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1 Odor discrimination in terrestrial and aquatic environments in California sea
2 lions (*Zalophus californianus*) living in captivity

3

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18

19 **ABSTRACT**

20 Pinnipeds, as any mammal species, use multimodal signals, including olfactory ones, to
21 ensure vital functions. Thus, some pinniped species seem able to use olfaction in both social
22 and foraging contexts and to discriminate between different odors in air including both natural
23 and artificial odors, but studies on that topic remain scarce. Here, we studied the olfactory
24 capabilities of California sea lions living in captivity at La Flèche Zoo (France) in both
25 terrestrial and aquatic environments. We used two categories of odors: social odors (from
26 familiar individuals of the same group, unfamiliar individuals from another Zoo, animal
27 zookeepers and a terrestrial carnivore) and non-social odors (food and odors identified as
28 repellents in certain vertebrates). Several behavioral parameters were measured and analyzed
29 as the number and duration of contact with the odor, mouth openings, vocalizations (air only)
30 and air bubble production (water only). Our results, although limited by the low number of
31 animals monitored (n=5), suggest that California sea lions are able to discriminate between
32 different odors both in the air and under water. In the aquatic environment, the process
33 allowing the perception of odors remains to be characterized. Applications to this work could
34 be considered in captive conditions as well as in the wild.

35 **Keywords (6):** California sea lion, *Zalophus californianus*, olfaction, discrimination, aquatic
36 environment, social/food odors

37

38 **1. Introduction**

39 In pinnipeds, the auditory system is considered as highly developed. Indeed, the majority
40 of pinnipeds vocalize in a variety of social contexts, and the importance of acoustic signals in
41 social recognition has been demonstrated [1,2]. For instance, vocalizations are involved in
42 mother-pup recognition, territorial defense and rival assessment [1,2]. Regarding olfaction,
43 pinnipeds, like all marine mammals, have long been considered ‘microsmatic’, i.e., having a
44 poorly developed sense of smell [3]. However, some studies conducted in captivity and in the
45 wild have pinpointed that olfaction plays a significant role in regulating the behavior of
46 certain pinniped species, and that some of these species (at least) may perceive and
47 discriminate olfactory stimuli at very low concentrations [4,5]. Regarding mother-young
48 recognition, the mother accepts or rejects the young after intense sniffing in various pinnipeds
49 species [1,6]. It has been demonstrated in Australian sea lion (*Neophoca cinerea*) that mothers
50 are able to discriminate the odor of their pup from that of another one [7].

51 Olfactory signals are also important in social interactions occurring during the
52 reproductive period. Male New Zealand fur seals (*Arctocephalus forsteri*), for example, sniff
53 the facial and perineal regions of females, suggesting an olfactory examination of the female’s
54 reproductive status [8]. Chemical analyses have shown that the odors of young Australian sea
55 lions vary with age but not depending on the sex or body region. Body odors therefore appear
56 to carry intraspecific information [9]. In addition, there is no similarity in chemical profiles of
57 mothers and juveniles, thus ruling out the assumption of ‘phenotype matching’ in the process
58 of olfactory recognition of pups by their mothers in this species [10]. However, a link between
59 chemical profiles and genetic proximity has been found in Antarctic fur seal (*Arctocephalus*
60 *gazella*) mother-pup pairs [11]. Moreover, it has been shown in Australian sea lions that the
61 chemical profiles of individuals from two distinct colonies were different [10] and under the
62 influence of the environment and/or genetic variability (even close colonies show high genetic

63 variability [12]). In addition to its involvement in social behavior, olfaction seems to be used
64 by some pinnipeds in their search for food. Thus, harbor seals (*Phoca vitulina*) find areas of
65 high forage value by using odor cues for spatial orientation and food detection [4,13].

66 Cape fur seals (*Arctocephalus pusillus pusillus*) discriminate between different
67 monomolecular odors [14] and are able to memorize an odor stimulus that became significant
68 by learning, even a few weeks after learning exposure. Another study highlighted that the
69 sense of smell plays an underestimated role in regulating the behavior of these animals since it
70 has been shown that these fur seals are able to discriminate between aliphatic odor [5]. A
71 recent study also indicates that adults and sub-adults can discriminate between the odors of an
72 adult male and a non-specific control odor, i.e., the odor of an alga present in the environment
73 (Charrier et al., unpublished data).

74 While on land some pinnipeds appear to use olfaction to display a diversity of behaviors,
75 the use of this sensory modality has never been demonstrated in the aquatic environment.

76 In this context of major gaps that still exist in the literature concerning olfactory
77 capabilities of pinnipeds and their role both in the context of foraging and social interactions,
78 the aim of our study was to test and compare, in terrestrial as in aquatic environments, the
79 ability of captive California sea lions (1) to discriminate between food, social and repellent
80 odors, and (2) to initiate the assessment of the use of olfaction under water. We carried out the
81 experiments on captive animals, knowing the limits and advantages related to this context, in
82 order to obtain results which could allow us in the future to continue this study in a natural
83 environment and thus to have a comparative approach.

84

85 **2. Materials and methods**

86 *2.1 Animals and housing conditions*

87 Five captive-born California sea lion (*Zalophus californianus*) males (one intact and four
88 castrated) living at La Flèche Zoo (La Flèche, France) were involved in our experiments. The
89 sea lions had two separate enclosures at the zoo, one for the day and one for the night. They
90 were locked into their indoor enclosure before the closure of the park (5:30 p.m. during the
91 period of our study, conducted in February-March 2020). This building consists of a medical
92 room where training and treatments were carried out as well as six individual pens. Animals
93 went to their outdoor enclosure in the morning if the weather allowed it. Their outdoor facility
94 comprised a concrete beach and a chlorinated pool.

