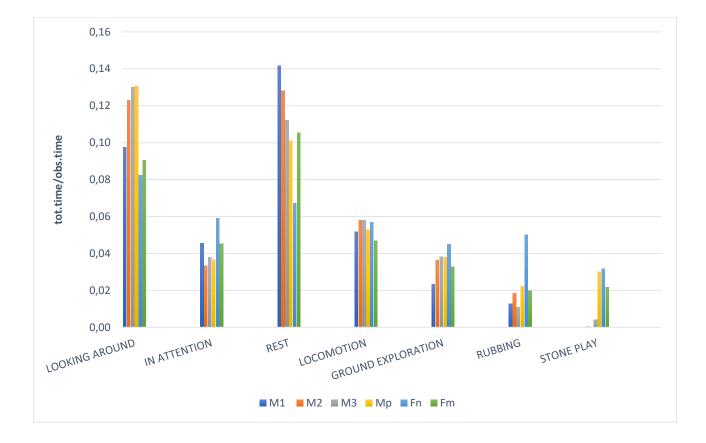


Figure 5) The chart shows the amount of time dedicated to each individual behaviour (behaviours with >0,03 at least for one animal). The values are calculated as follow: total seconds per behaviour/total seconds of observations (including OOS)

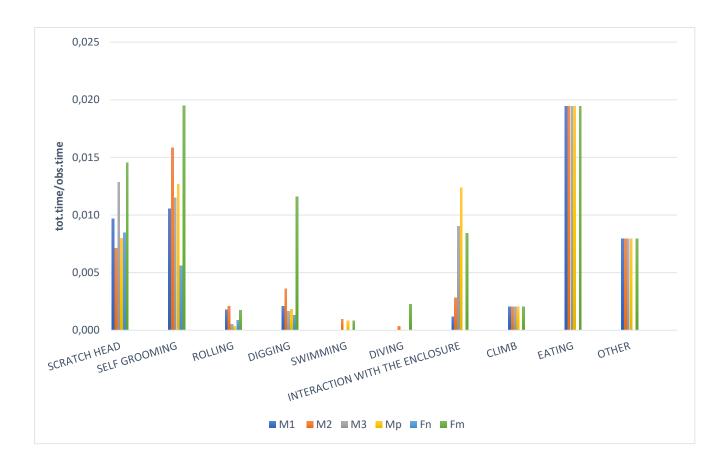


Figure 6) The chart shows the amount of time dedicated to each individual behaviour (behaviours with <0,03 for all animals). The values are calculated as follow: total seconds per behaviour/total seconds of observations (including OOS)

As for visibility, the same comparisons can be done for behavioural allocations, showing that at Cappeller zoo the most performed behaviours were Resting (6.7%-14%) and looking around (8.2-13.1%). In attention was performed in a range between 3.3% and 5.9%, while swimming was almost never seen (0-0,09%). Similarly, in *Bandoli et al. (2023)* resting was counted as 7.93% for the female and 12.17% for the male, while vigilance was at 15.88-17.51%. In this case, swimming was observed a bit more often, reaching the 3.42% and 3.46% of the total time. On the contrary, in *Cuculescu-Santana et al. (2017)* the otters were engaged in similar inactive behaviours only for 4.6% of the time, showing instead higher percentages for Swimming (33.4%) and Maintenance (15%). These evidences seem to comply with the theory proposed by *Bandoli et al. (2023)* according to which the major differences in otter's visibility and time budgets might be due to enclosure's design, even though further studies are necessary in order to consider also the effect of the group composition. Indeed, outdoor enclosures equipped with enriching furniture, shelters and vegetations, offer to animals more opportunity to hide from visitor's eyes and therefore, as suggested by *Eaza Husbandry guidelines*, are necessary for this elusive species' well-being.

