Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

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Lekbeteende hos Urialer (*Ovis orientalis bocharensis*)

Comportament del joc en Urials (*Ovis orientalis bocharensis*)


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**MSc Animal Science**



**Studentarbete** **Uppsala 2018** **Nr. 758**
**Sveriges lantbruksuniversitet**
**Institutionen för husdjurens miljö och hälsa**

***Student report*** ***No. 758***
***Swedish University of Agricultural Sciences***
***Department of Animal Environment and Health***

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**Credits:** 30 credits
**Level:** A2E
**Course title:** Degree project in Animal Science
**Course code:** EX0803
**Course coordinating department:** Dept. of Animal Environment & Health

**Place of publication:** Uppsala
**Year of publication:** 2018
**Cover picture:** Irene Guanyabens Garrido
**Title of series/** Studentarbete Sveriges lantbruksuniversitet
**Part number:** Institutionen för husdjurens miljö och hälsa, nr 758
**ISSN**: 1652-280X
**Online publication:** https://stud.epsilon.slu.se
**Keywords:** play behaviour, ural lamb

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# 1. Summary

## *1.1. English*

Welfare constitutes of health, physiology, behaviour and reproduction. It becomes very important when a species suffers in any of these parameters and thus, the assessment of welfare should be carried out. Play behaviour has been identified as a positive behaviour performed when satisfactory circumstances are present. In order to assess welfare through play in domesticated animals it is essential to know the natural behaviour of the ancestral species. The purpose of this study was to evaluate and describe the ontogeny of play behaviour in Urial sheep lambs, the ancestor of our domesticated sheep, during their first weeks of life. With this, also to contribute in the conservation of this vulnerable mountain ungulate and the maintaining of its predator-prey relationship.

The study was conducted in Nordens Ark and two female lambs born on the 3rd of May 2018 were the objects of it. They were bottle-fed because of the lack of the mother. An ethogram and a check sheet were used, and continuous focal observations were carried out during three periods: two, four and six weeks old. Each period consisted of two days of observations and three sessions of 2 hours per day (in exception of week two which only had two sessions): morning, midday and afternoon. Lambs stayed indoors with 2 hours session outdoors at week two, and outdoors at week four and six.

The results at week two were very different from week four and six, showing a higher amount of play behaviour at week two which later greatly decreased at week four and six. Locomotor play was found to be the predominant type of play throughout the six weeks, with jump and run being the prevalent play behaviours. Manipulating objects was another prevalent play behaviour and its frequency was seen to increase from week two to four. However, the most prevalent behaviour performed by the lambs along the study was to sit or lie down, showing an abnormal juvenile behaviour pattern.

Play behaviour in these lambs was less complex, contained less elements than that of domesticated lambs. This was totally surprising as domestication generally leads to a less complex behaviour than found in the wild type. It was concluded that sheep were under some stress situation (i.e. predators, lack of the mother, lack of natural habitat) or under-fed, and hence, displayed less play

behaviour as would have done in normal circumstances. The lack of the mother influenced the results, and it is believed that the peak of play behaviour at week 2 could be explained by the motivation to move into an open and bigger area.

## *1.2. Swedish*

Välfärd utgörs av hälsa, fysiologi, beteende och reproduktion. Om någon av dessa parametrar avviker bör man göra en bedömningen av välfärden. Lekbeteende har identifierats som ett positivt beteende som utförs när omständigheterna är goda. För att kunna bedöma välfärden hos domesticerade djur genom att studera deras lek är det viktigt att känna till förfädernas naturliga lekbeteende. Syftet med denna studie var att utvärdera och beskriva lekens ontogeni hos Uriallamm, förfäderna till vårt tamfår, under de första veckorna av livet. Detta kan indirekt bidra till bevarandet av detta bergslevande klövdjur, som är hotat i det vilda, och därmed även bevarandet av den naturliga rovdjur-bytesrelationen.

Studien genomfördes på Nordens Ark med två tacklamm födda den 3 maj 2018. De föddes upp med flaska på grund av att mamman inte tog hand om dem. Ett etogram och ett protokoll användes, och kontinuerliga fokaldjursobservationer utfördes under tre perioder: vid två, fyra och sex veckors ålder. Varje period bestod av två dagar observationer och tre sessioner á 2 timmar per dag (med undantag för vecka två som endast hade två sessioner): morgon, middag och eftermiddag. Lamm hölls inomhus med 2 timmars utevistelse vid vecka två och utomhus under vecka fyra och sex.

Resultaten vid vecka två skilde sig kraftigt från vecka fyra och sex, med en högre frekvens lekbeteende vid två veckas ålder. Lekfrekvensen minskade kraftigt vid vecka fyra och sex. Lokomotorisk lek visade sig vara den övervägande typen av lek under sex veckor, med hopp och springande som de vanligaste lekbeteendena. Manipulera föremål var ett annat vanligt lekbeteende och dess frekvens sågs öka från vecka två till fyra. Det mest framträdande beteendet som utfördes av lammen under studien var dock att ligga ner, vilket är ett onormalt beteendemönster hos lamm.

Lekbeteendet hos dessa lamm var mindre komplext, dvs innehöll färre beteendeelement än hos domesticerade lamm. Detta var helt överraskande, eftersom domesticering i allmänhet leder till ett mindre komplext beteende än det man ser hos vildtypen. Slutsatsen var att lammen befann sig i en stressituation (brist på moder, brist på mjölk, avvikelse ifrån naturlig livsmiljö, närvaro av rovdjur mm) och därför visade mindre lekbeteende än de skulle ha gjort under normala omständigheter. Avsaknaden av mamman påverkade med stor sannolikhet resultaten, och troligen kan frekvensen av lekbeteendet vid vecka 2 förklaras av motivationen att komma utomhus på en större yta.

# 2. Introduction

## *2.1. Background Animal Welfare*

Welfare could be described by many characteristics. The Five Freedoms (Brambell, 1965) is one of the first documents considering the basic principles to assure the welfare of animals under human control. Based on the needs that are considered basic to assure the welfare of animals, they include:

- ✓ Freedom from thirst, hunger and mal- nutrition – by access to fresh water and diet to maintain full health and vigour.
- ✓ Freedom from discomfort – by providing a suitable environment including shelter and a comfortable resting area.
- ✓ Freedom from pain, injury and disease – by prevention or rapid diagnosis and treatment.
- ✓ Freedom to express normal behaviour – by providing sufficient space, proper facilities and company of the animal's own kind.
- ✓ Freedom from fear and distress – by ensuring conditions which avoid mental suffering.

(Marchewka, 2015)

The Welfare Quality ® project, targeted to develop assessment of farm systems, uses four principles similar to the ones mentioned above. In short, the main principles of animal welfare are good feeding, good housing, good health and appropriate behaviour (Temple et al., 2011).

Four of the five freedoms show a big concern on negative concepts (such as pain, hunger, discomfort or distress) and the way to alleviate them. However, there is one which could also suggest the idea of increasing a positive aspect, such as the expression of the normal behaviour, where we could include play behaviour among others. Although, it does not explicitly value a positive aspect since natural behaviour also involves some unpleasable states (Yeates and Main, 2008). Moreover, Marchewka (2015) affirm that public concern about the welfare of animals has been experiencing a dramatic increase in the last decade partly because of new knowledge arising from research on animal perception and sentience, signifying, as well, principally from the negative aspects of welfare.

On the other hand, some authors have been talking about welfare as “happiness” (Webster, 2005), “liking”, “wanting” and “pleasure”. Thus, is evident that methodologies for welfare assessment are essential (Yeates and Main, 2008).

Yeates and Main (2008) suggest ethograms and observation of wild conspecifics as a useful tool to get information. An ethogram consists of a formal description of a species behavioural catalogue or a major segment of it (McDonnell and Poulin, 2002). Therefore, they can be used in order to investigate and describe first of all the normal behaviour of a wild species and use it as a comparison to assess behaviour and welfare in other species.

With this, the use of behavioural markers may be effective, but not exclusive. These are defined as *activities that are more liable to be performed when animals are in positive affective states* (Yeates and Main, 2008). Play has been considered to correspond with positive affect (Manteca Vilanova, 2002) and differing levels of display could be used as a behavioural marker (Boissy et al., 2007; Yeates and Main, 2008; Temple et al., 2011). If we can notice the presence of positive affect, like play, considered as a reward for animals (Boissy et al., 2007; Anderson et al., 2015), it can be an indication, at the same time, that negative emotions are reduced, since play behaviour is displayed when the basic needs are fulfilled (Boissy et al., 2007). Thus, it can be used as assessment of welfare.

In the same way, the frequency and type of play may be affected by other factors (i.e. type of housing, feeding, management) and the decrease or absence of play could be a sign of unsatisfactory, stressful or negative experiences (Held and Špinka, 2011; Špinka, 2012; Mintline et al., 2013). In the study of Mintline et al. (2013) they tested whether play behaviour such as bucking, running and head-related behaviours was reduced after hot-iron disbudding in dairy calves (*Bos taurus)* after 3h and 27h. The calves were assigned with 6 different treatments, including a group without drugs. Last group of calves spent less time playing than the other ones. The results indicated that the disbudding could suppress the duration of play 3h, but not 27h, after the procedure because of pain.

In contrast, it does not necessarily mean that higher amount of play is always a positive aspect, as it has been detected an increase of play performance in stress situations as well (Dugatkin, 2014). For example, running could be expressed both because of fear or positive excitement, and high

levels of plasma cortisol could reflect both stressful and grateful events (Wemelsfelder and Farish, 2004). Moreover, this technique of welfare assessment is not exclusive and should be complemented with other constituents of welfare, such as physiological markers (Yeates and Main, 2008) or stress (pain, fear, agitation) (Wemelsfelder and Farish, 2004).

Thusly, it seems more sensible to assess welfare with the presence of the positive affects of welfare than just the lack of the negative ones (Yeates and Main, 2008). Hence, play behaviour in mammals as a positive input for animal welfare, has become subject of attention by the ethologists and zoologists (Berger, 1980; Burgdorf and Panksepp, 2006).

Likewise, using "what animals want" behaviours, what they would choose themselves, it seems a good way to assess welfare (Mellor, 2014). This supports the concept of play behaviour as a welfare assessment, as is something voluntary in animals, they perform it just because they want to (Dugatkin, 2014). We can consider play as a "what the animal wants" behaviour.

To really know about the "appropriate behaviour" mentioned in the fourth principle of Animal Welfare ®, we need to first evaluate and study the natural behaviour of the ancestral and wild species.

During the domestication, the species undergoes different changes that could have a possible effect on the animal welfare. For instance, morphological changes, especially due to the selection of breeds, as in the example of the chick *(Gallus gallus domesticus)*. If we think of two chicks of the same species and age, with the difference that one is destined for meat whereas the other is the ancestral one (*Gallus gallus*), the chick destined for meat has a problem of animal welfare, since it has been selected for extreme growth and appetite. As the meat type chickens grow, their weight cause leg pain, sudden death etc. (Manteca Vilanova, 2013).

Based on this, we can consider the domestication as another factor implied in animal welfare thus another reason to study the natural ancestral behaviour to later compare with the domesticated species and evaluate whether is normal or not.

## *2.2. Introducing Urials*

Urial, Shapu or Gad (*Ovis vignei; Bovidae; Artiodactyla)* are the names given by the local people of Pakistan for an endangered wild sheep of a medium size. (Ahmed, 1997; Siraj-ud-Din et al., 2016b).

