Dolphin Welfare Assessment under Professional Care: 'Willingness to Participate', an Indicator Significantly Associated with Six Potential 'Alerting Factors'

Fabienne Delfour [1,2,*], **Tania Monreal-Pawlowsky** [3], **Ruta Vaicekauskaite** [4], **Cristina Pilenga** [5], **Daniel Garcia-Parraga** [6], **Heiko G. Rödel** [2], **Nuria García Caro** [7], **Enrique Perlado Campos** [8] and **Birgitta Mercera** [1]

[1] Parc Asterix, 60128 Plailly, France; birgitta.mercera@parcasterix.com
[2] Laboratoire d'Ethologie Expérimentale et Comparée UR 4443, Université Sorbonne Paris Nord, 93430 Villetaneuse, France; heiko.rodel@univ-paris13.fr
[3] International Zoo Veterinary Group, West Yorkshire BD21 4NQ, UK; T.monreal@izvg.co.uk
[4] Klaipeda University, H. Manto 84, 92294 Klaipeda, Lithuania; ruta@fox-zooconsulting.com
[5] Zoomarine Italia, 00071 Rome, Italy; cpilenga@zoomarine.it
[6] Fundación Oceanografic, Eduardo Primo Yufera 1B, 46013 Valencia, Spain; dgarcia@oceanografic.org
[7] Barcelona Zoo, 08003 Barcelona, Spain; encmmarins@bsmsa.cat
[8] Mundomar, 03503 Benidorm, Spain; Kike@mundomar.es
* Correspondence: fabienne_delfour@yahoo.com or fabienne.delfour@parcasterix.com

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Abstract: In dolphinaria, dolphins and their trainers build relationships and bonds due to the nature, closeness and repeatability of their interactions, hence training sessions are deemed appropriate to evaluate dolphin welfare. Qualitative Behavioural Assessments (QBAs) have been used to study human–animal relationships and are included in several animal welfare assessments. We introduce here the first QBA aiming to analyse dolphin–trainer interactions during training sessions in terms of dolphin welfare. Our results show that "Willingness to Participate" (WtP) was significantly associated to six other parameters: high-speed approach, high level of excitement, high number of positive responses to trainers' signals, rare refusal to perform certain behaviours, rare spontaneous departure behaviours and fast approach once the trainer entered into the pool. Therefore, we suggest using WtP and those "alerting factors" when assessing dolphin–trainer interactions under professional care. The evaluation should also consider the time of day, the dolphin's age, trainer experience level, the nature of the training sessions and to a lesser extent the sex of the dolphins, as contributing and modulating factors. The factor eye contact has been used in various HARs studies and has been proven to be a valid indicator in welfare research works, hence potentially deserving further research. These results demonstrate the pertinence and feasibility of this approach, the ease of use of this methodology by professionals in zoo/aquarium settings and the appropriateness of the obtained results within the holistic frame of animal welfare.

Keywords: human–animal interaction; qualitative behavioural assessment; welfare; dolphins

1. Introduction

In zoological settings, Human–Animal Interactions (HAIs) happen routinely between keepers and the animals they care for, as these interactions occur due to the natural process of managing and taking care of the animals [1]. The nature of these interactions, their regularity, their duration, the identity and emotional state of the keepers involved as well as the personality, affective state and individual peculiarities of each animal [2–4] may all have an important impact, positive, negative

or neutral, on human–animal interactions and on animal welfare as a whole [5–7]. Both positive and negative keeper–animal relationships are potential indicators to assess animal welfare [7–11]. Animal keepers have the ability, the role and the ethical obligation to ensure that their interactions and their relationships with the animals under their care serve to maximize the positive impact on their welfare [12,13]. Human Animal Relations (HARs) as a possible outcome of regular HAIs may involve affiliative contentment for the animals [6,10]. Additionally, positive HARs have the potential to increase the performance of species-specific behaviours while reducing abnormal behaviours [14]. These relationships can also reduce the stress of exposure to unfamiliar zoo visitors [1,15,16]. Consistency, as well as knowing each particular animal individually, appears to be important elements in HARs [11]. Ninety-two percent of zoo professionals have a bond with at least one of the animals they work with [7].

Animal welfare concepts and theories should be included in a holistic approach [17] and therefore several frameworks have been set up (i.e., five freedoms [18]; Welfare Quality® [19–21]) using extensive lists of behavioural, physiological and cognitive parameters in quantitative and qualitative assessments. For instance, whether or not an animal is approaching humans, including the way it approaches, have been proposed as possible welfare indicators [2,5,6,22–24]. In farm animals, HARs have been used to assess the level of avoidance and fear towards humans [25].

Qualitative assessments are appropriate tools to use when evaluating animal welfare; they collect descriptive data complementary to quantitative ones [26]. According to Wemelsfelder et al. ([27,28] in Patel et al., [29] (p. 2) Qualitative Behavioural Assessment (QBA) is " . . . a 'whole-animal' methodology, which integrates observations of the dynamic emotionally expressive aspects of an animal's movement and posture (i.e., an animal's 'style' of behaving), using qualitative descriptors such as relaxed, lively, anxious or agitated". QBAs were historically developed for farm animals with the goal to assess their welfare. They were largely tested for consistency, reliability and biological validity in a range of livestock species [29–32]. QBAs have been shown to be a suitable method for investigating the quality of HARs [33] and an effective method to study animal's experiences and welfare. QBAs have also been effective to measure the impact of HAIs on individual animals under human professional care [34]. For example, a QBA studying the stable responses of domestic horses (*Equus caballus*) to human contact found that horses scored as calm showed less avoidance and responded less aggressively and fearfully to human approach than horses perceived as uneasy or alarmed [35]. How animals perceive humans and the fear they may feel towards them depend on their past experience [1,6]. Behavioural studies have also suggested that the actions of keepers can affect animals' fear responses as well as the resulting human–animal relationships [2]. As for zoo animals, short duration of human interactions with Western lowland gorillas (*Gorilla g. gorilla*) helped focus the interest of the animals on their conspecifics while preserving social behaviours' rules [36]. However, informal interactions of zoo staff with chimpanzees (*Pan troglodytes*) and gorillas resulted in higher levels of agonistic behaviour, suggesting greater arousal and stress in these animals [37].

Therefore, QBAs offer a method to disentangle the complex links between humans and animals [35] and of course they are employed in zoological park settings to understand the relationships between zookeepers and animals under their care [1]. Different QBAs appraising behaviour and personalities of terrestrial species have been validated as useful methods for cross-institutional comparisons [13,38–40]; this has facilitated the application of multi-institutional welfare studies into zoo research worldwide [12,13,41,42].

