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Evaluation of live fish as an echolocation enrichment for the bottlenose dolphin (*Tursiops truncatus*)

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Nyckelord
Keyword
Echolocation, Bottlenose dolphins, Sonar, Enrichment, Live fish, Goldfish, Welfare, Zoo animals

Content

1 Abstract ... 3

2 Introduction ... 3

3 Material & methods ... 6

3.1 Overview of methodology ... 6

3.2 Experimental procedure ... 7

3.3 Location and animals ... 8

3.3.1 Fish and fish handling ... 9

3.4 Equipment ... 9

3.5 Data analysis ... 10

3.5.1 PCL analysis ... 10

3.5.2 Behavioural analysis ... 11

3.5.3 Statistical analysis ... 12

4 Results ... 13

4.1 PCL data ... 13

4.1.1 Clicks and click trains ... 13

4.1.2 Beam ... 16

4.1.3 Buzz and beam core ... 17

4.2 Behaviour data ... 18

4.2.1 Events ... 18

4.2.2 Habituation ... 19

4.2.3 Duration ... 21

5 Discussion ... 23

5.1 PCL Data ... 23

5.1.1 Clicks and click trains ... 23

5.1.2 Beam 25
5.2 Behavioural observation data 27
5.2.1 Number of behaviours 27
5.2.2 Habituation 28
5.2.3 Duration of the apparent sonar behaviours 29
5.3 PCL data versus behavioural observations 30
5.4 Ethical aspects 30
5.5 Conclusions 32
6 Acknowledgement 32
7 References 33

1 Abstract

Bottlenose dolphins (*Tursiops truncatus*) kept in zoos and dolphinarias rarely get an outlet for their echolocation abilities as their pool environment is often quite barren. Not much research has been carried out on enrichments promoting echolocation for dolphins in human care. In the present study a setup with live fish was compared to a setup with air-filled floats (providing strong sonar targets, similar to the swim bladders of large fish) and a control setup. A PCL (porpoise click logger) was used to record the echolocation click trains produced by the dolphins and aimed at the three setups. Behavioural data was also collected from video footage. Both the PCL data and all the behavioural observations indicated that the fish setup was more interesting than the float and the control setup, for the dolphins to echolocate towards. However, there were some contradictions with some parameters, where the floats and control seemed to be more interesting. This was probably due to the location of the PCL hydrophone in relation to the floats and fish, and not because the dolphins had a real bigger interest in these setups. To increase the possibility for dolphins to perform more echolocation in human care and increase their welfare, live fish can be recommended as echolocation enrichment.

2 Introduction

A challenge every zoo faces is to always improve the environment and welfare for their animals and to allow them the opportunity to perform their natural behaviours. To achieve this different environmental enrichments may be used. However several conditions and objects in the wild might be hard to replicate and that is why the staff in the zoos constantly have to be creative in order to find artificial substitutes that can stimulate important, species-specific behaviours in their animals (Carlstead and Shepherdson, 2000).

There has been a lot of research in this area especially in primates. As primates are very intelligent animals (Matsuzawa, 2009; Tomasello and Call, 1997) they need high-quality cognitive stimulation and thus as diversified environments as possible. However many of the enrichments used to stimulate cognition in zoo-housed primates are not found in the wild, such as mirrors (for self-recognition) (Povinelli *et al*., 1997), or differ in both appearance and content, such as artificial “termite mounds”, made of concrete or wood logs, which allows the primate to perform similar behaviours as in the wild, i.e. preparing browse sticks to probe the holes, but instead of termites offering e.g. honey (Celli *et al*., 2003; Hopper *et al*., 2015) or yogurt.

Not only primates have high cognitive abilities. The bottlenose dolphin (*Tursiops truncatus*) is considered very intelligent, highly social, and proven highly trainable for public presentations and research tasks (Clark, 2013). There is not as much research in providing enrichments for dolphins as in primates, partly because it is more difficult to provide enrichments in the pools since water quality is an important issue and enrichments may interfere with water circulation, or not being able to withstand chlorine. This results in dolphin pools often being quite barren. There has been some research in the use of training as one type of enrichment as it stimulates the cognitive abilities in the dolphins (Clark, 2013; Delfour and Beyer, 2012). However there have only been two studies previously on providing and evaluating enrichments for echolocation purposes for the dolphins (Berglind, 2005, Van Zonneveld, 2015).

