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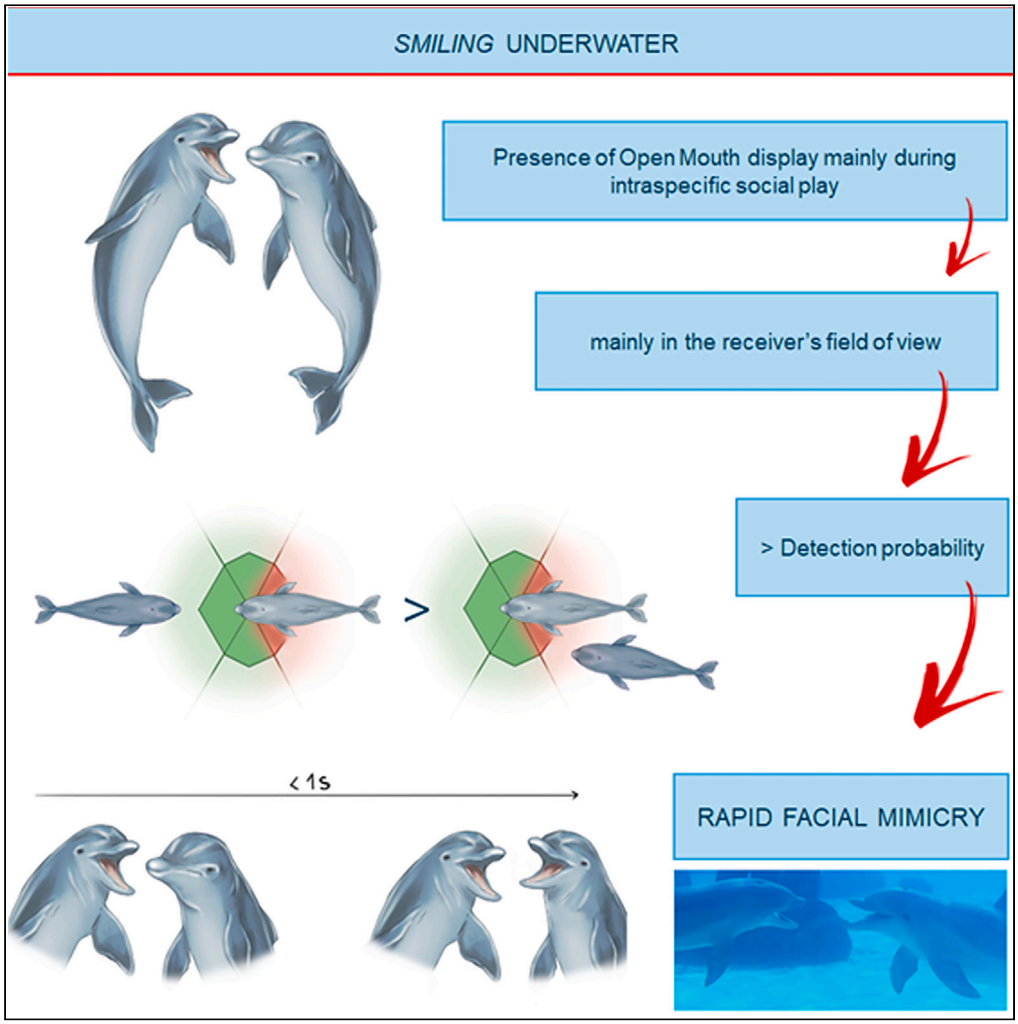

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livio.favaro@unito.it (L.F.)
elisabetta.palagi@unipi.it (E.P.)

Highlights

Play facial displays are widespread among mammals but unexplored in cetaceans

Bottlenose dolphins emit open mouth displays (OM) during their playful interactions

Detecting an OM evokes a mirror response in the playmate (rapid facial mimicry)

OM and RFM in dolphins are similar to playful facial signals in terrestrial mammals

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Smiling underwater: Exploring playful signals and rapid mimicry in bottlenose dolphins

Veronica Maglieri,[1] Federica Vantaggio,[1] Cristina Pilenga,[2] Martin Böye,[3] Alban Lemasson,[4,5] Livio Favaro,[6,7,*] and Elisabetta Palagi[1,8,9,*]

SUMMARY

Play is a widespread behavior present in phylogenetically distant taxa that, in its social form, relies on complex communication. Playful communication has been largely neglected in marine mammals. We focus on playful visual communication in bottlenose dolphins. The open mouth (OM) display was mainly emitted during social than during solitary play and occurred more frequently when the sender was in the receiver's field of view, suggesting that animals are attentive to the playmate's attentional state. Detecting an OM evoked the same facial display in the receiver, a result that strikingly matches with those obtained on cooperative social primates and carnivores. It is difficult to know whether such similarities derive from shared evolutionary pathways (*homology*) or from evolutionary convergence (*homoplasy*), as both have been suggested for play behavior. The pervasive presence of OM and rapid mimicry in the mammal phylogenetic tree indicates the relevance of visual mechanisms in shaping complex communication.

hominem non expavescit ut alienum, obviam navigiis venit, adludit exultans, certat etiam et quamvis plena praeterit vela Pliny the Elder[1]

INTRODUCTION

Play is a widespread behavior present in phylogenetically distant taxa, with data available both for invertebrates[2–4] and vertebrates.[5–7] For this reason, play is considered a polyphyletic behavioral trait for which we need to distinguish between origins and current functions.[8] Despite its broad distribution, the definition of play has always been challenging. Burghardt[9] suggested some criteria that a behavior must fulfill to be recognized as free play. The behavior must not have any obvious and immediate function, it must be spontaneous and autotelic, it must contain incomplete, exaggerated, and modified patterns that can be repeated in a non-stereotyped way. Finally, play occurs when subjects do not suffer acute stress.[9,10] There are different kinds of play according to (i) the use of motor patterns (e.g., running, somersaulting, and pirouetting), (ii) the number of players (e.g., solitary or social play), and (iii) the presence of objects (e.g., object play manipulation).[9,11]

From a cognitive viewpoint, managing a social play session requires complex communicative abilities (e.g., self-handicapping, role-reversal, and visual signal processing), especially when the session is punctuated by motor patterns borrowed from an agonistic domain.[12] For this reason, fine-tuned communication is the key for a social play session not to degenerate into real aggression.[7] To explain the evolution of signals, Tinbergen[13] proposed the ritualization theory. According to this theory, signals evolve from specific functional behaviors by developing highly stereotyped motor actions that better serve their new functions. During the ritualization process, functional behaviors (ordinary precursor behaviors) are removed from their original context and transformed into extraordinary, derived behaviors to convey a message to the receiver. Following Tinbergen's theory, it is broadly accepted by the scientific community that the origin of the open mouth derives from a process of ritualization resulting from the modification of the biting action.[7,14–21] This process would have deconstructed the entire biting sequence, causing the loss of the actual contact part and leaving only the preparatory phase, that is, the ritualized version of the intention to bite (open mouth, OM). The relaxed open mouth of several social carnivores,[19,22–24] the play face of monkeys,[25,26] and laughter in humans and great apes[27] are all visual ritualized signals that have an essential role in communicating the playful mood of the interacting subjects.