Zoos generally emphasize the need to objectively and scientifically assess the welfare of their animals [43]. In most cases, welfare studies require a direct approach from humans to animals (to study the reactions of the animals themselves) and such a methodology is therefore not directly applicable or suitable for most zoo-housed species due to safety and ethics [29]. However, marine mammals, and especially bottlenose dolphins (*Tursiops truncatus*), are trained in close contact with their keepers and thus their daily management implies direct contact between humans and animals. For most zookeepers, training is a very important tool during the daily care of the animals to stimulate

their mental and physical exercise and to facilitate important husbandry and medical procedures. The latter have both significant impact on animal health and welfare. Numerous methods can be used to condition behaviour, but training through positive reinforcement is the reference to be used in modern zoo practice and a standard practice in EAAM facilities (i.e., European dolphinaria) [9]. Husbandry procedures may sometimes involve some discomfort for the animals (e.g., some veterinary interventions) and they usually rely on trust between the animal and the trainer [9,44,45]. The general effect of positive reinforcement has been proven to be positive on HAIs [46,47], reducing fear of the animals towards humans, thus improving their welfare [48].

Many dolphinaria are actively involved in assessing the welfare of their animals [49–51]. The European Association of Aquatic Mammals Welfare Committee has launched a project to scientifically assess bottlenose dolphin welfare, called Dolphin-WET (Welfare Evaluation Tool). This tool uses approximately 70 indicators; the majority has already been scientifically validated in dolphins or in other species, and some indicators are currently being tested (e.g., [52]). The study presented here is part of this ongoing project. QBAs have not yet been fully implemented for marine mammals, although previous studies already proposed qualitative evaluation of certain behavioural components, such as the animals' "Willingness to Participate" (WtP: [53]). Most of the questions we asked have been researched in other species [2,7,53–55]. Previous studies have investigated some aspects of dolphin–trainer interactions and relationships. For instance, using classical conditioning, Clegg et al. [56] showed that dolphins anticipated human–animal non-feeding interactions more than toy provision and their anticipatory behaviour frequency was correlated with their participation in the event, suggesting that these marine mammals perceive HAIs and toys as rewarding situations. More recently, Welsh and Ward [57] showed that HAIs had an impact on mother-calf dyad interactions (i.e., encouraging interactions), even though they could not state if these were positively or negatively perceived by the animals.

Inquiring about an animal's relationship towards a human presupposes that the animal is able to recognize the targeted human, that it comprehends its gestures and that it understands the human's attentional and emotional states. Dolphins' cognitive abilities have been quite extensively studied and indeed it has been shown that bottlenose dolphins recognize their trainers individually using visual cues [58], they understand their trainers' attentional states [59] and they follow several types of human pointing gestures [60,61].

Our work presents the first animal-based QBA on trainer-dolphin interactions during training sessions and discusses its implications for assessing the animals' welfare. We analysed the variation of several behavioural parameters (i.e., willingness to participate, speed of approach, response to trainers' signals) according to various environmental factors (e.g., time of day, nature of training sessions, individual versus group training sessions), trainer experience level and dolphin individual features (i.e., age and sex). Finally, we cross-validated the parameters listed above with a recently successfully validated welfare indicator (WtP), which has been shown to be related with early changes in health status in bottlenose dolphins [53].

2. Methods

2.1. Involved Dolphinaria

During the winter of 2019–2020, a QBA protocol was implemented in five European facilities (in three different countries) housing bottlenose dolphins (*Tursiops truncatus*). This study adhered to the ASAB/ABS Guidelines for the Use of Animals in Research and was reviewed and accepted by the scientific and animal welfare committee of Parc Asterix. This study was conducted in accordance with the Declaration of Helsinki.

Four participating facilities were accredited by the European Association for Aquatic Mammals (EAAM) and three were accredited by the European Association of Zoos and Aquaria (EAZA) at the time of the study. All follow standard guidelines using exclusively Positive Reinforcement

Training (PRT) during which dolphins receive fish and/or other secondary reinforcers after performing conditioned behaviours; there was no punishment or negative outcome for cases of the animals leaving the trainers during the training sessions or for not performing the asked behaviour [46]. The animals were fed daily with a variety of fish and cephalopod species during multiple training sessions at participating facilities. The total amount of fish fed per day to each dolphin varied with age, sex and reproductive status and was determined in cooperation with the veterinary and nutritionist teams in each facility. Diets are designed to guarantee proper nutritional intake and maintain optimum body condition. Specific "free-feeding" sessions occurred daily in each facility; it operated on the basis that if the animals chose not to perform behaviours in the daily training sessions, their full daily fish ration would be offered to them at the end of the day. During the entire observations for this study, food was provided in a similar manner in each facility. Parc Astérix (Plailly, France) housed eight dolphins, but only seven were included in this study (Table 1). Dolphins were in an outdoor pool connected to two indoor pools, and they had always free access to all pools during the study period. In Barcelona Zoo (Barcelona, Spain), three male dolphins were housed in four pools connected by channels and all of them participated in the study (Table 1). At Mundomar (Benidorm, Spain), two males and five females had unrestricted access to five connected pools, six of them participating in the study (Table 1). Oceanogràfic (Valencia, Spain) housed 16 dolphins in an outdoor facility consisting of six pools of variable sizes and interconnected by variably opened or closed gates throughout the day (Table 1). In this facility, animals were usually divided in small or medium social subgroups (2 to 10 animals) to facilitate access to at least two pools at all times. Group size, individuals or locations within the dolphinaria were alternated periodically to promote variability. All 16 individuals participated in the study. Finally, Zoomarine Roma (Pomezia, Italy) had 11 dolphins; 10 participated in the study subdivided in three groups (Table 1). Each group was kept in one to two pools, varying through the days of observation. The study group therefore included a total of 43 bottlenose dolphins (32 adults: 18 females and 14 males, and 11 juveniles: 5 females and 6 males). In each facility, data were gathered during a time period consisting of three consecutive weeks in February 2020. Two facilities (Barcelona Zoo and Oceanogràfic) were open to the public and the latter had three public presentations per day, while the other three facilities were closed to visitors during the study period.

Table 1. Overview of the participating facilities and description of the animals involved in the study. The range span of occurring ages within each sex and age class is given in years. Note that the total number of observation sessions available for analysis was often lower as sometimes not all parameters were recorded (details in Table 2).