Echolocation is a big part of the dolphin's life in the wild. As light waves rapidly are absorbed even in clear water the dolphins can only use their vision at very short distances under the water surface, and have to rely more on their hearing and echolocation skills at depth, in murky waters and at night (Dubrovski, 2004). Dolphins use echolocation for various purposes such as orientation, and detecting and catching prey (DeLong *et al*., 2014). Using echolocation enables dolphins to discriminate between different preys and other objects (Harley *et al*., 2003; Helweg *et al*., 2003; Kloepper *et al*, 2014). Dolphins often predate on fish which hide in seagrass beds, such as pinfish, pigfish, mojarra and mullet (Rossman *et al*., 2015). In such an environment the ability to discriminate between the fish and the seagrass through echolocation is advantageous. When echolocating towards the fish it is the swim bladder (which is air filled) of the fish which provides the strongest echo (Rossman *et al*., 2015). From the returning echoes the dolphin can determine the location of the fish, the distance to it, by measuring the time it takes for the echo to return (Harley *et al*., 2003; Helweg *et al*., 2003; Kloepper *et al*., 2014) and identify the fish species and size by analysing amplitude "highlights" and the frequency composition of the echo (Au, 1993).

When a dolphin echolocates it produces series of ultrasonic clicks, 50–150 µs in duration (Au, 1993; Helweg *et al*., 2003), with a power spectrum ranging from a few kHz up to 150 kHz. If these clicks hit an object echoes bounce back to the dolphin

(DeLong *et al*., 2014). The dolphin does not transmit the next click until the echo from the previous one has returned. The time between clicks in a train is called the inter-click-interval (ICI). This usually includes a lag time between a received echo and the generation of the next click. The lag time is generally between 20 and 40ms (Au, 1993). When dolphins are searching for prey or travelling the ICI is usually 40-60ms (Nuuttila *et al*., 2013), but in the final stage of fish catch, the ICI decreases to below 10ms, sometimes down to 2-3ms; this is called a "buzz"; now there is no lag time. Buzzes have been observed in most Odontocetes, including the bottlenose dolphin and the harbour porpoise (*Phocoena phocoena*) (Nuuttila *et al*., 2013; Verfuss *et al*., 1999; Verfuß *et al*., 2005).

Odontocetes (including dolphins) generate sounds, whistles and clicks, in their nasal passage by pushing pressurized air through the two sets of phonic lips (Ridgway *et al*., 1980; Amundin & Andersen, 1983), which are located just below the blowhole (Cranford *et al*., 1996; Cranford *et al*., 2011). Cranford *et al*. (2011) found that the production of whistles requires twice the amount of nasal air volume that it takes to produce click sounds. The main source of echolocation clicks is thought to be the phonic lips on the right side of the nasal passage, which is also the bigger one of the two. However recent studies have found that sonar clicks in the bottlenose dolphin may also be generated by the left set of phonic lips and sometimes by both at the same time (Cranford *et al*., 2011). The echolocation clicks from the phonic lips are transmitted through the fatty melon which is located on the forehead of the dolphin. The melon functions as an acoustic lens and shapes the sounds into a narrow beam (Au, 1993; Au *et al*., 2012; Cranford *et al*., 2011; Cranford *et al.*, 2014; Lemerande, 2002; Starkhammar *et al.*, 2010) which is directed towards the object of investigation. The central part of the beam which is directed directly towards the object is called the beam core, while the part of the beam which is on both sides of the core beam is called the beam periphery. The clicks in the beam core are dominated by high frequencies, often >100kHz, whereas those in the periphery contain lower frequencies (Au, 1993).

When the echoes return they are picked up through a thin walled area in the caudal part of the lower jaw (called the acoustic window or pan bone) (Cranford *et al*., 2008; Mooney *et al*., 2015), and guided to the tympanoperiotic complex (TPC) (Cranford *et al*., 2010) through the mandibular fat body (Cranford, *et al*. 2011).

Dolphins have excellent hearing and can hear frequencies from 100 Hz to 150 kHz. The range at which dolphins can hear is 12 octaves, which is the widest frequency range among all the animal species (Au, 2004). As bottlenose dolphins possess a longer cochlear channel and have three times more ganglion cells than our human ear, they have the ability to discriminate and hear higher frequency sounds and also to detect weak signals in a noisy environment (Au, 1993).

Although echolocation is such an important natural behaviour and used for many different vital processes in the lives of dolphins, they cannot get much outlet for this behaviour in captivity as their pool environment usually is quite barren and static. Except for pool walls and floor, and the trainers interacting with them in the water, there is not much in the pools which would return echoes to the dolphins and hence stimulate to acoustic investigation.

The aim of my master project is to find out if enrichment designed to stimulate echolocation would be used by the dolphins at Kolmården Wildlife Park and to assess if live fish would be preferred as a sonar target over air-filled floats (currently used as echolocation enrichments at Kolmården Wildlife Park), which mimics the sonar target of the swim bladder in a fish, and control (empty water containers). If these enrichments are used by the dolphins, the prediction is that the dolphins will echolocate more and aim more echolocation click trains towards the fish setup than towards the other two setups.

3 Material & methods

3.1 Overview of methodology

There were two enrichment types to be tested: live gold fish and a string of air-filled floats. They were contained in three soft-plastic water-filled bags, arranged around a click detector (an Aquaclick 100 Porpoise Click Logger, PCL; Aquatec Group Ltd, UK) that logged the sonar click trains the dolphins generated to investigate the content of the bags. These enrichments were tested against empty bags which was the control.

