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**Departamento de Producción y Sanidad Animal, Salud Pública Veterinaria y Ciencia y
Tecnología de los Alimentos**



**Social play in *Otaria flavescens*:
description of activity patterns and
analysis of its functional meaning**

TESIS DOCTORAL

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SUMMARY

Play fighting is the most common form of social play in mammals (Fagen, 1981; Bekoff & Byers, 1998; Burghardt, 2005; Pellis & Pellis, 2009; Norscia & Palagi, 2016). Its functions may differ according to the species under study, players identity and other factors such as social structure, dominance relationships or level of inter-individual tolerance (Poirier *et al.*, 1978; Bekoff & Byers, 1998; Palagi, 2007; Cordoni, 2009; Ciani *et al.*, 2012; Cordoni & Palagi, 2016). This behaviour covers a broad range of behavioural patterns that can be exclusive of the play domain or can be borrowed from other functional contexts (Burghardt, 2005; Petru et al., 2009). Some of these patterns act as play signals since their performance specify the harmless intention of the players, thus avoiding escalating into aggression and making the session successful (Bekoff, 1995; Burghardt, 2005; Waller & Dunbar, 2005; Mancini, *et al.*, 2013; Cordoni *et al.*, 2016).

Although play fighting has been studied for over a century in both human and non-human animals, quantitative data on marine mammals are still scarce. The South American sea lion (*Otaria flavescens*) is one of the most sexually dimorphic species of pinnipeds (Cappozzo *et al.*, 1991). It has an extremely polygynous mating system and high levels of both intra- and inter-sexual competition (Campagna & Le Boeuf, 1988; Riedman, 1990; Fernández-Juricic & Cassini, 2007). Despite its particular features, play in this species has never been studied and nothing is known about its modality, its distribution among players and its functions. Here, we studied not only the behavioural repertoire of play fighting in this species, but the role of the *Relaxed Open Mouth* as a

play signal and the possible functions of play fighting according to its distribution among players.

We observed a group of South American sea lions (*Otaria flavescens*) housed at the *Oceanogràfic* aquarium (Valencia, Spain). The group was made up of one harem and was composed of 6 males (1 adult and 5 juveniles) and 11 females (8 adults and 3 juveniles). Observations were carried out for 14 months from May 31st 2013 to July 25th 2014. Data collection was preceded by a 6-month training period in which data were gathered by using a video camera following the *ad libitum* sampling method (Altmann, 1974). During this period the observer listed and described all the behavioural patterns that could be displayed during play interactions. Later on, during the 14-month data collection, videos were collected following the focal animal sampling method (Altmann, 1974). Each focal sample lasted 6 minutes and animals were randomly selected throughout the day. A total of 29h of recordings was compiled. Parametrical and non-parametrical statistic tests were employed to analyze data.

We recorded 36 behavioural patterns from which 12 were exclusive of the playful domain, 16 were classified in other categories but could be also displayed during play interactions and 8 were exclusive of other domains. On the other hand, as it occurs in many other carnivore species, the *Relaxed Open Mouth* (ROM) seems to be used as a playful signal for successfully managing the play sessions. In this species, ROM is disentangled from the biting action and is reciprocated by the players, especially if they share good relationships. ROM did not vary as a function of the number of subjects involved in the session and it was more reciprocated during dyadic sessions, that is when the players have the highest probability to engage in a face-to-face interaction. ROM reciprocity was independent from the level of asymmetry

characterizing each session, but it was fundamental to prolong the play session thus increasing play success.

Additionally, play is restricted to juveniles and is mainly expressed in males, as expected in a species showing a high degree of sexual dimorphism. Even though playful interactions were punctuated by competitive behaviours, animals played in a highly symmetric way and were able to adjust their competitive playful interactions in a flexible manner, thus reducing the risk of escalation at a minimum level. They were highly selective in choosing their playmates also by limiting the number of players per session and by playing more with age-matched companions and friends. Taken together all these factors are probably at the basis of the low level of escalation recorded during the study. This result is predictive of the high ability and motivation of these animals to engage in play behaviour which could have a role not only in the acquisition of dominance status, but also in establishing and maintaining social relationships, an unexpected role in a highly competitive species.

RESUMEN

El juego de lucha es el juego social más común en mamíferos (Fagen, 1981; Bekoff & Byers, 1998; Burghardt, 2005; Pellis & Pellis, 2009; Norscia & Palagi, 2016). Sus funciones pueden variar dependiendo de la especie de estudio, de la identidad de los jugadores y de otros factores como la estructura social, las relaciones de dominancia y el nivel de tolerancia social de los individuos (Poirier *et al.*, 1978; Bekoff & Byers, 1998; Palagi, 2007; Cordoni, 2009; Ciani *et al.*, 2012; Cordoni & Palagi, 2016). Este comportamiento comprende una amplia gama de patrones comportamentales que pueden ser exclusivos del contexto de juego o que pueden ser prestados de otros contextos funcionales (Burghardt, 2005; Petru' *et al.*, 2009). Algunos de estos patrones pueden actuar como señales de juego, ya que mediante su realización indican la naturaleza lúdica del contexto, evitando así que se produzca una agresión y haciendo de este modo que la sesión de juego sea exitosa (Bekoff, 1995; Burghardt, 2005; Waller & Dunbar, 2005; Mancini, *et al.*, 2013; Cordoni *et al.*, 2016).

Aunque el juego de lucha ha sido estudiado a lo largo de un siglo tanto en humanos como en otros animales, en mamíferos marinos los datos cuantitativos son todavía escasos. El león marino sudamericano (*Otaria flavescens*) es una de las especies de pinnípedos que presenta un mayor dimorfismo sexual (Cappozzo *et al.*, 1991). Tiene un sistema de apareamiento poligínico extremo y elevados niveles de competición intra e intersexual (Campagna & Le Boeuf, 1988; Riedman, 1990; Fernández-Juricic & Cassini, 2007). A pesar de estas particularidades, el comportamiento de juego nunca se ha estudiado en esta especie y no sabe nada acerca de su modalidad, de su distribución entre los jugadores, ni tampoco de sus funciones. En este estudio no solo investigamos

el repertorio comportamental del juego de lucha en esta especie, sino el rol que la “*Boca abierta relajada*” tiene como señal de juego y las posibles funciones que tiene el juego de lucha según su distribución entre los distintos individuos.

Durante nuestro estudio observamos un grupo de leones marinos sudamericanos (*Otaria flavescens*) albergados en el acuario *Oceanogràfic* (Valencia, España). El grupo estaba compuesto de un harem con 6 machos (1 adulto y 5 juveniles) y 11 hembras (8 adultas y 3 juveniles). Las observaciones se llevaron a cabo durante 14 meses, desde el 31 de mayo del 2013 hasta el 25 de julio del 2014. La recolección de datos fue precedida de un periodo de entrenamiento de 6 meses en el cual se recogieron los datos mediante una cámara de video siguiendo el método de muestreo *ad libitum* (Altmann, 1974). Durante este periodo el observador describió y clasificó todos los patrones comportamentales que se dieron durante las interacciones de juego y aquellos que eran susceptibles de darse durante este comportamiento. Durante los 14 meses de recogida de datos se hicieron videos siguiendo el método de muestreo focal (Altmann, 1974). Cada focal tenía una duración de 6 minutos y se reunieron un total de 29 horas de grabación. Los sujetos se eligieron de manera aleatoria a lo largo del día. Para analizar los datos se llevaron a cabo tanto pruebas paramétricas como no paramétricas.