Facility		Females				Males				*N* Sessions Observed	
		N Dolphins		Range of Age		*N* Dolphins		Range of Age			
	N total	Juv.	Ad.	Juv.	Ad.	Juv.	Ad.	Juv.	Ad.	Total	Per Dolphin
Parc Astérix	7	2	3	2–4	20–46	0	2	-	35–37	70	10
Mundomar	7	0	5	-	12–45	1	1	4	33	68	10
Oceanogràfic Valencia	16	1	8	2	13-40	2	5	6–10	14–35	52	1-10
Zoomarine Italia	10	2	2	3–8	18	2	4	5–10	21–36	110	10–12
Barcelona	3	0	0	-	-	1	2	8	17–22	23	7–8
Total	43	5	18	-	-	6	14			323	-

2.2. Information Used on the QBA

The QBA developed included an explicit methodology which was implemented in each facility with the goal to gather data on HAIs, in this case dolphins interacting with their trainers. A pre-defined form was filled in by observers who were all familiar with dolphin training procedures and the dolphins being studied and who were observing and rating a particular training session with one or several dolphins ('individual session' and 'group session'). Observers were different from the trainers

performing the training being scored. These observers were then familiar to the dolphins, who were not participating directly in the training sessions. Daily management of the dolphins in the facilities included in this study encompasses different types of sessions, during which trainers are interacting with animals. These include 'medical training', 'new behaviour learning session', 'public presentation', 'routine training session' and 'mixed training session'. Only training sessions were evaluated, while free feeding or enrichment sessions were discarded from this study. Each training session routinely starts with dolphin trainer(s) approaching the pool and immediately after the dolphin(s) swimming towards them (or not). Due to the obvious variation in this dolphin behaviour we took the opportunity to rate the dolphins' speed (or probability) of approach, see details below. The training sessions happened at different times of the day, which we grouped in three different time bins: 'morning' (from start of the day to noon), 'midday' (from noon to 2 pm) and 'afternoon' sessions (after 2 pm).

The selection of the sessions to be assessed was semi-randomised, depending on trainers' availability. After a selected session, the trainer working with the animal(s) was requested to help the person in charge of the observation to fill in the pre-defined form by giving information on the behaviours and the reactions of the dolphins he/she interacted with during the session considered. The information initially collected by this form included: the speed of the approach of the dolphin when the trainer got in position at the edge of the pool ('fast approach', 'delayed approach' or 'no appearance' of the animal(s)), the behaviour of the dolphin when the trainer entered the pool in cases when the session included the trainer working in the water ('fast', 'delayed', 'no appearance') and the occurrence and categorized frequency of eye contact between dolphin and trainer during the session ('rare', 'frequent', 'none'). We also collected information on the level of excitement of the dolphins, the number of positive reactions from the dolphin to the requested behaviours, the possible refusal to perform them and the possible leaving of the dolphin during the session.

However, we had to re-group some categories to only two, due to the rare occurrences of one of the original categories (i.e., fast/slow to qualify dolphin approach; high/low and intermediate to qualify dolphin excitement and eye contact/no eye contact). Finally, the dolphin's Willingness to Participate (WtP), which is on a focal animal-based five-point Likert scale, with the ordinal categories representing incremental grades of the dolphin's motivation and enthusiasm during training sessions [53], was also assessed. WtP ranges from zero (no contact) with a dolphin not present during the entire session to four (excellent) when a dolphin was correctly performing all asked behaviours and was highly motivated. Moreover, we wanted to see which elements of trainer participation (i.e., based on our detailed scores of different contexts) finally contributed to the variation in WtP score (which is based on an overall impression of trainers).

Additionally, for each assessed session, basic data were collected, such as trainer identity and her/his years of experience, dolphin individual characteristics and identity (name, sex, age), the nature of the session and the time of day as described above.

2.3. Statistical Analysis and Sample Sizes

Statistical analyses were done with the programme R, version 3.6.2 (R Development Core Team, Vienna, Austria [62]). Samples used for analyses were repeated measurements (observation sessions) of different behavioural parameters (see details in Table 1). The maximum number of observation sessions was 323 although the available number of sessions for analysis was sometimes lower (min. 99 sessions), as it was not always possible to quantify all behavioural parameters during each session (see details in Table 2).

Table 2. Effects of different predictor variables on dolphins' behavioural parameters quantified during training and show sessions. Repeated measurements from 43 dolphins from five different facilities. The number of observation sessions (*N*) is given for each analysis. Significant effects are highlighted in bold.

Behavioural Parameters	N	Predictors	χ^2	df	P
(a) Speed of approach	322	Sex *S*	1.552	1	0.213
		Age class *A*	0.385	1	0.535
		Trainers' experience *TE*	0.219	1	0.640
		Ind./group session *IG*	2.148	1	0.143
		Nature of session *NS*	2.284	4	0.684
		Time of day *TD*	3.418	2	0.181
		A × IG	4.504	1	**0.034**
(b) Excitement level	320	Sex *S*	1.585	1	0.208
		Age class *A*	9.615	1	0.002
		Trainers' experience *TE*	1.486	1	0.223
		Ind./group session *IG*	0.135	1	0.713
		Nature of session *NS*	5.754	4	0.218
		Time of day *TD*	4.576	2	0.101
		TE × IG	6.532	1	**0.010**
(c) Positive responses to commands	203	Sex *S*	0.428	1	0.513
		Age class *A*	3.387	1	0.066
		Trainers' experience *TE*	0.190	1	0.663
		Ind./group session *IG*	1.182	1	0.277
		Nature of session *NS*	82.615	4	**<0.001**
		Time of day *TD*	7.530	2	**0.023**
(d) Refusal to perform a behaviour	321	Sex *S*	0.293	1	0.588
		Age class *A*	2.311	1	0.128
		Trainers' experience *TE*	2.858	1	0.091
		Ind./group session *IG*	1.493	1	0.221
		Nature of session *NS*	10.382	4	**0.034**
		Time of day *TD*	0.179	2	0.915
(e) Leaving during session	322	Sex *S*	0.795	1	0.372
		Age class *A*	14.231	1	**<0.001**
		Trainers' experience *TE*	0.162	1	0.687
		Ind./group session *IG*	2.218	1	0.136
		Nature of session *NS*	0.898	4	0.925
		Time of day *TD*	1.523	2	0.467
(f) Eye contact with trainer	323	Sex *S*	0.436	1	0.509
		Age class *A*	0.310	1	0.578
		Trainers' experience *TE*	0.205	1	0.651
		Ind./group session *IG*	0.493	1	0.483
		Nature of session *NS*	3.528	4	0.474
		Time of day *TD*	3.348	2	0.188
(g) Approach speed when trainer enters pool	99	Sex *S*	0.093	1	0.761
		Age class *A*	0.446	1	0.504
		Trainers' experience *TE*	1.913	1	0.167
		Ind./group session *IG*	0.001	1	0.998
		Nature of session *NS*	0.001	4	0.999
		Time of day *TD*	1.063	2	0.587
(h) Willingness to participate	323	Sex *S*	0.001	1	0.999
		Age class *A*	0.001	1	0.999
		Trainers' experience *TE*	0.053	1	0.818
		Ind./group session *IG*	3.706	1	0.054
		Nature of session *NS*	4.151	4	0.386
		Time of day *TD*	8.647	2	**0.013**
		S × A	4.058	1	**0.044**