Each setup was tested for 4 hours during a day. It was fixed under a floating platform in front of underwater panels, making it possible to view and film the behaviour of the dolphins when they interacted with the setup.

3.2 Experimental procedure

Before each test day the PCL (figure 3c) was activated. This was done manually by opening the unit and turning on a power switch on the circuitry board. The PCL was then connected to a computer to sync its internal clock to internet time. After activation the unit was re-assembled again. The PCL was inserted into a plastic tube situated in the centre of the setup (figure 3b) and fixed in place by a metal rod. Three transparent soft plastic water bags were permanently tied to the plastic tube (figure 3b). The whole setup was then put into the water and towed out to the test site in the "Laguna" and fixed by a rope under a floating platform (figure 1 and 3a). The setup was exposed to the dolphins for 4 consecutive hours each test day but due to training and other activities these four hours were at different times during the day, i.e. sometime between 08.30- 16.30.

After the completed four hours the setup was loosened from the platform and towed back and brought out of the water. The PCL was removed from the setup, rinsed with fresh water, dried and then opened, and switched off so the memory microSD card could be removed. The data collected on it was transferred to a laptop.

During the whole test a GoPro Hero 3 camera was used to film the behaviour of the dolphins in the vicinity of the setup.

Three setups were used, a control, floats and fish (the setups are explained further in the "Equipment" section). The order in which these were offered to the dolphins was decided according to a semi-random schedule with at least every setup tested once every week (there was 5 test days every week). Each test setup was deployed a total of 7 times during the whole test (table 1). Due to technical problems with the PCL, these were distributed over a total of approximately 4 months, from September to December 2015.

Table 1. The dates of each test session throughout the collection period.

Day	1	2	3	4	5	6	7
Control	11 Sep	16 Sep	25 Nov	1 Dec	7 Dec	10 Dec	15 Dec
Floats	15 Sep	17 Sep	26 Nov	2 Dec	4 Dec	8 Dec	14 Dec
Fish	14 Sep	18 Sep	21 Sep	30 Nov	3 Dec	9 Dec	16 Dec

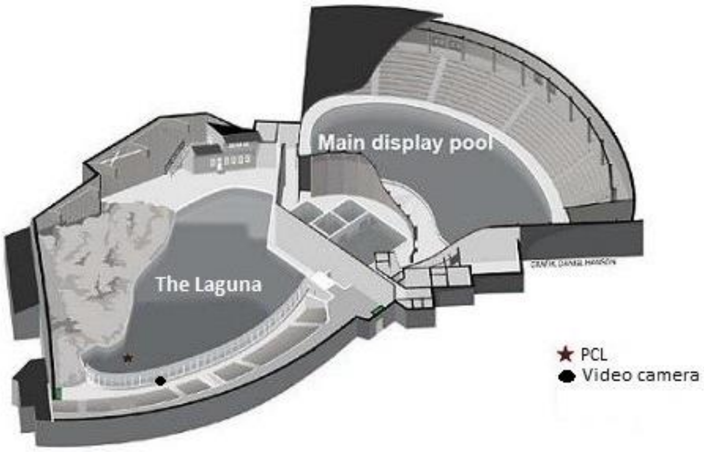

Figure 1. The three pool facility at Kolmården Wildlife Park. Red star marks the location of the setup. Black circle marks the location of the video camera used for collecting behavioural data. Picture cited from Van Zonneveld, 2015.

3.3 Location and animals

The present study was approved by the animal experimentation ethics committee in Linköping (reference number 28-15). The study included eight (1 male, 7 females) Atlantic bottlenose dolphins (*Tursiops truncatus*) kept at Kolmården Wildlife Park, Sweden. Their ages ranged from 3 months to 32 years. The dolphin facility consists of three pools: an 800m^2 main display pool, where some of the dolphins participated in trained public show programs, a non-public 130 m^2 holding pool, provided with a lifting platform, making it possible to beach selected dolphins for medical examination and/or treatment and the 900m^2 "Laguna" (figure 1 and 2), an exhibit where the visitors can observe the dolphins through underwater panels and where this study was carried out. The water depth in the Laguna varies between 3 and 6m. The total water volume of all three pools is 6400 m^3.

The number of dolphins in the "Laguna" differed from day to day due to factors connected to shows and husbandry and social circumstances (e.g. to split the calf and mother from the rest of the group for some privacy in the "Laguna"). Some days the gate between the pools were open and the dolphins were allowed to swim freely between the three pools, while on other days the gate was closed with 3 or 4 dolphins separated in the "Laguna" throughout the test. There were also occasions where the gate was closed during a few hours and later opened during the same session (the time being closed and opened varied). The number of dolphins and the time any dolphin spent in the pool were taken into consideration when analysing the data.

