

## Research Paper

## Do bottlenose dolphins display behavioural response to fish taste?

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## ABSTRACT

The chemosensory abilities (i.e. taste, smell and trigeminal perception) of odontocete cetaceans are still widely unknown. However, a better understanding of their potential use of these senses would not only improve our knowledge of their behavioural ecology, but also allow us to develop behavioural enrichment strategies for captive odontocetes using sensory stimulation. While studies on taste bud anatomy and taste receptor genes in these animals have provided useful information, ultimately behavioural experiments are crucial to assess whether odontocetes use their sense of taste in water. Go/no go and conditioning experiments in bottlenose dolphins (*Tursiops truncatus*) have previously shown that they can perceive basic tastes, but it is still unclear whether they are able to detect food-related chemical mixtures. We thus designed a spontaneous choice experiment using floating taste diffusers in order to test whether captive bottlenose dolphins could detect and display attraction behaviours towards a natural fish taste stimulus. Four dolphins, two adult males and two juvenile females, were involved in the experiment. Our results show that the juvenile females interacted with the fish taste diffuser significantly more than with the control. However, the adult males did not seem to make a clear choice. Also, the juvenile females showed a significantly greater level of motivation towards the test, spending more time interacting with the diffusers and holding them in their open mouth more often than the adult males. These findings corroborate previous behavioural studies suggesting that taste perception is functional in bottlenose dolphins, at least in young individuals. They also suggest that the taste of their natural prey could be attractive to them. Finally, the methodology used in this study proved to be easy to implement in captive odontocetes and will allow for investigating further their use of taste in feeding and social contexts without the need for conditioning experiments that require long periods of training. This experimental design could also be included in behavioural enrichment initiatives in captive marine mammals.

## 1. Introduction

Animals gain information about their environment through a variety of different sensory modalities. In captive animals, knowledge of a species' sensory capabilities is important for a number of reasons. Various sensory stimuli (or a lack thereof) can act as significant stressors for captive animals (Morgan and Tromborg, 2007). In addition, sensory enrichment using biologically relevant stimuli may be used to stimulate 'natural' behavioural expression in captive animals (Mills et al., 2010; Wells, 2009), and it may be possible to incorporate natural sensory stimuli into cognitive enrichment strategies.

Various species of toothed whale (odontocetes), such as beluga whales (*Delphinapterus leucas*), killer whales (*Orcinus orca*), and, in particular, bottlenose dolphins (*Tursiops truncatus*), are held in captivity in zoos and aquariums around the world (Clark et al., 2013). While we are accumulating increasingly detailed knowledge about the sensory

systems of these animals, in particular audition and vision (see Kremers et al., 2016a; for a recent review) very little is known about the chemical senses (taste, smell and trigeminal perception) in odontocetes. In terrestrial mammals, these senses are involved in a wide range of biological functions such as foraging, reproduction and orientation. This is also true for many marine vertebrates including fish, turtles, birds and pinnipeds (Grassman and Owens, 1987; Hara, 1994; Nevitt et al., 2004; Pitcher et al., 2011; Stoffel et al., 2015).

In contrast, odontocetes are thought to have very limited chemosensory abilities (Lowell and Flanigan, 1980). For example, these animals lack the canonical neuroanatomical structures necessary for olfaction as found in other vertebrates, e.g., olfactory epithelium, bulbs, nerves and tract (Jacobs et al., 1971; Oelschläger and Buhl, 2008), although these structures may be present embryonically (Oelschläger and Buhl, 2008). In addition, odontocetes also appear to have a poor sense of taste. Juveniles possess small numbers of taste buds, which

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diminish in number or even totally disappear in adults (Behrmann, 1988; Komatsu and Yamasaki, 1980; Kuznetsov, 1990; Li, 1983; Shindo et al., 2008; Werth, 2004; Yoshimura and Kobayashi, 1997). Moreover, they usually swallow their food whole (Perrin et al., 2009) and appear to lack functional taste receptor genes for four of the five basic vertebrate tastes (sweet, bitter, umami, sour, and salt; odontocetes only have functional salt taste receptor genes) (Feng et al., 2014; Liu et al., 2016; Zhu et al., 2014). Because these animals are thought to have such limited chemosensory abilities, very few studies have focused on this sensory system in comparison to the large number of studies performed on other modalities like audition or vision (e.g., Supin et al., 2001; Thomas et al., 1992; Thomas and Kastelein, 1990).