Registramos 36 patrones comportamentales de los cuales 12 fueron exclusivos de la categoría de juego, 16 de otras categorías pero se llevaron a cabo también durante las interacciones de juego y 8 patrones que fueron exclusivos de otros contextos. Por otro lado, tal y como ocurre en otras especies de carnívoros, la “*Boca abierta relajada*” (ROM) parece que se utiliza como señal de juego para manejar de manera exitosa las sesiones. En esta especie esta expresión facial está desprovista de la acción de mordida y es recíproca entre los jugadores, sobre todo si comparten una buena relación. Como se predijo, esta expresión no varió en función del número de jugadores de la sesión y fue

más recíproca durante las sesiones diádicas, que es cuando los jugadores tienen una mayor probabilidad de participar en interacciones cara a cara. La reciprocidad de esta señal fue independiente del nivel de asimetría que caracterizó cada sesión, pero fue fundamental para prolongar la duración del juego, incrementando así su éxito.

Por otro lado, el juego se limita a los individuos jóvenes, se inhibe en adultos y, como es de esperar en una especie que muestra un gran dimorfismo sexual, se da principalmente en machos. Aunque las interacciones de juego estuvieron intercaladas por comportamientos agresivos, los animales jugaron de manera simétrica y fueron capaces de ajustar las interacciones de juego competitivas de una manera flexible y así reducir el riesgo de agresiones al mínimo nivel. También fueron muy selectivos en la selección de sus compañeros de juego limitando el número de jugadores por sesión y jugando con compañeros de edades similares y con los que existe una relación social estrecha. La unión de todos estos factores es probablemente la razón por la que se registró un nivel muy bajo de agresiones durante el estudio. Este resultado muestra la alta motivación y habilidad de estos animales para participar en el comportamiento de juego de lucha, el cual puede tener un rol no solo en la adquisición del estatus de dominancia, sino que también en el establecimiento y mantenimiento de las relaciones sociales, rol inesperado en una especie tan altamente competitiva.

INTRODUCTION

1.1 The Pinnipeds

1.1.1 Classification, distribution and evolution

Pinnipeds are a clade of marine mammals derived from the terrestrial arctoid carnivorans from which they split up about 50 million years ago during the Eocene (Hammond, 2012). The name Pinnipedia (from Latin *pinna* “fin” and *pes, pedis* “foot”) was proposed more than one century ago and makes allusion to carnivores of semi-aquatic life with well-developed fin-shaped limbs and feet (Scheffer, 1958). Due to the physiological, morphological and ecological adaptations to the marine environment, many authors have placed this clade as a suborder within the order Carnivora, or even as a separate order (e.g., Eisenburg, 1981; Riedman, 1990). However, other authors still defend the pinnipeds’ position inside the Arctoidea infraorder (e.g., King, 1983; Arnason *et al.*, 2007).

The clade of Pinnipedia is made up of 33 extant species. They are found along coastal areas, and despite many of these species have restricted distributions, the group as a whole can be found from the Arctic to the Antarctic regions (Riedman, 1990). Pinnipeds are grouped in two superfamilies: Phocoidea and Otarioidea. The first one is made up of one family, Phocidae, which includes earless seals (hereafter, seals). The second one is made up of two families, Otariidae which consists of eared seals (sea lions and fur seals) and Odobenidae which only includes the walrus (Riedman, 1990; Berta, 2009). The origin of these superfamilies is controversial and is still under debate (Repenning, 1977; Arnason *et al.*, 2006; Yonezawa *et al.*, 2009). Some scientists state

that both superfamilies share a common ancestor (monophyly), while others postulate that they evolved from two different carnivore lineages (biphily). The first lineage would have evolved from a “bearlike” ancestor in the late Oligocene and would have given rise to otariids and odobenids. On the other hand, the second lineage would have descended from an “otterlike” ascendant in the middle Miocene and would have led to the emergence of phocids (Riedman, 1990; Berta *et al.*, 2006; *Figure 1*).

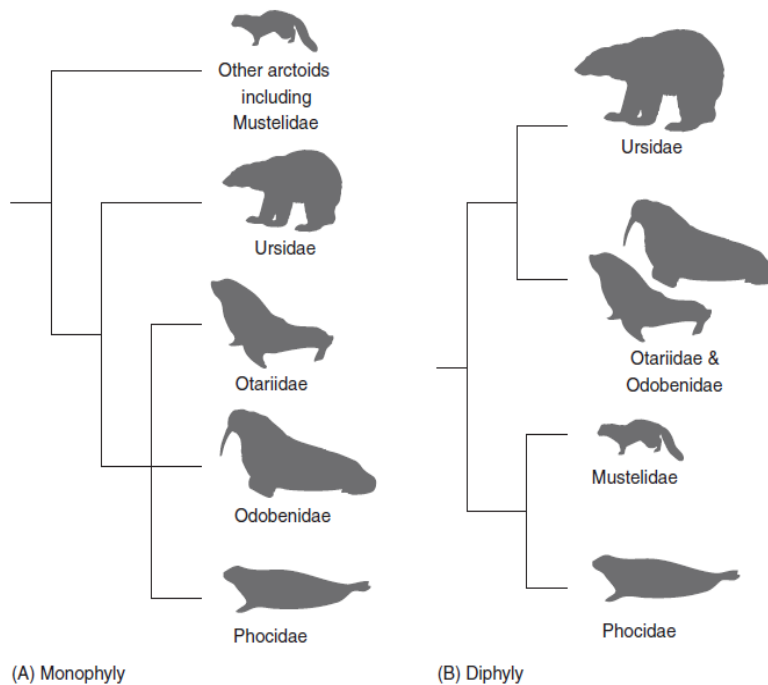


Figure 1 - Alternative hypotheses for the evolution of pinnipeds (from Berta *et al.*, 2006). (A) Monophyletic view with ursids as the closest pinniped relatives. (B) Diphyletic view in which phocids and mustelids are united as sister taxa as are otariids, odobenids, and ursids.

Those scientists who support the monophyletic view have based their arguments on studies at a molecular level of different living species (e.g., Sarich, 1969; de Jong & Goodman, 1982; Arnason & Widegren, 1986; Delisle & Strobeck, 2005; Arnason 2006;

Berta *et al.*, 2006). On the contrary, the authors who sustain the biphyletic origin have centred their arguments on comparative anatomical studies of living pinnipeds or on fossil records (e.g., McLaren, 1960; King, 1966; Tedford, 1976; Repenning, 1976, 1980). Despite the numerous studies performed at an anatomical level, it is worth noting that it can be misleading to assess the evolutionary and taxonomic relationships between two animals by observing their anatomical similarities (Lowenstein, 1985). In fact, two species may have acquired similar morphological traits driven by the action of similar environments. Moreover, two different lineages may share the exact morphological feature which evolved in each lineage at different times. Because of that, and given the reliability of the molecular analyses, the monophyletic view seems to prevail.