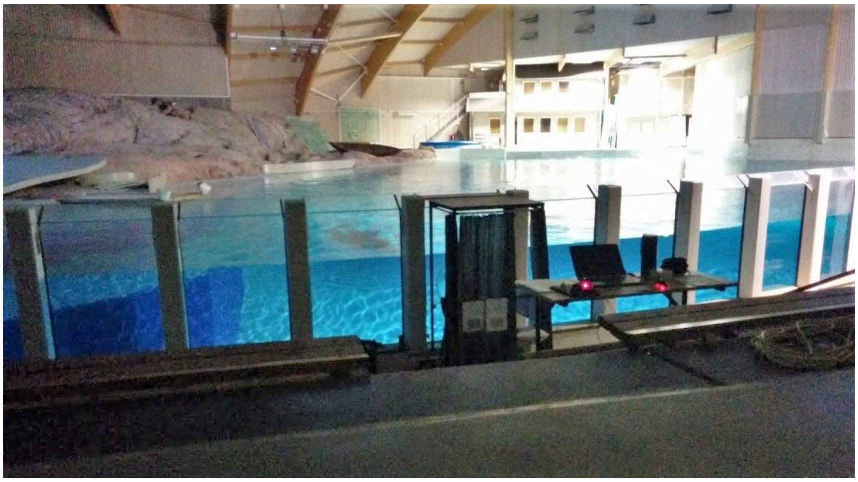

Figure 2. A picture of the "Laguna". The booth in the middle of the picture was used for behavioural observations; it was provided with an air-filled plexiglass cupola, offering a fish-eye lens effect. The picture is taken from a previous study; the table as well as the computer in the foreground were not used during the present study.

3.3.1 Fish and fish handling

Twenty-one goldfish (*Carassius auratus*), approximately 10-15cm in length, were used as sonar targets in one of the test setups. When not used in the test they were kept in a 3.9m^3 indoor pool in an adjacent house. Before an observation period, nine of these fishes were put in the test setup, three fish in each of the three soft plastic bags (see figure 3a and 3b). Between observation sessions, these fish were kept in the bags and were provided with regular feeding. The water in the containers was oxygenated by an air pump (MARINA 200, Hagen Deutschland Gmbh Co, Germany) and replaced regularly. The pump output was branched with plastic tubes, with an air stone connected at the end of each plastic tube submerged in the water in each of the bags. Prior to a session, the containers with the fish were provided with oxygen pills in order to supply the fishes with oxygen during the 4 hour test session.

3.4 Equipment

The test setup consisted of three 20 litre transparent (to sound and vision) soft plastic bags arranged around a PCL (porpoise click logger; AquaClick 100, Aquatec, UK; fig 3b) contained inside a plastic tube (figure 3b and 3c). The PCL recorded the sonar clicks directed towards the plastic bags. The bags were either filled with just water (control), live fish (three fishes in each container; see below) or with air-filled, hard-shelled plastic P20 floats (oval shaped, measuring 60x20mm, providing strong sonar targets, similar to fish swim bladders). The containers and the PCL were as mentioned earlier fixed under a floating platform in the Laguna (fig 3a).

Figure 3. The test setups used in the present study. In figure 3a, a dolphin is echolocating towards the fish setup. In figure 3b, the control and the floats setup is shown. Arrow show the location of the PCL inside the orange plastic tube. Figure 3c show the PCL; the black rod inside the blue square is the hydrophone, which was pointed downward in the setups.

3.5 Data analysis

3.5.1 PCL analysis

The recordings collected by the PCL were transferred to a computer using a custom-made software called AquaClick µSD Reader. It was then analysed using another custom-made program called AquaClickView (Aquatec group Ltd., UK; http://www.aquatecgroup.com). This software is written to extract the typical, narrow-band harbour porpoise clicks (Villadsgaard *et al.*, 2007), but it also displays and extracts broadband dolphins clicks. Since in this situation the only possible click source was the dolphins, all clicks were accepted for the analysis.

The PCL does not record the full time function of the clicks, but only, based on the click envelop, logged a timestamp, the click duration and the peak amplitude through two narrow-band filters with centre frequencies at 60 and 130 kHz. A ratio between the amplitude in these two filters was used to distinguish between beam core and periphery beam clicks: the former has a ratio >1 (130kHz>60kHz) and the latter a ratio <1 (130kHz<60kHz). The time stamps were used to calculate the inter-click-interval (ICI).

These parameters were then exported as csv files, which were imported into Excel where all further processing and the statistical analyses were performed.

Click trains with an ICI below 10ms were termed "buzzes", and is associated with close range inspection of objects and final phase of fish capture (Verfuss *et al.*, 1999; Miller, 2008). All click trains were classified into four ICI/ratio classes: buzz&beam core (ICI<10ms; ratio >1), beam core≠buzz (ratio>1; ICI>10ms), buzz≠beam core (ICI<10ms; ratio<1), ≠buzz≠beam core (ICI>10ms, ratio<1).