[1]Department of Biology, Unit of Ethology, University of Pisa, Pisa, Italy
[2]Zoomarine Italia, Torvaianica Pomezia, Rome, Italy
[3]Centre de Recherche et d'Étude pour l'Animal Sauvage, Planète Sauvage, Port-Saint-Père, France
[4]Université Rennes, Normandie University, Centre National pour la Recherche Scientifique, EthoS (Éthologie animale et humaine), Rennes, France
[5]Institut Universitaire de France, Paris, France
[6]Department of Life Sciences and Systems Biology, University of Turin, Turin, Italy
[7]Stazione Zoologica Anton Dohrn, Naples, Italy
[8]Natural History Museum, University of Pisa, Pisa, Italy
[9]Lead contact
*Correspondence: livio.favaro@unito.it (L.F.), elisabetta.palagi@unipi.it (E.P.)
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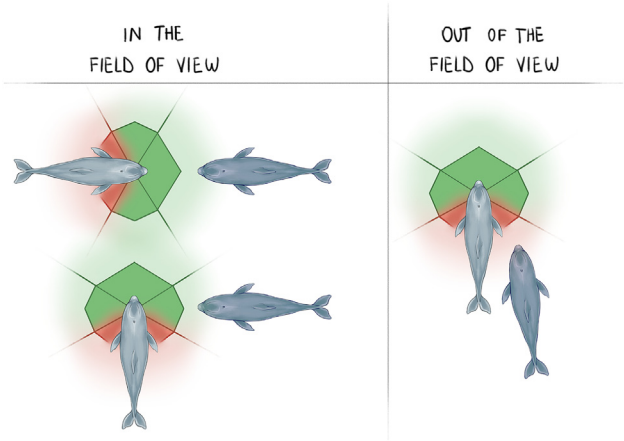

Figure 1. Being IN or OUT of the field of view of the playmate

Scheme illustrating the visual field (120°) of bottlenose dolphin.[49,53] The left part of the figure represents the position of the sender when it is in the field of view (green area) of the receiver. The right part of the figure represents the position of the sender when it is out of the field of view (red area) of the receiver. According to the position of the sender with respect to the receiver the stimulus can be classified as detected or not detected. Drawings by Fosca Mastrandrea.

Relaxed open mouths and play faces often involve mimicry that includes the automatic and very fast replication of others' facial expressions (i.e., rapid facial mimicry, RFM[27]). In humans, reactions start within the first 500 ms after the perception of the stimulus, indicating that facial mimicry is outside of conscious awareness and voluntary control.[28] Therefore, RFM can be defined as a reflex reaction.[29] By following the same criterion already used for humans, RFM has been explored and demonstrated in several non-human mammals, and it has a role in improving motor synchronization between playmates.[27] RFM is a valuable measure to assess the automatic ability to share the same mood as the original emitter (i.e., emotional contagion).[30,31]

Although several studies have investigated play and its communicative modules in terrestrial mammal species,[7] marine mammals remain largely neglected.[32]

The "anatomical smile", the frontal eyes, the round head, and their propensity to play are all features that make dolphins one of the most popular species.[33] The propensity to play, in particular, makes the bottlenose dolphin (*Tursiops truncatus*) an ideal model for investigating playful communication. Bottlenose dolphins are extremely playful at all ages,[34,35] and regardless of their age, they often engage in locomotor/acrobatic, object, and social play. Aerial performances (e.g., leaps, breach, flips[36]), percussive behaviors (e.g., striking the water surface with the tail flukes[37]), and playing with natural/artificial waves (surfing play[37]) are only a few examples of locomotor/acrobatic solitary play. Moreover, bottlenose dolphins often play with simple and floating objects, which can have a natural (e.g., sponges, corals, sticks, and bubble rings) or anthropic origin (e.g., plastic bags and floating buoys).[37–39] Importantly, bottlenose dolphin playfulness is also social, since aerial, acrobatic, and object play are frequently performed with groupmates and, under human care, animals spontaneously produce them with no need for reinforcement by trainers.[34,40] Bottlenose dolphins start playing socially in the first weeks of life.[36,41] The mother is the first playmate and then the calf expands its social network and starts playing with other conspecifics, preferring unrelated companions of similar age.[41–44]

Despite the human perception of their "anatomical smile" as a friendly feature,[45] the role of facial communication in managing playful encounters remains unexplored in dolphins, although there is evidence that they rely on the visual sensory modality in their social life.[46–49] Here, by recording the free activities (outside the training and feeding sessions) of captive bottlenose dolphins, we explore the presence and possible functions of open mouth display (OM) during solitary, interspecific (human-dolphin), and intraspecific free play (spontaneously evoked according to the second Burghardt's criterion,[9]) by testing the following hypotheses.

Hypothesis 1

If OM is a signal mediating playful interactions, we expect it to occur more often during inter- and intra-specific social play than solitary free play (prediction 1). Indeed, the signal optimization theory predicts that specific signal features have evolved to maximize the probability of success in modifying receivers' behavior.[50] For example, when the subjects interact at a close distance, the possibility of detecting subtle visual cues enhances the use of facial expressions whose emission needs to be adjusted by the sender to increase detection probability.[51,52] Therefore, we expect OMs (Video S1, S2, S3, and S4) to be produced by the dolphins when the sender is in the field of view of the potential receiver (prediction 2) (Figure 1) compared to the other two patterns, which are similar in the mouth motor execution: rostrum touch and attempt to play bite. During a rostrum touch (RT), the animal touches with its rostrum on a part of the conspecific's body or an object with either open or closed mouth (Table S2, Video S5; Figure S1). During an attempt to play bite (BiTe), a dolphin rapidly opens and closes its mouth while lunging at the partner trying to reach its body (Video S6, Figure S1).