Two species are identified: *Ovis vignei* (Urial) and *Ovis orientalis* (Asiatic mouflon) (Nadler et al., 1971; Rezaei et al., 2010; Siraj-ud-Din et al., 2016b; Yeomans et al., 2017). However, despite that there are differences in morphology and chromosome numbers (Urial: 2n=58; Asiatic mouflon: 2n=56), Valdez et al. (1978) and Pichler et al. (2017) classify them as a single entity, *Ovis orientalis*. Nevertheless, it has several sub-species considered as Urial type sheep including *O. orientalis arkal, O. orientalis vignei, O. orientalis punjabiensis, O. orientalis bocharensis,* and other considered as an Asiatic mouflon type including *O. orientalis isphahanica, O. orientalis laristanica, O. orientalis cycloceros* and *O. orientalis gmelinii.* In the present study we will focus on the Bukhara Urial (*Ovis orientalis bocharensis*).

Going back to the origins of domestic sheep, the wild Urial (by then *O. ammon vignei*) was first domesticated in the Aralo-Caspian basin, spread around the Middle East and Europe, which would later hybridise with other lines (Nadler et al., 1971). Thus, we know Urial (*Ovis orientalis)* is the true ancestral species for domesticated sheep (*Ovis aries)* as other authors also suggest (Rezaei et al., 2010; Bashari and Hemami, 2013; IUCN, 2015).

They are gregarious gathering small groups seen to be of an average of seven Urial (in Punjab Urial), depending on the season. Mixed groups are most common during the rut, whereas, divided all-male and nursery groups (only ewes, lambs and yearlings) are typical during the lambing season (Awan et al., 2006a). They are sexually dimorphic: adult males weight about 40-60kg with large curly horns of 63.5-105.5cm length. Adult females weigh 25-40 kg and have horns in a slender upward slightly curved shape about 12 cm long (Figure 1) (Valdez et al., 1978; Ahmed, 1997; Farhadinia et al., 2014). The females of Punjab Urial give birth to one or two lambs in early April (Ahmed, 1997; Awan and Festa-Bianchet, 2006; Awan et al., 2006a; Awan et al., 2008), and during late October – early November, the rut takes place (Schaller and Mirza, 1974; Awan et al., 2006a). The study of Awan et al. (2006a) shows the single births were more common, a twins' percentage of 18% of the total and an average litter size of 1.18. Their face is generally greyish, belly and legs

are creamy-white and the body fur is a reddish-grey colour (Ahmed, 1997). They are classified as grazers on grasses and shrubs (Schaller and Mirza, 1974; Awan et al., 2006b; Bang et al., 2018; Siraj-Ud-Din et al., 2018), as they use to habit an altitude below 1.500m (Awan et al., 2006a).

**Figure 1. Adult Urial *(O. vignei bocharensis)* female and male.**

The Urial is widely distributed in Asia Minor (Rezaei et al., 2010) in the Mediterranean region (IUCN, 2015). Most of the habitat is arid sub desertic, comprising on barren lands with soil, sands, stones and rigid steep rocks. The other part of the natural habitat contains scattered stunned vegetation comprising small trees, shrubs, herbs and grasses of diverse kinds.

Unfortunately, the Urial is endangered in its natural habitat, directly related to the increase of the human populations. The threats are: destruction of the mountain habitats due to the fuel wood, the extraction of medical plants with unsustainable methods, grazing of the domestic livestock (Awan et al., 2006a; Bang et al., 2018) and poaching. The hunting of Urials is considered illegal (Mallon, 1991; Bazyan et al., 2016; Siraj-Ud-Din et al., 2016a; Siraj-Ud-Din et al., 2018). No data is available about the status of the poaching, although Ayaz et al. (2012) determined in their study that the Urial population was still decreasing due to illegal shooting and Siraj-Ud-Din et al. (2018) state the illegal hunting is still one of the major threats, and the greatest short-term one appears to be the poaching of the lambs to keep them as a pet (Awan et al., 2006a) and for meat (Michel, 2010). Thus, this species is declining by at least 30% over three generations (IUCN, 2015).

Based upon all these threats, the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Animals (2015) has included and registered all the subspecies of Urial as vulnerable under criterion A2cde assessed by Valdez, R. (IUCN, 2015) (Figure 2).


Figure 2. *Ovis orientalis* IUCN Red List (available at: www.iucnredlist.org/details/15739/0)

Other species of mammal co-habit in the same area such as the snow leopard (*Panthera unica),* Persian leopard (*Panthera pardus saxicolor*), Himalayan lynx *(Felis lynx),* flare-horned markhor (*Capra falconeri falconer)* among others (Farhadinia et al., 2014; Siraj-Ud-Din et al., 2018).

The trophic interactions between the endangered Persian leopard and the vulnerable mountain ungulate, Urial, are affected by the declining status of both species. The leopard diet consists of 12 different species including Urial, Persian wild goat (*Capra aegagrus),* Eurasian wild pig (*Sus scrofa*), and diverse range of carnivores. However, the median ungulates ranged 10 to 40 kg of body weight such as Urials, are preferred (Farhadinia et al., 2014) and hence, Urials have an important role to play in the ecosystem.

## *2.3. Play behaviour*

Play is considered either to be an ignored behaviour or just assumed to exist along other important behaviours over the time (Burghardt, 2005a). However, more interest has risen by the ethologists and zoologists since is demonstrated to be useful as welfare assessment (Berger, 1980; Burgdorf and Panksepp, 2006).

Play behaviour has been defined in many ways. One of the most cited authors is Marc Bekoff, who proposed the following:

> "Play is an apparently purposeless postnatal motor behaviour that has been mould in response of the selection pressures, likely consequences of specie's adaptive syndrome. If the activity is directed toward another living being, it is called social play". (Bekoff, 1984)

It has also been similarly defined by Xavier Manteca Vilanova and Sue Mc Donell as "a behaviour that seems to have no function and that englobes motor features and modified behaviours from the normal ones (Manteca Vilanova, 2002; McDonnell and Poulin, 2002). Not all species engage in play but is described in most mammals and many birds. Octopuses have also shown play behaviour. It is often most evident in young individuals (kittens, puppies, lambs) (Manteca Vilanova, 2002; Burghardt, 2005b; Mintline et al., 2013; Dugatkin, 2014).

When people have to label a behaviour as play, they define it as something that "we can not say exactly what it is, but we know when we see it" (Dugatkin, 2014). To standardly distinguish play, Burghardt (2005c) believes in 5 criteria. All five criteria have to be simultaneously satisfied for a behaviour being described as play but just one attribute met into each criterion is needed.

1. *The first criteria for recognizing play is that it is some variant of the view that play behaviour is not serious, not of immediate use, and not necessary for survival. The performance of the behaviour is not fully functional in the form or context in which is expressed, that is, it includes elements, or is directed toward stimuli, that do not contribute to current survival.*
2. *The second criteria for recognizing play is that the behaviour is spontaneous, voluntary, intentional, pleasurable, rewarding, reinforcing or autotelic (done for its own sake).*
3. *The third criteria for recognizing play is that it differs from the "serious" performance of ethotypic behaviour structurally or temporally in at least one respect: it is incomplete (generally through inhibited or dropped final elements), exaggerated, awkward or precocious; or it involves behaviour patterns with modified form, sequencing or targeting.*
4. *The fourth criteria for recognizing play is that the behaviour is performed repeatedly in a similar, but not rigidly stereotyped, form during at least a portion of the animal´s ontogeny.*
5. *The fifth criteria for recognizing play is that the behaviour is initiated when an animal is adequately fed, healthy, and free from stress (e.g., predator threat, harsh microclimate, social instability), or intense competing systems (e.g., feeding, mating, predator avoidance). In other words, the animal is in a "relaxed field."*

In short, the criterion are: 1. Limited immediate function, 2. Endogenous component, 3. Structural or temporal difference, 4. Repeated performance, 5. Relaxed field.

When does the play behaviour start? In puppies, four stages of development are defined: neonatal stage, transition stage, socialization stage and youth stage. The second stage lasts from the end of the second week till the end of the third week of age (Day 12-21) and is then when the behaviour of play and the environmental exploration begin (Manteca Vilanova, 2002).

Why is play selected for? Three major sources of modern play theory have been proposed: The Surplus Energy Theory, play only exists with extra energy; The Instinct-Practice Theory, play as a training for future; and Recapitulation Theory, considered as a behaviour needed in the past that evolved into play (Burghardt, 2005b).

Ethologists generally delineate three different types of play: object, locomotor and social play (Dugatkin, 2014). Both, object and social behaviour can be locomotor at the same time. For instance, chasing / being chased is a social behaviour, but when performing it, the animals also run (locomotor).

### 2.3.1. Object play

When the activity is directed toward an inanimate object such as sticks, rocks, leaves, feathers, fruit… or an animate object such as the tail or any part of the body of an animal by manipulating and interacting with such objects (Bekoff, 1984; McDonnell and Poulin, 2002; Dugatkin, 2014).

It can be easily confounded with exploratory behaviour, especially when a novel object is presented. However, there are some clues described to identify and differentiate both. Exploring (Figure 4) appears to answer the question “what it is?”, whereas during object play (Figure 3) the animal seems to try to determine the “what it can do with this object?” question. The function of

this type of play could be associated with a practice where the animal can learn something new that will benefit in short or long term (Dugatkin, 2014).

**Figure 3. Urial lamb manipulating a feather (object play).**

**Figure 3. Urial lamb interacting with a feeding trough (exploration behaviour).**

### 2.3.2. Locomotor play

It is also known as rotational play. It includes any acrobatic activity while in motion at any gait (McDonnell and Poulin, 2002; Dugatkin, 2014). The animal may gambol, run, jump (Figure 5), roll, pivot (Figure 6) among others and can occur either in a social group or in a solitary individual.

There are different theories about its function. Firstly, it is suggested to be a training of motor skills (Špinka et al., 2001) for the adult life. It could be associated with adult locomotor movements to escape from predators. Secondly, it could provide a better understanding of the surrounding, where are things in relation to one another while seen to develop a greater differentiation of the muscle fibre type (Dugatkin, 2014).

**Figure 5. Urial lamb jumping.**

**Figure 4. Urial lamb pivoting / rapid turning.**

### 2.3.3. Social play

It is the activity of playing with others, directed toward an individual (Bekoff, 1984). General social behaviour also includes laying with others (Figure 8). It includes mounting, chasing (Figure 7), butting, sniffing others, balancing on an animal, etc.

Some of the possible functions for this behaviour are the following: long-lasting bonds, fine-tune skills (related to fighting, hunting, mating) and development of cognitive skills (Bekoff, 1984; Dugatkin, 2014).

In Bighorn sheep (*Ovis canadienses)* males it has been described a greater contact play than in females, suggesting its function could be to prepare males for the aggressive interactions for reproductive interest (Berger, 1980).

**Figure 7. Urial lambs chasing / being chased.**

**Figure 8. Urial lambs cuddling together.**

One of the aims in ethology is to investigate the ontogeny of a behaviour, its development and its underlying mechanisms, in the life of in individual (Burghardt, 2005a; Martin and Bateson, 2007).

# 3. Aim

The Urial sheep is the ancestor of our domesticated sheep but has been studied very sparsely. Now this species is seriously endangered and any information about natural behaviour and how to manage these sheep in the zoo environment is valuable for conservation. As Bazyan et al. (2016) stated, we need to take advantage of the captive-rearing animals that will lead to a reintroduction to asses important elements including behaviour, nutrition, genetics, physiology, reproduction and pathology. Moreover, the evaluation and assessment of play behaviour has rarely been done for captive mammals specifically endangered wild sheep.