The extent to which odontocetes can perceive chemical signals, however, is still controversial. For example, while most of the taste receptor genes (except those implicated in salt taste) are pseudogenized in the bottlenose dolphin (Feng et al., 2014), this species has a well-developed gustatory thalamus (Kruger, 1959). Behavioural observations have suggested that bottlenose dolphins, as well as other dolphin species, have taste preferences and can potentially perceive chemical signals from congeners' secretions including faeces, urine and blood (Dudzinski et al., 2009; Gubbins, 2002; Muraco and Kuczaj, 2015; Norris et al., 1994). Indeed, in bottlenose dolphins, some of these biological secretions (urine and faeces) can induce an autonomous physiological response (such as an increase in heart rate, respiratory rate and skin conductivity) when they are irrigated as dilute solutions in the oral cavity (Kuznetsov, 1990).

Concerning the perception of the five basic tastes, experiments using go/no go operant conditioning in bottlenose dolphins indicate that these dolphins could detect at least four of them (sweet, salt, bitter and sour) at thresholds similar to those of humans (Friedl et al., 1990; Kuznetsov, 1990). Kuznetsov (1990) termed this ability "quasi-olfaction" instead of gustation, mainly because the dolphins seemed to perceive in their oral cavity some compounds that are usually perceived through olfaction in terrestrial mammals. In a recent preliminary study, food preference was also tested in the same species (Kremers et al., 2016b), the results of which suggest that bottlenose dolphins can discriminate artificial fish flavour from shrimp flavour or water. However, while these aforementioned studies using conditioning and/or go/no go experiments inform us about the ability of bottlenose dolphins to perceive some chemical compounds, they give limited information about whether or not these compounds will naturally influence their behaviour.

In this study, we implemented an experiment using captive bottlenose dolphins where the animals were free to spontaneously interact with two devices (floating diffusers containing ice cubes consisting of either natural fish mixture or a control solution) without any conditioned behaviour. This innovative, direct choice experiment was based on this species' spontaneous tendency to play with floating objects (Greene et al., 2011). We hypothesized that if the dolphins can discriminate the taste diffuser from the control, they would interact with them differently. Furthermore, if they perceive the food-related stimulus (fish mixture solution) as biologically relevant, the animals should display more attraction/interaction behaviours towards the taste diffuser compared to the control. Bottlenose dolphins were the focus of this study because most of the previous behavioural (conditioning) experiments on odontocetes' chemoreception have been performed on this species. As well as providing fundamental biological information, this study on the chemoreceptive capabilities of captive bottlenose dolphins could contribute to improve the welfare of such animals in captivity and above all, to contribute to the conservation and management of wild populations.

## 2. Materials and methods

### 2.1. Study animals and facilities

This study was performed at Marineland, Antibes, France, and involved four captive-born bottlenose dolphins (*Tursiops truncatus*). Two of the dolphins (A1 and A2, aged 22 and 24 years old, respectively) were adult males and the other two were juvenile females (J1 and J2, aged 7 and 4, respectively). These animals were kept together in a social group in a main lagoon pool (43 × 40 m with a maximum depth of 4 m) with another eight-year-old male which was not included in the experiment because of its tendency to destroy floating objects. None of the dolphins housed in the lagoon pool were part of the daily shows, which made them available for behavioural experiments.

### 2.2. Chemical stimuli

A natural fish taste stimulus (Fish) and a control stimulus (Control) were used. Atlantic mackerel (*Scomber scombrus*) was chosen as the fish stimulus because this species had already been included in the diet of the dolphins, thus preventing a neophobic reaction. At the same time, as the dolphins' regular diet consisted mostly of capelin (*Mallotus villosus*) and sprat (*Sprattus* spp.), we expected the dolphins to have a higher level of motivation towards a less-common fish stimulus (e.g., Epstein et al., 2007). The Fish solution was prepared by mixing 500 g of fresh mackerel in commercial spring water (Cristaline®, Saint Yorre, France) to a final volume of 1300 ml. This fish mixture was then filtered through a 200 µm sieve and 10 ml of red dye (natural beetroot extract, Terre Exotique, Rochecorbon, France) was added to produce a homogenous and uniformly coloured solution. For the Control solution, the spring water was coloured with the red dye in the same concentration as the fish solution. Ice cubes were prepared by freezing 100 ml of each solution at −20 °C for at least 72 h, which allowed the destruction of potential parasites in the fish mixture.

Two stimulus presentation devices, or 'diffusers' were built using modified commercially available floating chlorine diffusers for outdoor swimming pools, painted either red or blue (RAL 3000 and RAL 5019 respectively, Nuance, Motip Dupli, GmbH, Hassmersheim, Germany) to assist identification during video analysis (Fig. 1). The hue of these two colours was chosen because they had identical brightness, so avoiding a possible visual bias (bottlenose dolphins are cone monochromats; Fasick et al., 1998). During each experimental trial, each diffuser contained one 100 ml ice cube that was not visible through the narrow openings at the bottom. A rubber band was added to the screw-on cap so it remained tightly in place during manipulation by the subjects. In order to habituate the dolphins to the diffusers prior to the start of the trials, they were introduced while empty to the dolphins' pool for five minutes twice a day on the two consecutive days before the start of the trials.