For a long time, it was thought that the Otariidae family was composed of two subfamilies, the Otariinae (sea lions) and the Arctocephalinae (fur seals) (Boness, 2009). Both subfamilies are morphologically similar although sea lions tend to be heavier. Despite that, the substantial distinction and the reason why they were classified in this way is their pelage difference. Fur seals are characterized by the presence of underhair while sea lions are not (Boness, 2009). Nevertheless, recent molecular and anatomical studies show evidence that this classification may not be appropriate (Berta & Deméré 1986; Wynen *et al.*, 2001; Arnason *et al.*, 2006). In fact, some authors have proposed other classifications (e.g., Arnason, *et al.*, 1995; Berta & Churchill, 2012), but until further studies support them, the classification of the Otariidae family will remain debatable.

Sea lions originated in the North Pacific region and it is thought they crossed into the Southern Hemisphere around three million years ago (Berta & Sumich, 2005). There are actually six extant sea lion species (Wolf *et al.*, 2007; Berta & Churchill, 2012). Two of them still occur in the Northern Hemisphere (the Steller sea lion,

Eumetopias jubatus, and the California sea lion, *Zalophus californianus*), while the other four are found only in the Southern Hemisphere (the Galapagos sea lion, *Zalophus wollebaeki*; the Australian sea lion, *Neophoca cinerea*; the New Zealand sea lion, *Phocarctos hookeri*; and the South American sea lion, *Otaria flavescens*) (Figure 2).

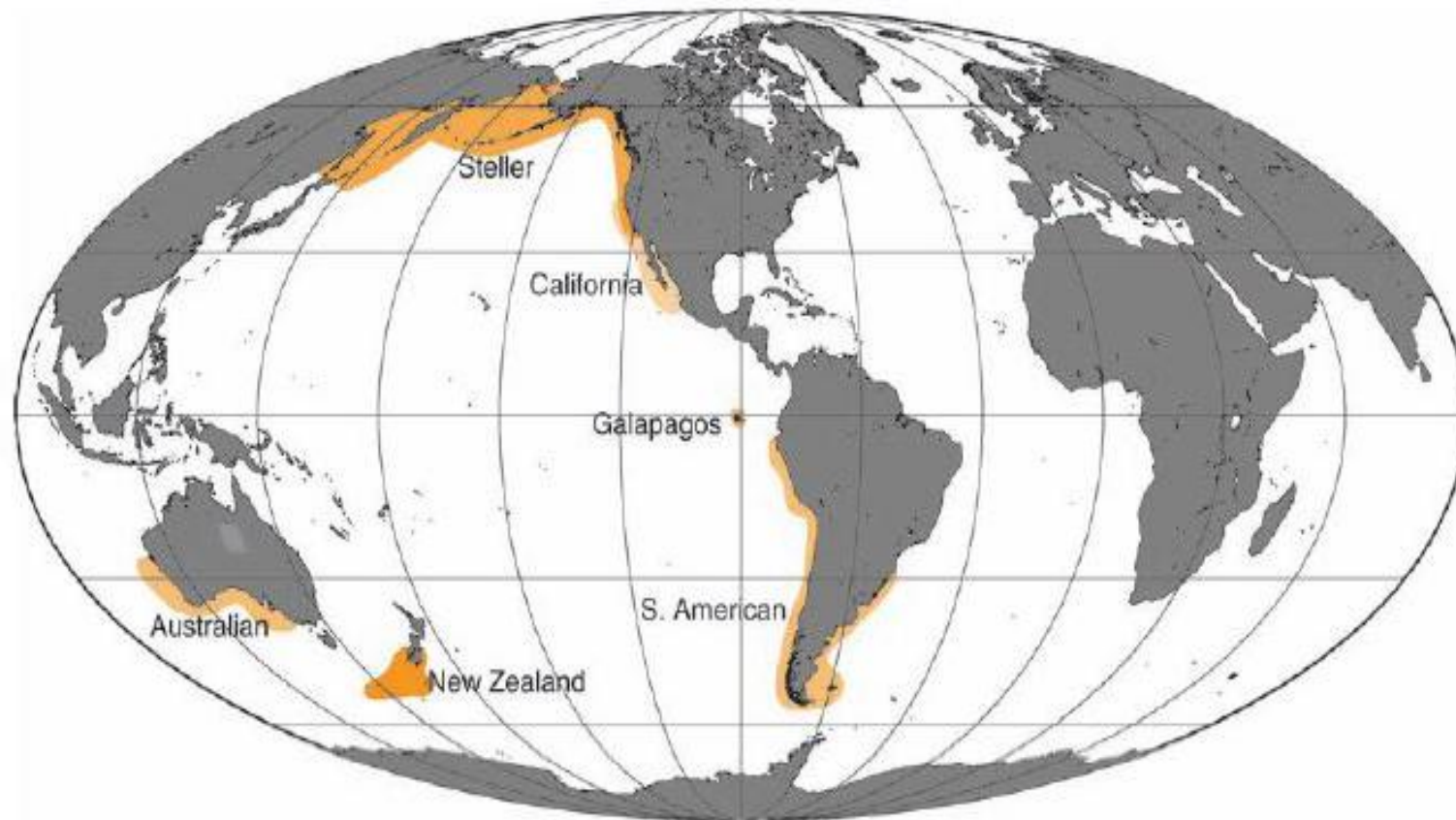


Figure 2 - Distribution of sea lions. Based on Riedman (1990). (From Berta *et al.*, 2006).

1.2 The South American sea lion (*Otaria flavescens*)

1.2.1 Distribution and nomenclature

The South American sea lion has one of the widest distributions among all sea lions species. Its geographical distribution covers both the Pacific and the Atlantic coasts of South America, from Zorritos in Peru to Torres in Brazil (Vaz-Ferreira, 1982), although some colonies have been registered up to Rio de Janeiro (Vaz-Ferreira, 1965; Carvalho, 1975; Vaz-Ferreira, 1982; Pinedo, 1990; Rosas *et al.*, 1994)) (Figure 3).

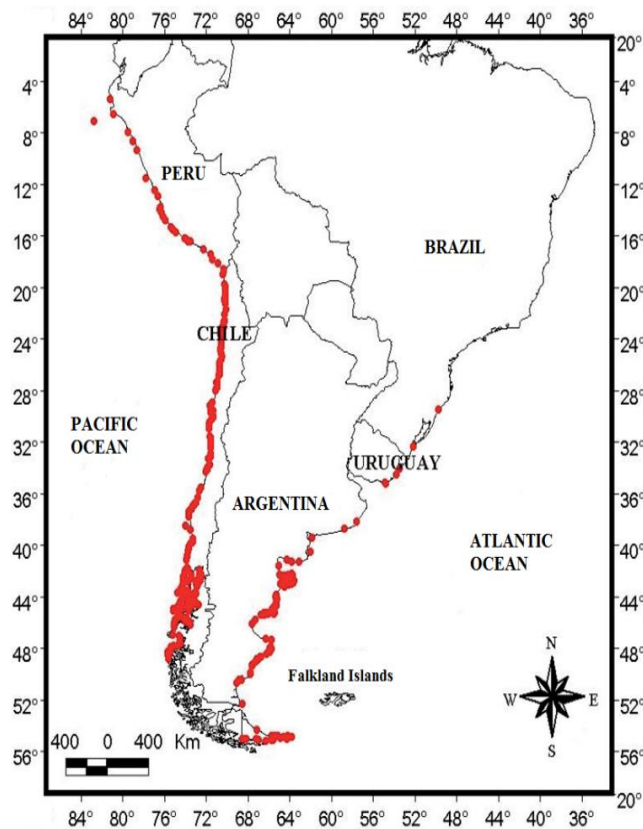


Figure 3 - Distribution of the South American sea lion (*Otaria flavescens*). (Modified from Crespo *et al.*, 2012).