The purpose of this study is to evaluate and describe the ontogeny of play behaviour in wild Urial (*Ovis vignei bocharensis*) lambs in captivity in order to (1) help assessing domestic sheep's welfare based on the occurrence of play; (2) contribute in the conservation of the vulnerable mountain ungulate which will on a long term aid the conservation of the endangered predators, such as the Persian leopard (*Panthera pardus saxicolor).*

### *3.1. Research questions*

The main aim of this study is to investigate which is the ontogeny of play behaviour in Urial lambs during their first months of age as this has, to my knowledge, never been done before.

a. What elements of play do the Urial lambs display and how does Urial play compare with play in domestic lambs?
b. How would environmental factors such as space, climate/weather, time of day, vicinity of predators (leopards and humans) affect play behaviour in urial lambs?

A second aim for this study is to get further knowledge about this endangered wild sheep in order to contribute in its conservation. Furthermore, more information is needed about Urials to achieve an effective management of predator-prey and preserve the trophic interactions in their ecosystem.

### *3.2. Hypotheses*

No hypotheses have been done before the study, since play behaviour has not been extensively studied in any wild sheep before. However, I would expect a complex play behaviour as domestication generally leads to a less complex behaviour than found in the wild type.

## 4. Materials and Methods

This study was conducted over one and a half months starting two weeks after the Urial lambs were born in early May until they were 6 weeks old in mid-June 2018.

### *4.1. Study localization and housing*

The Urial lambs were studied at Nordens Ark (Ark of the North). It is a zoo located on the 383-hectare *Åby Manor* in Bohuslän, on the west coast of Sweden, not too far from the border to Norway (latitude: 58° 26' 16.79" N; longitude: 11° 26' 3.59" E).

During the first two days of observations, when the lambs were two weeks old, they were housed indoors in a straw-bedded enclosure with steel hurdles of approximately 25$m^2$ dimensions during most of the day and outdoors just from 11.30h to 13.30h, in a small part of the Urials' enclosure

(65 $m^2$, see Figure 9) together with two adult ewes (one of them, the mother). The pen had 3-4 straw-bales for the lambs to play with.

All the other observations were carried out outdoors, in half of the Urials enclosure (see Figure 9) with the other adult females (N= 6 for ewes, born in 2007, 2008, 2009, 2010, 2015-mother- and 2017). The two males were separated in the other half of the enclosure (left in the map Figure 9) for the proper adaptation of the lambs with the female group. The habitat features in the Urials' enclosure include coniferous (*Pinus sylvestris*, *Picea abies*) and large deciduous trees (mainly *Fagus sylvatica* but also *Quercus robur* and *Betula spp)*; big rocks; dried leaves on the ground.

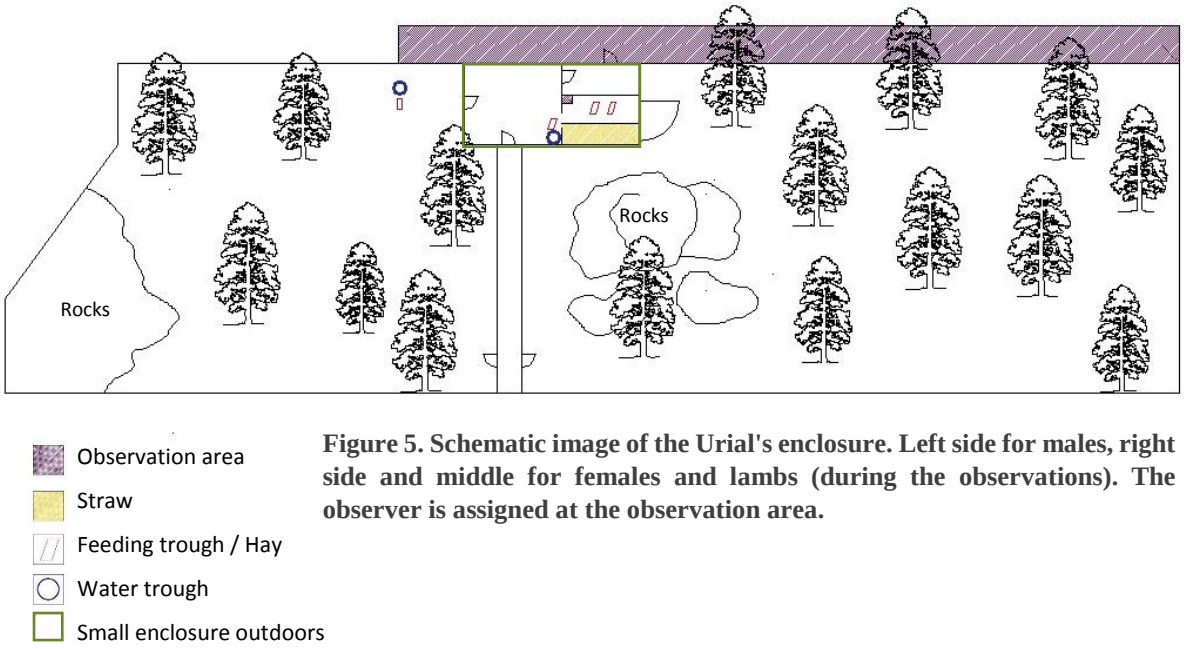



**Figure 5. Schematic image of the Urial's enclosure. Left side for males, right side and middle for females and lambs (during the observations). The observer is assigned at the observation area.**

## *4.2. Animals*

This study was conducted over one and a half months starting two weeks after the Urial lambs were born in early May 2018. One male lamb was born the 29th of April but died a few days after birth because of lack of nutrition. Two female twin lambs were born on the 3rd of May and despite the early critical problems of dehydration of one of them, they survived and hence became the

objects of the study. In connection with the ear tagging the mother ewe stopped to show normal maternal behaviour. Despite she was not aggressive with the lambs, they had to be bottle fed by the zookeepers during a period of 12 weeks. As mentioned before, both lambs were tagged by different colour plastic ear tags on the right ear, yellow and orange, to allow their identification (Figures 10 and 11).

**Figure 6. Yellow ear tagged Urial lamb (female)**

**Figure 7. Orange ear tagged Urial lamb (female)**

## *4.3. Behavioural observations*

### 4.3.1. Pilot study

The periods of time for the observations were selected through a pilot study the day before starting the first observations (15th of May, Day 12) to focus observations when the lambs where more active and the feeding hours. Continuous focal observations (Martin and Bateson, 2007) throughout the day were used for the pilot study for a total of 11 hours and a pencil and paper were used to annotate the overall of activity. A second pilot study was necessary before starting the second period of observations, on the 29th of May (Day 26), due to changes on the management, both environment and feeding hours, of the lambs. Continuous focal observations (Martin and Bateson, 2007) were used for 6 hours and the same material and design as the first pilot study were used. Some adaptations with the observations were also done during the third period, although no pilot study was carried out before.

The Urials were familiar with the indoor enclosure and the big enclosure before starting with the observations or the pilot studies, as they were moved into the closing when they were born (hence 2 weeks before starting observations) and moved into the big enclosure with the other females when they were three weeks old (hence, one week before starting with the following observations).

### 4.3.2. Feeding hours

When the lambs were born, they drank from the mother for two days, hence, they were able to drink the colostrum.

During the first observations the feeding hours for the lambs were 6, 11, 16 and 21h (every 5 hours), although during the pilot study the feedings were every 4 hours (6,10, 14, 18 and 22h). After the first feeding of the day the lambs showed to be tired. Throughout the second period of observations the feedings for the lambs were at 7h and 16h. They were, then, together with the adults which were feed at 8.30h and 15.30h. During the last observations, the feedings were supposed to be at 7.45h and 15.30h, but they varied a bit depending on the day. From Wednesday 13$^{th}$ of June a second feeding was added in the afternoon at 16.30h (3 feedings in total/day), and once the observations were finished, they put a milk bar for the lambs in the enclosure. The lambs were offered 2.5dL of milk replacer each one with every bottle feeding, during the pilot study. Later, the amount of milk was different depending on the number of feedings per day and the judgment of the zookeepers on how much were the lambs drinking (Table 1). Water was available ad libitum at all times.

The replacement milk was the same as used in domestic lambs (*Konnect Pontus, Lantmännen*).

**Table 1. Feeding hours and diet offered for the lambs and adults during the observations.**

| | Feeding lambs | Offered | Feeding adults | Offered |
|---|---|---|---|---|
| Pilot study 1 | 6, 10, 14, 18, 22 h | 2.5 dL replacement milk / feeding / lamb | Not relevant | Not relevant |
| 2 weeks old | 6, 11, 16, 21 h | Depending of nº feedings | Not relevant | Not relevant |
| 4 weeks old | 7 & 16 h | Depending of nº feedings | 8.30-15.30h | Carrots, pellets (Hormoden), browse (Salix, Maple, Rowan) and fresh grass (hay ad libitum) |
| 6 weeks old | 7.45 & 15.30h | Depending of nº feedings | 8.30-15.30h | Carrots, pellets (Hormoden), browse (Salix, Maple, Rowan) and fresh grass (hay ad libitum) |

After the observations periods included in the study, the milk bar was tried out during few days but without good results. The lambs did not learn how to use it properly, therefore it was withdrawn. They were bottle-fed again until they turned 11 weeks (Day-81 on 22nd July 2018) when they were weaned.

### 4.3.3. Main study

Observations were carried out during two consecutive days every second week, when the lambs were two, four and six weeks old, i.e. in three observational periods. The time of observations was adjusted to maximise the play observed. The first observations on the 16$^{th}$ and 17$^{th}$ of May (2 weeks old, Day 13 and Day 14) were performed in two sessions of two hours each day: at 11.30-13.30 hours and 16.30-18.30 hours. The second week, during the days 30$^{th}$ and 31$^{st}$ of May (4

weeks old, Day 27 and Day 28), the observations were carried out in three sessions of two hours each day from 7.15-9.15 hours, 11.30-13.30 hours and 16.30-18.30 hours. Third and last observations on the the days 12$^{th}$ and 13$^{th}$ of June (6weeks old, Day 40 and Day 41) were performed in three sessions of two hours each day at 8h-10 hours. 11.30-13.30 hours and 16-18 hours. As mentioned, the periods of time for the observations were selected through a pilot study realized the day before starting with the first observations, according to the lamb's activity and the feeding hours. They were readapted from the second week considering the feeding hours for the lambs or the adult ewes, as the zookeepers were interacting with them. Focal continuous observations (Martin and Bateson, 2007) were used for all the sessions.

The field observations and pilot studies were done directly by the same person on all the recording days and sometimes with the help of photos from a camera (Nikon 3.6X, 18-105mm) to assure the differentiation of the lambs when situated far from the observer. The observer stood or sat quietly outside the lamb pen and inside the little Urials enclosure during the first observations (2 weeks old) and stood or sat quietly or walked along the outside of the Urials enclosure during the other observations (4 and 6 weeks old), trying not to disturb or attract the sheep or lambs. The behaviours of each were annotated completing a protocol in the form of a check sheet following an ethogram (Table 2 and 3) on a phone, tablet or paper. The lambs were continuously observed for 2 hours every time completing the protocol in 10 minutes periods, without a pause, making a total of 24 periods/day (4h with 10min observation periods) in the first observations (2 weeks old) and a total of 36 periods/day (6h with 10min observation periods) during each of the other observations (4 and 6 weeks old). There was no rest along the 2 hours of observation to ensure no play was missed. Both lambs were easily observed at the same time, since the lambs were mostly synchronized. Each performance of behaviour was written as an A or a B, depending on which lamb performed it, A for the yellow tagged lamb and B for the orange tagged lamb. Noteworthy, the duration of each behaviour was not considered, but only the frequency. The behaviours were written down at every transition of behaviour. The following information was also complied: weather (with temperature, ºC), surrounding/group and site. Images to the lambs were taken mostly by a digital zoom camera Nikon D3400 equipped with a lens 18-105 mm.