### 2.3. Experimental design

Each spontaneous choice experiment consisted of a four-minute trial conducted in an experimental pool (11 × 11 m, 2.5 m deep) that was separated from the lagoon pool by a stainless steel gate. In order to participate in a trial, each dolphin was asked by a trainer to enter alone the experimental pool using a visual signal (pointing arm), while the others remained in the lagoon pool. A total of 48 trials were undertaken by testing each individual during 12 sessions across 10 days. The trials were run mostly during the late morning between 10:00 and 13:00, but also during the afternoon between 15:00 and 16:30. The dolphins were fed in the lagoon pool a light meal of fish (a quarter of their daily ration) on average 108 min (range: 15–213 min) before their participation in the experiment, which replaced the routine training sessions. The temperature of the air and water varied between 14 and 17 °C and 15–17 °C, respectively. The trials were performed with the assistance of

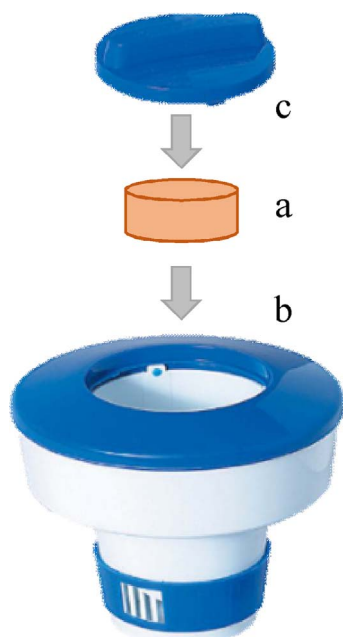


Fig. 1. Schematic view of the floating diffuser used in the spontaneous choice experiment. (a) 100 ml ice cube of taste (fish mixture) or control solution. (b) Modified PVC chlorine dispenser with (c) screwable cap reinforced with a rubber band.

6 trainers although two trainers were involved in only one trial each. We utilized two established command signals used by the trainers for many years, 'free-time' (the trainer indicates the training session has stopped) and 'retrieve' (the trainers ask the dolphin to retrieve an item in the pool). At the beginning of each trial, the dolphin stationed in front of a blind-folded trainer (starting place), while the two diffusers (Fish and Control) were placed by an experimenter at the opposite side of the pool equidistant from the starting place. The identity of the dolphin, the colour of the Fish and Control taste diffusers (red or blue) as well as their position (to the left or to the right of the subject) were all chosen in a random balanced order to avoid a side- or colour-preference effect. Once the trainer gave the dolphin the signal for free time and walked away, the dolphin was left alone to freely interact with the two diffusers for the whole duration of the trial. The movements and the behaviour of the dolphin during each trial were recorded using two GoPro digital cameras (GoPro Hero 4 Black Edition, GoPro Inc., San Mateo, CA, USA) placed facing in opposite directions above the pool. Additional environmental data were also recorded to control for their respective influence on the dolphins' behaviours, including their prandial state (i.e., the amount of time since their last meal), the weather conditions (presence of rain or wind > 10 km/h), and the identity of the trainer. After four minutes, the trainer came back and, again using signals, asked the dolphin to retrieve both diffusers. The dolphin was rewarded with one fish upon retrieving the diffusers and the keeper asked to transfer to the lagoon pool after the gate was opened. The presence of a remaining ice cube of a similar size in both diffusers was then checked to confirm that there was no temperature difference around the two diffusers during the experiment.

#### 2.4. Data collection

The video recordings were analyzed by a blind experimenter. Firstly, trials deemed as invalid due to a protocol problem (e.g., a diffuser was opened by the dolphin or became stuck in the pool skimmer, or the trainer used an incorrect command signal sequence) were identified and discarded. For the remaining 37 valid trials (10 trials each for A1 and A2, 9 and 8 for J2 and J1, respectively), the number and duration of the behavioural interactions with each diffuser were measured. These interactions were divided into three categories:

(1) 'bite' (the dolphin holds the diffuser in its mouth); (2) 'push' (the dolphin pushes the diffuser using its rostrum); and (3) 'throw-follow' (the dolphin throws the diffuser away using its mouth and then swims back to it). The total exploration time was defined as the total time of interaction with both diffusers and was used as an indicator of the exposure of the dolphins to both the stimulus and the control. The taste preference (P) was calculated as the difference in the duration of all the interactions between the Fish and the Control stimuli. The number of switches between the diffusers (where the dolphin goes from one diffuser to the other) was also measured as a potential indicator of the dolphins' interest in finding the chemical cue.