95 In addition, the animals returned to their indoor enclosure daily in the afternoon to be taken
96 out individually, or in pairs, to participate in training. During these daily trainings, which
97 lasted about 20 minutes per sea lion, animals were rewarded with fish and received petting
98 and praising from their zookeepers. During the day, animals were fed individually (in the
99 indoor enclosure or during training) and collectively (several times a day). The food varied
100 according to the season: fatty fish in winter (*Clupea harengus*) and lean fish in summer
101 (*Merlangius merlangus*), but animals could also get sprat or roach in their rations. The
102 amount of fish distributed per day varied from one individual to another (depending on their
103 weight weekly checked).

104 This present study complies with the European Union Directive on the Protection of
105 Animals Used for Scientific Purposes (EU Directive 2010/63/EU) and also with current
106 French laws. Animal husbandry and care were under the management of the animal
107 zookeepers of La Flèche Zoo, France.

108

109 *2.2 Odor stimuli*

110 The tested odors were of animal, human or vegetal origin. All were collected and presented
111 on sterile compresses (Mercurochrome, 20x20 cm, 100% cotton) by the experimenter or one
112 zookeeper wearing nitrile gloves. The three types of odor used were:

113 - Animal odors collected a) from the California sea lions at La Flèche Zoo and from
114 other California sea lions, castrated and intact males and females, at Beauval Zoo (France):
115 the zookeepers rubbed the animal's head, neck and body with a sterile compress during
116 45s in the morning in the outdoor facility; b) from the male tiger (*Panthera tigris*) at La
117 Flèche Zoo: the zookeepers rubbed during 30s with one hand the tiger's body through the
118 enclosure fence as it approached; c) from herring (oily fish much appreciated by sea lions)
119 and whiting (lean fish less appreciated): the experimenter rubbed the compress during
120 45s directly on thawed fish defrosted for 10 min intended for feeding the animals.

121 - Human odors collected from two of the four zookeepers dedicated to the sea lions at
122 La Flèche Zoo. In our first experimental session, the zookeepers rubbed a sterile compress
123 on their own face, neck and underarms during 30s. In our second session, two of the
124 zookeepers wore a 100% cotton white t-shirt the night before the experiment. The t-shirts
125 were collected in the morning and kept in an individual plastic bag until the experiment (3-
126 4h later).

127 - Vegetal odors from organic essential oils of camphor (Herbes et Traditions, France) and
128 black pepper (Pranarôm, France) known to trigger repulsion in terrestrial mammals
129 [common vole (*Microtus arvalis*) and snowshoe hare (*Lepus americanus*)] [15,16] but also
130 in a pinniped species, the harbor seal (Campagna S., personal observations): the
131 experimenter poured 5 drops of essential oil on a sterile compress.

132 The collected odors were either used directly in the experiments or stored in the freezer in
133 Ziploc bags for further presentations. Freezing was considered to allow a long-term odor
134 retention with minimal degradation [17,18].

135 All these odors were separated into two categories: social odors (sea lion, tiger and zookeeper
136 cues) and non-social odors (food/attractive and vegetal/repellent cues). Within the social
137 odors, some were considered as familiar (California sea lion from La Flèche Zoo and their
138 zookeepers), others as unfamiliar (California sea lion from Beauval Zoo, Saint Aignan,
139 France, and the tiger from La Flèche Zoo). Among the familiar odors, those of the two
140 zookeepers were sub-divided into "zookeeper 1", who was the first to be recruited in the
141 aquatic team at La Flèche zoo (12 years of seniority) and "zookeeper 2", who was the last to
142 be recruited in the team (3 years of seniority). Finally, the odors of the five sea lions were also
143 divided into two categories as follows: "affinity -" vs. "affinity +" when the odor was from a
144 group member with which the target animal displayed the least vs. the most affiliative
145 interactions according to the zookeepers, respectively.

146

147 *2.3 Experimental set-up*

148 Animals were tested in two types of environment, terrestrial and aquatic. Two sessions
149 (session 1 and session 2) were carried out, 18 days apart, in order to limit the animals'
150 habituation to the experimental devices and testing procedure.

151 In the aerial environment, 14 conditions were tested during session 1: 10 conditions with one
152 target odor vs. water (herring, whiting, zookeeper 1, zookeeper 2, familiar conspecific,
153 unfamiliar conspecific, pepper, camphor, affinity -, tiger vs. water) and 4 conditions with two
154 target odors (herring vs. whiting, zookeeper 1 vs. zookeeper 2, familiar vs. unfamiliar, affinity

155 - vs. affinity +). The “familiar vs. water” condition was identical to the “affinity + vs. water”
156 condition.

157 During session 2, 6 conditions were tested: 1 condition with a single target odor (camphor vs.
158 water) and 5 with two target odors (herring vs. whiting, zookeeper 1 vs. zookeeper 2, familiar
159 vs. unfamiliar, affinity - vs. affinity +, herring vs. herring + camphor). All experiments took
160 place in the indoor enclosure, in each sea lion individual pens.