Interestingly, Cuculescu-Santana et al. (2017) observed a significant relation between seasonality and otters' behaviour, showing that the two males spent significantly more time swimming in the summer, with water temperatures around the 18°C and air temperature around 16-17°C, then in winter, with water temperatures at 11°C. The study also demonstrated a decrease in the time budget dedicated to resting, vigilance, maintenance, and aggressive interactions in summer compared to winter. In summer the 87%-92.4% of the total time was dedicated to active behaviours, including 32%-34% of active behaviours on land, 15-17% maintenance and 5%-8% affiliative social interaction. In winter, the percentage of active behaviours counted as 68.1-81%. Although in my research temperature was not monitored, the average temperature in the study area exceed 30°C therefore, according to Cuculescu-Santana et al. (2017), behavioural allocations were expected to be much different. Even in Bandoli et al (2023) it was possible to notice an almost significant correlation between the behavioural allocation for vigilance and swimming with temperature and humidity. In details, the model explained how, with increasing ambient and water temperature, the animals were less engaged in vigilance and more in swimming. Looking at inactive behaviour and in attention categories, the results of my analysis were much more similar to the one of Bandoli et al. (2023) than to Cuculescu-Santana et al. (2017), even considering the much higher environmental temperature in the latter study. However, the time Cappeller's otters spent swimming was even less than in Bandoli et al (2023). Although more accurate temperature measurement throughout the day is needed, this comparison of the three studies shows that temperature variations, especially across the day, may indeed play a role in the animals' activity levels. However, the large discrepancies between the percentages of activity, especially concerning the time spent swimming, could mean that many other factors may exert a greater influence, such as animals' personality and age, group composition, daily animal management (Bandoli et al., 2023) or even other water parameter not yet considered.

When pointing out differences in results between studies, it is equally important to consider differences in settings, such as seasonality, observation schedule, and sampling rules. *Cuculescu-Santana et al. (2017)* and *Harrop and Cuculescu-Santana (2021)* carried out their researches in different seasons but for only few days each and with variable timing. Overall, they recorded the otters from 12:40 to 16:40 in the first case, and from 10:30 to 16:10 in the second, covering therefore less time compared to my study and *Bandoli et al.(2023)*, who focused the observations along the whole park opening hours, from 9 to 19 and within a period of 14 days between July and September. In additions, *Bandoli et al. (2023)* used a focal continuous sampling rule, while the other two studies were based on instantaneous and one-zero focal sampling.

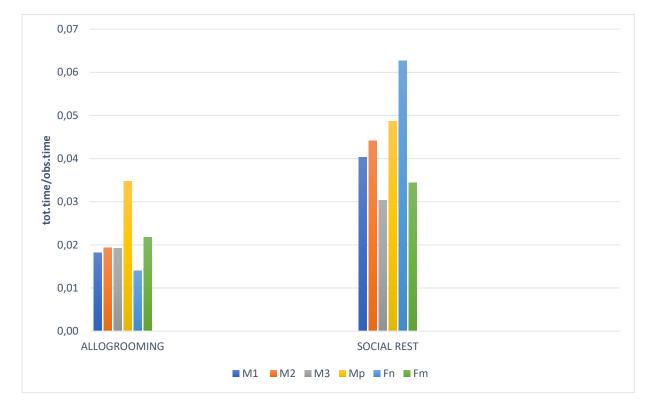


Figure 7) The chart shows the total time dedicated to social behaviour (>0.03 at least for one animal). The ratio are calculated as follow: Total seconds per behaviour/ total observation time (including OOS).