The behaviours recorded during the observations are assembled and defined in the Ethogram (see Table 2 and 3).

**Table 2.** *Ethogram: Definitions of recorded play behaviours (modified after Anderson et al. 2015, Augustsson 2014, Chapagain et al. 2014).*

| **Recorded play behaviours** | |
|---|---|
| **Behaviours** | **Definitions** |
| **Race** | Running in a group, commonly back and forth in the same area repeatedly. It involves at least 3 participants, which includes the adult ewes. |
| **Run** | Gait faster than walking. Quick movement of the legs systematically toward a direction but with no apparent destination. |
| **Jump** | The animal elevates the body from the ground several times in a row, through a springing motion by the legs. The body is rather stiff, and no twists are included. The behaviour is commonly preceded by running. |
| **Gambol** | The animal jumps and twists its body and head at the same time repeatedly. |
| **Pivot / Rapid turn** | A quick, stiff, rotation horizontally through a little jump on the same spot. This makes the animal face another direction. |
| **Chase** | One animal is pursued by another to reach or pass it, usually by running at high speed. |
| **Being chased** | The animal is subjected to the behaviour described above. |
| **Mount** | The animal raises its forelegs and chest against the rear of another animal. It rests the forelegs on the back of the animal and pushes |

| | |
|---|---|
| | the chest against the back. Usually, it is very brief since the other lamb removes itself quickly. |
| **Being mounted** | The animal is subjected to the behaviour described above. |
| **Butt** | Two animals push with their foreheads to each other. It can be unidirectional or reciprocal. Including from the side, rear and front. |
| **Being butted** | The animal is subjected to the behaviour described above. |
| **Manipulate object** | The animal interacts with an item in some way by chewing, butting, shaking (with their mouth), picking it up and dropping it repeatedly (with their mouth), sniffing or pushing it with the head or paws. The item can be for example a stick, a stone, a small bush, high grass, the fence, a feather or a plastic cord. |

Running activities were considered to be play if they occurred without obvious attempts to avoid possible "predators" or when they were not scared of an apparent noise.

Other behaviours were recorded as laying down, eating, grooming or standing/walking.

**Table 3.** Ethogram: definitions of general recorded behaviours in lambs (modified after Anderson et al. 2015, Augustsson 2014, Chapagain et al. 2014).

| **Recorded general behaviours** | |
|---|---|
| **Behaviours** | **Definitions** |
| **Lie/sit** | The rear, belly or side of the body are placed on the ground or in contact with the ground. They can sometimes groom or eat while |

| | |
|---|---|
| | laying down and perform a social behaviour if laying together with other lamb. |
| **Eat/drink** | Putting roughage or water in the mouth or chewing with the mouth. |
| **Walk/stand** | The body is raised up and all the feet are in contact with the ground, lifting them in pairs simultaneously and moving the body. Without any step in case of standing. The behaviour of exploring the surrounding area (wall or ground) by using the nose may be performed. |
| **Groom** | The animal moves its hind feet repeatedly against the body or licks/bites the fur. In addition, it can rub the body against a fence, rock or vegetation. |
| **Social** | Keeping nose in contact and/or sniffing within five centimetres of any part of the body of other lamb. Commonly while laying down together. |
| **Others** | Other behaviours not mentioned resulted as pawing before laying down, following adult ewes or a zookeeper, vocalization, stretching, urination and defecation. |

# 5. Data Analysis

All the data collected in the different devices or paper was put in the same Excel file. From the focal observations the frequencies of the behaviours run, manipulate objects, jump, butt and being butted, chase and being chased, mount and being mounted, pivot/rapid turn, gambol, lie/sit, groom, stand/walk and "others" were calculated per lamb for each observation session. The amount of As (yellow tag behaviours) and Bs (orange tag behaviours) recorded on the protocol were transformed into numbers. The duration of a behaviour was not registered, only the frequency. The total amount of performances for both lambs per each behaviour and each session (morning, midday and afternoon) during the two days of period observations (2, 4 and 6 weeks old) was added. The results from each lamb were summated and with this, three graphs were created showing the three more-common play behaviours during the three periods of different age (2, 4 and 6 weeks old). Moreover, with the total amounts of behaviours a pie chart was built showing the percentage of each different behaviour.

Besides, the total amount of play behaviours, excluding lie/sit, groom, stand/walk and others, were summed for each lamb, each session (morning, midday and afternoon) and each day of observations (2 days/period). This was used to relate with the volume milk fed per day and lamb. The average of the total amount of performances between both lambs was calculated and used to create a table relating it with the climate of the session (temperature, ºC).

Behaviours were also summarized and sorted into behaviour groups: locomotor play, object play and social play. A linear graph was originated to show the variability of these three groups along the age.

Some of the numerical values were standardized into averages with their standard errors.

# 6. Results

## *6.1. Human interference*

There were visitors at the zoo every day of observations from 9 to 17 hours in the afternoon. Depending on the day there was a different amount of people visiting the Urials. By the beginning and the end of the day, there was less people. Hence, the observations performed during the morning (7-10h) and during the afternoon (16-18.30h) had a lower level of human interferences.

At 2 weeks of age the lambs were affected by the public only when going outdoors at midday (11.30-13.03h). Furthermore, during the last period of observations (6 weeks old) it was noticed a less amount of children / scholar groups as the academic year was finished. The public used to walk along the fence and/or stand still in front of the Urial sheep (Figure 12) while talking and sometimes shrieking (children).

**Figure 9. Visitors at the Urial sheep facilities (children)**

**Figure 8. Lamb following a zookeeper in the Urial sheep facilities**

Secondly, the zookeepers were visiting the sheep at least 3 times a day in order to feed the lambs or the adults. They would enter the enclosure and interact with the lambs by feeding and checking their health. As the lambs were bottle fed they were tame, following the zookeepers around (Figure 13). Intermittently during the day, the quads and four-wheelers from the staff were driving along next to the facilities. Then, the lambs could at times wake up if laying down, scare and run.

## *6.2. Play as an effect of week (age) and diurnal rhythm*

The observations during 2 weeks of age showed a high amount of play performance (an average±SE of 382.5±63.5 recordings/lamb for both days of observations) in comparison with the other ages of 4 and 6 weeks of age, which later decreased (average±SE of 64±9 recordings/lamb and 56±3 recordings/lamb, respectively).

The most commonly observed behaviours found throughout the 3 periods of observation were Run (with an average±SE play behaviours/lamb and percentage: 2w=79±9, 15%; 4w=19.5±2.5, 9%; 6w=20, 9%), Jump (with an average±SE of recordings/lamb and percentage: 2w=139±25, 26%; 4w=12.5±2.5, 6%; 6w=14.5±0.5, 8%) and Manipulate Objects (with an average±SE of play behaviours/lamb and percentage: 2w=70.5±.3.5, 13%; 4w=24.5±3.5, 11%; 6w=18.5±0.5, 8%). The percentage is a result from the general behaviour observations (including other conducts than play) (Figure 14, 15 and 16). Two of the play behaviours mentioned previously are considered as locomotor play (run and jump) and the other one as Object play. Nevertheless, when the lambs were 2 weeks old, the play behaviours of Chasing/Being Chased, Pivot/Rapid Turn and Mount/Being Mounted also showed a greater prevalence among the other play behaviours, with the percentages of 11%, 4% and 2%, respectively (Figure 14). Self-grooming was seen considerably along all the observation periods.

The most common action carried out at 2 weeks of age was to jump (26%) and lie/sit at the age of 4 and 6 weeks (28% and 31%, respectively). Point out that i.e. while sitting it was counted just as 1 action until a transition into another activity was seen. Therefore, this does not reflect the time the lambs were sitting or lying down.

The play behaviour of “Balancing on an ewe” was absent during all the observations.