161 During session 1, the experimenter placed the compress impregnated with the target odor or
162 control odor (pool water) at the end of a small metal rod (35 cm * 5 cm) previously cleaned
163 with alcohol. The compress was attached to the metal rod with a clamp, which was also
164 cleaned with alcohol. The rod was then presented to the animal through the bars of its lodge
165 for 30s after the animal's first contact with the compress. The animals had the opportunity of
166 direct physical contact with the compress but they could not remove it from the rod. Each
167 animal was tested with the two stimuli of a same condition sequentially. The order of odor
168 presentation was random for each condition but remained the same for all animals in the
169 session. The time interval between the two stimuli presentations was 30s. In session 2, the
170 target and control odors were presented simultaneously to the animal at the end of two
171 identical metal rods, each odor being separated by 40 cm. The position of the target and
172 control odor, right or left, was randomly chosen but remained the same for all animals in the
173 session.

174 In the aquatic environment, 2 conditions were tested during session 1 only, with one odor
175 (water vs. herring, water vs. familiar). Conditions with food odors were carried out during
176 session 2, but unfortunately without exploitable results. The experiments took place in the
177 outdoor pool. The protocol was the same as in the terrestrial environment, except that the
178 animal was tested in the presence of a zookeeper for safety reasons. The experimenter stood at
179 the edge of the pool with the metal rod just below the water surface and the zookeeper joined

180 him with the animal; the zookeeper remained close to the experimenter without giving any
181 order to the animal. We expected that sea lions would approach the device by curiosity. Their
182 behavior was then observed for 30s after the first contact with the compress. Within a test, the
183 time interval between the two stimuli presentations was 30s. Each condition involved the five
184 sea lions. Regardless of the environment, individuals were tested separately one after the other
185 in a random order, and they were tested twice a day with at least a 4-hour interval (morning
186 and afternoon) for each session in a random environment (either in the air in the morning and
187 in the water in the afternoon or conversely). The compresses were changed between each
188 animal. The experimental device was cleaned with water between each animal and cleaned
189 with alcohol between each testing condition. Animals were not rewarded following the
190 experiment, but at the end of the session after all animals have been tested.

191

192 *2.4 Behavioral responses*

193 The olfactory discrimination abilities of the sea lions in both terrestrial and aquatic
194 environments were studied by quantifying several behavioral parameters recorded during the
195 tests: the number and total duration a) of snout contacts with the compress, b) of vocalizations
196 (terrestrial environment only), c) of bubble production (aquatic environment only), and d) of
197 mouth openings. These variables were quantified *a posteriori*, during the analysis of video
198 sequences recorded (GoPro Hero 3 camera) throughout the duration of the tests. Each 30-sec
199 video was analyzed frame by frame using Avidemux software.

200 During session 1, the camera was attached to one end of the metal rod, with the compress
201 attached at the other end, and thus the head of the animal was clearly visible on the video.

202 During session 2, the camera was attached to the experimenter's chest so that the two
203 compresses were in the camera field.

204

205 *2.5 Statistics*

206 The statistical analyses were performed using RStudio (R version 3.5.0). A PCA analysis was
207 performed on our 6 behavioral parameters [number and duration of contacts, vocalizations (air
208 only), bubble production (aquatic only), mouth openings]. This allowed us to obtain a
209 composite score of the behavioral response for each test performed on each individual. These
210 composite scores were then used in mixed linear models as a "response" variable, allowing us
211 to compare the responses obtained with the control odors to those obtained with the target
212 odors, but also to compare the responses between two target odors. The odors, the odor
213 categories (human, animal, vegetal) and categories of odor tests (social, non-social) were
214 coded as "fixed factors". The order of presentation and individuals were coded as "random
215 factors" to take into account repeated measures. Interactions between these different factors
216 were implemented in the model or removed from the model if not significant (the model was
217 then recalculated). When a significant effect was obtained, post-hoc tests were carried out by
218 running the same models with data subsets allowing paired comparisons and sequential
219 Bonferroni corrections were applied to account for multiple comparisons (Holm's procedure).

220

221 **3. Results**

222 All the behavioural responses obtained during our different tests can be found in
223 Supplementary Table 1.

224 *3.1 Tests in the terrestrial environment*

225 In the aerial environment, the PCA indicated that PC1 (first principal component) explained
226 66.3% of the variability and had an eigenvalue > 1 . The PC1 scores obtained for each test

227 were therefore used for further analysis. Moreover, all behavioral variables were positively
228 correlated to PC1 with the same magnitude (0.37 to 0.44). Strong reactions were therefore
229 represented by positive and high PC1 scores.

230 Analysis of the composite scores showed that the factor interaction “odor x test type” was
231 non-significant ($\chi^2 = 2.75$, $p = 0.6$), but odor ($\chi^2 = 114.22$, $p < 0.001$) and test type ($\chi^2 = 90.58$,
232 $p < 0.001$) showed a significant effect on the responses. Since the test type had a significant
233 effect, the data were analyzed separately for each of them.

234 *3.1.1 Session 1*

235 Post-hoc analyses between the two odors of the same category revealed significant
236 differences, even after Bonferroni - Holm corrections (Figure 1A, B, C).