Its nomenclature has always been controversial. Currently, two scientific names are still in use: *Otaria flavescens* (Shaw, 1800) and *Otaria byronia* (de Blainville, 1820). The first one was given after the examination of a two-foot-long yellowish specimen of an otariid collected in the Strait of Magellan and later stored in the Leverian Museum, London. Given the immature physical features of that individual, some authors stated that this identification was uncertain and suggested that the holotype could have been from a fur seal as well (*Arctocephalus australis* or *Arctocephalus gazella*) (e.g., Oliva, 2007; Berta & Churchill, 2012). Nevertheless, other authors compared such characteristics in the three species and expounded that the only species who inhabited that area and whose pups possessed the same features (body length, yellowish fur color and ear size), was indeed the South American sea lion (Rodriguez & Bastida, 1993). The second specimen was an adult otariid skull stated to have been collected on the Tinian Island, Marianas Archipelago, and then stored in the Royal College of Surgeons of London. No otariids have ever been seen in this geographical range, but as de Blainville visited the Falkland Islands on the same trip, many scientists think that the specimen he collected was later mistakenly labelled in the museum (e.g., Berta & Churchill, 2012). As both holotypes were lost and there is no way to prove that the labelling of the second specimen was in fact a mistake, the use of an adequate nomenclature will probably remain troublesome. Here, in order to be consistent we will use the name *Otaria flavescens*, as the International Commission on Zoological Nomenclature (1985) stipulates that a new scientific name can only substitute another when it is proved that the previous one is erroneous (Rodriguez & Bastida, 1993).

1.2.2 Ecology

1.2.2.1 Circa-rhythms

O. flavescens presents an annual cyclic pattern mainly associated to the breeding activity, which is composed of a breeding and a non-breeding season (Sepúlveda *et al.*, 2001). The reproductive period extends from the middle of December to the middle of March, and the non-reproductive period encompasses the other months of the year (Aguayo & Maturana 1973; Vaz-Ferreira, 1975). During the breeding season animals concentrate in the breeding sites and establish reproductive colonies (Hamilton, 1939). Later, they migrate to the resting sites or haul-outs, where there is a lower level of aggregation and remain there until December, when they return to the breeding sites (Sepúlveda *et al.*, 2001). The circannual rhythm is not the only cyclical pattern which has been observed in this species, as circadian rhythms have also been reported (Sepúlveda *et al.*, 2001, 2012). During the non-breeding season (fall and winter), most individuals remain in the haul-outs during daylight hours and spend the rest of the day in the sea. However, during the breeding season (austral summer), adult males and females usually remain in the territories (due to their reproductive constraints) and juveniles stay on land early in the morning and during the afternoon. These circadian rhythms have been suggested to be influenced by the fluctuations of temperature and the feeding patterns of their prey (Sepúlveda *et al.*, 2012; Carlens *et al.*, 2006; Andrews-Goff *et al.*, 2010).

1.2.2.2 Feeding habits

The South American sea lion is thought to be an opportunistic species which changes its diet depending on the season and on the geographical location (Suárez *et al.*, 2005). Because of that, it feeds on a wide variety of prey, consuming mainly pelagic and demersal fishes, but preying on benthic species as well (Suárez *et al.*, 2005).

Teleost fishes, cephalopods, and crustaceans are the main basis of its diet (Vaz-Ferreira, 1982; George-Nascimento *et al.*, 1985; Koen Alonso *et al.*, 2000), but it has also been seen to prey on fur seals and penguins (Riedman, 1990; Koen Alonso, *et al.*, 2000).

This species does not have terrestrial predators. The only species who feed on South American sea lions are the white shark (*Carcharodon carcharias*) and the killer whale (*Orcinus orca*) (Riedman, 1990). The former preys on them when sea lions are in the sea, but the latter may additionally do it performing a stranding technique when they are resting on the shore. This tactic has only been seen described at the Valdés Peninsula, Argentina. Killer whales approach slowly to the coastal lines without being noticed by sea lions and grasp them quickly while returning to the sea (Riedman, 1990; Vila *et al.*, 2008). While adult sea lions have developed anti-predatory behaviours, pups are more vulnerable, as they practice their diving skills when they are on the shore (Vila *et al.*, 2008).

1.2.2.3 Threats

O. flavescens is the most abundant marine mammal that inhabits the southern coasts of South America (Cappozzo 2002). The laws of the countries in which it lives have helped to protect this species by creating numerous reserves and protected areas at their breeding and non-breeding sites. However, the main threat of this species is the fishery industry. There is an overlap of the areas used by both sea lions and fishing fleet which makes them compete for the same fish resources (Koen Alonso *et al.*, 2000). The development of the industrial fishing in some areas is reducing the abundance of pelagic resources that this species needs and consequently the number of sea lions in these areas is decreasing (Dans *et al.*, 2003). In contrast, the fishery industry complains about the economical costs that this species provokes as it damages its nets and feeds on its fish

(Sepúlveda *et al.*, 2013). In fact, to avoid the economical losses, fishermen in some countries intentionally kill this species (Northridge, 1984).

1.2.3 Reproduction

The South American sea lion is one of the largest and most dimorphic species of otariids. At birth males are 10% heavier than females (Cappozzo *et al.*, 1991), and they are up to five times heavier as adults (Hamilton, 1939) (*Figure 4*). This species is characterized by a delayed sexual maturation with females reaching their sexual maturity faster than males. Females give birth for the first time at 4–5 years of age, while males become sexually mature around the age of 4–7 years (Grandi *et al.*, 2010). This delay in male maturity is especially pronounced, as it is in other highly polygynous species where males have an intense male-male competition and postpone reproduction until they are old and large enough to effectively compete for mates and resources (Riedman, 1990; Grandi *et al.*, 2010).

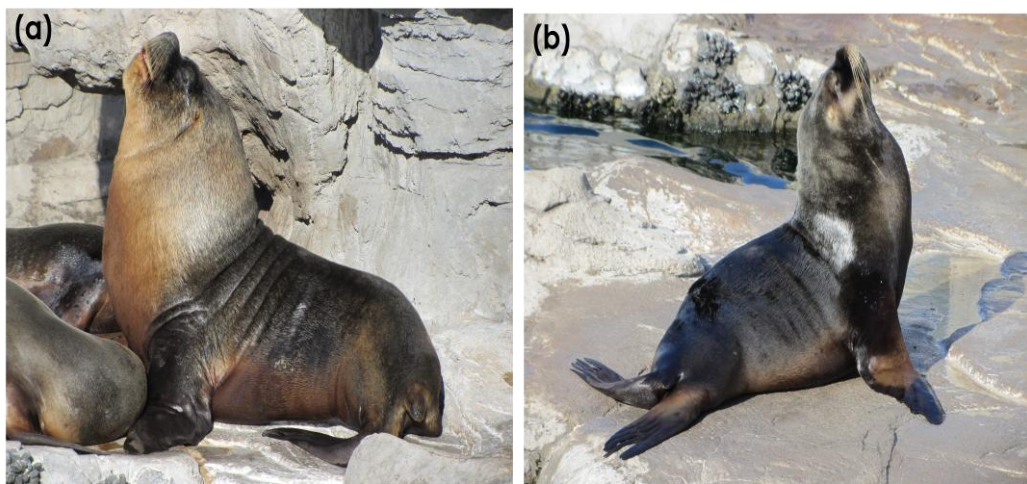


Figure 4 - Sexual dimorphism between an adult male (a) and female (b) of *O. flavescens*. (Photos by C. Llamazares-Martín).