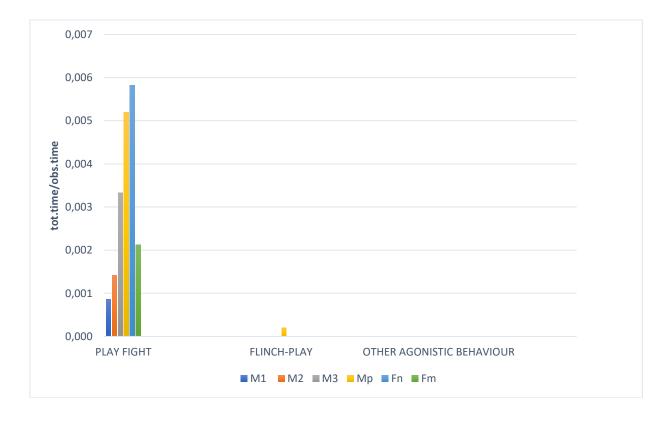
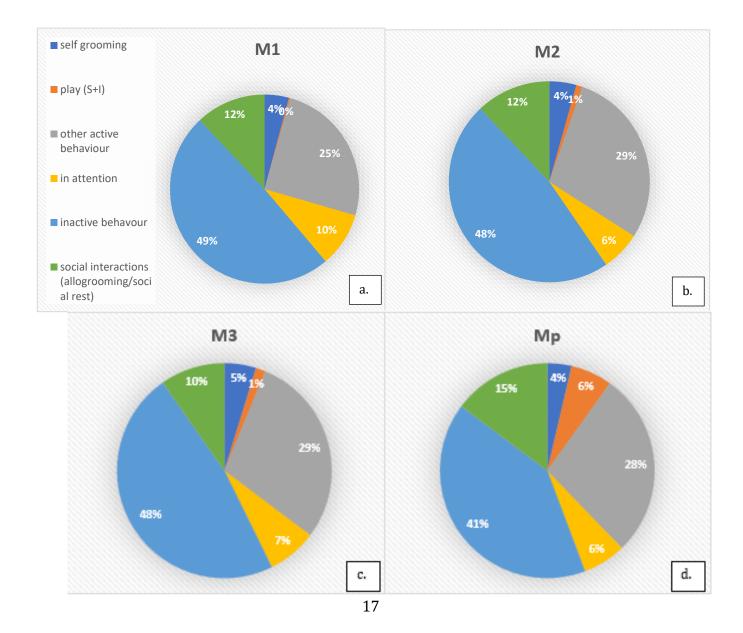


Figure 8) The chart shows the total time dedicated to social behaviour (with < 0.03 for all the animals). The ratio are calculated as follow: Total seconds per behaviour/ total observation time (including OOS).

The otters were recorded to perform social behaviours in a range between 5% and 9% on the total recording time, therefore confirming the values obtained by *Cuculescu-Santana et al. (2017)*. During the whole observation time there weren't any recordings of agonistic behaviour, suggesting a positive and balanced social environment. The result obtained in this study and in *Cuculescu-Santana et al. (2017)* were much higher than those of *Bandoli et al (2023)*, maybe due to a possible influence of the different group composition. Indeed, at Pistoia Zoo the otters were not related while in both Cappeller Zoo and Blue Reef Aquarium Tynemouth, the animals were members of the same family.

3.2 Individuals' variability and activity levels



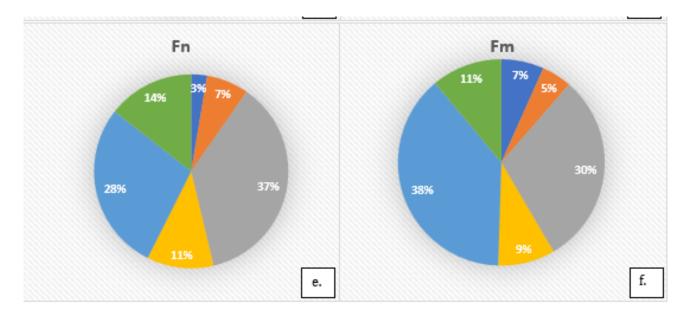


Figure 9) The pie charts show the time allocation per behavioural category on each animal's visibility time. a. M1; b. M2; c. M3; d. Mp; e. Fn; f. Fm. SELF GROOMING: self-grooming, scratch head. PLAY: stone play, play-fight. OTHER ACTIVE BEHAVIOURS: swimming, diving, locomotion, climb, rolling, rubbing, digging, eating, other interaction with the enclosure, ground exploration, other. IN ATTENTION: in attention. INACTIVE BEHAVIOUR: Rest, Looking around. SOCIAL INTERACTIONS: allogrooming, social rest