Multifactorial analyses by GLMM for binomial data (a, b, d, e, f), GLMM for Poisson distributed data (c), and by a cumulative-link mixed-effects model (g), all of these including the identities of the facilities, dolphins and trainers as random intercept factors. All two-way interactions of dolphins' sex, age class, the years of experience of the trainer with all other predictor variables were calculated, although only significant interactions are given in the table. Non-significant interaction terms (*p* > 0.05) were removed from the models before these were re-calculated. For (f, g), no interaction terms were tested due to the low number of cases (f) during which no eye contact with the trainer occurred (binomial response variable: 14 out of 323 observations), and (g) during which fast approaches (compared to slow ones) of the dolphins towards the trainer occurred once the trainer entered the pool (nine out of 99 observations).

We analysed the effects of a set of six predictor variables (see Table 2): dolphins' sex (factor with two levels) and age class (factor with two levels), the number of years of trainers' experience (covariate), whether the training was done individually or in a group (factor with two levels), the nature of the session (factor with five levels) and the categorised time of day (factor with three levels) on a set of dependent variables (see below). The trainers' experience ranged from less than 1 to 41 years. The distribution of this covariate was strongly right-skewed, as only two of the trainers had work experience of more than 20 years (i.e., 33 and 41 years). Thus, for analysis, these cases of more than 20 years of work experience were categorized as '> 20'. We considered all two-way interactions of sex, age class and trainer experience with all other predictor variables. Non-significant interactions were stepwise removed from the models before these were recalculated.

Analyses were done by multifactorial modelling, always running a separate model per dependent variable (see Table 2). The following dependent variables, the 'speed of approach' (fast versus delayed approach), 'excitement level' (high versus low excitement), 'refusal to perform a behaviour' (yes/no), 'leaving during session' (yes/no), 'eye contact with trainer' (yes/no) and 'approach speed when trainer enters pool' (fast/slow-or- absent) showed a binomial structure. For their analysis, we applied generalised linear mixed-effects models (GLMM) for binomial data (with a logit link) using the R package lme4 [63]. The dependent variable "positive responses to commands" was a count of the occurrence of behaviours and thus was analysed by a GLMM for Poisson distributed data with a square-root link (R package lme4). The dependent variable "willingness to participate WtP", an ordinal index on a Likert scale (zero to four; see details above), was analysed by a cumulative-link mixed-effects model (with a logit link) using the R package ordinal [64]. All of these models included the identity of the dolphin as a random factor to account for individual-based repeated measurements. All models also included the identity of the facility and the identity of the trainer as further random factors to account for effects of same origin [65]. Furthermore, as the GLMM for Poisson distribution showed indications of overdispersion, we included a further case-level random factor [66]. For GLMM for Poisson distribution, we also verified homogeneity of variance by plotting the fitted values versus the residuals [65].

3. Results

During this study, a total of 323 sessions were collected from the five participating facilities. These sessions included the assessment of behaviours from 43 individual dolphins interacting with 32 trainers (see Table 1 for number of sessions per facility and per dolphin).

3.1. Effects of Different Training Conditions and Individual Features on Behavioural Parameters

3.1.1. Dolphins' Speed of Approach in Presence of the Trainer

The probability that a dolphin approached with fast speed (compared to the merged categories "delayed approach and "no appearance") in response to the approach of the trainer at the beginning of any session was significantly affected by dolphins' age in interaction with the type of training session, i.e., whether the training was carried out individually or in a group of animals (Table 2 (a)). Post-hoc analyses revealed that the probability of a fast-speed approach by the dolphin towards the trainer was significantly higher for adult animals in individual training versus group training sessions (Figure 1b). In contrast, there were no significant differences for juveniles between individual and group sessions (Figure 1a).
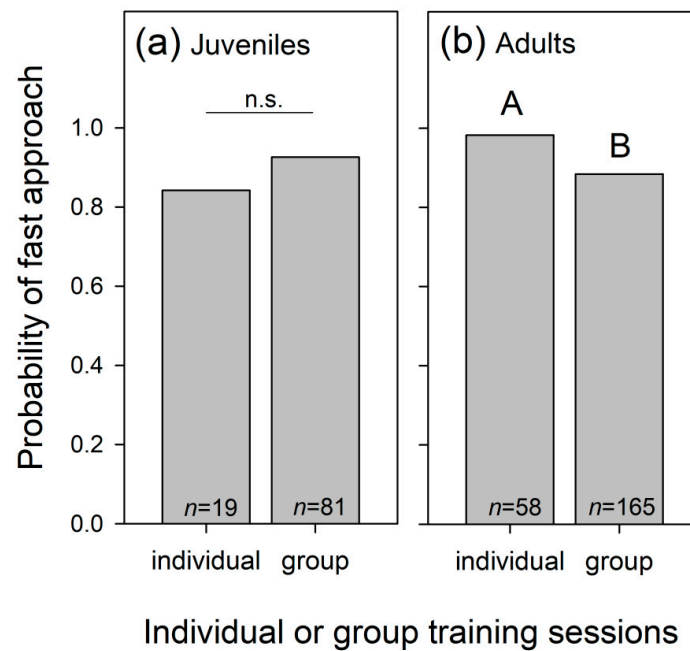
Figure 1. Comparison of probabilities that dolphins showed fast approach towards the trainer(s) during individual and group training sessions in (**a**) juvenile and (**b**) adult individuals. Sample sizes in the bars indicate the number of observation units. Significant post-hoc comparisons within the two age classes ($p < 0.05$; pairwise GLMM for binomial data) are indicated by different capital letters over the bars; see Table 2 (a) for details on statistics. n.s. means not significant.