#### 2.5. Statistical analyses

Statistical analyses were carried out using R 3.1.2 (R Development Core Team 2015). Arithmetic means and  $\pm$  standard errors are presented. The data were tested for normality using a Shapiro–Wilk test prior to all analyses. Non-normal distributions were transformed using either square-root (exploration time and number of switches) or arcsine (proportion of biting behaviour) transformations. The effects of the trial session and the prandial state on the behavioural parameters were analyzed using Pearson's correlation coefficient and the effects of both environmental (weather conditions, prandial state) and protocol (presentation side and colour of the Fish diffuser, identity of the trainer) variables were assessed using Kruskal Wallis' H-test. The differences in these parameters between the two age/sex groups (adult/male and juvenile/female) were tested using two-tailed Student's *t*-tests. Taste preference was tested against the theoretical value of zero using a Student's *t*-test.

#### 2.6. Ethical approval

This experiment was approved by the French national ethical committee (Comité Consultatif National d'Ethique, permit APAFIS #5392-2016051717525952v3) and was in accordance with the European directive 86/609/CEE. The subjects' participation in research was entirely voluntary, with the experiment described here replacing routine training sessions.

### 3. Results

#### 3.1. Environmental and protocol variables

None of the measured behavioural parameters (preference, time of exploration, and number of switches) was significantly affected by any of the environmental and protocol variables (Table 1).

#### 3.2. Behavioural interactions

Bite was by far the most common behavioural interaction, accounting for on average  $70.0 \pm 4.1\%$  of the total interactions across the four dolphins. The two juvenile females displayed bite behaviour significantly more frequently than the two adult males (81% of the interactions vs. 60% in the juvenile females and adult males, respectively;  $t = 2.37$ ;  $df = 32.5$ ;  $P = 0.02$ ; Student's two-tailed *t*-test on arcsines, Fig. 2a). The push behaviour was observed more frequently in adult males (31.4% vs. 13.6%;  $t = 2.39$ ;  $df = 22.4$ ;  $P = 0.03$ ; Student's two-tailed *t*-test on arcsines, Fig. 2b) while there was no difference in the throw-follow behaviour between the two groups ( $t = 1.35$ ;  $df = 28.6$ ;  $P = 0.19$ ; Student's two-tailed *t*-test on arcsines, Fig. 2c).

#### 3.3. Total exploration time

The four dolphins manipulated both diffusers extensively, with an average of  $19.2 \pm 2.1$  contacts per trial. On average, total exploration time was significantly higher in the juvenile females than in the adult

**Table 1**

Influence of environmental and protocol variables on the dolphins' behavioural parameters. *P*-values are presented for each statistical analysis. Two-sample Student's *t*-test have been used for means comparison between two categories (Presence of rain or wind, side and colour of the fish diffuser). Kruskal-Wallis H-test was used for multiple categories (keepers) and Pearson's correlation for numeric data (prandial state, i.e. time since the last meal).

Environmental & protocol variable	Statistical test	Behavioural parameter		
		Preference	Exploration time	Switches
Rain	Two-samples <i>t</i> -test	0.07	0.64	0.11
Wind	Two-samples <i>t</i> -test	0.78	0.56	0.83
Keeper	Kruskal Wallis' H-test	0.43	0.69	0.84
Prandial state	Pearsons' correlation	0.11	0.89	0.34
Side of diffuser	Two-samples <i>t</i> -test	0.52	0.68	0.64
Color of diffuser	Two-samples <i>t</i> -test	0.63	0.41	0.71

males (153 s per trial vs. 82 s per trial, respectively;  $t = 2.81$ ,  $df = 32.5$ ;  $P = 0.008$ ; Student's two-tailed *t*-test on square roots, Fig. 3a). The exploration time did not change throughout the sessions in both groups (Pearson's  $r^2 = 0.003$  on square roots,  $P = 0.76$ ; Fig. 3b).

### 3.4. Taste preference

On average, the taste preference *P* (i.e. the difference in the time spent with the Fish diffuser and the Control) was  $18.4 \pm 17.1$  s across the four animals but this did not differ significantly from 0 ( $t = 1.079$ ;  $df = 36$ ;  $P = 0.29$ ; one sample Student's two-tailed *t*-test). However, we observed a significant difference in taste preference between the two age/sex groups. With the mature males, no difference was found in the time spent with each diffuser ( $t = 1.014$ ;  $df = 19$ ,  $P = 0.32$ ; one sample Student's two-tailed *t*-test) while the two juvenile females showed a significant preference towards the Fish diffuser: they interacted about one minute more with the Fish diffuser than with the Control diffuser (107.8 vs. 45.3 s;  $t = 2.345$ ;  $df = 16$ ;  $P = 0.03$ ; one sample Student's two-tailed *t*-test) resulting in an average taste preference *P* of  $62.5 \pm 26.7$  s (Fig. 4). Similar results were obtained when considering the number of contacts with the floaters instead of the time spent interacting with them (data not shown). There was no significant change in taste preference through the different trial sessions in adult males or in juvenile females (Pearson's  $r^2 = 0.10$ ;  $P = 0.18$  and  $r^2 = 9.10 \times 10^{-4}$ ;  $P = 0.91$ , respectively) (Fig. 5).