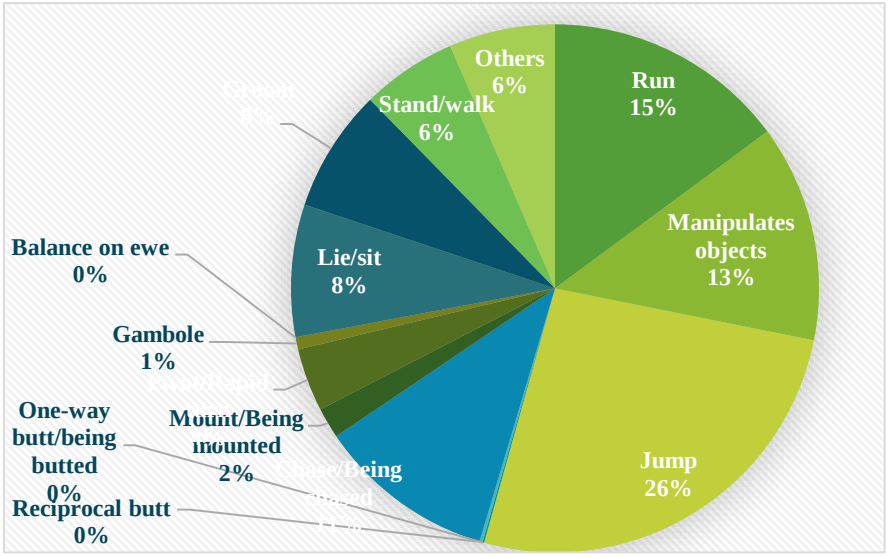



**Figure 10. Overview behaviours at 2 weeks old for both lambs**

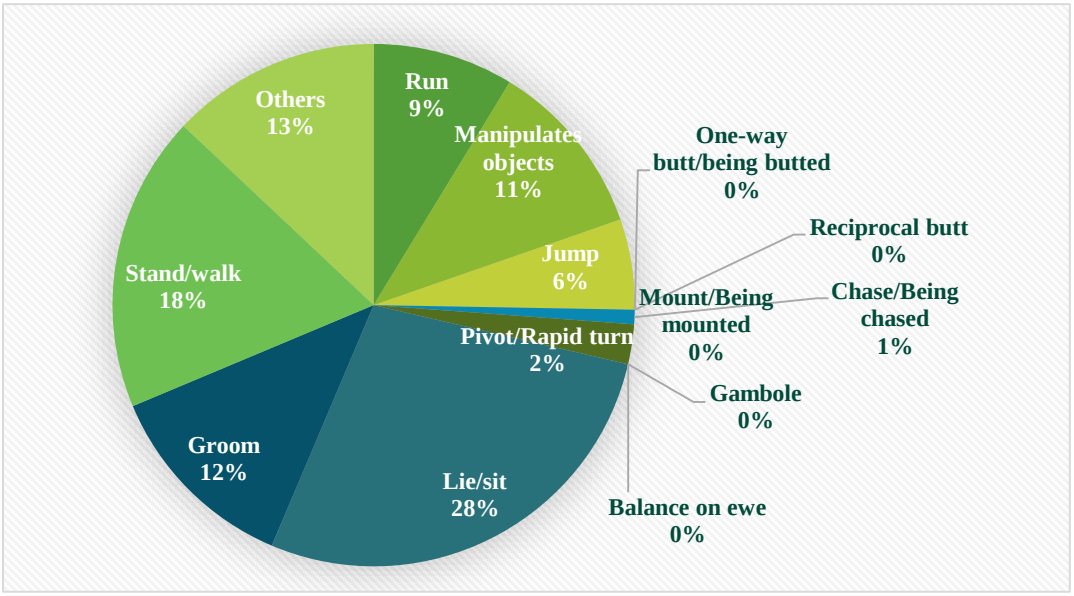



**Figure 11. Overview behaviours at 4 weeks old for both lambs**

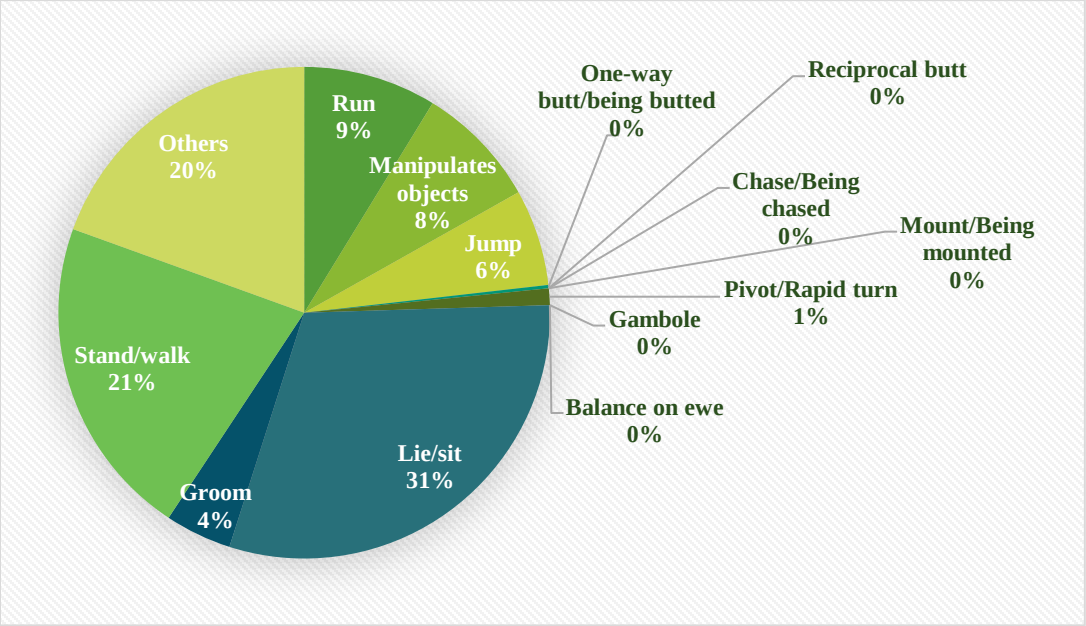



**Figure 12. Overview behaviours at 6 weeks of age for both lambs**

The different play behaviours were categorised in: locomotor play, social play and object play. Among the categories of play, locomotor play was identified as the most common play category throughout the sessions (Figure 17). A substantial difference in frequency of play was found between week 2 and 4-6 of age (Figure 17). From week 2 to 4 of age, the play category of locomotor and social play decrease (from a 63% to a 59%; from 18% to 3%, respectively) whereas the relative frequency of object play increases from 18% to 38%. From week 4 to 6 of age, the locomotor play increases to 66%; the social play keep decreasing to 1%; and the object play start decreasing to 33%.

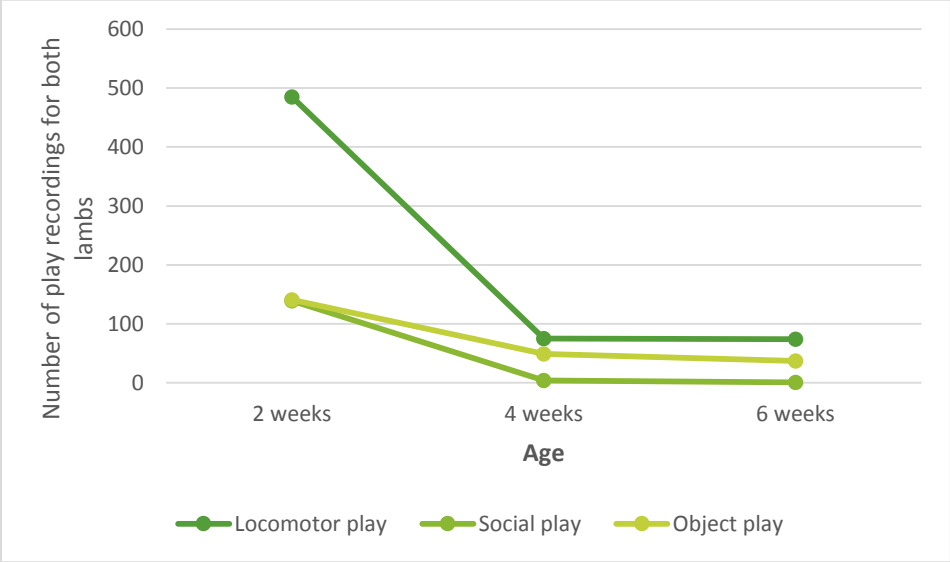



**Figure 13. Type of behaviour- age**

Regarding the different sessions throughout the day (Figure 18, 19 and 20), the results show that jump had the highest peak of play behaviour among all ages and it was presented on the midday session at the age of 2 weeks.

When considering the total amount of play activities from 2 weeks of age to 6 weeks of age, the preferable activity during the morning was to run and both during midday and afternoon was preferable to jump. The second week of age had very different outcomes which could mask the real interpretations. Thus, if we exclude it (considering only weeks 4 and 6 of age), during the morning, run was still the activity performed the most as the second week had no morning session of observations. During midday, manipulating objects was the preferable activity and running was the preferable activity during the afternoon.

Manipulating objects is the action that they performed with less variation along the day during the observations at the ages of 2 and 4 weeks old. The play that turned out to be less variable along the day at 6 weeks of age was running.

During the first observations (2 weeks old) the lambs were generally more active at midday, when they were going out with the other Urial ewes with a total of n=708 performances of play. When

they were 4 weeks old they were more active during the afternoon with a total of n=61 performances of play. Finally, at 6 weeks of age, the lambs showed more activity during midday again with a total of n=55 performances of play.

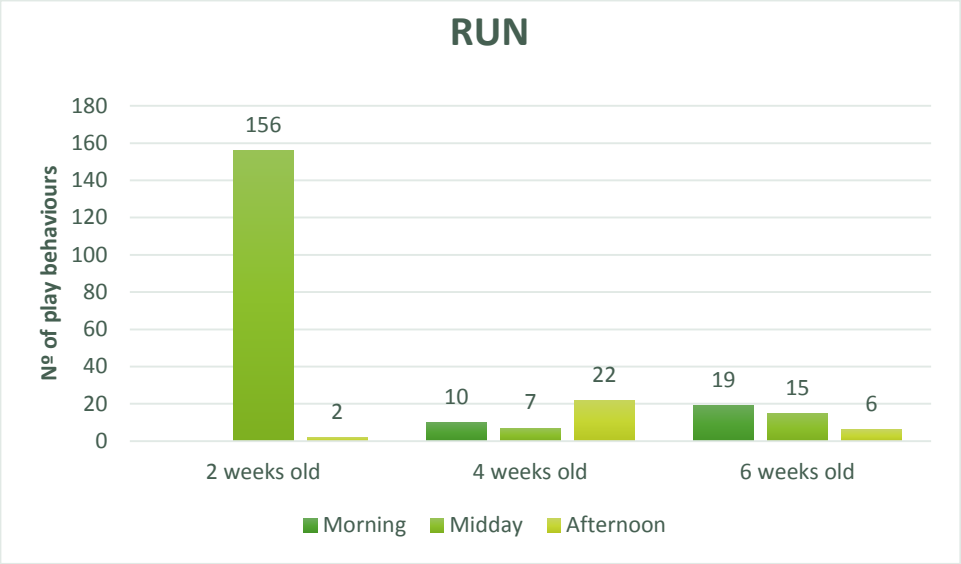



**Figure 14. Ontogeny of run behaviour troughout the 6 weeks of age.**

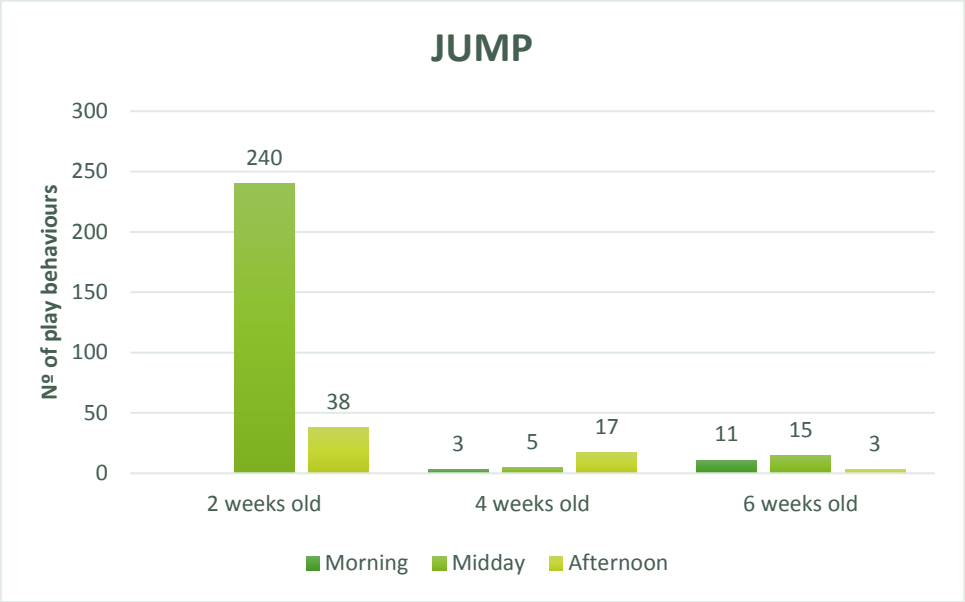



**Figure 15. Ontogeny of jump behaviour troughout the 6 weeks of age.**

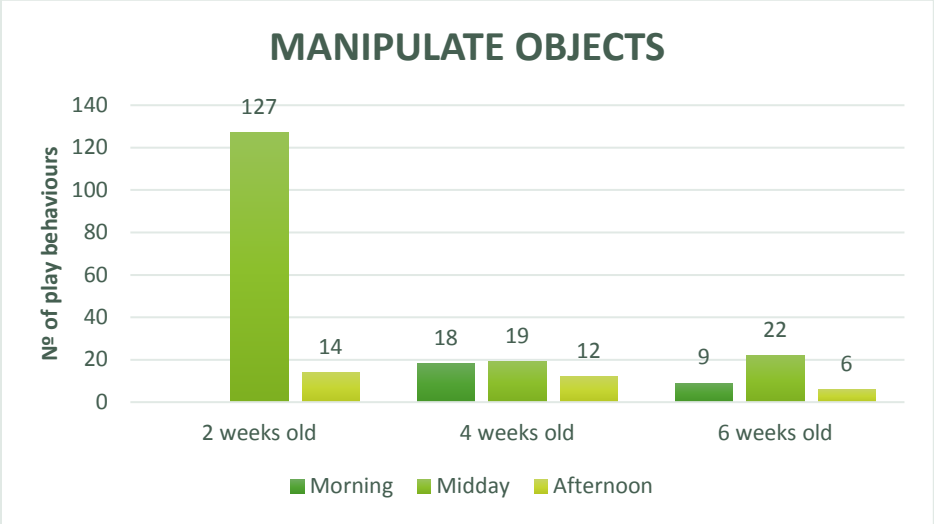



**Figure 16. Ontogeny of manipulate objects behaviour troughout the 6 weeks of age.**

## *6.3. Play as an effect of temperature*

Most of the days during the observations, the weather was constant, warm (16 to 28ºC) and sunny (13 days out of 16), except for 3 morning sessions with temperatures from 12 to 13.5ºC. Despite the sunny days, the Urials enclosure was not receiving big amount of sunlight due to the high trees and their abundant leaves, which were producing a dim atmosphere.