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Hypothesis 2

If, as it occurs in humans and other mammals,[31] OM can rapidly evoke a mimicry response in the playmate RFM, we expect receivers to engage in an OM within 1 s after detecting the triggering stimulus compared to a control condition (playing but not detecting the OM emitted by the playmate) (prediction 3).

RESULTS

Descriptive results

We extracted 837 free play sessions from 80 h of videos spread over 60 working days. The videos included 22 subjects belonging to four social groups (Table S1): 79 locomotor/acrobatic solitary play sessions, 398 social interspecific, and 360 social intraspecific sessions. On a total of 567 play biting events only 25 were directed to the area expanding from the rostrum to the anterior part of the pectoral fin (4.41%). Since a dyad of particularly playful subjects (Indie, Zeus) was over-represented in our sample (about 50% on the total), with the aid of randomization software (https://www.randomizer.org/) we randomly reduced about the 50% of data points relative to that specific dyad to cope with a possible pseudo-replication of the data thus obtaining 65 locomotor/acrobatic solitary play sessions (spread over 25 days by 18 subjects), 240 social interspecific (spread over 37 days by 21 subjects), and 185 social intraspecific dyadic sessions (spread over 30 days by 17 subjects).

The patterns recorded during the study period are shown in Table S2. We recorded a total of 1,288 OM events from 17 subjects during both inter- and intraspecific social play (only 1 OM was performed during solitary play). To cope with a possible pseudo-replication of the dataset, we randomly reduced the sample to test prediction 2, obtaining 157 OM (spread over 27 days by 15 subjects), 75 RT (spread over 19 days by 12 subjects), and 99 BT (spread over 22 days by 15 subjects). The same procedure was also applied to test prediction 3, obtaining 224 OM events (spread over 30 days by 16 subjects).

Contrary to real fighting, during play each subject allows the playmate to counterattack with the consequence that patterns such as chasing/fleeing, biting, and tail slapping can be expressed by players throughout the entire session, thus making this activity highly bidirectional. The OMs were never accompanied by "violent vertical head motions" as described by overstrom[54] (p. 102) during aggressive encounters. Although we observed only 15 highly directional aggressive interactions (charging/fleeing, violent head hitting), during such events we never recorded any OM from either the aggressor or the victim. Jaw claps have been reported to be common during real aggression. During a jaw clap, dolphins can generate loud sounds by rapidly snapping their jaws shut. This action produces an acoustic signal that can travel over great distances[55] (p. 40), *"dolphin(s) may open and shut jaw(s) rapidly, directed at a dolphin(s).* We only observed two jaw clap events (one during intra- and one during inter-specific social play), and they were never associated with OM. OMs were never observed during relaxing periods (e.g., parallel swimming or upside-down swimming).

Prediction 1

The type of play affected the number of OMs emitted (GLMM with Poisson error distribution, full ≠ control model, likelihood ratio test [LRT]: $\chi 2 = 129.000$, df = 4, $p < 0.001$). OM was less frequent during solitary play compared to interspecific (Tukey test: t-ratio = −2.84; df = inf; $p = 0.011$) and intraspecific social play (t-ratio = −4.552; df = inf; $p < 0.001$) (Figure S2). Moreover, OM was more frequent during intraspecific (92.3% of cases) than interspecific social play (t-ratio = −7.899; df = inf; $p < 0.001$; Table 1).

Prediction 2

During the intra-specific play, the type of display (OM, RT, and BT) had a significant effect on the probability of being emitted in/out of the receiver's field of view (GLMM with binomial error distribution, full ≠ control model, LRT: $\chi 2 = 49.172$, df = 4, $p < 0.001$, Table 1). OM was more frequently performed in the visual field of the receiver compared to RT (Tukey test: t-ratio = −4.884; df = inf; $p < 0.001$), and BT (t-ratio = −6.082; df = inf; $p < 0.001$). No differences were found between RT and BT (t-ratio = −1.037; df = inf; $p = 0.554$) (Figure 2). The 89.17% of OMs were emitted in the visual field of the receiver.

Prediction 3

We never recorded two subsequent OMs from the same subject within 1 s, which is the time gap within which (by definition) an OM reply from the receiver can occur. During the intra-specific play, the possibility of detecting the OM stimulus affected the probability of OM replication by the receiver (GLMM with binomial error distribution, full ≠ control model, LRT: $\chi 2 = 11.384$, df = 1, $p < 0.001$; Table 1; Figure 3) that was 13 times (odds ratio = 13.142) higher in the case of visual detection than in non-detection of the stimulus. On a total of 199 OM events perceived by the receiver, the cases of OM replication were 66 (33.16%); on a total of 25 OM events not visually detected, OM was replicated only in one case (4%).

DISCUSSION

Since ancient times, humans have always been attracted to dolphins. In the *Naturalis Historia* 1, the first encyclopedia of the world, we can find a story about the friendship between a boy and a dolphin (Book 09, paragraph 01–28). As a whole, the key to such empathic attraction mainly resides in the friendly appearance of these "smiling" animals[33] (p. 69). However, facial communication has not been systematically explored in dolphins, although their visual acuity does not hinder their reliance on this sensory modality[53] to navigate their social[46,48,56] and physical world.[47,57] Due to their limited facial muscle movements,[58] the study of facial expressions in dolphins is obviously challenging.[33] Here, we

Table 1. Estimated parameters (Coeff), standard error (SE), and results of the likelihood ratio tests (χ^2) of the models