237 The responses displayed by sea lions to food odors (fish) were significantly stronger than
238 those displayed to our control (water; $\chi^2 > 35.38$, $p < 0.001$) (Figure 1A). Responses to human
239 odors were also significantly stronger compared to water ($\chi^2 > 9.24$, $p < 0.01$). Concerning
240 responses to other animal odors, those displayed towards tiger odor did not differ significantly
241 compared to water ($\chi^2 = 0.01$, $p = 0.91$). For the tests involving odors from conspecifics,
242 behavioral responses to “affinity - “were weak and not significantly different from water ($\chi^2 =$
243 1.72 , $p = 0.57$) but responses to familiar and unfamiliar odors were significantly higher or
244 tended to be higher than those to the control (familiar vs. water: $\chi^2 = 6.26$, $p = 0.06$;
245 unfamiliar vs. water: $\chi^2 = 15.90$, $p < 0.001$) (Figure 1B, C). For plant odors, the results did not
246 reveal any significant differences in responsiveness with the control (camphor or pepper vs.
247 water: $\chi^2 < 3.26$, p values > 0.28) (Figure 1D).

248

249 Please, insert Figure 1 here

250

251 Post-hoc analyses on PC1 scores for the two-odor tests did not reveal any significant
252 differences (affinity – vs. affinity +: $\chi^2 = 2.21$, $p = 0.28$; familiar vs. unfamiliar: $\chi^2 = 0.49$, $p =$
253 0.49 ; zookeeper 1 vs. zookeeper 2: $\chi^2 = 3.00$, $p = 0.27$; herring vs. whiting: $\chi^2 = 3.35$, $p =$
254 0.27) (Figure 2). However, before applying the Bonferroni-Holm correction, PC1 scores
255 tended to be higher for the odor of herring compared to those of whiting ($p = 0.07$) as well as
256 for the odor of zookeeper 1 compared to those of zookeeper 2 ($p = 0.08$).

257

258 Please, insert Figure 2 here

259

260 *3.1.2 Session 2*

261 Post-hoc analyses on PC1 scores (Figure 3A, B) showed that only the behavioral responses
262 obtained with herring were significantly stronger than those obtained with herring + camphor
263 ($\chi^2 = 0.02$, $p = 0.02$) (Figure 3A).

264 The responses obtained for all other tests (Figure 3A, B) were not found significantly different
265 (herring vs. whiting: $\chi^2 = 1.21$, $p = 1.00$; water vs. camphor: $\chi^2 = 0.02$, $p = 1.00$; affinity - vs.
266 affinity +: $\chi^2 = 1.24$, $p = 1.00$; familiar vs. unfamiliar: $\chi^2 = 0.18$, $p = 1.00$; zookeeper 1 vs.
267 zookeeper 2: $\chi^2 = 0.38$, $p = 1.00$).

268

269 Please, insert Figure 3 here

270

271 *3.2 Tests in the aquatic environment*

272 In the aquatic environment, only 3 out of 5 sea lions approached our experimental device
273 (metal rod) and were thus included in our analysis. Two conditions were tested: water vs.
274 familiar and water vs. herring. During the odor investigations, the number and duration of
275 contacts did not vary between the target odors (familiar or herring) and the water (Figure 4A).
276 However, while the number of contacts did not vary significantly between the herring odor
277 and water, the duration of contacts for herring was nearly three times longer than for water
278 (Figure 4B).

279 During the tests, we also observed some animals' production of bubbles and openings of the
280 mouth. The mouth opening occurred twice during the presentation of herring odor for one
281 individual, while the bubble production was observed for two sea lions with a total of 6
282 occurrences (3 in response to water, 2 to herring and 1 to the familiar odor).

283 Due to the small number of animals approaching our device (n=3), no statistical tests could be
284 done on these data. However, according to our observations and the PC scores, the behavioral
285 responses to herring seemed to be stronger than to water (Figure 5A, B). In contrast, no clear
286 difference was observed between the responses displayed to the familiar odor and to water.

287

288 Please, insert Figures 4 and 5 here

289

290 **4. Discussion**

291 Our study aimed to determine whether captive California sea lions displayed spontaneous
292 behavioral responsiveness or preferences for certain odors depending on their nature/category
293 and the medium in which the odor stimuli were presented (air vs. water). The results showed

294 that California sea lions responded strongly to some odors, both in the air and under water,
295 demonstrating effective olfactory abilities for social and non-social odors.

296

297 *4.1 Odor perception in the air*

298 In the aerial environment, when a fish odor - herring or whiting in our conditions - was
299 presented sequentially to the individuals (session 1), one of the responses that we considered
300 as behaviorally relevant, i.e., vocalizations, was significantly more often displayed by sea
301 lions than during presentation of the control stimulus (water); this was not observed for any
302 other odor stimulus compared to water (Figure 6).

303 Please, insert Figure 6 here

304 Sea lions appear therefore able, as expected, to selectively respond to fish using olfaction.
305 Interestingly, the behavioral variable contributing the most to the fish vs. water discrimination
306 was their vocal production and not sniffing (i.e., olfactory investigation) as we should have
307 expected. Moreover, vocalizations were only produced by the sea lions during the
308 presentation of fish odors, and can be interpreted as a high-motivation trait. According to the
309 zookeepers, the sea lions have a preference for herring, an oily fish, over whiting, a lean fish.
310 Although this preference did not emerge significantly in our experiments, one may note that
311 when the two with odors were presented sequentially, vocalizations were only observed for
312 herring. Further trials including additional individuals would thus be necessary to corroborate
313 this preference. Such food preference illustrated by stronger behavioral responsiveness to the
314 preferred fish smell suggests that pinnipeds could use olfactory cues during foraging
315 activities. Laska and colleagues (2008) demonstrated that captive Cape fur seals were able to
316 discriminate the odors of two different fish mixtures, whatever these mixtures were highly
317 different (i.e., from different species) or weakly different (i.e., one with, one without fish oil).