The relationship between the climate and the amount of play behaviour it is not very clear. The highest peak of temperature (28ºC) does not coincide with the lowest peak of play behaviour (Figure 21). However, it seems there is a slight relationship with more play when the temperature decreases and vice versa. Controversy, when we analyse the dispersion graph (Figure 22), excluding the temperatures and play behaviours of week 2, we can surprisingly clearly see a tendency line that indicate the lambs tended to increase their play performance along with an increasing temperature.

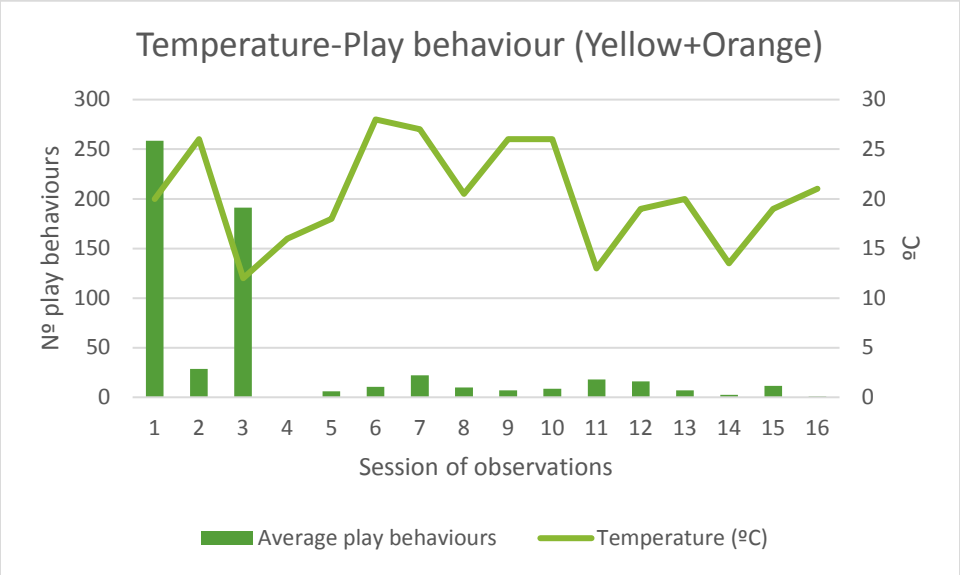



**Figure 17. Relationship between temperature (ºC) and average of play behaviours for both lambs during each session of observations.**

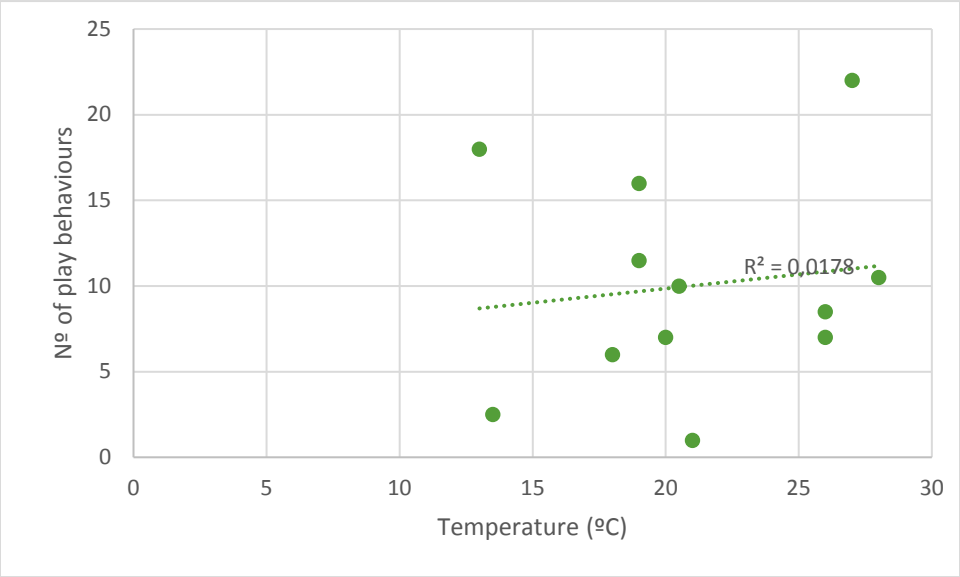



**Figure 18. The tendency to play in relation to the prevailing temperatures during the observations at week 4 and 6.**

## *6.4. Play as an effect of milk intake and growth*

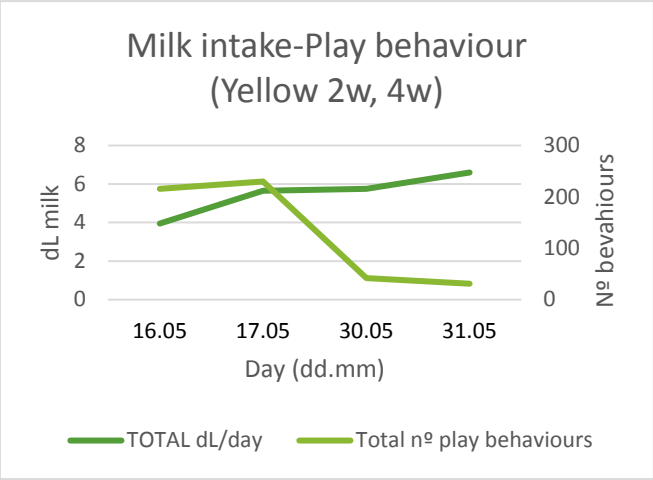



**Figure 23A. Relationship between the milk intake and the total amount of play behaviour during week 2 and 4 of age for the yellow tagged lamb.**

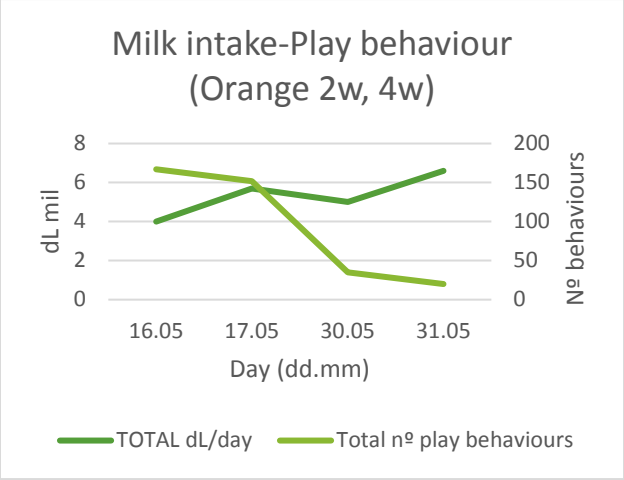
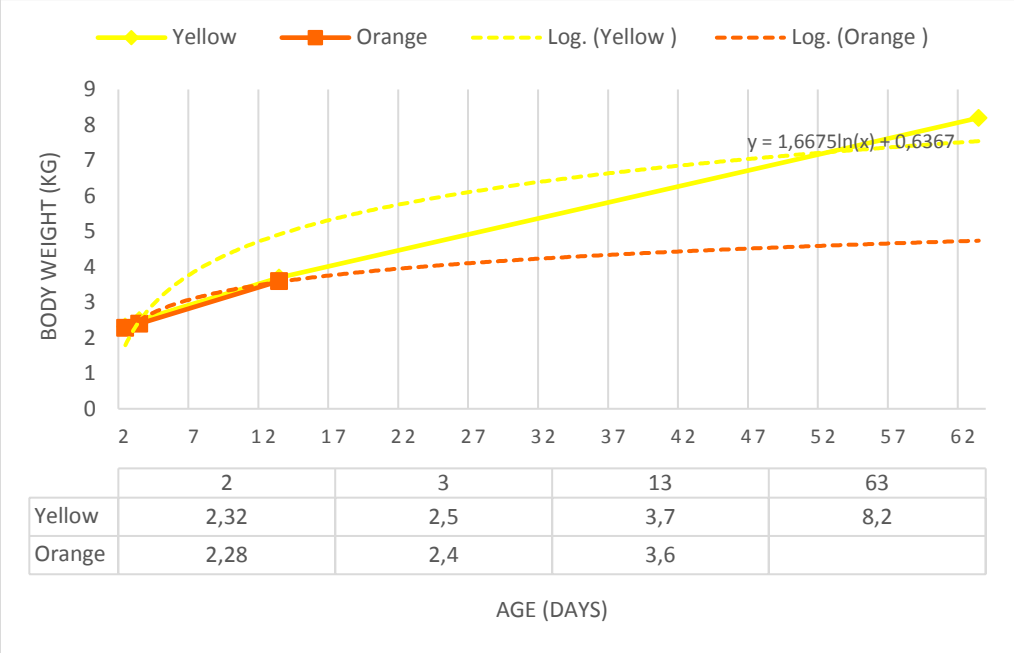



**Figure 19B. Relationship between the milk intake and the total amount of play behaviour during week 2 and 4 of age for the orange tagged lamb.**

The graphs (Figures 23A and 23B) show the amount of milk in dL the lambs drank per day of observation during weeks 2 and 4 of life and its relationship with the recorded play behaviours on that day. There is no data available for the intakes during week 6 of age. We can see a modest progressive increase of the intake of milk as the lambs grow up (from 3.95 to 6.6 dL/day, yellow tag; from 4 to 6.6 dL/day, orange tag) and at the same time the number of feedings was reduced from four to two while the play behaviour decreases. There is very little variation between both lambs as they had a similar intake pattern along the days.




| | 2 | 3 | 13 | 63 |
|---|---|---|---|---|
| Yellow | 2,32 | 2,5 | 3,7 | 8,2 |
| Orange | 2,28 | 2,4 | 3,6 | |

**Figure 20. Growth curve of the Urial lambs.**

During the week two of age, the growth curve (Figure 24) shows to be steeper whereas afterwards it goes flat. We can just affirm this in the Yellow lamb curve, as there is no data for orange lamb weight after two weeks old.

### *6.5. Other behaviours recorded*

- "Pawing to initiate play": it was seen that one of the lambs would paw around the ground where the other lamb was already laying down. Then, the second lamb would wake up.
- Information gathering / Exploration: The nose is in contact with the ground, the fence / wall or an object *(modified after Anderson et al. 2015).*

# 7. Discussion

The main aim of this study was to investigate the ontogeny of play behaviour in captive Urial lambs.

To evaluate the behaviours and interactions during the six weeks of life of the Urial lambs, I analyse each result's section.

### *7.1. Play as an effect of week (age) and diurnal rhythm*

The main finding in this study was that the lambs played a lot during the first observation period at 2 weeks of age and hardly at all during the second and thirds observation periods at 4 and 6 weeks of age.