Fixed Effects	Coeff	SE	χ^2	df	*P*
Prediction 1 (GLMM) – OM is performed more during social play sessions (supported)					
Intercept	−4.425	1.071	N/A	N/A	N/A
Tested variables					
Sex sender	0.253	0.316	0.642	1	0.423
Age sender	−0.015	0.015	0.950	1	0.330
Type of Play	–	–	76.927	2	**<0.001**
Type of Play$_{[INTERSPECIFIC]}$	2.927	1.018	–	–	–
Type of Play$_{[INTRASPECIFIC]}$	4.626	1.016			
Control variables					
Duration	0.015	0.001	250.551	1	<0.001
Group	–	–	1.528	3	0.674
Group$_{[2]}$	−0.284	0.506	–	–	–
Group$_{[3]}$	−0.187	0.842	–	–	–
Group$_{[4]}$	0.249	0.328	–	–	–
Time slot	–	–	13.844	7	0.054
Time slot$_{[10-11]}$	−0.292	0.178	–	–	–
Time slot$_{[11-12]}$	−0.134	0.180	–	–	–
Time slot$_{[12-13]}$	0.277	0.180	–	–	–
Time slot$_{[13-14]}$	0.211	0.204	–	–	–
Time slot$_{[14-15]}$	−0.189	0.185	–	–	–
Time slot$_{[15-16]}$	0.208	0.336	–	–	–
Time slot$_{[16-17]}$	−0.084	0.422	–	–	–
$N_{sessions}$ = 490, N_{sender} = 23, N_{dates} = 48, Variance for the random factors: $ID_{player1}$ = 0.185 ± 0.430 SD, Dates = 0.204 ± 0.451SD					
Prediction 2 (GLMM) – OM is performed more in the receiver's field of view (supported)					
Intercept	−0.460	0.452	N/A	N/A	N/A
Tested variables					
Sex sender	−0.509	0.408	1.559	1	0.212
Age sender	0.016	0.017	0.888	1	0.346
Type of display	–	–	39.756	2	**<0.001**
Type of display$_{ROSTRUM\ TOUCH}$	0.343	0.331	–	–	–
Type of display$_{OPEN\ MOUTH}$	2.144	0.353	–	–	–
Control variable					
Group	–	–	6.444	2	0.040
Group$_{[2]}$	−1.399	1.348	–	–	–
Group$_{[4]}$	0.743	0.366	–	–	–
Time slot	–	–	7.858	7	0.345
Time slot$_{[10-11]}$	−0.183	0.521	–	–	–
Time slot$_{[11-12]}$	−0.882	0.539	–	–	–
Time slot$_{[12-13]}$	−0.075	0.512	–	–	–
Time slot$_{[13-14]}$	0.022	0.553	–	–	–
Time slot$_{[14-15]}$	0.471	0.543	–	–	–
Time slot$_{[15-16]}$	0.396	0.515	–	–	–
Time slot$_{[16-17]}$	−0.078	0.661	–	–	–
$N_{displays}$ = 331, N_{sender} = 17, N_{dates} = 33, Variance for the random factors: $ID_{player1}$ = $1.657e^{-8}$ ± $1.287e^{-4}$SD, Date = $3.101e^{-12}$ ± $1.761e^{-6}$SD					

(*Continued on next page*)

Table 1. *Continued*

Fixed Effects	Coeff	SE	χ^2	df	*P*
Prediction 3 (GLMM) – Presence of Rapid Facial Mimicry (supported)					
Intercept	−1.899	1.136	N/A	N/A	N/A
Tested variable					
Stimulus detection	2.576	1.058	5.930	1	**0.015**
Control variables					
Age sender	0.015	0.024	0.393	1	0.531
Age receiver	−0.044	0.032	1.937	1	0.164
Sex sender	−0.029	0.614	0.002	1	0.962
Sex receiver	0.252	0.580	0.183	1	0.669
Group	–	–	7.965	2	0.019
$Group_{[2]}$	−0.252	$1.244e^{5}$	–	–	–
$Group_{[4]}$	−1.374	0.87	–	–	–
Time slot	–	–	6.146	7	0.523
$Time\ slot_{[10–11am]}$	−1.338	0.622	–	–	–
$Time\ slot_{[11–12am]}$	−0.550	0.609	–	–	–
$Time\ slot_{[12am–01am]}$	−0.747	0.592	–	–	–
$Time\ slot_{[01–02pm]}$	−0.319	0.598	–	–	–
$Time\ slot_{[02–03pm]}$	−0.566	0.724	–	–	–
$Time\ slot_{[03–04pm]}$	−0.133	0.659	–	–	–
$Time\ slot_{[04–05pm]}$	−0.836	0.917	–	–	–

$N_{displays}$ = 224, N_{sender} = 16, $N_{receiver}$ = 17, N_{dates} = 30, Variance for the random factors: $ID_{PL1} = 4.490e^{-12} \pm 2.119e^{-6}$SD; $ID_{PL2} = 1.072^{-11} \pm 3.275e^{-6}$SD; Dates = $2.517e^{-9} \pm 5.930e^{-5}$SD

Significant *p* values of the tested variables are shown in bold; df = degree of freedom; n/a = not applicable.

demonstrate the presence of a facial display (OM, Figure S1A) in this species, and we show that this display is mainly expressed during intraspecific free play (Figure S2) and when the sender is in the field of view of the receiver (Figure 2).

Although OM in dolphins cannot assume the morphological variability observed in primates and terrestrial social carnivores, its performance modality such as social playful-context specificity and emission in the receiver visual field resembles that already reported in other mammals.[27,59] We can interpret the presence of these displays in bottlenose dolphins as a result of the optimization of the information transfer.[60] Animals can use a variety of sensory modalities to convey messages that influence the behavior of the receivers. However, selective pressures can favor modalities that optimize the information transfer according to the characteristics of the environment.[61] Dolphins have evolved one of the most complex vocal communication systems in the animal kingdom,[62] where narrowband whistles mediate both individual recognition[63] and social interactions.[64–66] The complexity of their vocal communication system has been explained in light of their fission-fusion and multi-level social organization, as well as the turbidity and poor underwater visibility of many waters where dolphins can be found.[67] Acoustic signaling also exposes the emitters to the risk of predation[68] and eavesdropping, which has been demonstrated during dolphins' intraspecific vocal interactions.[69] Under good visibility conditions and when subjects are in proximity, the visual modality can be thus favored,[49,60] making the role of facial expressions relevant for rapidly exchanging information at a dyadic level.[7,65] During cooperative tasks, dolphins can successfully reach the goal by whistling and coordinating visually, in a multimodal process.[70] From an adaptive perspective, shifting from acoustic to visual modality can be a particularly effective strategy in social play, during which playmates' vigilance against predators is significantly reduced.[11] A recent study demonstrates that in belugas, subjects modify the melon shape according to different social contexts and the recipient's attention, thus suggesting the importance of the visual cue in this taxon.[71] Flexibly adjusting the emission of a facial expression according to others' attention is one of the building blocks of intentional communication.[71,72] Through an experimental study, Xitco et al.[56] have demonstrated that bottlenose dolphins were responsive to the attentional state of a human receiver when they needed to communicate the location of a food resource that they could not reach by themselves. When the human experimenter turned their back to the animal, the dolphin did not engage in any pointing behavior. The results suggest that, as occurs in great apes,[73,74] dolphins are attentive to the receiver's attention when they engage in interspecific visual communication.