All other predictor variables considered (sex of the dolphins, years of experience of the trainers, time of day and nature of the session, including any interaction between these factors) did not significantly affect the speed of dolphins' approach (Table 2 (a)).

3.1.2. Dolphins' Level of Excitement

The probability that dolphins showed a high level of excitement (in contrast to a lower level) during a training session was significantly higher in juveniles than in adults (Table 2 (b) and Figure 2a). Furthermore, the probability of showing a high excitement level significantly depended on the trainers' experience in interaction with the type of training session, i.e., whether the training was carried out individually or in a group (Table 2 (b)). Post-hoc analyses revealed that during individual sessions, dolphins showed a significantly higher probability of high excitement level when interacting with trainers who had fewer years of experience than with more experienced trainers (GLMM for binomial data: $\chi^2_1 = 9.385$, $p = 0.002$) (Figure 2b). However, there was no significant association between dolphin excitement levels and trainer experience during group sessions ($\chi^2_1 = 0.634$, $p = 0.426$) (Figure 2c). All other predictor variables or interactions between them did not show significant effects (Table 2 (b)).
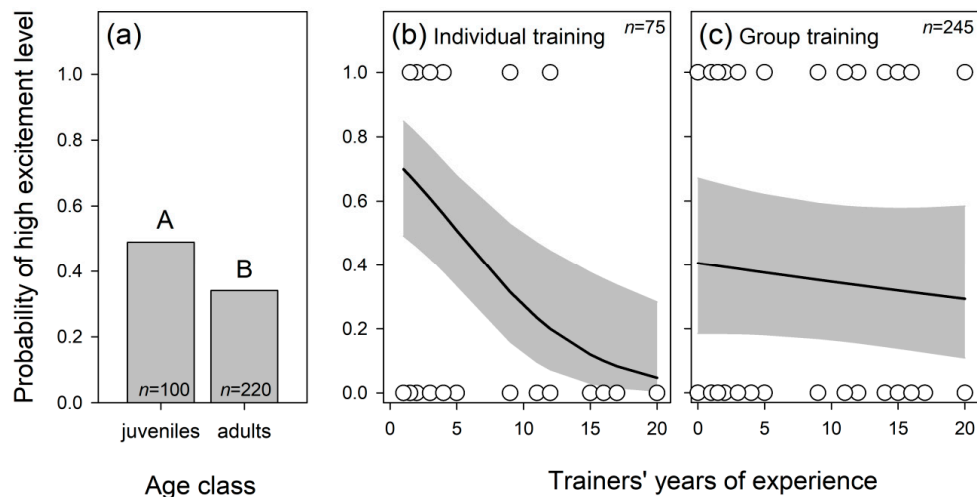
Figure 2. Probability that dolphins showed high excitement levels during the training (**a**) in relation to age class and depending on the trainer experience level (quantified in years of practice) during (**b**) individual and (**c**) group training sessions. The different capital letters over the bars in (**a**) indicate significant differences ($p < 0.05$) between age classes (statistics in Table 2 (b)). Logistic regression lines (with 95% confidence intervals indicated as grey areas) are based on parameter estimates (GLMM for binomial data) resulting from the interaction between trainers' experience and group or individual training (statistics in Table 2 (b)). Sample sizes shown (**a**) in the bars and (**b,c**) in the different figures indicated the number of observation units.

3.1.3. Dolphins' Positive Responses to Trainers' Signals

The dolphins' positive responses to trainers' signals showed a significant increase during the day, with significantly lower positive responses during morning sessions compared to afternoon sessions (Table 2 (c); post-hoc statistics in Figure 3a). Furthermore, the nature of the session was decisive, as there were significantly higher numbers of positive responses during public presentations compared to medical training, training of new behaviours and routine sessions (Table 2 (c); post-hoc statistics in Figure 3b). All other predictor variables including their interactions did not show any significant effect on this behavioural parameter (Table 2 (c)).
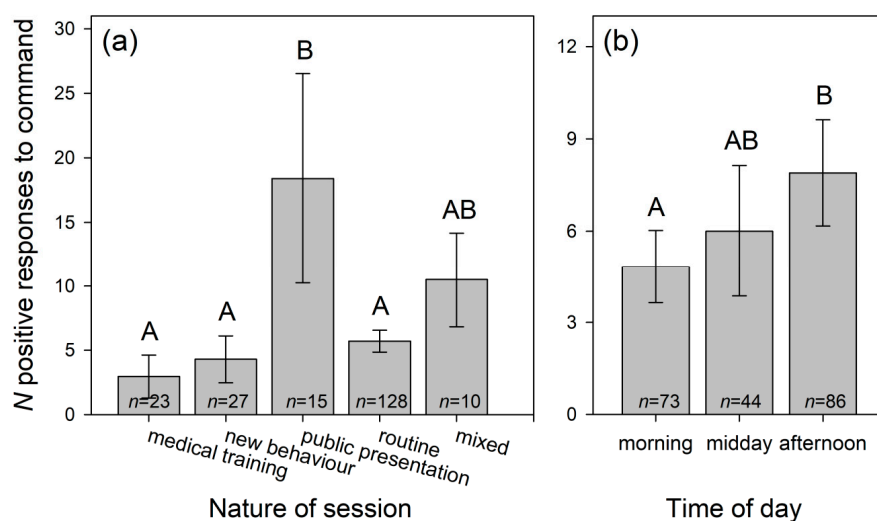
Figure 3. Number of dolphins' positive responses to trainers' commands (**a**) during different natures of sessions and (**b**) during different times of the day. Significant post-hoc comparisons between the different categories ($p < 0.05$; pairwise GLMM for Poisson distributed data) are indicated by different capital letters over the bars; see Table 2 (c) for details on statistics.