Considering visitor's point of view, the most visible animal was, in terms of seconds of visibility on the total recording time, Mp, followed by M2, Fm, M3 and M1 (Table 3). The young female, Fn, had a visibility percentage equal to M2 but the total recording time was lower because of the transfer to a new zoo. In details, all the animals seemed to follow a similar pattern of visibility (Figure 4) and, considering animals and days as random effects, the statistical model confirmed the significance of hours to explain the different chances to see or not the otters across the day (Wald chisq. Test p value=3.487e-06). Even though statistical analysis is still ongoing, according to the data collected and considering parks' opening hours, it's possible to say that the moment in which a visitor could have more chances to observe all the animals, it's between 11:00 and 12:00 or after 17:00. Is equally advisable to avoid a tour at 13:00 because the average animal visibility is at the minimum (5%-20%). Similarities among individuals were visible as well in time budgets, especially between M1, M2 and M3. Considering these otter's visibility time, the 48%-49% was dedicated to Inactive behaviours, hence Looking around and Rest, whereas 25%-29% of the visible time was for active behaviours. The percentage of inactivity was lower for Mp and Fn, 41% and 28% respectively, while the percentage dedicated to play, social and individual, increased significantly (6%-7% against 0%-1%). Halfway between these two groups there is Fm, who exhibited inactive behaviours for the 38% of the visible time, similar play and social interactions (5%) of Mp and Fn, but slightly higher percentages for self-grooming (7%) and attentiveness (9%) than the male offsprings (Figure 7).

In general, it was possible to notice more activeness and social interactions in Mp and Fn, especially at 11:00 and 12:00, while feeding related behaviours and vigilance were higher close to 8:00 and 16:00-17:00(Figure 8). While microchip reading before transfer confirmed that Fn correspond to the youngest female that was born in 2022, the same cannot be said for the other male otters. Indeed, due to management and stress-related issues, it was not possible to scan animals' microchip. However, considering the higher levels of activity and play, my hypothesis is that Mp was Fn's litter brother. This could explain the percentage's diversity among the animals of the same family. In fact, activity levels, play and interactions with the surrounding environment are often affected, in many species, by biological factors such as age (*Head et al., 1997; Ward et al., 2023*).



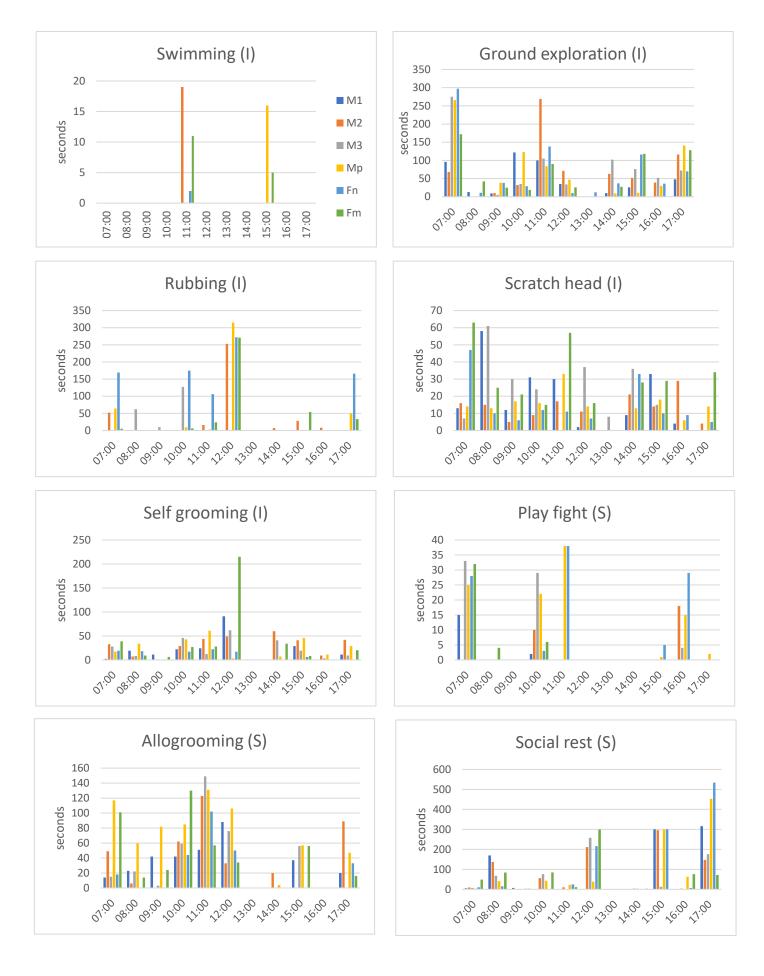


Figure 10) The charts show the total seconds each animal spent performing each behaviour in every hour of observation. I. individual behaviour; S. Social behaviour