One possible explanation of our results could be that the orientation of the OM merely coincides with the best direction for delivering an acoustic signal to the receiver. Thus, the visual stimulus would not be the reason for OMs. However, although previous studies demonstrated that head position plays a role in determining the directivity of dolphin vocalizations,[75] no evidence suggests that opening the mouth can affect the directionality of the signals. In dolphins, vocalizations are not produced by vocal cords but by vibrating membranes

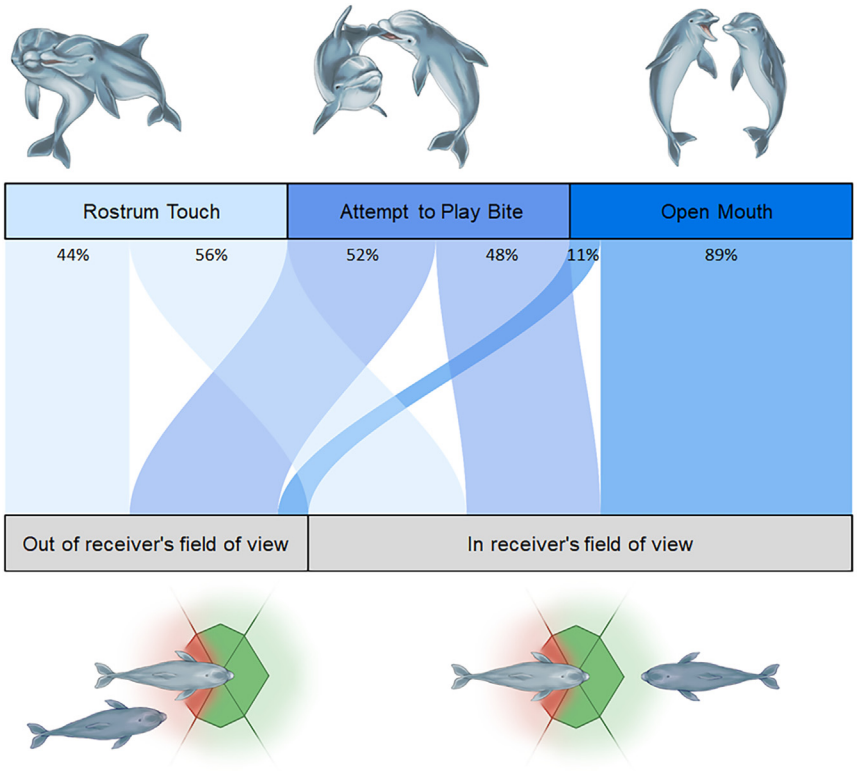

Figure 2. Emission of Rostrum Touch, Attempt to Play Bite and Open Mouth as a function of the position of the receiver respect to the emitter

Alluvial plot showing the percentage of the emission of each display (rostrum touch, light blue; attempt to play bite, medium blue; open mouth, dark blue) as a function of the position of the sender respect to the receiver (OUT or IN the field of view). In the central drawing we reported an actual bite to help reader understand the action, since an attempt to bite is a dynamic action difficult to illustrate in a static image. Drawings by Fosca Mastrandrea.

in the nasal passages located behind the melon.[76] The directivity in the vocal emission is allowed by this same melon which, thanks to its relative mobility, plays the role of an acoustic lens.[77] As supporting evidence, echolocation clicks, with their highly directional beam patterns,[78] are emitted with the mouth closed, even when associated with head scanning movements.[79] In our study we miss acoustic recording and further studies should investigate the possible role of mouth opening during multimodal playful interactions. However, the fact that OM can occur with or without any mimicry seems to rule out the idea that OM is a simple behavioral reflex associated with sound reception.

In addition to the visual cue, since the rostrum is highly sensitive to the pressure variations generated by water movements,[80] we cannot exclude that tactile cues can also play a role during the emission of OM and its replication (RFM). Although further studies are needed to investigate the possible role of vocalizations and tactile cues in mediating intraspecific playful interactions (multimodal nature of OM signal), dolphins seem sensitive to conspecifics' attentional state while emitting playful facial expressions. Due to the perceptual laterality of dolphins,[46,48] future investigations should also explore whether the sender actively "selects" a specific side of the receiver's visual field while emitting OM.

The first description of RFM in dolphins is probably the most remarkable result of this study (Video S2). One could argue that animals are just producing OM at the same time by chance since they are engaging in the same activity/context. However, this interpretation does not explain why the probability of replicating others' OM within 1 s is 13 times higher when the receiver detects the previous visual stimulus compared to when the detection is prevented. The replication rate of OM in dolphins falls within the range of replication rate obtained in the studies of some carnivore species (e.g., 0.27 in meerkats,[19] 0.39 in sun bears[81]).

One could hypothesize that our definition of OM does not describe a signal (*sensu* Tinbergen[13] and van Hooff and Preuschoft[21]) but rather a simple preparation to bite. If the OM emitted by the receiver after perceiving the OM from the playmate is a maneuver to prevent bites by the opponent, we would expect that OMs emitted in front of the receiver, not followed by an OM response, would translate into bites or attempts to bite. Our results show that 89.17% of OMs were emitted within the receiver's field of view, evoking an OM in the receiver in 33.16% of cases. In the 66.84% of cases that did not involve an OM response, the subject performing the OM never bit the playmate. Moreover, focusing exclusively on play bites, out of a total of 567 play biting events recorded, only 25 were directed to the area expanding from the rostrum to the anterior part of the pectoral fin. Although this consideration does not completely rule out the possibility that some OM responses could fall into the defensive domain, our results are compatible with Tinbergen's ritualization theory.[13] The ritualization process could