To explain and try to understand the large difference of play behaviour among week 2 and 4-6 of age there are two possible options/questions we can pose: were the lambs playing more at 2 weeks of age, or were they supressing play at 4-6 weeks of age? One may not exclude the other. Might be this kind of behaviour normal for wild Urial sheep? Likely none of them is what is natural for wild sheep because of other interfering factors. Below, I discuss the potential resolutions to explain each hypothesis related to the results obtained regarding to that difference.

### 7.1.1. Influencing factors

#### 7.1.1.1. Housing

Studies have demonstrated that exposure of a lamb to an open-field arena proves to be a potent stimulator of behavioural responses such as play (Moberg and Wood, 1982; Chapagain et al., 2014). Increasing the space allowance has also been found to increase the occurrence of play behaviour in calves and in piglets (Boissy et al., 2007).

During the first 2-3 weeks of age, the lambs were used to go to a larger enclosure at the same time daily. Hence, anticipation behaviour could have been expected before the lambs were going outdoors as it is seen in other studies with domestic lambs, in the form of increased duration of locomotor behaviour (walking), sniffing or vocalizations (Chapagain et al., 2014; Anderson et al., 2015). However, we don't know whether this anticipation behaviour was shown by the lambs as the observations started when going out after feeding, but according to the zookeepers they noticed somehow "the lambs seemed to know they were going outdoors to play".

Hence, the fact that during the first observations (2w) they were playing outstandingly more during 11.30-13.30h could be explained as a possible previous motivation to play while being confined in a smaller area (indoors) and an increased occurrence of play behaviour once the space is expanded (outdoors enclosure) (Moberg and Wood, 1982) where they played during almost the whole 2 hours expressing the intrinsic rewarding properties of play (Chapagain et al., 2014)

Continuing with it, Špinka et al. (2001) predicted that those species that move between different habitat types perform more locomotor play than the others that stay in a stable environment since they will have to cope with environmental changes, and showing as well an increase in play frequency. If we extrapolate this to an individual level, we could suggest that lambs could have experienced this sudden change in the environment when going outdoors, hence, more unpredictability, novel events (Wemelsfelder and Farish, 2004) and higher expression of locomotor play.

#### 7.1.1.2. Stress

While trying to explain the play behaviour at 4-6 weeks of age, we can mention that the environmental stress in general (increased predation, poor habitat quality, food...) leads to the level of play being one of the first behaviours that is reduced (Špinka et al., 2001; Dugatkin, 2014).

Play appears to be suppressed in situations of danger in the environment, i.e. detecting predators. While playing, animals self-handicap, they put themselves in a position very different from that in which they normally find themselves, where dominant animals allow subordinates to defeat them during play fights, for instance, by inhibiting force of their pushes (Špinka et al., 2001; Dugatkin, 2014). Thus, play could be affected, when they spent all day in the Urials enclosure, by the human visitors or the Amur leopard (*Panthera pardus orientalis)* situated in front of the Urial's enclosure, detecting them as dangers or "predators", and hence, play was reduced.

If animals can "afford" play is because they receive parental care, comprising the provision of milk and a degree of protection from predators (Špinka et al., 2001). Thus, another reason why less play was expressed, in exception of the second week of life in the new environment, might be because no parental care and no feeling of parental protection was present.

Conversely, a study of Moberg and Wood (1982) said there were no differences in behaviour from lambs being deprived of the normal maternal care but from the absence of any social stimulation. They raised male lambs under three different social conditions (isolation, with peers or with their mothers). Differential rearing conditions significantly affected the lambs' behavioural response in short term (14 days of age) to a novel environment. Isolate-reared lambs spent less time moving in the arena than the other groups (Moberg and Wood, 1982). In the present study, our lambs were hand-reared together as siblings, thus, compared to the previous study they would act as the peer-reared lambs, which showed to have no impact in the change of behaviour regardless of the lack of a mother. Hence, more research with lambs and a normal maternal behaviour should be carried out to figure out whether they show the same play behaviour.

In several species of wild animals, it has been observed that play behaviour decreases or even disappears when the animal is in an inadequate habitat. Likewise, there are studies that show that situations of chronic stress decrease the frequency of the play in felines in captivity (Manteca Vilanova, 2002).

The Urial sheep is a grazer, which means they feed on growing grass and pasturage for large parts of the day (Collins, 2012). The part of the Urials' enclosure where the ewes and lambs were kept in was basically made up with trees and rocks with a minimal part of growing grass, probably due to the scarce sunlight that arrives to the ground (Figure 25). If they were not properly showing their natural behaviour of grazing, providing a stress situation, we could suspect an effect on other behaviour as well, such as play (Manteca Vilanova, 2002; Dugatkin, 2014).

**Figure 21. Urial's enclosure. Notice there is no grazing area available for the sheep**

Moreover, in a study testing how nutrition was affecting ewes during gestation and later lamb development and feeding behaviour, showed that with higher pasture allowance (10-12 kg dry matter/100kg BW/day) during gestation, the lambs tended to graze more than lambs from the low pasture allowance (5-8 kg dry matter/100kg BW/day) ewes (Freitas-de-Melo et al., 2018). Therefore, not just because there are not many chances of grazing in the Urial enclosure, but also

because the mother did not have a great grazing availability this could affect the natural grazing behaviour of the lambs.

In a study carried out by Berger (1980) about the social ontogeny of wild Bighorn sheep (*Ovis canadienses)* it was found that the frequency of specific motor actions differed between individuals inhabiting in two different habitats, desert and mountain. Play occurred at early ages in both but then tapered off in frequency in the desert environment. The main reason could be that they had to face certain hazards in the desert (injury because of cactus), perhaps the heat stress minimized the energy consumption through a reduction of play activity. Moreover, it was seen a greater feeding with milk in the desert which could also suggest less time playing. That supports the possible effect on behaviour of the Urials by the environment where they live. Another possible explanation suggested for the lack of observed play in desert living Bighorn sheep might be that it occurred at night. In fact, the Urial lambs were seen to be more active on some occasional days observed around 22 hours (Figure 26). This data was not included in the main study since this period of activity was not seen as early as the pilot study. Hence, a future study of play behaviour including night observations would be advisory to conduct. Augustsson (2014) also suggests further investigation whether the evening darkness triggers the play, as European Mouflon (*Ovis aries orientalis*), cousin to the Urial, is crepuscular.

**Figure 22. Urial lambs active at night**

When managing captive wild animals, it is important to give them a “natural life”, resembling their normal environment in the wild to be able to express its species-specific behaviours. Although, it

is not always possible to replicate the wild environment, it is very important to provide living circumstances that promote health and nutrition (Mellor, 2014).

Therefore, an extensive grazing or pastorally area in the enclosure would be a great way to offer them more opportunities to engage in their natural behaviours that may be accompanied by positive effects.

Self-grooming was seen frequently during all the observation periods. Self-grooming, defined by Boissy et al. (2007) as "the maintenance of the one's own body surface through licking, scratching and rubbing the fur or through wallowing; bathing to remove the dirt and the ectoparasites and as well as by thermoregulation", appears to be more frequent in novel or stressful situations. Along these lines, can we suggest that the lambs were under stress and hence, they expressed more self-grooming and less level of play?

Conversely, some authors have considered mutual grooming or self-grooming as play behaviour (McDonnell and Poulin, 2002). And it might also be a result because of the lack of maternal care and grooming.

The most common action carried out by the lambs at week 4 and 6 was lie/sit (31% of the total). While laying down or sitting, the lambs were mostly together. As suggested by Temple et al. (2011) in a study with Iberian pigs (*Sus scrofa domestica)*, the different types of social behaviour, not just social play, is related to relieve from stressful experiences. Therefore, possibly our lambs performed more social contact such as laying down together or following each other in order to reduce the negative effects of stressful events.

#### 7.1.1.3. Nourishment

Another important factor to contemplate is the nutrition. Numerous studies investigate the diet characteristics of wild adult sheep (Awan et al., 2006b; Elves de Souza et al., 2017; Bang et al., 2018), but since we do not know too much about lactation in wild sheep and weaning period in lambs, it is likely that we make mistakes when hand-rearing a wild sheep lamb. However, also domesticated sheep of older breeds could be used to gain knowledge about natural suckling behaviour of wild sheep.

While body weight increases, the nutritional requirements for maintenance of sheep augment at the same time. Thus, it will lead to longer and/or more frequent feeding periods required. During the early stage of life, rumen will develop linked to the body weight increase (Elves de Souza et al., 2017). The domestic lambs should start eating solid diet at 2 weeks of age to start developing the rumen (Herrera, 1997). However, if they do not begin by then, there could be a problem with the rumen development related to a lower body weight increase. The Urial lambs showed behaviour of hay biting from 2 weeks old.

Because play behaviour is not essential for survival (Boissy et al., 2007; Mintline et al., 2013), young animals reduce or eliminate play in unfavourable situations, i.e. young ungulates such as calves and fawns have shown a decrease in play behaviour when confronted with a shortage of milk or during weaning (Mintline et al., 2013). Stone (2008)´s study evokes that a decrease in play in squirrel monkeys was linked to low food availability and low energy budgets. Animals will just enhance in play behaviour when other basic needs are met (Boissy et al., 2007).

We can see a modest progressive increase of the intake of milk as the lambs grow up, while the play behaviour decreases, with the same pattern in both lambs.

We can suggest the lambs were not enough fed to meet their needs and hence, they suppressed play. This could be because of an insufficient amount of milk provided, too few feeding bouts, to early weaning, wrong composition of the milk or a combination of several factors.

The milk yield is not known for wild sheep or Urials. When studying the artificial lactation in domestic sheep (Herrera, 1997) it is stated that it can be given *ad libitum* with the aim of maximum development of the lamb, or restricted if the aim is to reduce the costs. With a good substitution milk *ad libitum* it is said the lambs should gain 350-450 g daily. If we calculate the daily gain of weight for Yellow tagged lamb from Day 2 to Day 3 (when the growth is faster for this lamb according to the graph X) through the equation of the growth curve, we obtain a daily gain of 149g/d. This is less than half what they should gain according to Herrera (1997). The weaning criteria should be the age of the lamb not the weight, being advised to not wean a lamb before the 6 weeks of age. In Norwegian reindeer (*Rangifer tarandus*) the lactation is 24-26 weeks long (Gjøstein et al., 2004).

Moreover, in Norwegian reindeer, the milk composition changes with the lactation stage (Gjøstein et al., 2004) and our lambs were fed always the same kind of milk. Although it must be said that the lambs in their original habitat do not have to survive to the arctic winter as Norwegian reindeer does and reindeer are much bigger than sheep.

Thereby, first of all, more research in wild sheep's lactation should be performed in order to contribute to the conservation of this species.

There is a positive correlation with variation in body weight and periods on other activities. Hence, more variation or increase in the body weigh will lead to higher number of periods resting or interacting. On the other hand, the greater feed intake, the less time spent on other activities. (Elves de Souza et al., 2017).

Play is also seen to be most frequent and / or intense during periods of rapid allometric growth (Špinka et al., 2001). With the growth curve of the yellow lamb, and an expected similar curve for the orange lamb, we could suggest the lambs played more during their peak of growth when the curve is steeper and the growth is faster.

The "*surplus energy hypothesis*" advocate that when animals obtain better nutrition, will have to let off the energy excess in play (Špinka et al., 2001; Burghardt, 2005a). Consequently, even though the lambs may have had the right amount of milk/alimentation for growing up and surviving, as they may have lacked in the excess of energy, they did not use it to play and hence they showed a lower display of play.