The gestation is characterized by a delayed implantation in which the blastocyst stage stops and remains inactive for up to several months. Later on, the egg implants in the uterine wall and begins the full fetal development. The time spanning from the copulation to the parturition is around 12 months (Campagna, 1987). This reproductive feature is linked to the ecological factors needed for reproduction, and makes females' reproductive cycle be synchronic and occur every austral summer (Campagna, 1985). Days after giving birth one single pup, females come into oestrus and mating occurs.

1.2.4 Mating system

As in other species in which males do not provide parental care, the mating system of *O. flavescens* responds to the needs of females for reproduction and nursing (Davies, 1991). This species has a polygynous mating system in which males indirectly attract females to the breeding territories by monopolizing first the most suitable sites for their reproduction and thermoregulation (“resource-defense polygyny”; Emlen & Oring, 1977) (Campagna & Le Boeuf, 1988). Once females have arrived and established in a male territory, this type of polygyny switches as the male directly monopolizes those females by herding them and preventing other males from getting closer (“female defense polygyny” or harem; Emlen & Oring, 1977) (Campagna & Le Boeuf, 1988). Nevertheless, some studies suggest that the geographical distribution and the topography of the breeding sites may influence the convergence of both types of polygynous systems or even the presence of a “lek-like system” (Cassini & Vila, 1990; Soto & Trites, 2011).

1.2.5 Social behaviour during the breeding season

Males establish breeding territories by arriving sooner than females at the breeding sites and engaging in male-male agonistic interactions to control and monopolize such territories (Riedman, 1990). These interactions are characterized by the exchange of agonistic vocalizations, threats, bites, pushes and chases (Riedman, 1990; Acevedo *et al.*, 2003). The winner of the contest remains in the territory becoming the resident or dominant male, whereas the loser leaves the territory becoming a satellite or peripheral male. The resident male establishes its breeding territory when females arrive from the sea. Then, in order to preserve its territory, it prevents females' departure by herding them (Riedman, 1990). The maneuvers it performs include locking them with the neck and body, grasping them with its jaws and holding, shaking and hurling them into place (Campagna & Le Boeuf, 1988). Dominant males of this species are the only sea lions which successfully sequester females (Campagna & Le Boeuf, 1988). On the other hand, satellite males try to obtain mates by entering into their breeding groups and trying to seize females (Campagna *et al.*, 1988a).

Being females highly synchronous, they start giving birth some days after their arrival at the breeding sites (Campagna, 1985). This increases the number of the individuals clustered in the harems and subsequently it leads to a reduction of the space females need for nursing their pups. Nursing is a critical period in which females not only need sufficient space to feed their pups, but the quality of such space is also essential. Access to shade areas is of a great importance for thermoregulation during hot hours and an access close to the sea is crucial for both feeding trips and thermoregulation (Soto & Trites, 2011). Due to the scarcity of these resources agonistic interactions occur. Females threat other females by performing lunges with the mouths opened, but they also threat and attack unfamiliar pups by performing bites into the air

or by grasping them and throwing them (Vila & Cassini, 1990; Fernández-Juricic & Cassini, 2007).

Males fast during the breeding season to avoid the possibility of losing their breeding territories while foraging (Riedman, 1990). Nevertheless, females fast only during the perinatal period (i.e., the first week after parturition), that is when an uninterrupted nursing of the pups is needed to let them store energy and begin their growth properly (Riedman, 1990). Given that after this period females need to restore body fat, they begin making feeding trips to the sea by alternating two days on land nursing their pups and two days foraging (Campagna & Le Boeuf, 1988). This period of females' absence is critical for the pup's survival and, therefore, for females' reproductive success (Campagna *et al.*, 1988b).

When females are foraging, pups are especially vulnerable to social threats (Campagna *et al.*, 1988b). Females that remain on land perform aggressive behaviours towards unfamiliar pups (threatens and bites) and infanticide by adult and juvenile males can also occur (Campagna *et al.*, 1988b; Vila & Cassini, 1990). However, the most frequent cause of pups' death during this period is the squashing by heavier individuals (Le Boeuf & Briggs, 1977; Cassini, 1985).

In order to meet their pups when returning from the sea, females have to cope with the strong harassment of peripheral males which perform coercive behaviours to retain and mount them (Cappozzo *et al.*, 2008b). Once peripheral males approach, females may avoid them by returning to the sea, or may slowly approach to their respective breeding territories by stopping and lying down thus making the mount difficult (Campagna & Le Boeuf, 1988). Other times females face them by fighting back and biting their snouts, necks and flippers (Campagna & Le Boeuf, 1988).

Additionally, these females can perform agonistic behaviours towards unfamiliar pups before finding theirs, and they can also receive threats by the females who are already in the breeding territories (Vila & Cassini, 1990).

Aggressive behaviours diminish at the end of the breeding season (Pérez-Alvarez *et al.*, 2013), that is when females have been copulated, males begin their leave, and mothers remain with their pups and nurse them until they are able to swim long distances (Sepúlveda *et al.* 2001, 2009; Acevedo *et al.* 2003).

1.3 What is play?

Play is an intriguing behaviour which has been studied for over a century in both human and non-human animals (Fagen, 1981; Groos, 1898, 1908; Pellegrini & Smith, 2005). Despite it is easy to recognize when two or more individuals are playing, many efforts have been made to find a universal definition for this behaviour. The difficulty to find the appropriate definition and the reason why it has been the focus of numerous debates (e.g., Pellegrini & Smith; 2002; Burghardt, 2005) derives from the fact that it includes many behavioural patterns which differ between species and which are found in other functional contexts as well (e.g., predatory/anti-predatory, sexual, affiliative or agonistic) (Burghardt, 2005; Petru *et al.*, 2009).

Generally, three categories of play are distinguished: locomotor-rotational (Burghardt, 2005), object (Wilson & Kleiman, 1974) and social play (Fagen, 1981). These categories, even if useful, may sometimes overlap and intermixed making the definition of play even more difficult (Wilson & Kleiman, 1974; Fagen, 1981; Burghardt, 2005). For example, locomotor-rotational or object play can be performed both in a solitary and social manner (Burghardt *et al.*, 2016). The fact that some behavioural patterns can be borrowed from other functional contexts or can be displayed

during different types of playful interaction, proves that the distinction between play and non-play contacts lays down on the way by which such patterns are performed and not on the patterns themselves (Martin & Caro 1985; Pellis & Pellis, 1998).