### 3.5. Switches

The dolphins switched between the two diffusers  $2.4 \pm 0.49$  times per trial on average with no difference found between the two age/sex categories ( $t = 0.92$ ,  $df = 28.2$ ;  $P = 0.37$ ; Student's *t*-test (two-tailed) on square roots). However, we observed a progressive decline in the number of switches through the trial sessions (Pearson's  $r^2 = 0.17$ ;  $P = 0.01$ ).

### 3.6. Correlations between behavioural parameters

We observed only two correlations between the measured behavioural parameters in the two age/sex groups. Preference was negatively correlated with the number of switches in juvenile females (Pearson's  $r^2 = 0.23$ ;  $P = 0.05$ ) as well as with the exploration time in adult males (Pearson's  $r^2 = 0.21$ ;  $P = 0.04$ ) (Table 2).

## 4. Discussion

In previous studies on taste perception in bottlenose dolphins, only pure taste compounds such as citric acid, sodium chloride or sucrose (Friedl et al., 1990; Kuznetsov, 1990) or artificial flavours (Kremers et al., 2016b) were tested. In contrast, here we report for the first time the perception of a complex, food-related taste in bottlenose dolphins,

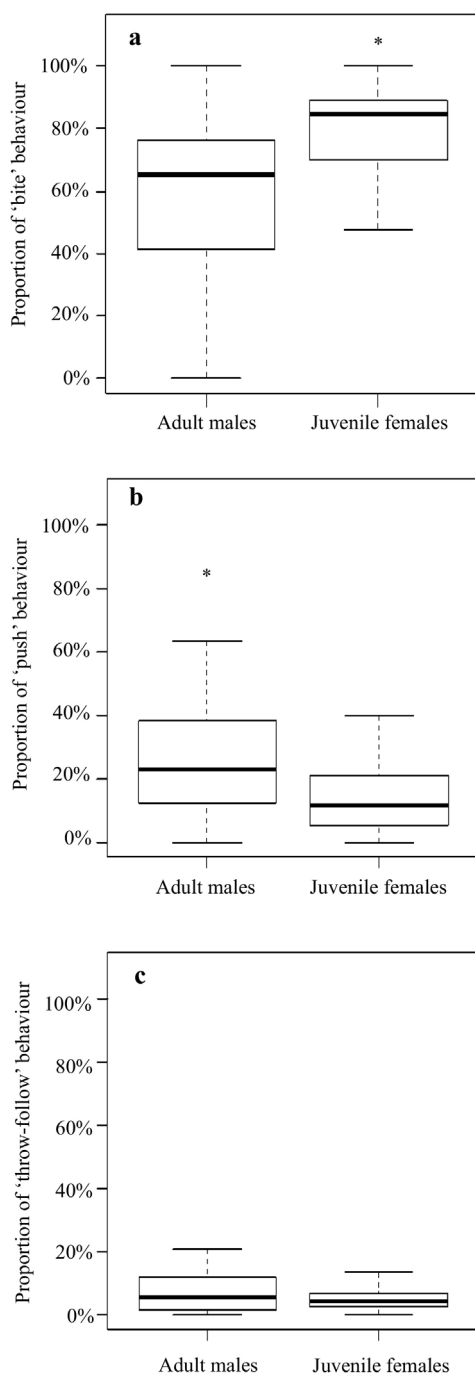
at least in one age/sex class. Indeed, our results strongly suggest that the juvenile females we tested discriminated the fish taste, as they had significantly more contacts and higher time of interaction with the Fish diffuser than with the Control diffuser. It is also noteworthy to mention that the trials were performed without conditioning and using single individuals, allowing a free spontaneous exploration of the stimuli without any social bias due to hierarchical structure in captive animals.