### 3.1.4. Dolphins' Refusal to Perform Particular Behaviours

Dolphins' refusal to perform a requested behaviour was significantly associated with the nature of the session (Table 2 (d)). Such refusal occurred with a significantly higher probability during mixed sessions compared to medical training, public presentations and routine training (post-hoc statistics in Figure 4). There was no significant effect on this behavioural parameter by other predictors including their interactions (Table 2 (d)).
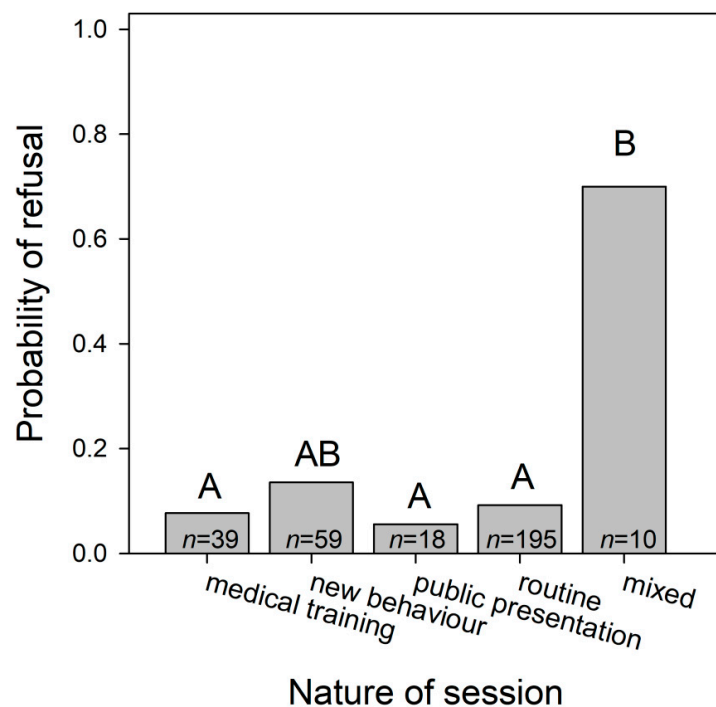
Figure 4. Comparison of probabilities that dolphins refused to perform a requested behaviour during different nature of training sessions. Significant post-hoc comparisons between session types ($p < 0.05$; pairwise GLMM for Poisson distributed data) are indicated by different capital letters over the bars; see Table 2 (d) for details on statistics.

3.1.5. Dolphins' Leave Trainer during Trainer-Dolphin Sessions

The probability that dolphins left during a training session was significantly affected by the age of the animals (Table 2 (e)). Juvenile dolphins left their trainers two times as often compared to adult dolphins (29% versus 13.5% of sessions) (Figure 5). All other predictor variables including their interactions were not significant (Table 2 (e)).
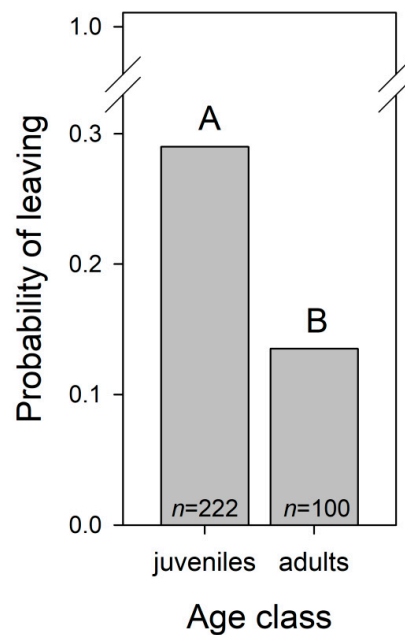
Figure 5. Comparison of probabilities that juvenile and adult dolphins left during the training session. Significant differences, as given in Table 2 (e) (*p* < 0.05), are indicated by different capital letters over the bars.

3.1.6. Dolphins' Eye Contact with Trainers

The probability that dolphins displayed eye contact with the trainers during the sessions did not show significant associations with any of the predictor variables tested in this study (Table 2 (f)).

3.1.7. Dolphins' Approach Speed Once the Trainer Entered the Pool

The probability that dolphins approached the trainer with fast speed (opposed to slow speed) did not show significant associations with any of the predictors tested in this study (Table 2 (g)).

3.1.8. Dolphins' Willingness to Participate (WtP) during Training

Dolphins' Willingness to Participate (WtP) in sessions with their trainers showed significant changes related to the time of day (Table 2 (h)), as dolphins were willing to participate more actively during midday sessions compared to morning sessions (post-hoc statistics in Figure 6a). WtP scores during the afternoon sessions were intermediate and did not differ significantly from the scores recorded in the morning or during midday (Figure 6a).

Furthermore, WtP scores were significantly affected by the interactive effects of dolphins' age class and sex (Table 2 (g)). There was no significant difference between sexes for juvenile dolphins (post-hoc statistics in Figure 6b), whereas adult females showed a significantly higher willingness to participate than adult males (Figure 6c).
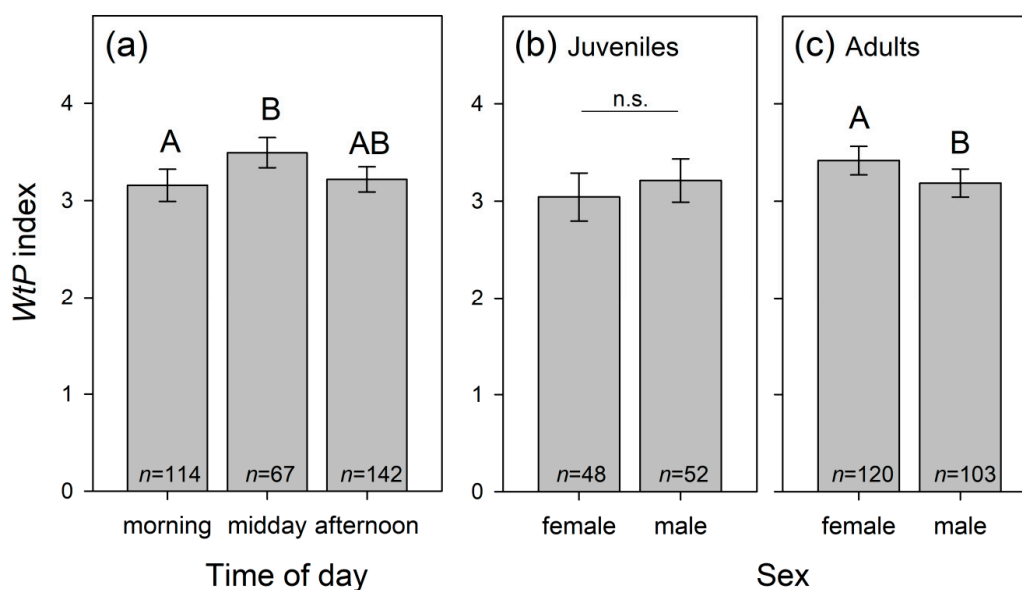
Figure 6. Comparison of WtP scores, expressing the dolphins' willingness to participate in the training, between (**a**) different times of the day, and between males and females in (**b**) juveniles and (**c**) adults. Significant post-hoc comparisons between different times of day and between sexes ($p < 0.05$; pairwise cumulative-link mixed-effects models) are indicated by different capital letters over the bars; see Table 2 (h) for details on statistics. n.s. means not significant.