Some authors stated that play is a non-functional behaviour, but, considering its energetic and survival costs (Fagen, 1981), it is more likely that play is a functional behaviour with no easily recognizable benefits (short- or medium/long-term benefits) (Pellegrini *et al.*, 2007). Recently, Burghardt (2005, 2011) listed five criteria for recognizing play behaviour across the different species:

- i. it may be incompletely functional in the form or context in which it appears because it does not seem to contribute to current survival;
- ii. it may be spontaneous, voluntary, rewarding, pleasurable and autotelic (“done by its own sake”);
- iii. it may differ from other behaviours in its form (e.g., exaggerated, uncompleted, or disordered) or in its timing (e.g., occurring early in life);
- iv. it may be performed repeatedly, but not in an abnormal or stereotypic form (e.g., distressed rocking, or pacing);
- v. it may be initiated when animals do not have to cope with heavy social or environmental stressors (e.g., predator threat, food shortage, or extremely hot temperatures).

1.3.1 Play fighting

Play fighting is the most common form of social play in mammals (Fagen, 1981; Bekoff & Byers, 1998; Burghardt, 2005; Pellis & Pellis, 2009; Norscia & Palagi, 2016); indeed, it is widely reported in primates, carnivores, rodents and artiodactyls (Bekoff, 1995; Smith, 1997; Pellegrini & Smith, 2005; Palagi, *et al.*, 2016; Bauer & Smuts,

2007; Cordoni, 2009; Pellis & Pellis, 2009, 2016). During play fighting, two or more animals exchange competitive actions that may be similar to those found in serious fights (Palagi *et al.*, 2016). Role reversals and/or self-handicapping behaviours may appear to give the playmate a chance to counterattack and consequently to prolong the session (Petrù, *et al.*, 2009). A self-handicapping behaviour is the act by which an animal puts itself into unnecessarily disadvantageous positions or situations being more vulnerable to attacks by their opponents (Bauer & Smuts, 2007). This disadvantage is mainly due to the inhibition of its own strength and to the performance of certain movements or postures that are physically demanding (e.g., avoidance of quadrupedal locomotion) or that restrict its sensorial perception (e.g., eyes closing or head rotation) (Petrù, *et al.*, 2009). Role reversals occur when dominant individuals in the non-play context appear in subordinate roles during play letting the playmate takes the offensive position (Bauer & Smuts, 2007). These reversals are facilitated by the performance of self-handicapping behaviours by the dominant individual.

Numerous benefits have been proposed for engaging in play fighting behaviour:

- i. creating and strengthening social bonds between conspecifics, often functioning as an “ice breaker” (Smith, 1997; Pellis & Iwaniuk, 2000; Antonacci *et al.*, 2010; Palagi, 2011);
- ii. acquiring and improving individual physical, cognitive and social skills (self- and social-assessment, Smith, 1982; Pellegrini, 1988; Byers & Walker, 1995; Thompson, 1998; Pellis & Iwaniuk, 1999,2000);
- iii. improving the encoding and decoding of emotions (MacDonald & Parke, 1984; Palagi *et al.*, 2015, 2016);
- iv. establishing dominance relationships (Symons, 1978; Cordoni, 2009);

- v. training animals to cope with the unexpected situations (Špinka *et al.*, 2001);
- vi. limiting aggression and increasing tolerance around food (Palagi, 2007; Palagi *et al.*, 2004, 2006; Norscia & Palagi, 2011).

The functions of play are not the same in all the species and, within the same species, play may acquire different role/s in relation to the players identity (e.g., age, sex and size) (Bekoff & Byers, 1998), environmental conditions (Palagi *et al.*, 2016; Palagi & Fouts, 2016), context (e.g. pre-feeding time Palagi *et al.*, 2004, 2006, 2007), social structure, level of inter-individual tolerance and dominance relationships (Poirier *et al.*, 1978; Palagi, 2007; Cordoni, 2009; Ciani *et al.*, 2012; Cordoni & Palagi, 2016).

Play distribution follows a bell-shaped developmental curve, starting in infancy, peaking in juvenility and decreasing at puberty (Fagen, 1981). The possible ultimate explanation for the reduction of play frequency in adulthood might be the increase in competition and aggression during play bouts, which can make the activity particularly risky (Caine, 1986; Pusey, 1990; Palagi, 2007). This hypothesis is supported by comparative studies that focus on phylogenetically close species characterized by different levels of social tolerance. The frequency of play between adults covaries with their social tolerance independently from their shared evolutionary history (*Pan* spp., Palagi 2006; Palagi & Cordoni, 2012; *Macaca* spp., Ciani *et al.*, 2012). In highly tolerant species adult play fighting can be used for strengthening social relationships (Pellis & Iwaniuk, 1999, 2000). On the other hand, in species showing crystalized and fixed hierachical relationships and a high aggressive tendency, adult play is inhibited (Palagi, 2006). This could be due to the high risk that such behaviour implies and also to the scarce importance of play fighting in establishing social relationships that have been already established via other kinds of serious behaviours (e.g., agonistic conflicts).

Gender differences in play fighting vary as a function of sexual dimorphism, reproductive strategies and roles covered by males and females in the group (Fagen, 1981, 1993; Byers & Walker, 1995; Power, 2000). In gorillas (*Gorilla gorilla*), a species characterized by a marked sexual dimorphism and a strong male dominance, immature males play more frequently than immature females and prefer to engage in play fighting with other males (Palagi *et al.*, 2007). However, the primacy of males in play fighting decreases in those species that are sexually monomorphic (Box, 1975; Stevenson & Poole, 1982, for young *Callithrix jacchus*; Cleveland & Snowdon, 1984, for young *Saguinus oedipus oedipus*; Pedersen *et al.*, 1990, for young *Crocuta crocuta*; and Cordoni, 2009, for adult wolf *Canis lupus lupus*) or characterized by female dominance (Palagi, 2006, for adult *Pan paniscus*; Palagi, 2009, for *Lemur catta*).

1.3.2 Play in Pinnipeds

Pinnipeds form a heterogeneous group in which the species inhabit a wide range of habitats and present different mating systems with different degrees of clustering and levels of competition. From the 33 extant species of pinnipeds play has only been reported in few of them (*Phoca vitulina*, Wilson 1972, Wilson & Kleiman 1974, Renouf & Lawson, 1986, 1987, Renouf, 1993; *Halichoerus grypus*, Wilson, 1972; *Mirounga angustirostris*, Rasa, 1971; *Eumetopias jubatus*, Farentinos, 1971, Gentry, 1974; *Neophoca cinerea*, Marlow, 1975; *Phocarctos hookeri*, Marlow, 1975; *Arctocephalus forsteri*, McNab & Crawley, 1975; *Arctocephalus australis*, Harcourt, 1991).

In three of these species: the Australian sea lion (*Neophoca cinerea*), the New Zealand sea lion (*Phocarctos hookeri*) and the New Zealand fur seal (*Arctocephalus forsteri*), play behaviour was incidentally described since the aim of these studies was to describe social and non-social aspects of each species behaviour. In the Northern

elephant seal (*Mirounga angustirostris*) and the Steller sea lion (*Eumetopias jubatus*) play fighting behaviour was described and characterized according to the age- and sex-class combinations of the subjects involved and some hypothesis on the functional meaning of this behaviour were discussed. However, no statistical analysis was performed to corroborate such results. McNab and Crawley (1975) studied the developmental time course of the different play types in South American fur seal pups (*Arctocephalus australis*) during their dependence period (32 months). But even if they carried out some statistical analyses, their hypothesis were not sufficiently supported, as they only analyzed the distribution of the play types according to the age of the individuals and no other factors were analyzed to give them more support. Finally, the common seal (*Phoca vitulina*) is the pinniped species in which a more exhaustive analysis of play has been conducted. Firstly, play type behaviours and playful patterns have been described along with those of the grey seal (*Halichoerus grypus*) (Wilson, 1972) and secondly, correlational studies on the distribution of play according to demographic, social and ecological factors have been performed (Renouf & Lawson, 1987, Renouf, 1993). However play fighting behaviour in this species has never been reported.