On average, it's possible to observe that, while Looking around was performed across the whole day without significant variations, Rest was exhibited more towards the hottest hours of the day, thus at 11:00 and 12:00, even though with individual's variability. Both for Looking around and Rest, the different observation times resulted to be statistically significant to determine the chances to see the behaviour performed or not (p < 2.2e-16 and p = 0,0001). However, focusing only on the amount of time they were effectively recorded each hour, the significance was altered by other variables such as animal, sex, and day.

Among Active behaviours, the most registered was Locomotion (4.7%-5.8% on the whole observation time) and, as well as Looking around, it was almost uniformly represented by all the animal across the day, even though it was possible to notice higher percentages before morning feeding and in the late afternoon.

The otters were more attentive and alert towards the first hours of observation, thus between 7:00 and 9:00 but also at 11:00 and between 16;00-17:00. Even in this case, the statistical analysis confirmed the significance of hours in the different time allocated to the behaviour (p < 0,002). The reason might be found, as suggested also by Bandoli et al. (2023), in park daily maintenance activities, causing keepers repetitive passage and noise in front of otters' enclosure. In addition, these kinds of alert behaviours soon before feedings times has been suggested by many previous studies as Feeding anticipatory behaviours, usually expressed in this species with peaks in vigilance, begging and long calls (Cuculescu-Santana, et al., 2017; Harrop and Cuculescu Santana, 2021; Bandoli et al., 2023). Such increase in alertness results, as already observed by Harrop and Cuculescu-Santana (2021), in reduction of other active behaviours like swimming, play or self-grooming, as well as social interactions and rest. Indeed, Asian small-clawed otters are known to have a very high metabolism to cope with thermoregulation, especially in cold environments (Harrop and Cuculescu-Santana, 2021). While in nature the species spend up to 40-60% of it's time budget only foraging, in captivity the daily feeding portion should be split at least in two main meals (*Heap*, 2008), even though it seems that three main feedings, coupled with feeding enrichments and changes in the distribution method, are a good solution to decrease otter's anticipatory behaviour (Harrop and Cuculescu-Santana, 2021). At the same time, the peak of attention recorded at 11:00 could coincide with the increase in visitors number, who were mainly children from summer camps. Rossi et al. (2020) investigated the effect of visitors on the behaviour of three female Asian Small-clawed otters housed at Cattolica Aquarium.

In details, they observed a significant increase of frequency and duration of Out of sight, attention and sleeping during the aquarium opening period compared to the closed season, probably due to visitor presence. A similar analysis was carried out by *Bandoli et al. (2023)* across the day. In this case, they studied the effect of visitors' number and background noise with animals' behaviour, but the statistical analysis didn't show any significant correlation. These researches seems to demonstrate that visitors have an impact on animals, altering their behavioural patterns over the course of different seasons, however the presence of humans cannot be considered excessively invasive as it doesn't seem to affect animal's time budgets throughout the day. In any case, the topic should be analysed further in order to be able to attest with certainty whether visitors are a source of stress for these animals, as in that case this would made necessary a review of housing and management requirements.

In line with *Harrop and Cuculescu-Satana (2021)*, the feeding times were followed by gradual increase of other activities like stone play, self-grooming and rubbing until their peak at 12:00. The chi-square test confirmed the significance of hours in the amount of time Self-grooming was performed along the day (p < 0,006) while the same cannot be said for Play, including also the social one. Indeed, the test reported a significant difference in this behavioural occurrences and distribution mostly due to animal (p = 0,01) rather than hours (p = 0,11).