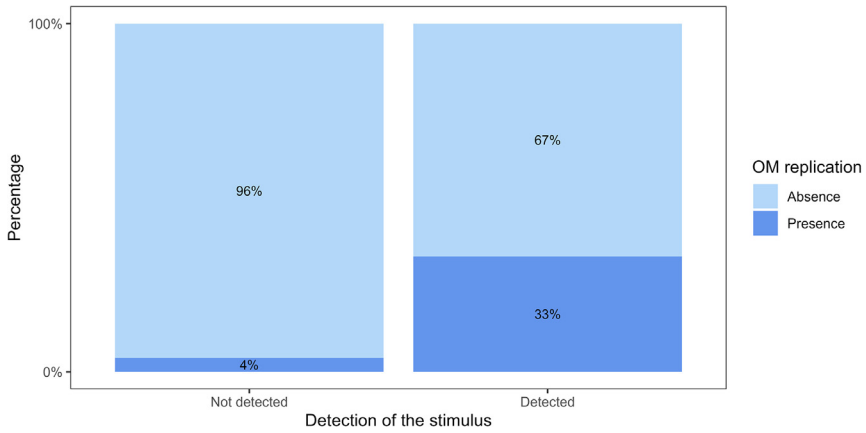

Figure 3. Percentages of OM emission by the receiver (<1 s) as a function of the detection of the stimulus emitted by the actor (detected/not detected)

have deconstructed the entire sequence of biting prevention thus leading to the loss of the original function of the behavior that now can acquire a communicative function.

Through RFM, playmates inform each other that the signal has been correctly perceived and decoded thus making the interactions more successful.[27] Sharing a similar propensity to engage in joint activities can be particularly adaptive in those cooperative species which need to coordinate their actions to reach common goals (e.g., alliances[35,82]). In this view, play in dolphins can be an excellent domain to explore complex communicative abilities, which can be successfully exported to other behavioral domains such as cooperative hunting[83] and reproduction.[84,85] Although our results on RFM derive from only three groups of bottlenose dolphins, they match with those obtained on cooperative social primates and carnivores.[27]

Such similarities might be ascribed to shared evolutionary pathways (*homology*) but also due to evolutionary convergence phenomena (*homoplasy*) as has been suggested for play behavior in general.[9,86] To understand the possible evolutionary pathways of such communicative traits, we should focus on those taxa supposed to share with cetaceans a recent common ancestor. The most accredited theory about the origin of cetaceans places the group phylogenetically close to the artiodactyls, the mammal order including deer, camels, boars, and hippos[87] for which data on play communication are not available. In conclusion, although we are far from understanding the evolutionary origins of play (monophyletic vs. polyphyletic) and the ability of animals to fine-tune their playful sessions, the pervasive presence of open-mouth signals and rapid mimicry in the mammal phylogenetic tree indicates the relevance of such visual mechanisms in shaping complex communication.

Limitations of the study

Exploring visual communication in dolphins is interesting as well as challenging due to the objective difficulties to address the topic in wild populations. Focusing on dolphins living under controlled settings offers unique opportunities to investigate some subtle communicative and cognitive cues that animals could engage in to manage their interactions. Studying these groups obviously implies limitations due to space confinement and reduced fluid arrangement of social groups. To increase the variability of our sample, we extended our observations to different groups. Yet, conducting assessments on additional captive groups could improve the statistical power to detect effects of other explanatory variables such as age and sex. We demonstrated that most of the OM displays are used by dolphins as playful visual signals; however, we cannot exclude the possibility that in some cases opening the mouth can occur defensively. Even though inter-observer reliability always scored high values, a future analysis of the moment-to-moment inter-animal movement dynamics can help clarify this issue. The extensive use of AI in behavioral analysis could help overcome this potential issue. Future studies should include eye-tracking techniques to better understand the dolphins' peripheral and binocular vision and ultrasonic audio recordings to investigate the possible role of multimodal communication during play. Finally, the differences between intra- and inter-specific play and the possible presence of RFM between dolphins and caretakers remain unveiled.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to the lead contact, 1. Elisabetta Palagi (elisabetta.palagi@unipi.it).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All the raw data used for statistical analyses have been uploaded as supplementary material.

- The code has been uploaded as supplementary material.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.
- All the raw data are submitted as supplementary files.

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AUTHOR CONTRIBUTIONS

E.P. and L.F. conceived and designed the experiment; E.P. and L.F. trained F.V.; F.V. collected the data and performed the video analysis; C.P. and M.B. provided logistic support and information about the dolphin management; E.P., F.V., and V.M., analyzed the data and ran statistics; E.P., F.V., L.F., and V.M., wrote the manuscript; E.P., C.P., F.V., L.F., V.M., A.L., and M.B. revised the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.110966.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
software BORIS88 (version 7.10.5-2021-05-12)	https://besjournals.onlinelibrary.wiley.com/doi/10.1111/2041-210X.12584	
R software	https://www.r-project.org/	

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Subjects

We filmed all the free activities (outside the training and feeding sessions) of 11 resident bottlenose dolphins (six males, five females; age range 1–38 years, Table S1) hosted at Zoomarine Rome, Italy (September-November 2020), and 11 resident bottlenose dolphins (seven males and four females; age range 1–34 years, Table S1) hosted at Planète Sauvage (Port-Saint-Père, France; August-September 2023). Video recordings at Zoomarine were collected by FV and a field assistant on 5 days per week (09.00–16.00). Video recordings at Planète Sauvage were collected by VM and EP on 5 days per week (09.00–17.00).

At Zoomarine, due to social and breeding management reasons, the animals were grouped into three mixed-age and mixed-sex groups (Table S1). Five conjoined outdoor pools (a total volume of 7.6 billion m^3 of water; water quality maintained in compliance with Dlg. N. 73/2005) hosted in alternation the different subgroups. Dolphins were daily fed with fish (from 4 to 18 kg) depending on the necessities of each individual. Food was provided during free feedings, training (5-30 min) or enrichment sessions. During the data collection, the park was closed to the public, with only trainers and experimenters allowed to stay in the proximity of the tanks. The animals underwent four short vet training sessions per day with their caretakers. Videos included all the activities of the visible animals when free to swim in the tank 1 (641 m^2, 2581 m^3, maximum depth 6.4 m). Indeed, the huge front wall window of this pool allowed a full underwater vision of the animals. Since observers were free to move along the window, the animals were always visible. This setting guaranteed recording all the possible solitary and social interactions. Being the observation point outdoors, the glass window separated humans from dolphins. It was possible for the animals to splash and throw water from their side to the other one, wetting the caretaker.