318 These findings clearly illustrate their sharp sense of smell. Another study, on captive harbor
319 seals, showed a high sensitivity for atmospheric DMS, a marker of high primary productivity
320 [4]. The perception and discrimination among fish odors in air and/or under water would thus
321 benefit pinnipeds to orient their foraging investigations and also to identify the preferred preys
322 during their foraging trips at sea.

323 Concerning the vegetal odors considered as "repellent" in other mammal species including a
324 pinniped one, the harbor seal (Campagna S., personal observations), the group of sea lions
325 involved in our study did not show distinct responsiveness to camphor or pepper vs. water.
326 Camphor and pepper may not have a strong repellent value for California sea lions. Both
327 these tested essential oils are known to have trigeminal properties in mammals. The
328 trigeminal system is differently activated depending on the nature of the odorants: some are
329 very active even at low concentration while others need very high concentration to activate
330 this system [19]. When trigeminal activation occurs, odorants may generate different
331 sensations such as "freshness" (peppermint, eucalyptus), "pain" (vinegar, acetone) or
332 "warmth" (alcohol, cinnamon) [19,20]. These sensations are linked to specific trigeminal
333 receptors activated by specific odorants [21]. In California sea lions, the lack of strong and
334 clear repellent effects of camphor and pepper observed here suggests that the trigeminal
335 system may not be activated by these odors, at least at the concentrations used in our
336 conditions, or that the trigeminal system is not involved for olfactory assessment in this
337 species. Further investigations using different concentrations of camphor and pepper, as
338 additional olfactory-trigeminal odors, would be needed to conclude on the effective role of the
339 trigeminal system on olfactory abilities in pinnipeds.

340 Another possible explanation of this absence of repellent effect is that the two stimuli were
341 tested at the end of our experimental sessions. Therefore, the animals were putatively

342 habituated to the procedure and this habituation effect may have impacted their
343 responsiveness.

344 However, and interestingly, the results from the “fish vs. fish + camphor” condition used in
345 session 2 highlighted that camphor may actually have a negative hedonic value in California
346 sea lions since animals displayed a reduced response to the herring with camphor odor.
347 Without having a powerful repellent value by itself in California sea lions, camphor essential
348 oil could therefore have a negative effect when it is paired with an attractive food odor. This
349 effect could be the result of interactions between molecules from the repellent and the food.
350 Again, a more in-depth study on that question would be useful to determine the putative
351 repellent effect of camphor in California sea lions and if this effect is effective over prolonged
352 periods. Indeed, a recent study has shown that the use of a chemical repellent, HaTe2, spread
353 on plants is not effective over time against a terrestrial mammal, the fallow deer (*Dama*
354 *dama*) [22].

355 The use of odors as pinniped repellents could have applications in the field, particularly to
356 prevent depredation caused by pinnipeds in aquaculture farms or in fishing traps [23], and
357 thus to limit their impact on fisheries – an impact experienced as very negative by fishermen.

358 The presentation of the odor of another carnivore, here a tiger (*Panthera tigris tigris*), did not
359 trigger particular responsiveness in sea lions. This clearly indicated that, in our conditions, sea
360 lions did not react to any odors presented in contrast to water. Here, this unfamiliar animal
361 odor did not appear to have any relevance to them. In the future, it will be interesting to test
362 pinnipeds for their responsiveness to odors from biologically relevant species such as
363 competitor or terrestrial predator. For instance, Cape fur seals could be tested with the odors
364 of jackals or hyenas, known to constitute some of their terrestrial predators [24].

365 Within the group of sea lions monitored at La Flèche Zoo, affinities between the five animals
366 were established and mostly based on their seniority at the zoo. While some individuals

367 displayed agonistic interactions between each other, in particular the animals called Tanguy
368 and Wally, this kind of interactions remained rare. This may thus explain the absence of
369 significance in their behavioral responses when they were tested with both odors "affinity +"
370 vs "affinity-". More data would be useful to further investigate the link that may exist between
371 odor-guided behavior and degree of affinity/dominance among conspecifics in these
372 pinnipeds.

373 Similarly, when tested with familiar and unfamiliar conspecific odors, the sea lions reacted
374 significantly more often to both odors compared to water, but no significant difference was
375 observed when these odors were presented in the same session (in sequential or simultaneous
376 testing). This result was quite surprising as we expected that sea lions from La Flèche Zoo
377 would have shown a stronger reaction to the odor of unfamiliar individuals for instance, but
378 this was not the case. Social olfactory communication is used in pinnipeds in several
379 interactions, and it has been experimentally shown that Australian sea lion mothers
380 discriminate between the odor of their filial pup and those of non-filial pups [7]. Another
381 study on Australian sea lions had shown that animals from two different breeding colonies
382 presented different chemical profiles, which are related to both genetic and environmental
383 differences [9]. Therefore, the absence of such discrimination observed in our conditions
384 could be explained by the fact that the odors of familiar and unfamiliar individuals were
385 considered as equally attractive to the tested animals. It is also possible that our low sample
386 size, a loss or change in odor quality related to the freezing/thawing of our samples can have
387 led to the lack of significant behavioral responses. However, the freezing/thawing effect is
388 unlikely since our samples triggered animal responses when tested vs. water. Moreover,
389 testing samples were frozen for 3-4 weeks only while other studies conducted in Australian
390 sea lions [9,10] and polar bears [18] showed a very good conservation of odors during
391 freezing and after thawing, even after several weeks or years.