Allogrooming were frequently performed along the day, but the general peak was registered at 11:00, usually associated with Rest (*Cuculescu-Santana et al., 2017*). On the contrary, social rest was observed less uniformly, with higher values at 16:00 and 17:00 but less frequently also in the morning at 8:00, 10:00 and 12:00.

Other minor behaviours such as diving, digging, rolling and climb were observed in a very limited number of occasions and therefore they were not further investigated.

3.3 Sociograms

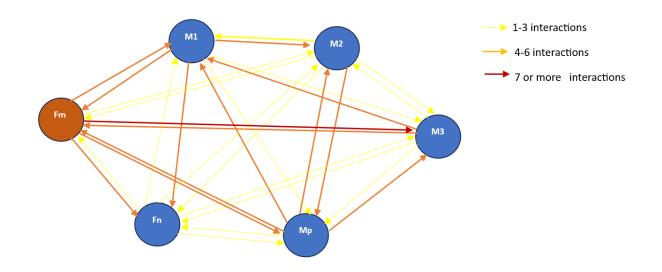


Figure 11) ALLOGROOMING sociogram. The graph shows the interactions among individuals. Each circle represents an animal while the arrows go from performer to reciver. Arrows' color indicate the number of interactions occured during the whole observation time. Fm's circle is orange to highlight her maternity on the rest of the group (blue circles).

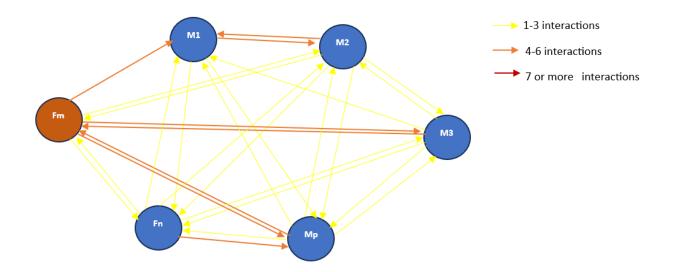


Figure 12) SOCIAL REST sociogram. The graph shows the interactions among individuals. Each circle represents an animal: Fm's circle is orange to highlight her maternity on the rest of the group (blu circles). Social rest interactions were calculated directionally, represented by the arrow, thus considering who moved to initiate the interaction and the individual chosen to rest with. The colour of the arrows indicates the number of interactions occurred.

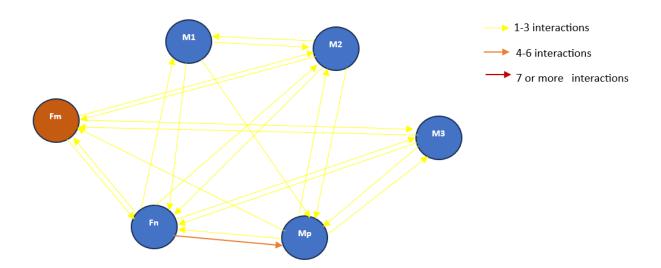


Figure 13) PLAY-FIGHT sociogram. The graph shows the interactions among individuals. Each circle represents an animal: Fm's circle is orange to highlight her maternity on the rest of the group (blu circles). Play-fight interactions were calculated directionally, represented by the arrow, thus considering who moved to initiate the fight and the individual chosen to play with. The colour of the arrows indicates the number of interactions occurred.

Social behaviours were recorded only between the 5%-9% of the total observation time, even though it's important to consider that they might have been expressed by the animal inside the dens, out of visitor and observers' sight.

Although the number of observations was too low to make statistically precise and reliable assessment, looking at the sociograms it was still possible to glimpse some pattern of individual preferences in terms of interactions.

Looking at the sociograms (Figure 11 to 13), the mother (Fm) was the one that started the highest number of allogrooming and social rest interactions (23 and 20), evenly distributed in terms of recivers, whereas she rarely triggered social play (4 interactions). On the contrary, the youngest female Fn had lower values for allogrooming and social rest (8 and 14 as source) but the highest for social play (11 as source). Interestingly, the preferred playmate of Fn seemed to be Mp, who received 6 out of her 11 total social play interactions as source. The less playful otter seemed to be M1, achieving only 2 interactions as receiver and performing 4 as source. Instead, M1 was the preferred target for social rest and the second for allogrooming, just after the mother. The other otters, thus M2 and M3, had similar middle values, both as source and as receiver in any social behaviours.