At Planète Sauvage, four conjoined outdoor pools (a total volume of 7.5 billion m^3 of water) hosted the animals. Dolphins were fed with fish (from 4 to 18 kg) per individual each day distributed during free feedings, training (5-30 min) or enrichment sessions. During the data collection, the park was open to the public and animals underwent to four short training sessions per day with their caretakers. Data were not collected during the training and feeding sessions. During the inter-species interactions, the low number of visitors stayed far from the window in the dark area of the arena, while the caretaker interacted with the animals while staying close to the window. Thanks to the camera zoom, the experimenter stayed far from the window. Videos included all the activities of the visible animals when free to swim in the tank 1 (1150 m^2, 4950 m^3, maximum depth 4.85 m) and tank 2 (330 m^2, 1280 m^3, maximum depth 4.85 m). Indeed, the huge front walls window of this pool allowed an almost full underwater vision of the animals. Since observers were free to move along the window, the animals were visible for most of the time. This setting guaranteed recording all the possible solitary and social interactions. Since the observation point was placed underground, there was no possibility for direct contacts between animals and caretakers (e.g., animals could not splash the caretaker).

The entire video collection was made using Panasonic HC-V180 cameras. All the free play sessions were always recorded underwater when the dolphins played and engaged in RT, BT, and OM. All the videos were recorded through the glass window and outside feeding and training sessions, which lasted about 15 min. A window always separated the dolphins and the caretakers, and for this reason, the animals never entered contact with the humans during their inter-species play sessions. This ruled out the possibility that the dolphins could open their mouth for begging food for the following reasons: they never received food outside of the training/feeding sessions, and it was not possible for them to receive food through the glass. During their free play animals never saw/received food.

Ethic statement

The research was purely observational, noninvasive, and it complies with the ASAB/ABS Guidelines for the Use of Animals in Research, the current Italian law and University regulations. Thus, no permit from the Bio-Ethical Committee of the University of Pisa (Italy) was needed.

METHOD DETAILS

Video analysis

Videos were analyzed frame-by-frame by FV using the open-source software BORIS[88] (version 7.10.5-2021-05-12). We categorized three types of free play: solitary session (including object/locomotor actions), interspecific social session (human-dolphin), intraspecific social session. For each play session, we annotated the identity of the subject(s) involved, the exact sequence, and the duration (seconds) of each behavioral

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pattern performed (Table S2). We defined the play sessions by applying the following criteria. A spontaneous solitary object play session started when the dolphin entered in contact with and manipulated an object present in the tank (e.g., inanimate objects such as toys or natural debris, wooden, sticks or leaves) or when the focal individual started producing air bubbles from the blowhole with a subsequent "bubble manipulation" such as biting or swimming inside bubbles.[36] A solitary locomotor play started with the first aerial/acrobatic pattern or with a percussive behavior on the water surface (e.g., tail slap).[37,89] A solitary play session ended when the dolphin ceased playful behaviors for at least 10 s or when it clearly started performing another activity (e.g., social play, resting). An interspecific play session started when, in the absence of food (animals never received food out of the training/feeding session), the dolphin spontaneously approached the caretaker just engaging in peek-a-boo, offering/throw objects, or splashing the caretaker. After the dolphin's approach, the caretaker was allowed to start engaging in playful actions such as running up and down, responding to peek-a-boo, jumping in different directions. An interspecific play session ended when the dolphin ceased play behaviors for at least 10 s or when it swam away from the caretaker and started another activity. While interacting with dolphins, caretakers were asked not to display facial expressions to avoid biasing the observations of spontaneous Open Mouth emissions by the dolphins toward human subjects. The motivation of dolphins to interact playfully with humans was not a concern in this study, nor whether these play sequences have the exact same value as social play. Finally, a spontaneous intraspecific play session started when a bottlenose dolphin engaged in a playful pattern toward a conspecific that responded with another playful pattern. We considered the end of an intraspecific play session when one of the bottlenose dolphins moved away, when a third subject interrupted/interfered with the ongoing session, when animals continued to swim together without performing other behaviors or when the animals were not clearly visible in the video.

During intra-specific play, playmates give each other the possibility to counterattack by engaging in self-handicapping (e.g., belly up, logging) and competitive patterns (e.g., pushing, pectoral and caudal rubbing) in an alternative way. The sequences of the patterns do not follow any precise and directional schemes as it occurs in aggressive and sexual domains. As a consequence, a playful interaction appears unpredictable, chaotic and unorganized with both players engaging in a sort of turn taking.

A play session was considered as new if it began after at least 10 s from the end of the previous one. This scoring criterion was intended to make our data comparable with those measured on a variety of terrestrial mammal taxa[26,59,90] and South American sea lions.[91] We need to take into account that this criterion may need revision as more information on play in different species will be added to the dataset.

A random selection of videos (about 20%) was analyzed to calculate the inter-observer reliability during the entire course of the video analysis at regular intervals. FV and VM scored the same videos and for each behavior under investigation the Cohen's kappa values were calculated.

Operational definitions

During an OM the animal maintains its mouth open never closing it in attempting to bite even if it could easily reach the body of a conspecific or also in the absence of any specific target. This pattern was recorded only during play. Therefore, we provide the definition of OM for each type of play recorded. In solitary play, during an OM the subject maintains its mouth open while engaging in acrobatic locomotor actions. In inter-specific social play, during an OM the animal maintains its mouth open never rapidly closing it in the direction of the caretaker. During intra-specific social play, the dolphin performing an OM avoids targeting with the mouth any part of the other's body never attempting to bite, although some occasional contacts can occur due to the dynamicity of the interaction considering that the subjects are close in space (Figure S1A; Video S1, S2, S3, and S4). Such targeting avoidance led us also to differentiate OM from an attempt to engage in mouth-to-mouth interaction, a pattern that has been observed in beluga calves and described as follows "*Two individuals gently clasp each other's mouth and then pull in opposite directions* (i.e., *like a handshake but with the mouth*")[92,93] (p. 743[92]). OM in bottlenose dolphins also differentiates from another mouth pattern described in orcas (*Orcinus orcas*): the gentle tongue bite[94] (p. 7). The authors defined the pattern as follows: "*Two animals standing face to face and touching their snouts. One of them opens his/her mouth and the other gently holds his/her tongue between the teeth*". The mouth interactions described in belugas and orcas involved mouth-to-mouth contact (*pulling* in belugas; *holding tongue* in orcas) with two subjects targeting their mouth reciprocally.

An OM started with the first frame in which the lower and upper jaws appeared separated and ended when the mouth appeared closed.