For each session the total number of clicks, the total number of click trains (i.e. click trains separated by a silent interval of ≥250ms), and the median ICI, was calculated,

Assuming that there would be more clicks recorded when there were many dolphins in the pool the data was normalized to number of clicks and click trains per dolphin. Since in some sessions the dolphins were free to leave, the distribution of number of dolphins over time in the Laguna was computed, and used to normalize the number of clicks and click trains per dolphin and hour.

When the setups were towed from the deployment site to the platform and back again it attracted a lot of interest in the dolphins, and rather intensive echolocation. These parts of the recordings were eliminated from analysis.

3.5.2 Behavioural analysis

Behavioural data was collected in order to contribute to the understanding of the dolphins' interest towards the different test scenarios (fish, floats, and control). The behavioural observations were made using continuous sampling from the video footage recorded by the GoPro camera (Hero 3) using Pocket Observer (Noldus Information Technology; www.noldus.com). The Pocket Observer was run on an Android tablet, and the behaviours were logged using an ethogram (table 2) with selected behaviour events (momentary observation) and states (measuring duration). The logged observations were later transferred from the Pocket Observer to Observer XT (Noldus Information Technology; www.noldus.com) from which the data was exported as excel files.

Table 2. The ethogram used for behavioural observations.

Behaviour	Description	Behavioural classification
Echo Swim	The duration investigating enrichment (possibly echolocating) while directing snout towards the enrichment while swimming by or towards the enrichment.	State
Echo still	The duration investigating enrichment (possibly echolocating) while directing snout towards the enrichment staying still close to the containers. No movement in any direction.	State
Biting setup	Manipulating enrichment by "biting" some part of the enrichment.	Event
Biting water	Swimming or being stationary and directing snout towards enrichment and jamming jaws together ("biting" water).	Event
Touching	Touching or pushing the enrichment with snout, head or other part of the body.	Event
Other	Playing with ball or other enrichment in the pool.	Event

3.5.3 Statistical analysis

All the data from the PCL and the behavioural data were arranged and analysed in Excel and MiniTab 17. As none of the data was normally distributed non-parametric tests were used; comparing the median number in the setups for significant differences. As the number of dolphins differed as well as the time the dolphins spent in the "Laguna" in each session, most of the data was divided by the number of dolphins and time. This is the reason why some data is presented as per dolphin and hour.

The Kruskal-Wallis test was used when testing for significant differences between any of the three tested scenarios. The Kruskal-Wallis multiple comparison test was then used to compare the control, floats and fish scenarios with each other in order to find where the significant differences could be. This was performed for both the sonar and the behavioural data. When analysing the difference between two sets of data in relation to a test scenario a Wilcoxon test was used. A Chi-Square Goodness of Fit test was also performed to investigate how the total number of clicks, click trains and the total average number of click trains per dolphin and hour differed from the expected value in each of the three scenarios.

To evaluate if there was a significant habituation effect in the number of “Echo swim” and “Echo still” a Regression Fitted Line Plot was performed.

4 Results

4.1 PCL data

4.1.1 Clicks and click trains

In table 3 the number of clicks and click trains (an ICI longer than 250ms was used to separate click trains) produced by the dolphins towards the three test setups are reported. There was no significant differences found, using a Kruskal-Wallis test, between the three test scenarios in the median number of clicks per session (Chi2=2.70; df=2; p=0.259), median number of clicks/dolphin (Chi2=2.99; df=2; p=0.224), median number of click trains (Chi2=3.23; df=2; p=0.198) or in the average of the median number of click trains/dolphin/observation hour (Chi2=1.25; df=2; p=0.534). However when performing a Chi-Square Goodness of Fit test on the total number of clicks (Chi2=27523; df=2; p<0.000), total number of click trains (Chi2=625.53; df=2; p<0.000) and the total average number of click trains per dolphin and hour (Chi2=56.65; df=2; p<0.000) in all sessions, significant differences were found. The control setup was similar to the expected value in both the total number of clicks and click trains, although it was lower than the expected for the average number of click trains per dolphin and hour. The float setup values were lower than the expected values and the fish setup values were higher than the expected values for all the variables (total number of clicks, total number of click trains and the average number of click trains per dolphin and hour).

Table 3. The median and total number of click and click trains generated by the dolphins in the three test scenarios. Bold numbers show higher values than the expected values (Chi2 Goodness of Fit test).

Test Variable	Control	Floats	Fish	Expected values (Chi2-test)
Median number of clicks per session	1042.0	245.0	1103.0	
Median number of clicks/session/ dolphin	164.7	71.4	155.3	
Median number of click trains/session	33.0	8.0	65.0	
Median average number of click trains per dolphin per hour	0.2	0.3	0.4	
Total number of clicks, all sessions	**27364.0**	7869.0	**46624.0**	(27286)
Total number of click trains, all sessions	**575.0**	128.0	**960.0**	(554)
Total average number of click trains per dolphin per hour	12.2	2.2	**47.5**	(21)

A significant difference was found (Kruskal-Wallis test) between the median number of clicks per click train and session in the three test setups (Chi2=19.42; df=2; p<0.000; figure 4). When performing the Kruskal-Wallis multiple comparison it was found that the median number of clicks per click train was significantly higher in both the floats (Z=3.7342; df=2; p=0.0002) and the control (Z=3.0689; df=2; p=0.0021) setup versus the fish setup. No significant difference was however found between the floats and the control; there was only a tendency for the floats to have a higher number of clicks/click train than the control (Z=1.9455; df=2; p=0.0517).