392 Finally, considering the responsiveness of sea lions to human odors, our results pinpoint that
393 animals were able to discriminate between zookeepers' odors and water, regardless of how
394 long the zookeeper have been working at the zoo, with higher responsiveness to them. Since
395 no significant differences were displayed in the sequential or simultaneous presentation of the
396 odors of the two zookeepers, sea lions might not react to one zookeeper more than the other
397 based only on the sense of smell. Nevertheless, and interestingly, even if the result was not
398 statistically significant, animal responsiveness to zookeeper 1 (the one who has been working
399 at the zoo for the longest time) tended to be higher than to zookeeper 2 (recently recruited)
400 during the sequential presentation of these odors. Such responsiveness to human odors
401 deserves also to be further assessed, for instance by comparing the odors of sea lions'
402 zookeepers vs. zookeepers of other animals from the same zoo and/or vs. unfamiliar people
403 (i.e., not working at the zoo). If sea lions display a positive and preferred responsiveness to
404 their own zookeepers' odors, these stimuli could be used as positive cues to reduce the stress
405 of the animals in certain situations such as veterinary procedures or transport.

406

407 *4.2 Odor perception under water*

408 In pinnipeds, the use of olfaction in the aquatic environment can not follow the same
409 perceptual processes as in the air since the animals cannot "sniff" under water, at risk of
410 drowning. In some other species such as the star-nosed mole (*Condylura cristata*) or the
411 American water-shrew (*Sorex palustris*), the use of olfaction under water is based on the
412 production of air bubbles, which are exhaled on objects of interest or on chemical tracks, and
413 then inhaled by the animals through the nose [25]. During our underwater experiment, we
414 observed the production of air bubbles via the nostrils during the test with herring, and also
415 during the presentation of the familiar odor and the water control. One may therefore assume
416 that sea lions could detect and perceive odor stimuli under water by producing air bubbles

417 towards an odor stimulus, which would then be recaptured by the nostrils or the mouth.
418 However, during our experiment, we did not observe any case of re-inhalation of air bubbles
419 via the mouth or the nostrils. Pinnipeds do not breathe under water, but they might exhale or
420 inhale the bubbles produced when in contact with olfactory stimuli. Another possibility is that
421 they might perceive the odor when the emitted bubbles pierce the surface of the water.
422 Anyway, the interest displayed by sea lions for the herring odor in our condition pinpoints
423 their ability to perceive certain odor cues in the aquatic environment. However, this interest
424 might have been triggered by taste perception rather than by odor. Further investigations are
425 required to clearly determine whether bubble sniffing could be used by some semi-aquatic
426 mammals, such as pinnipeds.

427

428 *4.3 Issues with the method and animal welfare*

429 In the wild, pinnipeds show high level of curiosity for novelty, approaching and sniffing any
430 new elements contained in the environment. This may have dramatic consequences when, for
431 instance, it led animals to become entangled in plastic debris (fishing lines and nets,
432 plastics...) [23]. When we started this study, we assumed that this natural curiosity of sea lions
433 would guarantee the interest in, and approach to, our experimental devices. Surprisingly, the
434 animals showed actually only a weak attention for our experimental devices, even though they
435 were introduced in their daily environment. This lack of interest in exploration of new
436 elements added in the environment may be a consequence of captivity or a consequence of a
437 lack of interest in enrichments. To stimulate their sea lions, zookeepers from La Flèche Zoo
438 regularly enrich the environment with ice cubes, balls, floating platform, food, but they have
439 noticed that the animals can get bored very quickly depending on the enrichment. In this
440 context, the use of odor enrichment may be relevant to improve attention of sea lions for
441 novel objects, at least during the periods of the day when they are not in contact with their

442 zookeepers. To this end, offering new, multi-sensory objects like floating platforms or
443 submerged structures, on which familiar sea lion sounds are broadcast and odors from
444 familiar food or social sources, or from the sea lions' natural environment are diffused could
445 be developed. Moreover, it would be interesting to test the response to novelty of captive sea
446 lions from other zoos, which have less training and/or contact with zookeepers than those
447 from La Flèche Zoo, in order to determine if the daily presence of the zookeepers reduces the
448 attention of the animals to other stimuli than those used in the training. Furthermore, familiar
449 or unfamiliar odors could be directly used to enrich the environment. Indeed, it was showed in
450 California sea lions that the introduction of different odors (i.e., familiar odors: soil, sand,
451 kelp and sardine oil; unfamiliar odors: orange, banana, vanilla extract and cinnamon) caused
452 an increase in habitat utilization with less time spent in the water [26] and, consequently, a
453 reduction in the majority of stereotypical behaviors [26]. A solution to enrich the environment
454 and improve the attention of sea lions for novel objects could then be to introduce odorized
455 objects during training periods, and to encourage zookeepers to promote interactions of sea
456 lions with novel objects as targets to play with, in both the indoor and the outdoor
457 environments.

458 Finally, the lack of differential behavioral responses towards the majority of stimuli
459 combinations tested in this study might be the results of the experimental approach, based on
460 spontaneous preferences and aversions. Therefore, these results do not allow us to draw firm
461 conclusions as to the perceptual or discriminatory limits of their sensory system, but at least
462 they gave us some ideas for future directions. To deeply examine the olfactory abilities of
463 pinnipeds in-air and under water, we have for instance planned to run operant conditioning
464 procedures that have been successfully used in pinnipeds [4,5,14] and that could help us to
465 validate our hypotheses.