For each play session, we checked for the presence of Open Mouth (OM; Cohen's kappa[95] $OM_{solitary\ play}$ = 1.00; $OM_{interspecific\ social\ play}$ = 0.90; $OM_{intraspecific\ social\ play}$ = 0.87).

During a Rostrum Touch (RT; Cohen's kappa = 0.84) the animal always targets and touches the conspecific's body with its rostrum with the mouth that can be open or closed (Figure S1B; Video S5). RT started when the rostrum of a subject touched the body of the conspecific and ended when the rostrum was no longer in contact with the other's body. While engaging in an attempt to play BiTe (BT; Cohen's kappa = 0.92), a dolphin rapidly opens and closes its mouth while lunging at the partner trying to reach its body (Figure S1C; Video S6). An attempt to play bite started when the subject targeted the other's body with the mouth open and ended with a rapid mouth closing. We never observed yawn-like behavior as defined by Enokizu et al.[96] who reported that this behavior was performed during animal inactivity periods.

Bottlenose dolphins have laterally positioned eyes, providing a 120° horizontal range of vision and 100° vertical range of vision with an estimated underwater visual acuity of 8.2 arcmin (at the best distance of 1 m).[49,53] To be classified as "detected by the receiver", a pattern (OM, RT, BT) had to be performed in the field of view of the receiver at approximately 1 m from its eyes (i.e., estimated from the rostrum to the dorsal fin) (Figure 1). To be classified as "not detected by the receiver", the pattern had to be performed outside the visual field of the receiver (Figure 1). All the unclear cases (about 2%) were discarded from the analysis.

Then, we focused on OM to verify the presence of Rapid Facial Mimicry (RFM), defined as the mirror response produced by the receiver within 1 s of the detection of the stimulus (Video S2). We defined as sender the first playmate that emitted an OM, and as receiver the other subject. For each OM performed, we noted: i) the possibility for the receiver to detect the OM (OM detected/OM not detected), ii) the presence/absence of an OM by the potential receiver within 1 s of the detection of the OM by the signaler. In accordance with the literature on humans[28,97] and other terrestrial mammals,[81] in cases where the receiver replicated the OM within 1 s after the detection of the stimulus, such event was defined as RFM; in cases where the receiver emitted the OM within 1 s without the detection of the previous stimulus, such events were used as a control.

To avoid pseudo-replication, two RFM events were considered as distinct only when the trigger OM occurred more than 1 s after the previous OM response. The Cohen's kappa values obtained by the two observers in scoring the detection/not-detection condition and the number of RFM events was 0.91 and 0.93, respectively.

Given that animals engage in different activities during their day routinely, to cope with a possible non-independence of our play session dataset, we used the hours of the day (time slots) as a control factor in our models. The hourly time slots were defined as follows: 9–10, 10–11, 11–12, 12–13, 13–14, 14–15, 15–16, 16–17.

QUANTIFICATION AND STATISTICAL ANALYSIS

Hypothesis 1 (Prediction 1)

To investigate whether OM was mainly expressed during social vs. solitary play sessions, we built a Generalized Linear Mixed Model (GLMM) with Poisson error distribution. Since it can be difficult to operationally define an OM when the animal is continuously biting an object, to be conservative as much as possible, we excluded from this analysis the play sessions with an object, considering only the locomotor solitary sessions and the intra- and interspecific play sessions (NSESSIONS = 490). The response variable was the total number of OM emitted during a play session, while the fixed factors were the age (expressed in years) and the sex of the sender, the type of play (i.e., locomotor solitary, social interspecific, social intraspecific), the duration of the play session, the time slot (9–10/10-11/11–12/12-13/13–14/14-15/15–16/16-17), and the group (1/2/3/4; Table S1). The identity of the play initiator (N = 22 dolphins) and the date of recording were included as random factors. We compared this model with a control one including the random factors and the fixed factors "duration of the play session", "time slot", and "group". We examined the Variance Inflation Factors (VIF[98]) by means of the R-package[99] performance v. 0.4.4. Fixed factors collinearity was low for all the variables considered: VIFmin = 1.14; VIFmax = 2.11. See Table 1 for the results.

Hypothesis 1 (Prediction 2)

To investigate whether OM was preferentially emitted in the visual field of the receiver, we built a GLMM with a binomial error distribution. Since during the inter-specific play, the dolphin could not move freely around the caretaker in the three dimensions, it was not possible to include this type of play to test this hypothesis. For this reason, this analysis focused only on intra-specific social play.

The response variable was "IN/OUT field of view" of the receiver. The fixed factors were the age, the sex of the emitter, the group, the time slot, and the type of display (for this analysis, we used a subset of randomly selected data, N_{OM} = 157, N_{RT} = 75, N_{BT} = 99). Fixed factors collinearity was low for all the variables considered: VIF_{min} = 1.07; VIF_{max} = 2.00. The identity of the sender and the date of recording were included as random factors. We compared this model with a control model including the random factors and the fixed factors "group" and "time slot". See Table 1 and Figure 2 for the results.

Hypothesis 2 (Prediction 3)

To investigate the presence of Rapid Facial Mimicry (RFM) we built a GLMM with binomial error distribution. As the caretaker were asked to avoid emitting any facial expression while playing with the dolphin, inter-specific play was excluded from this analysis.

In this model, for each OM emitted, we checked for the detection and the possible response by the receiver (N_{EVENTS} = 224). The response variable was the presence/absence of a mirror response, while the fixed factors were the detection of the stimulus (detected/not detected), the sex and the age of the sender and the receiver, the time slot, and the group. Fixed factors collinearity was low for all the variables considered: VIF_{min} = 1.02; VIF_{max} = 2.35. The interaction of the players' identities and the date of recording were included as random factors. We compared this model with a control one, including the random factor and all the fixed factors but the detection/not detection of the stimulus. See Table 1 and Figure 3 for the results.

We built all the models using the R-package *glmmTMB*[100] *v.* 1.2.5042. We tested the overall significance of the full models comparing them with control models[101,102] by means of the Likelihood Ratio Test (LRT[103]). The LRT was used also to test the significance of the fixed factors using the function *Anova* in the R-package *car* 3.0–10.[104] We performed all pairwise comparisons for the levels of the multi-level factor with the Tukey test[105] by using the R package *emmeans*.[106] All calculations were performed using R 4.0.3.[107]