In a family of ten captive Asian Small-clawed otters, *Lemasson et al. (2014)* observed similar patterns, with the mother being the preferred target for social rest and the father for affiliative interactions. Unlike this study, however, both Fm and M1 received social rest and allogrooming in a

fairly uniform manner from other individuals, so it is impossible with these data to agree with the statement that middle-aged individuals prefer their peers rather than their parents (Lemasson et al., 2014). It important as well to notice the different group composition of the two studies, underlying the presence of young of 0.5 years in Lemasson et al. (2014) while at Cappeller Zoo the youngest individuals were already 1.5 years old. In addition, another confounding effect might be found in the almost opposite sex ratio, with 8 females out of 10 otters in the other study, compared to 2 out of 6 at Cappeller Zoo. In addition, it is as well important to mention that at Cappeller zoo the father could not be included in the research because he suddenly died before the observations began. It is well known, both in captivity and in the wild, the strong relationship between an otter breeding pair, which is usually the dominant over the family (Lelias et al., 2021). When the original couple breaks up, the other members of the family disperse (Heap, 2008). Lemasson et al. (2014) observed an increased number of affiliative and resting interactions between the breeding pair and he defined it a "preferential bond". Analysing the data collected at Cappeller Zoo, it was not possible to notice this kind of exclusive relationship between the mother and any of the other individuals, suggesting therefore that no new couple was forming in that moment. However, to prevent any risk of dangerous inbreeding, the zoo manager, together with the veterinarian staff, decided to put Fm under fertility control.

4. CONCLUSIONS

In accordance with *Bandoli et al. (2023)* the factor that seemed to affect the most visibility and behavioural allocations was the observation time demonstrating how this information might be useful for zookeepers and zoo's owners to plan animals' management routine and visitors' activity. In general, the six Asian Small-clawed otter of Cappeller zoo were out of sight for almost half of the observation time, being therefore quite difficult to be seen to visitors. However, comparing the obtained visibility percentages with other studies, the high values did not seem strange. Indeed, considering the natural elusiveness of the species (*Rossi et al., 2020*), an enclosure that allows them to hide and rest far from visitors' eyes might be an element for good welfare. (*Heap,2008; Rossi et al., 2020; Bandoli et al., 2023*).

Although the first zoos' commitment is to guarantee high animal welfare standards, these structures have also another key role, which is the promotion of public education and awareness towards conservation issues. In these terms, the Asian small-clawed otter is for sure one of the most

charismatic species, and thus, by presenting its individuals in particular moment of the day or emphasising their individual character, it might be possible to catch visitors attention, involve their emotions and stimulate their sensitivity towards animal kingdom. The ability to attract the attention of the public and to communicate the needs of animals in an effective way, becomes especially important for those species that, as in the case of the Asian small-clawed otter, are victims of illegal trafficking as pets due to an inadequate and superficial knowledge of their actual needs and social media influence (*Otter Specialist Group, 2021*).

To plan visitors' tour, might be useful to keep in mind that during this study the otters were more visible at 11:00 or in the late afternoon, at 17:00. It is as well recommended to avoid a visit at 13:00 because the chances to see the animals are at minimum, at least in the season in which this study was done. Considering the high percentages of Out of Sight, another suggestion to managers might be to install remote cameras inside otters' dens, in order to allow their vision without violating their privacy.