3.2. Associations between WtP and Other Behavioural Parameters

Dolphins' Willingness to Participate (WtP) was significantly associated with most of the other behavioural parameters considered. Individuals with a higher WtP index approached the trainer with a significantly higher speed (cumulative-link mixed-effects model: $\beta = 2.301 \pm 0.691$ SE, $p < 0.001$), showed a significantly higher level of excitement during training ($\beta = 1.204 \pm 0.440$ SE, $p = 0.006$), showed a significantly higher number of positive responses to commands ($\beta = 0.674 \pm 0.244$ SE, $p = 0.006$), significantly refused less often to perform requested behaviours ($\beta = -2.127 \pm 0.611$ SE, $p < 0.001$), significantly left more rarely during the training sessions ($\beta = -2.865 \pm 0.534$ SE, $p < 0.001$) and significantly approached faster once the trainer entered into the pool ($\beta = 2.378 \pm 0.961$ SE, $p = 0.013$). However, there was no significant association between the WtP and the occurrence of dolphin's eye contact with the trainer ($\beta = 0.459 \pm 0.868$ SE, $p = 0.597$).

4. Discussion

The application of QBAs is relatively simple; they allow addressing questions that cannot be directly answered by other parameters related to the animals and are complementary to qualitative and quantitative measurements [67], and they can be routinely used by professionals. QBAs are traditionally used in studies on human–animal interactions (HAIs) and relations (HARs) [68]. We analysed the variation of several parameters already validated by other HARs studies (i.e., speed of approach, responses to trainers' signals) but also new ones (e.g., willingness to participate), according to environmental factors (time of day, nature of the training session, individual versus group training) and individual features of the trainers (years of training experience) and the dolphins (age and sex).

An animal's approach, or not, towards human(s) and the way the animal approaches have been already studied as possible welfare indicators [2,5,6,22–24]. Our protocol was not set up to study if dolphins distinguished the food provisioner (i.e., trainer) from a generally positive interaction (i.e., training session). In other words, trainers might have been associated with food even outside feeding sessions. Dolphin–trainer interactions are complex and how dolphins and humans experience these interactions, what they expect and what they feel remain unknown. Servais and Delfour [69]

discussed the benefits of considering this HAI under a social comprehension and inter-comprehension model as a whole and not to break it into different components. The main interest was to observe dolphins during specific human–animal interactions considered under this framework. One can also argue that dolphins' reactions might also depend on their hunger. During the study, observations were conducted after all animals had received their first early morning food ration. Food was provided in a similar manner in each facility and according to the EAAM's standard Guidelines their diets were calculated based on each individual animal needs. The hunger sensation of the animals remained unknown because of the impossibility of assessing it. However, information exists on dolphins' food intakes and their willingness to participate in training sessions [56]. Dolphins with a higher WtP score ate a higher percentage of their daily food and the WtP score was significantly lower up to three days before the weekly veterinary diagnosis of a decrease in health state but the percentage of daily food eaten did not show any significant change (Clegg et al. [56], p. 1).

Our analyses showed that level of dolphins' excitement during trainer–dolphin sessions was higher in juveniles compared to adults. This difference could be explained by young dolphins being more energetic, excitable and playful than adults [70].

The probability of a fast-speed approach by adult dolphins towards the trainer was significantly higher in individual training versus group training sessions, while there were no such significant differences for juveniles. Routine daily training sessions of dolphins under professional care happen in individual animal setups or in group sessions during which several animals are managed by one trainer. Our findings suggest that adult dolphins prefer being in individual interactions with one trainer. Since food was always provided, this could be due to their personality, the identity of the trainer they interact with, their mutual attention and/or the content of the training session. Trainer–animal interactions during daily management and routine care are recurrent and regular and therefore animals get to be well acquainted with their trainers, which typically lead to the development of specific HARs [29,71]. These relationships are reciprocal between specific individual animals and humans [5]. Further studies would be warranted to help identify contributing factors to positive and/or negative HARs (e.g., human and animal personality and age, nature of the interaction) in order to better understand the underlying processes of HARs.

4.1. Factors Explaining the Variation of the Different Behavioural Parameters Reflecting Dolphin Welfare

During individual sessions, dolphins were more excited when interacting with a junior trainer (less than five years of experience in dolphins training) than an experienced trainer. This could be because it is largely experienced trainers who conduct training sessions for complex behaviours and difficult medical examinations. Moreover, junior trainers usually ask dolphins for well-known and fully trained behaviours while experienced trainers develop new behaviours and/or improve existing behaviours. The nature of particular trained behaviours, the requested exercises and their level of difficulty vary greatly. The way to reward a dolphin (e.g., slow or fast, with or without enthusiastic sounds and gestures), or the perception and comprehension of the dolphin's behaviours, might vary according to the trainers' experience. Furthermore, adult dolphins might express their excitement in a different way (or level) compared to young individuals [70]. However, here, all age classes of dolphins appeared to be more excited in individual sessions when interacting with a junior trainer. During group sessions, no notable difference in dolphins' excitement according to the trainers' years of experience was detected.

During a group training session, a dolphin's interaction partners and/or sources of excitement do not depend only on its trainer and what he/she does or asks, but also on the presence of conspecifics. Moreover, in group sessions with one trainer, dolphins must compete for attention. In fission-fusion social structure such as that seen in bottlenose dolphins, relationships within group members may vary due to internal (physiological) and external conditions of the animals [72]. Under human care, social groups (spontaneous or imposed) are associations of particular individuals. However, individuals have different personalities, experiences and life histories, all of which can modulate their daily social

life [73–77]. While working in group sessions with dolphins, trainers may very well respect and build upon existing bonds between specific individual animals, but they also can temporary disrupt bonds between individuals when starting a session. Dolphins might have also some preferences for particular trainers and vice versa, and these zoo-anthropological preferences might modulate the HARs.