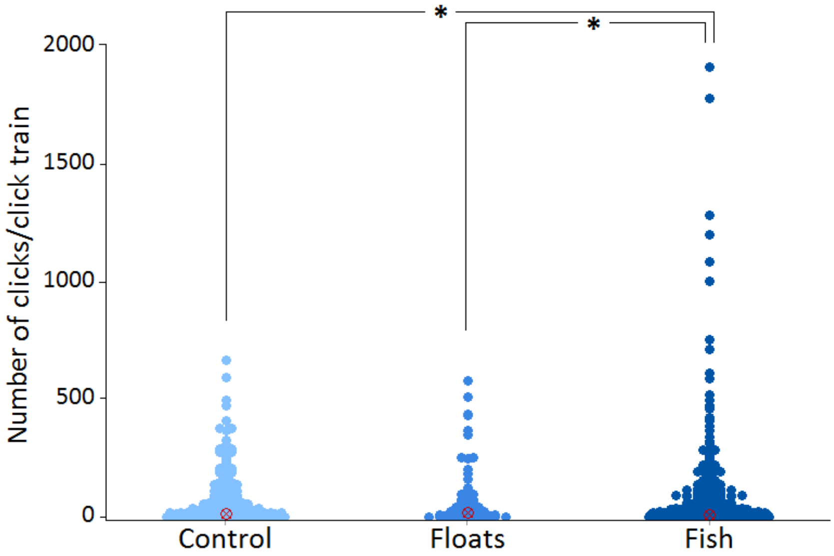

*Figure 4. The number of clicks/click train recorded in the control, floats and fish setups. Red circle marks the median. *= p<0.05.*

The median inter-click interval (ICI) per click train was also analysed (figure 5) and a significant difference was found between the three test setups (Chi2=61.53; df=2; p<0.000). The median ICI per click train was higher in the fish setup than in the control (Z=6.9616; df=2; p<0.0000) and the floats (Z=4.9920; df=2; p<0.0000). There was no difference between the control and the floats (Z=1.0629; df=2; p=0.2878).

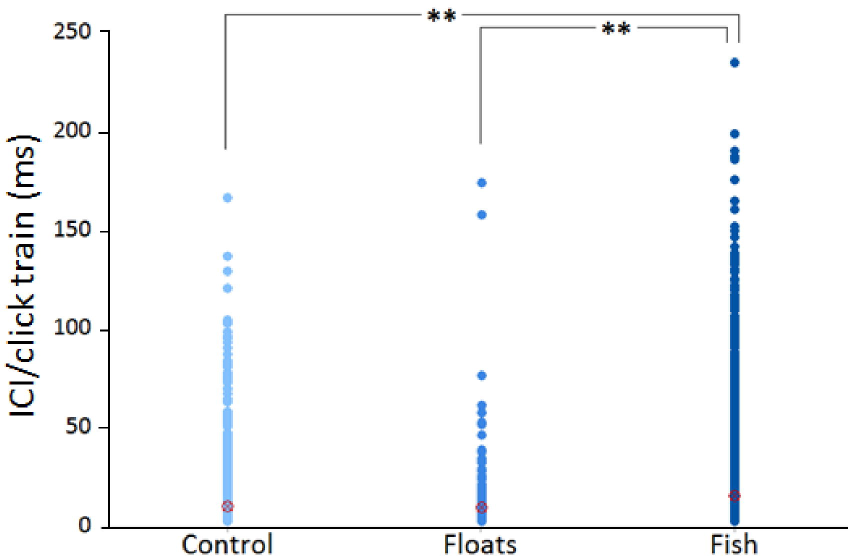

*Figure 5. Average ICI per click train (ms) in the control, floats and fish scenarios. Red circle marks the median. **= p<0.0001.*

After removing all obvious artefact Click length (CL) values (>500µs) from the data a significant difference (Chi2=8.89; df=2; p=0.012) was found in the CL between the test setups (figure 6). The median CL were both significantly longer in the control (Z=2.2272; df=2; p=0.0259) and fish setup (Z=2.9479; df=2; p=0.0032) than in the float setup. No significant difference was found in CL between the control and fish setup (Z=1.0806; df=2; p=0.2799).