1.4 Communication

Communication is essential for the development of complex social systems (Freeberg *et al.*, 2012; Palagi & Mancini, 2011). The communication involves a signal emitted by a sender and a response that is a behavioural modification enacted by the receiver after perceiving the signal (Wilson, 1975). Through this interchange of information both parties can influence one another in selecting adaptive behaviours in

response in order to achieve their own goals (Markl, 1983; Palagi & Mancini 2011). As exchanging information is potentially costly, the sender has to optimize the emission of the signal in order to minimize the costs and maximize the probability of a response by the receiver. In other words, a signal can evolve only if benefits outweigh costs for both the sender and the receiver (Gosling, 1986). For this reason, senders convey messages through signals driven by different sensory modalities. Specific actions, gestures, postures, facial expressions, odours or vocalizations can be all recruited to maximize signal detectability (Hebets & Papaj, 2005).

1.4.1 Metacommunication and the use of play signals

Gregory Bateson (1955) defined metacommunication as “communication which refers to communication”, meaning the act by which an animal simulates communicative actions and makes another animal aware of this simulation (Mitchell, 1991). Metacommunication is highly frequent during mammalian social play, a behaviour which relies on a huge behavioural repertoire (Bekoff, 1975). In play, the metacommunicative signals (hereafter, “play signals”) qualify the meaning of the consecutive actions specifying that “what follows is only play” (Bateson, 1955; Mitchell, 1991; Pellis & Pellis, 1996). Some of these signals are unique to play (e.g., play faces, play bow) (Petrù *et al.*, 2009; Palagi *et al.*, 2016). These signals derive from a process of ritualization in which the behavioural pattern was structurally modified and split up from its original function, thus acquiring a new function (Tinbergen, 1952). Other patterns which are borrowed from other functional contexts (i.e., predatory, antipredatory, agonistic, reproductive; Burghardt, 2005; Pellegrini, 2009; Pellis & Pellis, 2009) become signals when they are modified in their performance. The motor action pattern remains basically the same, but the action is exaggerated, slowed down, repeated, emphasized or interrupted. Hence, the modality of performance, more than the

actual motor acts that constitute the pattern, conveys the message of the playful intent (Fagen, 1981; Bekoff, 2001; Palagi, 2006, 2008). All these signals can be used either before or during a playful interaction, depending if they are used to solicit the session or to maintain it. During play fighting play signals punctuate the non-seriousness of the context in order to avoid the escalation of the playful session into a real aggression (Bekoff, 1995). Moreover, play signals and specially facial play signals (Henry & Herrero, 1974; Waller & Dunbar, 2005; Mancini, *et al.*, 2013) have also an important role in prolonging the playful interaction thus guaranteeing the success of the session (Burghardt, 2005; Waller & Dunbar, 2005; Mancini, *et al.*, 2013; Cordoni *et al.*, 2016).

1.4.1.1 Intentional and emotional play signals

There is still much debate whether the communicative mechanism of play facial signals is exclusively intentional or emotional. Some authors argued that probably both components are involved (Sherwood *et al.*, 2004, 2005; Cattaneo & Pavesi, 2014; Liebal *et al.*, 2014; Demuru *et al.*, 2015). When play signals unveil the emotional state of the player (e.g., arousal, self-rewarding), their occurrence and frequency should be completely disentangled from the type of play the animal is engaging in (i.e solitary, locomotor, contact, or dyadic/polyadic play) (Demuru *et al.*, 2015). Instead, when playful facial expressions are emitted by the sender according to the attentional state of the receiver (the so called *audience effect*, Russell & Fernandez-Dols, 1997), the intentional component of these signals cannot be completely discarded.

1.4.1.2 Efficacy of the play signals

The congruent reciprocity of the signal by the receiver can provide information not only about its attentional state, but also about the correct perception and decoding of the signal itself (Provine 1996; Palagi & Mancini, 2011; Palagi *et al.*, 2014). The

phenomenon of facial signal reciprocity generally peaks during face-to-face interactions, which are common during play fighting (Palagi *et al.*, 2014; Cordoni *et al.*, 2016). In highly competitive species, in which rough play fighting has a role in establishing and maintaining dominance ranking positions, the appropriate use of play signals and their reciprocity can become even more important to avoid any kind of escalation (Bekoff, 1995; Burghardt, 2005; Palagi, 2008).

1.4.1.3 The Relaxed Open Mouth facial expression

The *Relaxed Open Mouth* (ROM) is one of the most widespread playful facial displays in many species of Primates, Rodents and Carnivorans (lemurs, Jolly, 1966, Palagi *et al.*, 2014; monkeys, van Hooff & Preuschoft, 2003; apes, Palagi, 2006, 2016; rats, Panksepp & Burgdorf, 2003; foxes, wolves and coyotes, Fox, 1970; black bears, Henry & Herrero, 1974, Egbert & Stokes, 1976; otters, Poole, 1978; dogs, Palagi *et al.*, 2015; Cordoni *et al.*, 2016). It is a ritualized pattern (*sensu* Tinbergen, 1952) which derives from the typical biting action (van Hooff & Preuschoft, 2003). In ROM, the action of biting has been split up and the pattern includes only the movement of mouth opening with a total inhibition of the biting act (Andrew, 1963; Fox, 1970; van Hooff & Preuschoft, 2003; Palagi, 2006). This ritualized version of biting, that lacks of any harmful motivation, informs the receiver about the benign intent of the sender (Palagi *et al.*, 2014).

1.5 Aim of the study

Social play is shaped by the ecological (e.g., Palagi *et al.*, 2016; Palagi & Fouts, 2016) and social factors (e.g., Poirier *et al.*, 1978; Ciani *et al.*, 2012) of the species considered. The South American sea lion is one of the largest and most sexually dimorphic species of pinnipeds. The breeding season is a critical period where there is a high aggregation of animals and consequently, a strong competition for limited resources. Conflicts are not limited to adults, but involve individuals of all age- and sex-classes (Campagna & Le Boeuf, 1988; Riedman, 1990; Fernández-Juricic & Cassini, 2007). Males fight to monopolize females, while females have to cope with the strong male harassment. In addition to this, when pups are born, females perform aggressive interactions towards other females and their offspring in order to favour the survival of theirs. Female are not the only ones that perform agonistic interactions towards pups, infanticide by adult and juvenile males also occur (Campagna *et al.*, 1988b; Vila & Cassini, 1990). Because of these competitive characteristics in the mating system of the South American sea lion, this is a suitable model species to study different aspects of the play fighting behaviour.