During training sessions, usually dolphins responded positively to their trainers' signals but also refused to perform the commands and/or left during certain sessions. The number of breaks (i.e., spontaneous, voluntary departures by the animal) during a session is an important parameter to collect when assessing dolphin welfare since this could be a sign of negative wellbeing and/or affective state (e.g., disinterest, annoyance, boredom, lethargy, pain). Accordingly, the behaviour of animals towards humans has been frequently linked to their emotional, welfare and health states [2,6,23,24,54]. Our results showed that dolphins responded more positively during afternoon than morning training sessions and during public presentations when compared to medical and routine training, mixed training sessions, and training of new behaviours. A previous study showed that dolphins' social play behaviour was significantly more frequent and lasted longer in the morning than in the afternoon and was present before and after interactions with their trainers [78]. This could explain why in the present study dolphins responded less positively during morning versus midday and afternoon sessions. Moreover, dolphins responded more positively during public presentations than during other types of training, but only two dolphinaria out of five were open to the public during this study. During mixed sessions, dolphins answered more positively than during medical, routine training and sessions including the training of new behaviours. Furthermore, during mixed sessions, they also refused more often to perform a behaviour following their trainer's signal. Mixed trainings are sessions combining routine, new behaviours and/or medical training with several exercises of various levels of difficulty. Dolphins might therefore express different levels of interest and motivation to perform them, thus leading to an increase in the number of positive and negative (refusal) responses. Dolphins did not leave with a higher probability during mixed training sessions; they typically showed a lack of motivation by not performing the behaviour asked by the trainer. Juvenile dolphins left more often than adults during sessions. This could be explained by the fact that young dolphins are usually more energetic, curious, excitable, playful and distractible than adults [70].

Finally, we cross-validated the proposed behavioural welfare parameters with the validated welfare parameter Willingness to Participate (WtP: [53]). WtP is a focal animal 5-point Likert scale with the ordinal categories representing incremental grades of the dolphin's motivation and enthusiasm during training sessions (i.e., 0 (no contact) with a dolphin not present during the entire session to 4 (excellent) when a dolphin was correctly performing all asked behaviours and was highly motivated). WtP was recently successfully validated by showing that it could be used as an early indicator of health issues in dolphins, which is a key parameter of welfare [53]. The significant association of WtP with the other behavioural parameters considered in our study could be in part because the WtP index is a priori not completely independent from them. The fact that WtP is significantly associated with our (finer scaled) scores is not surprising, but it is interesting that WtP is significantly associated with *all of them* except eye contact (see our discussion on methodological concerns below). Moreover, in terms of welfare, this revealed that these behavioural parameters could be potentially used in a dolphin welfare assessment.

The WtP score varied along the day, as dolphins were willing to participate more actively during midday sessions compared to morning sessions. As discussed above, dolphins play more and longer in the morning than in the afternoon [78]. This could explain why during morning sessions dolphins had the lowest WtP score, as the animals were in a playful mood and were intensively engaged in intraspecific socializations. Other potential factors affecting these findings could be multiple, e.g., driven by events happening during the previous night or before the training started.

No significant difference between juvenile and adult individuals was found; only adult females had a higher WtP score than adult males. This could be due to the animals' personality and/or their reproductive status (e.g., cycling phase) that could have made the breeding males more easily distracted

than the females. Our results strengthen a previous finding about WtP as a potential behavioural measure of dolphin welfare and an indicator of early changes in the dolphins' health state [53].

In our analysis, we did not find any significant effects of the considered factors on the behavioural parameter "eye contact". Furthermore, this behavioural parameter was not significantly associated with the WtP score. One reason for this might be that, out of 316 observations available for analysis, only 14 showed no eye contact with the trainer. Thus, we cannot exclude that the non-significant results obtained were false negatives due to the strongly unbalanced data structure. However, we should consider that during the time frame of our study no dolphin suffered from severe injury, illness, lethargy, inappetence and/or experienced sharp pain. The very high occurrence of eye contact of the dolphin with the trainer concomitant with the absence of obvious and severe low welfare status could then be taken as an indication that the parameter "eye contact" is an important indicator of dolphin welfare. Alternatively, other environmental conditions (e.g., distractions by conspecifics or public) not considered here might lead to the rare cases where eye contact was not observed. Thus, since other works have shown the importance of eye contacts for horses' when humans approach [79] and in shelter dogs [80], we suggest conducting further studies to investigate with which other parameters the absence of eye contact could be associated. For shelter dogs, making eye contact with humans is enriching and increases their welfare. Horses seek human help through eye contact-seeking behaviour when involved in an unsolvable task [81]. Dolphins under human care [60,82,83] and in the wild [84] are attentive to and comprehend humans' gazes. Even if our results are not informative, we think that according to the existing literature, we should be cautious before evacuating the factor eye contact.

4.2. "WtP" and Potential Alerting Factors to assess Dolphin Welfare

In conclusion, our findings (i.e., QBA and the cross-validation of our proposed behavioural parameters) support the use of the validated indicator "Willingness to Participate" (WtP) [53] when assessing dolphin welfare along with the "alerting factors" [85]: "speed of approach" (trainer out of water and in water), "level of excitement" and "dolphins' answers during training" (positive answers and refusals/trainer's signals, spontaneous departure). "Welfare alerting indices do not directly reflect the animal's current welfare state, but they can direct attention in future assessment towards specific animal-based indices" (Harvey et al. [85], p. 6). The factor "eye contact" might be a parameter to consider, however with our experimental setting we could not validate it. When assessing the welfare of an animal during a dolphin–trainer interaction, if the individual presents a low WtP (i.e., an early indicator of health issues) and it is reluctant to approach the trainer, has a low level of excitement, shows a low number of positive responses to commands, refuses to perform certain behaviours, exhibits some leaving behaviours and potentially displays rare eye contacts with its trainer, those signals should alert the observer that this animal might have a diminished welfare state. Thus, we remind that it is time to look for other indicators to validate or invalidate these alerting factors.

Moreover, in our study, we showed that the dolphins' age class (juvenile versus adults), the time of day, the nature of the trainings (i.e., individual versus group trainings, mixed, new behaviour, medical training, public presentations), trainer experience level and to a lesser extent the sex of the dolphins, are all contributing and modulating the variance of the behavioural parameters studied, and are therefore worth being considered when setting up a dolphin welfare assessment. Once again, our results show the importance to combine animal-based and resource-based measures to objectively assess animal welfare at an individual level.

Our questionnaire is part of a larger dolphin welfare evaluation (Dolphin-WET, Welfare Evaluation Tool), which is planned to be conducted at least three times a year in each participating dolphinarium, as seasonality might be a further contributing factor. For instance, a previous study showed that social play in dolphins is lower in winter compared to spring and slow-intensity swimming is higher in winter than in summer [86]. Our QBA was implemented during the winter period, and by replicating it seasonally we will be able to validate its repeatability and consistency.

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