466

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472 Mammals".

473

474 **References**

- 475 [1] S.J. Insley, A. V. Phillips, I. Charrier, A review of social recognition in pinnipeds,
476 *Aquat. Mamm.* 29 (2003) 181–201. <https://doi.org/10.1578/016754203101024149>.
- 477 [2] I. Charrier, Mother--Offspring Vocal Recognition and Social System in Pinnipeds, in:
478 T. Aubin, N. Mathevon (Eds.), *Coding Strateg. Vertebr. Acoust. Commun.*, Springer
479 International Publishing, Cham, 2020: pp. 231–246. [https://doi.org/10.1007/978-3-030-](https://doi.org/10.1007/978-3-030-39200-0_9)
480 [39200-0_9](https://doi.org/10.1007/978-3-030-39200-0_9).
- 481 [3] R.E. Brown, The marine mammals: orders Cetacea, Pinnipedia, and Sirenia., in: R.
482 Brown, D. Macdonald (Eds.), *Soc. Odours Mamm.*, Oxford: Cl, 1985: pp. 723–731.
- 483 [4] S. Kowalewsky, M. Dambach, B. Mauck, G. Dehnhardt, High olfactory sensitivity for
484 dimethyl sulphide in harbour seals, *Biol. Lett.* 2 (2006) 106–109.
485 <https://doi.org/10.1098/rsbl.2005.0380>.
- 486 [5] M. Laska, E. Lord, S. Selin, M. Amundin, Olfactory Discrimination of Aliphatic
487 Odorants in South African Fur Seals (*Arctocephalus pusillus*), *J. Comp. Psychol.* 124
488 (2010) 187–193. <https://doi.org/10.1037/a0018189>.
- 489 [6] W.R. LOWELL, W.F. FLANIGAN Jr, Marine mammal chemoreception, *Mamm. Rev.*

- 490 10 (1980) 53–59. <https://doi.org/10.1111/j.1365-2907.1980.tb00233.x>.
- 491 [7] B.J. Pitcher, R.G. Harcourt, B. Schaal, I. Charrier, Social olfaction in marine mammals:
492 Wild female Australian sea lions can identify their pup’s scent, *Biol. Lett.* 7 (2010) 60–
493 62. <https://doi.org/10.1098/rsbl.2010.0569>.
- 494 [8] E.H. Miller, Social behaviour between adult male and female New Zealand fur seals,
495 *Arctocephalus forsteri* (Lesson) during the breeding season, *Aust. J. Zool.* 22 (1974)
496 155–173. <https://doi.org/10.1071/ZO9740155>.
- 497 [9] K. Wierucka, N. Barthes, B.J. Pitcher, B. Schaal, I. Charrier, R.G. Harcourt, Chemical
498 Profiles of Integumentary and Glandular Substrates in Australian Sea Lion Pups
499 (*Neophoca cinerea*), *Chem. Senses.* 44 (2019) 205–214.
500 <https://doi.org/10.1093/chemse/bjz008>.
- 501 [10] K. Wierucka, N. Barthes, R. Harcourt, B. Schaal, I. Charrier, B.J. Pitcher, Chemical
502 fingerprints suggest direct familiarisation rather than phenotype matching during
503 olfactory recognition in Australian sea lions (*Neophoca cinerea*), *J. Exp. Mar. Bio.*
504 *Ecol.* 517 (2019) 49–53. <https://doi.org/10.1016/j.jembe.2019.06.001>.
- 505 [11] M.A. Stoffel, B.A. Caspers, J. Forcada, A. Giannakara, M. Baier, L. Eberhart-Phillips,
506 C. Müller, J.I. Hoffman, Chemical fingerprints encode mother–offspring similarity,
507 colony membership, relatedness, and genetic quality in fur seals, *Proc. Natl. Acad. Sci.*
508 112 (2015) E5005 LP-E5012. <https://doi.org/10.1073/pnas.1506076112>.
- 509 [12] H. Ahonen, A.D. Lowther, R.G. Harcourt, S.D. Goldsworthy, I. Charrier, A.J. Stow,
510 The limits of dispersal: Fine scale spatial genetic structure in Australian sea lions,
511 *Front. Mar. Sci.* 3 (2016) 1–12. <https://doi.org/10.3389/fmars.2016.00065>.
- 512 [13] P.M. Thompson, D. Miller, Summer Foraging Activity and Movements of Radio-