Other interesting information that should be considered, are animals' time budgets during parks'opening hours and their levels of activity during visible time. This kind of information, compared to other studies in the wild and similar captive settings, might offer a bases to evaluate the presence of abnormal behaviour, welfare quality and eventually, the interference of external factors. On the bases of time budget and activity levels it's possible to plan animals'routine to keep them properly stimulated and to limit stereotypic behaviours. Finally, by knowing animals' routine it's possible to plan special guided tours in those moment in which there are more chances to catch peoples' attention and sensitivity. On average, when Cappeller's otters were visible, the most performed behaviours were resting and looking around (38%-49% of the total visible time). Other active behaviours such as locomotion, ground exploration and eating were visible between the 25-37% while playful moments, individual and social, represented only the 0.1-7% of otters' visible time. Interestingly, younger individuals like Fn and Mp showed higher percentages for active behaviour, play and social interactions compared to older individuals. In general, animals' behavioural allocations were affected significantly by observation time. Looking around and rest were quite uniformly distributed along the day while peaks of alertness were observed just before feeding times, respectively at 8:00 and 17:00. Another peak of attention was detected at 11:00, suggesting a possible effect of the increased number of visitors. Other active behaviour resulted to be more exhibited towards late morning and early afternoon, far from the feeding times. Allogrooming were frequently associated to resting and it was mostly observed around mid-day, social rest were uniformly distributed while, on the contrary, social play was observed only in few bouts before morning feeding, at 11:00 or after 16:00. Looking at this data, it is therefore suggested to propose

guided tours around 11:00, or in the late afternoon when the animals seem to be more visible and active. Abnormal behaviours and aggression were not detected however, the peaks of attention towards feeding times might be intended as Feeding anticipatory behaviour. As suggested by *Harrop and Cuculescu-Santana (2021)*, to decrease their occurrences may be useful to feed the animals three times a day, or to introduce scatter feeding to encourage foraging related behaviours, much higher in the wild than in captivity.

Lastly, another aim of this study was to analyse the social network inside Cappeller's otter family to understand if there were individual preferences, avoidance, or leadership interactions. Knowing this information is for sure essential for keepers to properly manage the group of animals during feeding times, but also in case of individual isolation for veterinary check or new introductions. Furthermore, social networks might offer as well interesting hints for animal characterisation and public attractiveness. Although the social interactions were not enough to ensure a statistical validity, it was possible to notice few behavioural patterns. Fm and M1 resulted to be the preferred individuals for social rest and allogrooming and they interacted almost uniformly with all the individuals of the family. On the contrary, Mp and Fm were the most playful. Finally, M2 and M3 showed intermediate values both as sources and recivers.

In conclusion, this study provides a general idea of captive Asian Small-clawed otters activity levels and social interactions, which can be used in the future as a reference and comparison to further investigate the topics in other captive groups. It is as well suggested to investigate more in deep the possible effects of environmental temperature and visitors on otter's behaviour, as well as to test the effect of an additional feeding time or feeding enrichment to decrease alertness and increase foraging related behaviours.

5. APPENDIX

4.1 Photos used for individuals' recognition



Figure 14) M1: largest body size, full dark brown fur underneath lower eyelids, dark brown "D" spot close to nose (right side)



Figure 15) Body sizes comparisons: the bigger one is M1 (on the left) and the other is Fm (on the right)



Figure 16) M2: large body size, light brown line underneath both eyes, dark brown-blended fur that runs towards mouth posterior edge



Figure 17) M3: complete dark areas underneath the eyes (like M1) but the mouth-area dark brown patch doesn't reach the nose. The body size is smaller compared to M1



Figure 18 and 19) Fn: slender body and thin neck, no brown fur underneath the eyes, little light brown spot just above right upper eyelid

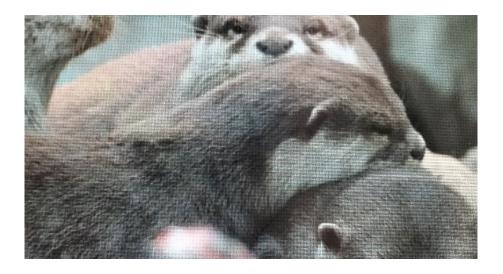


Figure 20) Mp: size very similar to Fm and smaller than the other males. Thin dark brown line under the right eye





Figure 21 and 22) Fm: Small body size compared to M1, M2 and M3 but bigger than Fn. Dark brown blended fur all around eyes' area (no lines nor precise circles like M1 and M3). Wide brown patch close to the nose



Figure 23) General pictures to compare body and face differences.

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