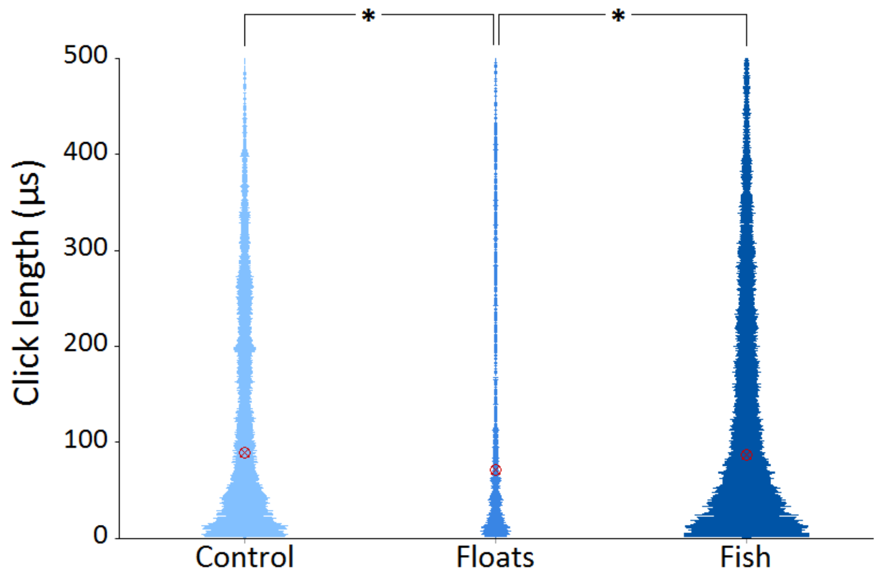

*Figure 6. Click length (µs) in the control, floats and fish setups. Red circle marks the median. *= p<0.05.*

4.1.2 Beam

When analysing to what extent the beam core was aimed at the test containers (i.e. counting the clicks where the ratio between the 130kHz and 60kHz filter click amplitudes was >1), and using a Kruskal-Wallis test, it was found that there were no significant differences between the three test scenarios (Chi2=1.83; df=2; p=0.400; figure 7). There was neither any significant differences between the three test scenarios in the extent the dolphins aimed the beam periphery towards the setups (i.e. ratio <1; Chi2=3.51; df=2; p=0.173). Using a Wilcoxon test, no significant differences were found between the median number of beam core and beam periphery clicks in the control (W=49; df=1; p=0.7015), the floats (W=61; df=1; p=0.3067) or the fish (W=48; df=1; p=0.6093) setups.

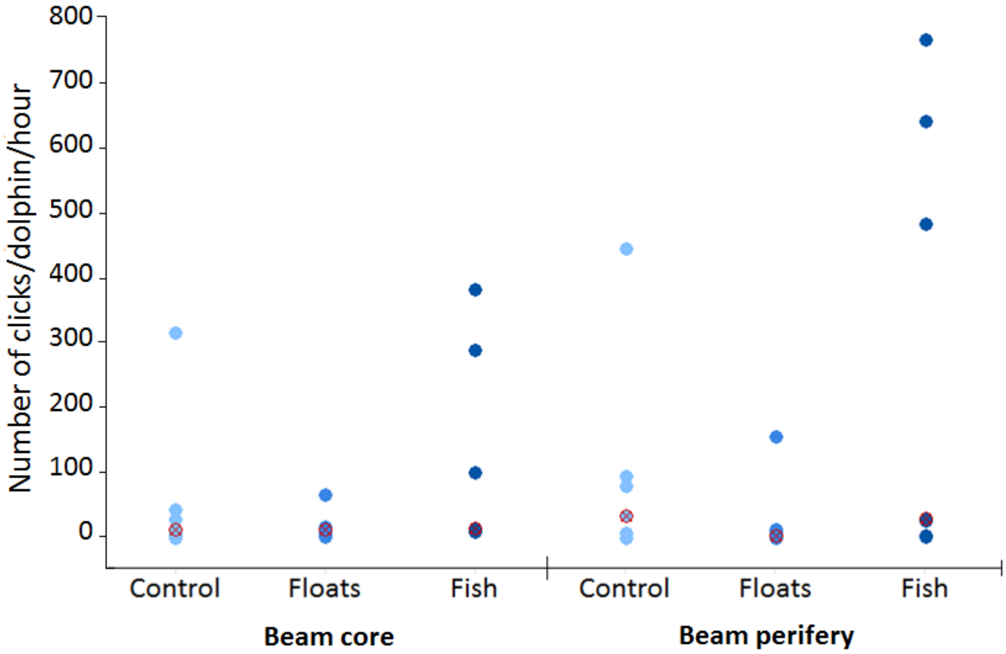

Figure 7. The number of beam core (ratio>1) and beam periphery (ratio<1) clicks recorded per dolphin and hour, during 7 sessions, in the control, floats and fish scenarios. Red circle marks the median.

4.1.3 Buzz and beam core

The median number of clicks in buzzes (ICI<10ms) vs. non-buzzes (ICI>10ms) and beam core and beam periphery clicks (amplitude ratio>1 and ratio<1, respectively) were analysed (figure 8). The number of clicks that were neither buzzes nor beam core (ICI>10ms; ratio<1; ≠buzz≠beam core clicks) were significantly higher in the fish scenario than in the floats scenario (Z=2.2039; df=2; p=0.0275; Kruskal-Wallis multiple comparison). No significant differences were found between the control and the floats (Z=1.0371; df=2; p=0.2997), or between the control and the fish (Z=1.1668; df=2; p=0.2433).