Regarding the compounds that are potentially perceived in the fish mixture, several hypotheses might be considered. First, although the salt concentration in the fish mixture (estimated to be 0.07 M based on Bhuiyan et al.'s (1986)) measure of the salt concentration in raw Atlantic mackerel) was slightly below the behaviourally-determined sodium chloride detection threshold reported in bottlenose dolphins (0.1–0.3 M) (Friedl et al., 1990; Kuznetsov, 1990) it was much higher than in the control solution ( $8.1 \times 10^{-4}$  M). This difference in salt concentration may thus have played a role in the discrimination, as the salt receptor genes have all been shown to be functional in the bottlenose dolphin (Feng et al., 2014). Moreover, trimethylamine (TMA) is also present at high concentrations in raw fish including mackerel (Ahn et al., 2014) and has been found to be perceived by bottlenose dolphins when perfused in their mouth at a concentration of  $3.4 \times 10^{-2}$  M (Kuznetsov, 1990). While the taste receptor type usually implicated in the detection of trace-amines in mammals has been lost in this species (Kishida et al., 2015), TMA may be perceived through another neural pathway such as the trigeminal nerve, as has been shown to be the case in humans and mice (van Thriel et al., 2006; Willis and Morris, 2013). While sodium chloride and TMA are two potential candidates, many other chemical compounds contained in the fish mixture may have contributed, either individually or in combination, to the taste discrimination in juvenile females.

In contrast to the juvenile female dolphins, the two adult males did not discriminate between the two diffusers. This could be linked to a lower chemical sensitivity related to either sex or age. In primates, for example, it has been suggested that higher taste acuity in females could be linked to a higher density of fungiform papillae on the tongue (Muchlinski et al., 2011). However, there has been no report of sexual difference in the gustatory structures in odontocetes so far, although unfortunately the sex of the individuals investigated is rarely mentioned in the few previous studies on taste perception in bottlenose dolphins. In the recent study by Kremers et al. (2016b), both male and female bottlenose dolphins were tested and the authors found no apparent differences between sexes regarding taste discrimination.

A more likely explanation for the apparent lack of discrimination between the fish mixture and the control in the adult males is age, given that the adult males were, on average, approximately four times older than the juvenile females. A similar decline in taste sensitivity has also been documented in other mammal species including humans and rats (Kurt Thaw, 1996; Mojet et al., 2001). Anatomical studies in bottlenose dolphins have suggested that taste buds progressively degenerate in adults as their presence was described in the pits located at the base of the tongue of a subadult and in 2–3 year-old calves, but not in mature





**Fig. 2.** Proportion of the total exploration time spent exhibiting 'bite' (a), 'push' (b) and 'throw-follow' behaviours in the two age/sex groups of dolphins. (a) The 'bite' behaviour was the most frequently observed, and was observed significantly more in the juvenile females ( $t = 2.37$ ;  $df = 32.5$ ;  $P = 0.02$ ; Student's two-tailed  $t$ -test on arcsines). (b) The 'push' behaviour was proportionally more frequent in the adult males than in the juvenile females ( $t = 2.39$ ;  $df = 22.4$ ;  $P = 0.03$ ; Student's two-tailed  $t$ -test on arcsines). (c) No difference was observed in the 'throw-follow' behaviour between the two groups ( $t = 1.35$ ;  $df = 28.6$ ;  $P = 0.19$ ; Student's two-tailed  $t$ -test on arcsines).

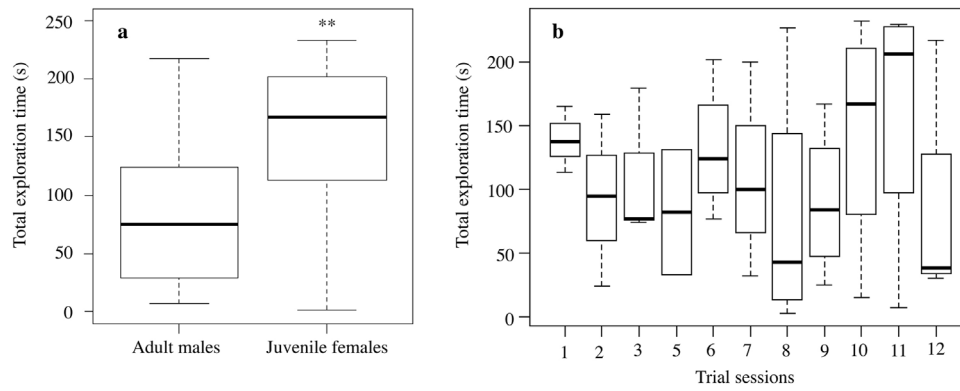
adults (Kuznetsov, 1990; Yoshimura and Kobayashi, 1997). While more anatomical studies are needed to determine the chronology of this process, the apparently greater taste sensitivity observed in young individuals may be of great ecological importance as it could allow them to taste milk during the 2-year lactation period, as well as to discriminate food as they learn how to catch live prey (Komatsu and Yamasaki, 1980). A similar process of progressive taste bud degeneration was also described in other species of toothed whale (Komatsu and

Yamasaki, 1980; Shindo et al., 2008). Therefore the discrimination abilities of the two juvenile females included in the present study, aged 4 and 7, may reflect a higher chemical sensitivity, possibly related to the presence of a larger number of functional taste buds. Taste thresholds have yet to be determined for juvenile bottlenose dolphins, but our results, as well as previous anatomical findings, suggest they are likely to be lower than those already reported in adults using go/no experiments (Friedl et al., 1990; Kuznetsov, 1990).