## *7.2. Ontogeny of different type of play behaviour*

The most significant behaviours found throughout the 3 periods of observation were two expressions of locomotor play: Run and Jump; and the object play of Manipulating Objects. It is seen in domestic lambs by a number of authors (Sachs and Harris, 1978; Augustsson, 2014) that females engage in locomotor play more frequently than males.

At 2 weeks old, the play behaviours of Chasing/Being Chased, Pivot/Rapid Turn and Mount/Being Mounted (social and locomotor play) also showed a greater prevalence among the other play behaviours. As we said before, sheep self-handicap when performing social play. This higher amount of social play at two weeks old could be explained if the lambs felt less threatened during that week, as they were spending more time in the indoors enclosure and could afford more self-handicapping.

When sorting the different play behaviours into groups, we could notice a decrease in the areas of locomotor and social play whereas the object play increases from week 2 to 4. From week 4 to 6 of age, the locomotor play increases, the social play keep decreasing and the object play start decreasing.

This first increase in object play appears to be important in Urial lambs as “manipulating objects” was seen as the activity they realized with less variation along the day (weeks 2 and 4). Augustsson (2014) only witnessed object play during the weeks 25-27 and very little, when studying play behaviour in different breeds of domestic sheep. Controversially, it has been noticed that lambs perform a greater amount of object play when reared artificially being more interested in investigating the environment because of the lack of the mother (Caroprese et al., 2006).

Jump had the highest peak of performance of all sessions, presented midday at the age of 2 weeks old. Likewise, among the categories of play, locomotor play was identified as the most common play category throughout the sessions. No major changes on the predominating type of behaviour are seen, however, the increase in object play could suggest similarities with the pattern Burghardt (2005c) mentioned, starting with locomotor play evolving into a predominant social and object play.

When excluding the outcomes of the second week of age and focussing on the main play behaviours recorded (Run, Jump and Manipulating Objects), during the morning and afternoon we identify run as the activity was more performed, and during midday, manipulating objects was preferably performed.

At 2 weeks of age the lambs were generally more active at midday, when they were going out with the Urial ewes. At 4 weeks old they were more active during the afternoon. Finally, at 6 weeks of age, the lambs were more active during midday again.

A general decrease in play behaviour was detected after week two until the end of the study at six weeks old. Sachs and Harris (1978) stated a bimodal trend to explain the ontogeny of play behaviour in domestic lambs (*Ovis aries*). Two peaks of play, around the third week of age and the seventh-ninth week. It was also marked a play behaviour depression between week five and six.

Comparing with the Urial lambs it could correspond if further observations would have been done to see whether there is a second peak around week seventh-ninth, as we could consider the first peak a bit earlier around week two-three. Also, it concords the lowest play display during week 3-5.

Ancestrally, the first peak has been suggested to coincide with the union of the little lambs to the nursery bands, a place near the lambing cliffs where the ewes and their lambs would gather to get some safety providing, as well, social contact and facility to start in play and social behaviour. The depression between peaks was explained as a coincidence with the migration of the ancestral sheep from the wintering to the summering grounds or the less production of milk from the ewes (Sachs and Harris, 1978).

The play behaviour of “Balancing on an ewe” was absent during all the observations. The animal jumps or climbs on top of the ewe's back and balances during a varied amount of time. Besides of play, it is seen to be associated with a feeding behaviour. When the lambs want to suckle from the ewe they jump to rise her up (Ewbank, 1967). The reason why they did not balance on an ewe could be supported by this theory as they were not suckling from an ewe.

### *7.3. Play as an effect of temperature*

During the observations, the weather was mostly constant, warm (16 to 28ºC) and sunny. Despite the sunny days, the Urials enclosure was not receiving big amount of sunlight due to the high trees and their abundant leaves which were producing a dim atmosphere.

The relationship between the climate and the amount of play behaviour seemed to be unclear. The highest peak of temperature (28ºC) does not coincide with the lowest peak of play behaviour (Fig. X) which we could have compared to Augustsson (2014)'s study whose results showed an decrease of play while an increase of temperature. On the contrary, we can surprisingly see a tendency line that indicate the Urial lambs tended to increase their play performance along with an increasing temperature.

This could be affected for other factors as the degree of satisfactory light conditions, so we can suggest that there is a correlation between the temperature and the play behaviour. Hence, when the weather seems to be warmer, the lambs seem to play more. The lowest peak of play happened a morning at 2 weeks of age when the lambs where housed indoors.

Such results should not surprise us as the climate in the natural habitat for Urial sheep is semi-arid with declared temperatures shown in the table below (Table 4).

**Table 4. Reviewed temperatures in the natural habitat for Urial.**

| | Time/Month | Area | Temperature |
|---|---|---|---|
| Schaller and Mirza (1974) | Daytime/October | Pakistan | <32ºC |
| Schaller and Mirza (1974) | Nigh time/October | Pakistan | 17-25ºC |
| Farhadinia et al. (2014) | Annual mean | North-eastern Iran | 14ºC |
| Hameed et al. (2012) | Average January | Pakistan | 5ºC |
| Hameed et al. (2012) | Average June | Pakistan | 40ºC |

With this, we can expect Urials adapted at such high temperatures, and we should not consider temperature as influential factor.

## *7.4. Other behaviours recorded*

- "Pawing to initiate play": it was seen that one of the lambs would paw around the ground where the other lamb was already laying down. Then, the second lamb would wake up. We could think this is an invite to play from one lamb to the other as is seen in horses (McDonnell and Poulin, 2002). However, the following behaviours were hardly ever showing play, but, the lamb that pawed, laying down in that area and the other lamb waking up laying down again near the place. Likewise, it was also seen how some of the adults where pawing the ground without any signs of play behaviour but sitting afterwards. It appears to be a way the sheep were testing the ground area where they wanted to lie. As the lambs were laying mostly all the time together, it was easy to think they were pawing the other lamb delivery though it was accidentally for the proximity to the sitting area.
- Information gathering / exploration: to put the nose on the ground, wall, fence or object *(modified after Anderson et al. 2015)* it is a behaviour that most species of animals are motivated to perform (Boissy et al., 2007). Urial lambs were mostly either sitting or walking meanwhile exploring. Could be because is a behaviour occurring until other more important are needed and thus, they were satisfied. It is differenced from object play because it answers the question "what is it?" instead of "what can I do with it?" (Dugatkin, 2014).

## *7.5. Limitations of the study and future research*

- During the observations, the duration of a behaviour was not considered, only the frequency. This would have been acceptable if shorter behaviours would have been predominant. However, previously to the study, we did not expect to see a prevalent behaviour of lying down or sitting, and hence, the observation method came out not being suitable. Even though the lambs woke up and sit quite frequently, the behaviour of lying

down or sitting was underestimated and we would have seen it even more abnormal if duration would have been considered.

- The human interference by the visitors and the zoo staff was a factor already expected before starting with the observations. Just comment that captive wild sheep in a zoo may be less fearful than expected in free wild sheep as, for example in the lambs, bottle feeding was provided manually every day by the zookeepers (Boissy et al., 2007).
- Also expected, was the captivity of the animals, as living in a zoo is never the same as in the natural habitat.
- There is a low significance throughout the study because of the little amount of lambs born or survived (n=2) taking part in the study.
- The studied lambs stopped being reared by the mother ewe from 2 days of age.
- When observing during the week 4 and 6, the lambs spent some time behind the rocks so no data was collected by then as the area for the observer did not reach behind the fence.

Future research or improvements proposed in this study are:

- More research with lambs and a normal maternal behaviour to figure out whether they show the same play behaviour or not.
- A grazing area in the enclosure would be a great way to offer them more opportunities to engage in their natural behaviours that may be accompanied by positive affect.
- More research in wild sheep's lactation in order to contribute with the conservation of this species.
- A future study of play behaviour including night observations. Ideally, to asses animals during both day and night, the direct observations could be complemented with cameras placed at several different locations of the enclosure.

# 8. Conclusions

The main conclusions of the project are:

- Among the different play behaviours, run, jump and manipulating objects were the most seen throughout the study. As for type of behaviour, locomotor play was prevalent higher

during all weeks. Along the day, they played depending on their management, including feeding hours or transfers from one place to another.

- Regarding to the ontogeny in Urial sheep, it showed a higher amount of play during week 2 of age and lesser during week 4 and 6. It probably was affected because of the lack of the mother. Therefore, more studies with normal rearing and maternal behaviour should be carried out to discover whether they show or not a similar behaviour. Moreover, the peak of play at week 2 of age was seen when the lambs were exposed to an open and bigger enclosure, likely increasing the motivation of play behaviour as seen in other studies.
- In matters of welfare, it was a bit of a concern what data showed. It appeared the lambs were under some level of stress and likely underfed. This stress could have been generated by the lack of a grazing area, the lack of the mother, predators / humans presence or the lack of nutrition itself.
- The temperature may be extreme for Sweden during Spring-Summer 2018. However, the lambs natural habitat consists of even higher temperatures for the same months of observations and hence, we should not consider this as a influencing factor.
- It has been suggested in other studies that the evening darkness could provoke the play, and in the present study it was also seen a possible peak of activity from 20-22 hours, hence, this supports the idea of a future study about play at night. However, this data was not collected and not included in the results.
- The lack of the mother affected to the expression of some play behaviours such as “balancing on an ewe”. This also affected with reference to the nutrition as the ewe’s lactation would probably be more available along the day (during 24h), longer in days and with different composition in each stage.
- One new, and surprising, finding was that the play behaviour in these lambs was less complex, contained less elements than that of domesticated lambs. This was totally surprising as domestication generally leads to a less complex behaviour than found in the wild type, but probably has to do with the rearing without the mother. Hence a new study needs to be performed where the lambs are reared by the mother. As seen in the domestic lambs (*Ovis aries*), lambs play complexly at 2w, which is more similar to our results at 2 weeks old for Urial wild sheep than results at 4 and 6 weeks old. Hence, we can suggest that in case the behaviour has been also affected for other factors (with the consequent

explanations previously exposed), the likely natural behaviour would be that expressed at week 2.

- On the other side, we can-not truly expect the wild to be similar to the domestic, but the domestic similar to the wild, as the later came first. However, this does not mean that we can-not go “backwards” and learn about behaviour and contribute with the Urial’s conservation from the observation of domesticated sheep.

Thereby, if we want to compare both species’ behaviours, further research in this field without the mentioned limitations should be involved.

# 9. Acknowledgements

The present project was supported and co-funded by SLU and Nordens Ark.

I would like to thank Senior Lecturer Jenny Yngvesson who has been my main supervisor during my stay in Sweden and have supported me during my hesitations and doubts along the process of

this study. I really appreciate and thank you for offering me this amazing opportunity of discovering animal behaviour world and trusting that I would be able to carry out this important project. Thank you for the constructive comments on earlier drafts. And thank you for this experience to travel across Sweden and live in a zoo.

I am thankful to Ewa Wikberg, for attending me on my first days at Nordens Ark and the time provided answering my multiple infinite questions about the lambs. To Camilla and Sofia, the main responsible zookeepers taking care of the Urial lambs, for their assistance, help and cooperation during the field work. And I am also very grateful to all the other staff at Nordens Ark that dedicated at least few seconds to help and assist me. I would also like to thank Nordens Ark for providing me accommodation throughout my observations and all the animals that made me enjoy it.

I express my gratitude to Claes Anderson for reading about my work done in this project.

Last but not the least, I thank the Urial lambs, the ones that have done the real work, as without them this study would have not been possible.

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