1.5.1 Description of the play patterns in *O.flavescens*

Despite the frequency with which social play appears in the South American sea lion, the study of this behaviour has never been performed. Hence, the behavioural patterns employed during play fighting are still unknown. Additionally, the social behaviour has mainly been studied from an ecological and reproductive point of view. Therefore, a complete and detailed ethogram of the social behaviour of this species has never been provided.

As previously mentioned, play fighting behaviour includes playful patterns and patterns borrowed from other functional contexts (Burghardt, 2005; Petrù *et al.*, 2009). In order to know the play fighting modality in this species and the variability in the behavioural patterns employed, here we provide a description of the patterns that may appear during the play sessions (exclusive or non-exclusive of the play context).

1.5.2 Functional meaning of the play fighting behaviour

As the functions of play fighting are related to the social factors of a given species (e.g., social structure, dominance relationships and social tolerance), several predictions have been generated in order to characterize this behaviour and its functional meaning.

1.5.2.1 Prediction 1

Adult play is sensitive to the degree of tolerance of a given species (de Waal, 1995; Palagi, 2006, Reinhart *et al.*, 2010; Palagi 2011) being usually inhibited in species characterized by strong adult competition and aggressive tendency (e.g., Palagi, 2006; Ciani *et al.*, 2012). If in sea lions adult play fighting is too risky and, at the same time, not so useful to mould hierarchical relationships that are maintained by serious behaviours (e.g. threatening, overt aggression, supplantation), a strong inhibition of this behaviour in adults is expected (**Prediction 1**).

1.5.2.2 Prediction 2

The motor training hypothesis predicts that play behaviour serves to accelerate the motor and cognitive skills needed when animals become adults (Fagen, 1981; Pellegrini, 2009; Berghänel *et al.*, 2015). If adult male fights are needed in the South American sea lion to attract and monopolize reproductive females, we expect that play

fighting is mainly performed by immature males, as it would act as a training tool (**Prediction 2**).

1.5.2.3 Prediction 3

Play fighting can be performed by two (dyadic) or more players (polyadic). If South American sea lion males must especially develop their cognitive and physical skills in order to successfully compete with other males in the future, polyadic play would be more effective in developing such skills, as males would simultaneously face different playmates. Nevertheless, as this species is highly competitive and polyadic play is more difficult to manage, engaging in this type of play would entail a higher risk of escalation into a real aggression than dyadic play. Hence, we expect that sea lions should engage less frequently in polyadic than in dyadic sessions (**Prediction 3a**). Additionally, we expect that, in order to reduce the risk of escalation, polyadic sessions have a lower duration than dyadic sessions (**Prediction 3b**).

1.5.2.4 Prediction 4

Play fighting is built on competitive elements which can make the playful sessions risky for the players (Pellis & Pellis, 1996). To limit the risk of escalation, we expect that animals make a selective choice of their playmates according to their relationship quality (the better the relationship quality, the higher the frequency of play; **Prediction 4a**) and their age difference (the less the players are mismatched, the higher the frequency of play; **Prediction 4b**).

1.5.2.5 Prediction 5

The 50–50 rule hypothesis (Aldis, 1975; Pellis & Pellis, 1998) predicts that players have to balance their offensive and defensive manoeuvres to give the playmate the possibility to counterattack. This strategy makes play successful (Bekoff, 2001). In

squirrel monkeys (*Saimiri sciureus*, Biben, 1998), hamadryas (*Papio hamadryas hamadryas*, Pereira & Preisser, 1998), and rats (*Rattus norvegicus*, Pellis & Pellis, 2009) stronger or more dominant individuals are able to limit their playful arousal thus increasing the probability that the play sessions lasts longer. However, the 50-50 rule does not fit for all species in which it has been tested. For example, many researchers demonstrated that, contrary to the expectations, dogs engage in unbalanced playful sessions (Bauer & Smuts, 2007; Ward *et al.*, 2008; Cordoni *et al.*, 2016). Obviously, the degree of asymmetry that characterizes the playful session can be influenced by several factors such as species, context and the relationship quality shared by the players (Bauer, & Smuts, 2007; Ward *et al.*, 2008) and this asymmetry will vary according to the different social functions of play (Ward *et al.*, 2008).

If, according to the motor training hypothesis, in sea lions play has a function in strengthening physical skills thus helping individuals in establishing dominance relationships, we can assume that play sessions show high levels of asymmetry (**Prediction 5a**). Moreover, if play fighting serves that purpose, we expect more competitive interactions in dyads who share a lower social relationship than in dyads who have stronger bonds (**Prediction 5b**). Finally, as competitive play is more risky than cooperative one, we predict that the sessions characterized by a higher level of asymmetry last less than those sessions that are more balanced (**Prediction 5c**).

1.5.3 The role of the *Relaxed Open Mouth* in the play sessions

During play fighting sessions are punctuated by play signals in order to avoid misunderstands and to make the sessions successful. This is particularly important in species with high levels of competition. Here we generate several predictions on the use of the ROM as a play signal in this species.

1.5.3.1 Prediction 6

If in South American sea lions ROM is a communicative signal, it should be less frequently followed by a biting action compared to a control pattern (*Attempt to bite*, PAB) (**Prediction 6a**). The interactive nature of playful signals predicts that they produce a behavioural change in the observers who generally reciprocate the signal perceived (Schmidt & Cohn, 2001). If ROM is an interactive signal we expect it to be reciprocated more frequently than its control (PAB) (**Prediction 6b**).

1.5.3.2 Prediction 7

If also in South American sea lions, ROM has an important role in communicating the motivation to play (Mancini *et al.*, 2013; Davila-Ross *et al.*, 2008; Waller & Dunbar, 2005), it should be performed during social play independently from the number of players involved in the session (**Prediction 7a**).

The effectiveness of a facial expression is measured by its interactive potential which generally reaches its climax during face-to-face interactions (Schmidt & Cohn, 2001). The more the response of the receiver, the more the efficiency of the signal. Therefore, if the interactive nature of ROM is confirmed (see **Prediction 6b**), we also expect that ROM should be reciprocated more when the session is characterized by a high frequency of face-to-face interactions as it occurs during dyadic play (**Prediction 7b**).

1.5.3.3 Prediction 8

If play in this species is characterized by a high level of competition that is useful for establishing dominance relationships (see **Prediction 5a**), we predict that ROM reciprocity should be more frequent in asymmetric sessions than in symmetric ones, as it would be necessary the punctuation and reciprocity of this signal to reduce

the risk of escalation into a real aggression (**Prediction 8a**). On the other hand, if sea lions limit the risk of engaging in a real fight by making a selective choice of playmates according to their social relationships (see **Prediction 4a**), we expect that ROM reciprocity should be higher between players who have exchanged affiliative patterns than between those who have not exchanged any kind (**Prediction 8b**).

1.5.3.4 Prediction 9

In many species it has been demonstrated that the interactive nature of ROM is essential for the success of playful sessions. For example, rapid mimicry (a mirroring response given within a second) significantly prolongs the play sessions (*Pongo pygmaeus*, Davila-Ross *et al.*, 2011; *Theropithecus gelada*, Mancini *et al.*, 2013; *Canis lupus familiaris*, Palagi *et al.*, 2015; *Macaca tonkeana*, Scopa & Palagi, 2016). For this reason, we expect that the reciprocity more than the mere amount of ROM is effective in prolonging it (**Prediction 9**).