- 513 Tagged Common Seals (*Phoca vitulina*. L.) in the Moray Firth, Scotland, *J. Appl. Ecol.*
514 27 (1990) 492–501. <https://doi.org/10.2307/2404296>.
- 515 [14] M. Laska, M. Svelander, M. Amundin, Successful acquisition of an olfactory
516 discrimination paradigm by South African fur seals, *Arctocephalus pusillus*, *Physiol.*
517 *Behav.* 93 (2008) 1033–1038. <https://doi.org/10.1016/j.physbeh.2008.01.019>.
- 518 [15] A.R.E. Sinclair, M.K. Jogia, R.J. Andersen, Camphor from juvenile white spruce as an
519 antifeedant for snowshoe hares, *J. Chem. Ecol.* 14 (1988) 1505–1514.
520 <https://doi.org/10.1007/BF01012422>.
- 521 [16] A. Schlötelburg, S. Bellingrath-Kimura, J. Jacob, Development of an odorous repellent
522 against common voles (*Microtus arvalis*) in laboratory screening and subsequent
523 enclosure trials, *J. Pest Sci.* (2004). 92 (2019) 677–689.
524 <https://doi.org/10.1007/s10340-018-1028-3>.
- 525 [17] S. Nilsson, S. Johanna, M. Amundin, C. Hartmann, A. Buettner, M. Laska, Behavioral
526 responses to mammalian blood odor and a blood odor component in four species of
527 large carnivores, *PLoS One.* 9 (2014). <https://doi.org/10.1371/journal.pone.0112694>.
- 528 [18] M.A. Owen, R.R. Swaisgood, C. Slocomb, S.C. Amstrup, G.M. Durner, K. Simac, A.P.
529 Pessier, An experimental investigation of chemical communication in the polar bear, *J.*
530 *Zool.* 295 (2015) 36–43. <https://doi.org/10.1111/jzo.12181>.
- 531 [19] J. Frasnelli, T. Hummel, J. Berg, G. Huang, R.L. Doty, Intranasal localizability of
532 odorants: Influence of stimulus volume, *Chem. Senses.* 36 (2011) 405–410.
533 <https://doi.org/10.1093/chemse/bjr001>.
- 534 [20] M. Laska, H. Distel, R. Hudson, Trigeminal perception of odorant quality in
535 congenitally anosmic subjects, *Chem. Senses.* 22 (1997) 447–456.

- 536 <https://doi.org/10.1093/chemse/22.4.447>.
- 537 [21] R.P. Filiou, F. Lepore, B. Bryant, J.N. Lundström, J. Frasnelli, Perception of trigeminal
538 mixtures, *Chem. Senses*. 40 (2015) 61–69. <https://doi.org/10.1093/chemse/bju064>.
- 539 [22] R.S. Stutz, L. Verschuur, O. Leimar, U.A. Bergvall, A mechanistic understanding of
540 repellent function against mammalian herbivores, *Ecol. Process*. 8 (2019) 25.
541 <https://doi.org/10.1186/s13717-019-0179-3>.
- 542 [23] E.M. Jepsen, P.J.N. de Bruyn, Pinniped entanglement in oceanic plastic pollution: A
543 global review, *Mar. Pollut. Bull.* 145 (2019) 295–305.
544 <https://doi.org/10.1016/j.marpolbul.2019.05.042>.
- 545 [24] I. Wiesel, Killing of Cape fur seal (*Arctocephalus pusillus pusillus*) pups by brown
546 hyenas (*Parahyaena brunnea*) at mainland breeding colonies along the coastal Namib
547 Desert, *Acta Ethol.* 13 (2010) 93–100. <https://doi.org/10.1007/s10211-010-0078-1>.
- 548 [25] K.C. Catania, Underwater “sniffing” by semi-aquatic mammals, *Nature*. 444 (2006)
549 1024–1025. <https://doi.org/10.1038/4441024a>.
- 550 [26] M.M. Samuelson, L.K. Lauderdale, K. Pulis, M. Solangi, T. Hoffland, H. Lyn,
551 Olfactory Enrichment in California Sea Lions (*Zalophus californianus*): An Effective
552 Tool for Captive Welfare?, *J. Appl. Anim. Welf. Sci.* 20 (2017) 75–85.
553 <https://doi.org/10.1080/10888705.2016.1246362>.
- 554

555 **Figures legends**

556 Fig. 1: Boxplots of PC1 scores based on (A) food tests, (B) human tests, (C) animal test and
557 (D) repellent tests with a single experimental odor during the first session in the aerial
558 environment. The fat horizontal line indicated the median; the upper and lower limit of the
559 box itself represented the third and the first quartiles; and whiskers indicated the maximum
560 and the minimum value. Finally, the asterisk represented a significant difference in the
561 behavioural responses between two tested odors (significant level at $p < 0.05$).

562

563 Fig. 2: Boxplots of PC1 scores according to the tests carried out with 2 odors during the first
564 session in the aerial environment. The fat horizontal line indicated the median; the upper and
565 lower limit of the box itself represented the third and the first quartiles; and whiskers
566 indicated the maximum and the minimum value.

567

568 Fig. 3: Boxplots of PC1 scores based on (A) food and repellent tests and (B) social tests
569 during the second session in the aerial environment. The fat horizontal line indicated the
570 median; the upper and lower limit of the box itself represented the third and the first quartiles;
571 and whiskers indicated the maximum and the minimum value. Finally, the asterisk
572 represented a significant difference in the behavioural responses between two tested odors
573 (significant level at $p < 0.05$).

574

575 Fig. 4: Barplots of (A) the numbers of contacts and (B) duration of contacts according to the
576 tests carried out and the odors tested under water.

577

578 Fig. 5: Boxplots of the (A) PC1 scores and (B) PC2 scores according to the tests carried out
579 and the odors tested under water. The fat horizontal line indicated the median; the upper and
580 lower limit of the box itself represented the third and the first quartiles; and whiskers
581 indicated the maximum and the minimum value.

582

583 Fig. 6: Boxplots of the numbers of vocalizations based on (A) food tests, (B) human tests, (C)
584 animal test and (D) repellent tests with a single experimental odor during the first session in
585 the aerial environment. The fat horizontal line indicated the median; the upper and lower limit
586 of the box itself represented the third and the first quartiles; and whiskers indicated the
587 maximum and the minimum value.

588