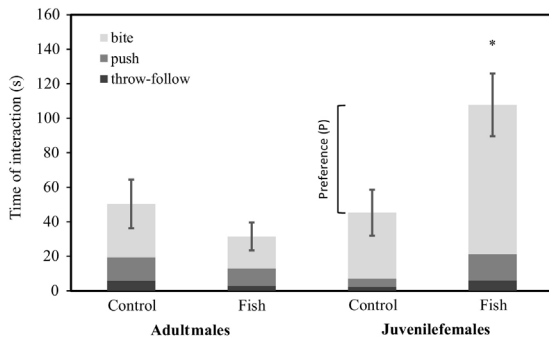
In addition to our discoveries regarding the ability of bottlenose dolphins to perceive a complex-food mixture using taste, the main feature of our study was to assess whether this chemoreceptive ability could influence their behaviour. For this reason, we chose to evaluate the spontaneous behavioural responses of the dolphins, instead of using operant conditioning techniques. Our results suggest that not only were juvenile females able to discriminate the fish taste from the control, but that they were also attracted by the fish mixture, as shown by the higher time of interaction recorded for these animals. We also observed a difference in behaviours between the adult males and the juvenile females that could explain partly the above-mentioned difference in perception between these two groups. Indeed, the juvenile females displayed a higher interest in the task in general compared to the adult males: they spent more time exploring the two diffusers and exhibited more biting behaviour. This result is not surprising as juvenile dolphins have previously been shown to engage in object play significantly more than adults (Greene et al., 2011). It may also explain why, on two occasions, one of the juvenile females (J1) was able to open the tightly screwed cap of the Fish diffuser and then swallow the remaining ice cube it contained. This happened only twice, so this unexpected event was not statistically exploitable and, as explained previously, the two trials in question were removed from the data analysis.

In general, both the adult males and the juvenile females showed a progressive decline in motivation to perform the experimental task, as suggested by the significant decline in the number of switches through the sessions. The number of switches may also be related to the performance in the discrimination task as it was negatively correlated to preference only in juvenile females; they tended to stick to their choice (i.e. switch less) when they found the Fish diffuser. This pattern appears similar to the "win-stay, lose-shift" strategy which involves a stimulus-reward association and is well documented in laboratory animals including mammals, birds and fishes (Emery, 2006; Evenden and Robbins, 1984; Heydarnejad and Purser, 2016). Wild bottlenose dolphins have been shown to use this behaviour during foraging: they tend to stay in patchy concentrations of nearshore preys until the local resource has been depleted and then travel in search of a new optimal site (Defran et al., 1999). In primates, a natural tendency to switch more between choices when the reward intensity decreases has also been described and linked to the stimulation of the parietal cortex (Kubaneck and Snyder, 2017), a brain region that is also well developed in bottlenose dolphins (Jacobs et al., 1971). Increasing the animals' motivation to play with the diffusers may therefore improve their performance throughout the entire duration of the experiment via an increase in their exposure to the taste stimuli. One simple method of achieving this could be to reward them at the end of each trial by giving them the tasty ice cube to eat.

This study demonstrated that our methodological design is appropriate to investigate bottlenose dolphins' chemosensory abilities, since the dolphins extensively manipulated the floating diffusers with their mouths. As it was also easy to implement, this experimental approach could be used as a complementary approach to operant conditioning experiments that require long training periods working with particularly attentive animals. Using the methodology described here, a wide range of putatively relevant chemical cues could now be screened, including food-related individual chemicals, or urine extracts that are believed to contain sex pheromones (Muraco and Kuczaj, 2015). The investigation of potential chemical deterrents would also be valuable for use in conjunction with pingers in the fishing industry (Hamer et al.,



**Fig. 3.** Total exploration time (time spent interacting with both Control and Fish diffusers) during the four-minute trials in the two age/sex groups of dolphins. (a) This behavioural parameter was significantly higher in the juvenile females than in the adult males ( $t = 2.81$ ,  $df = 32.5$ ;  $P = 0.008$ ; Student’s two-tailed  $t$ -test on square roots). (b) No significant evolution was observed throughout the trial sessions (Pearson’s  $r^2 = 0.003$  on square roots,  $P = 0.76$ ).



**Fig. 4.** Average time spent with the Control and Fish taste diffusers in adult males and juvenile females bottlenose dolphins over 12 sessions of four-minute trials. The preference was defined as the difference in time spent with the Fish and Control diffusers, and significantly differed from 0 only in juvenile females ( $t = 2.345$ ;  $df = 16$ ;  $P = 0.03$ ; one sample Student’s two-tailed  $t$ -test). The three different behaviours observed ‘bite’, ‘push’ and ‘throw-follow’ are detailed in light grey, grey and dark grey, respectively. Error bars represent the standard error of the mean.

2012).

From an animal welfare point of view, the present spontaneous choice protocol could also be used as part of an enrichment program for captive dolphins raised in a relatively sterile, captive environment with a low chemical diversity (Wells, 2009). Such sensory stimulations are now becoming increasingly encouraged in zoos and aquariums (Mills et al., 2010; Wells, 2009), and may be especially important in marine mammals that usually cannot exhibit their natural feeding behaviours as they are fed dead fish, mostly as a reward after the successful completion of a conditioned task (Clark, 2013). Rather than being

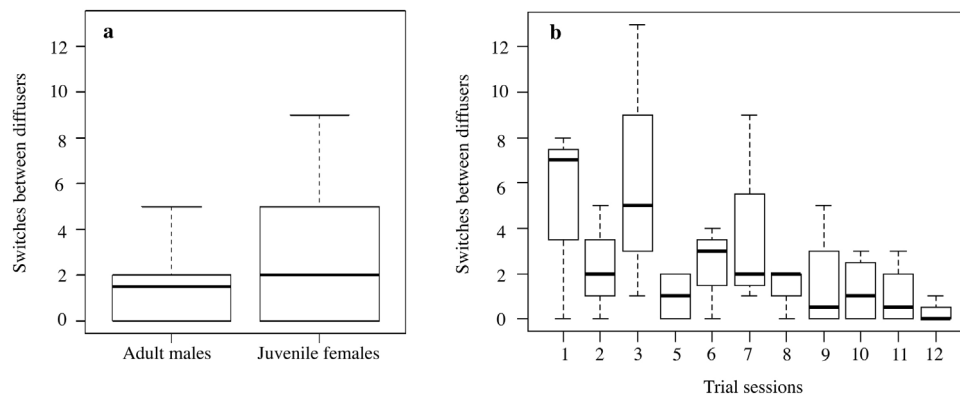
**Table 2**

Correlation between the three behavioural parameters measured in the dolphins. Pearson’s correlation coefficients and  $P$ -values are presented for each statistical analysis. Significant correlations ( $P < 0.05$ ) are highlighted in bold.

Behavioural parameters	Adult males		Juvenile females	
	Pearson’s $r$	$P$	Pearson’s $r$	$P$
Preference x Switches	-0.090	0.707	<b>-0.481</b>	<b>0.050</b>
Preference x Exploration Time	<b>-0.456</b>	<b>0.044</b>	0.306	0.233
Switches x Exploration Times	-0.007	0.975	0.430	0.086

dissipated throughout the entire captive environment, which could potentially be overwhelming and stressful to the animals, these chemical cues should be delivered as concentrated focal stimuli. This technique, using natural and artificial scents, has recently proved to be effective in decreasing pattern swimming and other stereotypical behaviours in California sea lions (Samuelson et al., 2016).

In conclusion, in this study we have designed and tested an innovative protocol to study the use of chemoreception in captive bottlenose dolphins. Our results corroborate with the very few previous behavioural studies on this subject (Friedl et al., 1990; Kuznetsov, 1990), suggesting that bottlenose dolphins can indeed perceive chemical compounds. In addition, we have shown that a fish mixture can be attractive, at least for juvenile females, which provides strong evidence that chemical stimuli could be involved in foraging behaviour in complement with other sensory modalities. Further studies are now required, focusing on wild dolphins that both live in contact with the complex mixture of chemicals present in the marine environment and exhibit natural foraging behaviours. Our team is currently implement-



**Fig. 5.** Number of switches observed during the four-minute trials in the two age/sex groups of dolphins. (a) No significant difference was observed between juvenile females and adult males ( $t = 0.92$ ,  $df = 28.2$ ;  $P = 0.37$ ; Student’s two-tailed  $t$ -test on square roots). (b) The number of switches progressively decreased throughout the trial sessions (Pearson’s  $r^2 = 0.17$ ;  $P = 0.01$ ).

ing field experiments using protocols adapted from the research on chemoreception in marine birds (e.g., Nevitt et al., 2004). We anticipate that, as it has been the case in birds (e.g. Baldwin et al., 2014; Caro et al., 2015), a combination of these behavioural approaches with further investigations on their neuroanatomical and molecular bases may reveal that chemosensory systems play a more significant role in the behavioural ecology of odontocetes (and indeed other cetaceans) than has been previously thought.

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