Furthermore there were no significant differences found between the test scenarios in the number of clicks that were in buzzes, but not in the beam core (ICI<10ms; ratio<1; buzz≠beam core clicks; Chi2=2.35; df=2; p=0.308), clicks that were in buzzes and in the beam core (ICI<10ms; ratio>1; buzz&beam core clicks; Chi2=0.83; df=2; p=0.660) or clicks that were in the beam core but not in buzzes (ratio>1; ICI>10ms; beam core≠buzz clicks; Chi2=3.36; df=2; p=0.186).

It was also investigated if there was a difference between the number of ≠buzz≠beam core clicks, buzz≠beam core clicks, buzz&beam core clicks and beam core≠buzz clicks, in each of the test scenarios. I.e. if the dolphins performed significant more in one of the four click types in e.g. the control scenario. However there was no significant difference found when comparing either of these four click types in the control (Chi2=0.85; df=3; p=0.830), floats (Chi2=3.18; df=3; p=0.365) or the fish (Chi2=1.00; df=3; p=0.801) scenarios.

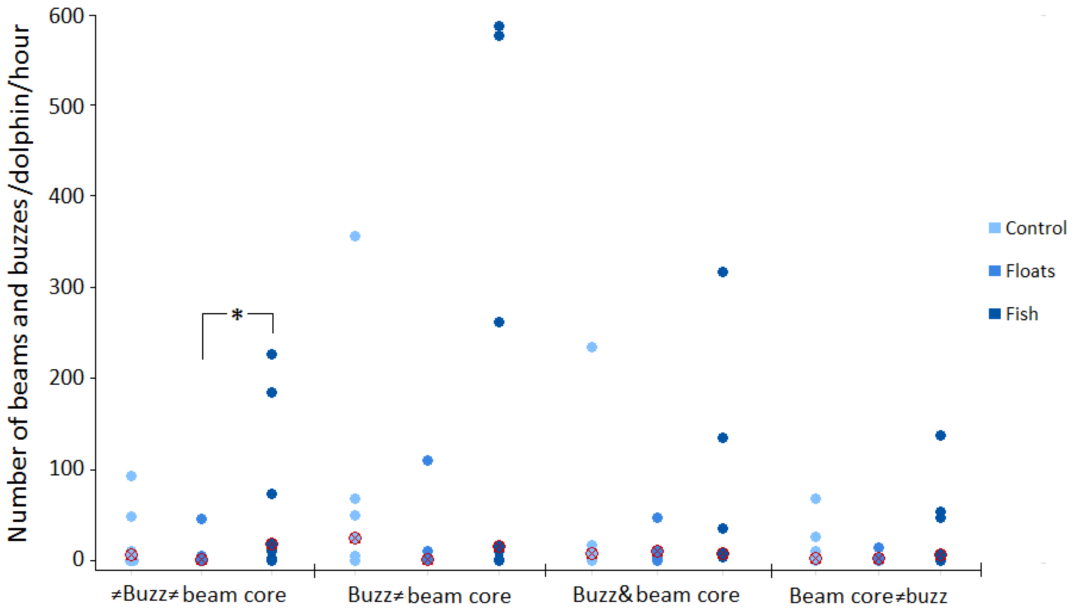

*Figure 8. The number of beam core (ratio>1) and buzz clicks (ICI<10ms) per dolphin and hour, during 7 sessions, aimed by the dolphins at the control, floats and fish setups. Red circle marks the median. *=p<0.05.*

4.2 Behaviour data

4.2.1 Events

It was found that there were no significant differences between any of the three scenarios in the behaviours “Biting set-up” (Chi2=1.69; df=2; p=0.430), “Touching” (Chi2=0.51; df=2; p=0.775) and “Other” (Chi2=3.47; df=2; p=0.177) (figure 9). However, a significant difference could be seen in the number of some of the behaviours counted per dolphin and hour. The dolphins performed significantly more “Biting water” towards the fish setup than towards the floats (Z=2.0731; df=2; p=0.0382), whereas there were no significant differences between the control and the floats (Z=0.5654; df=2; p=0.5718) or between the control and the fish (Z=1.5077; df=2; p=0.1316).

The dolphins performed significantly more “Echo swim” behaviours (Z=2.1106; df=2; p=0.0348) towards the fish than towards the floats, but there were no significant differences in this behaviour between the control and the floats (Z=1.1199; df=2; p=0.2628), or between the control and the fish (Z=0.9907; df=2; p=0.3218). Also the dolphins performed significantly more “Echo still” behaviours (Z=2.1106; df=2; p=0.0348) towards the fish than towards the floats, whereas no significant differences could be seen in this behaviour between the control and the floats (Z=1.3783; df=2; p=0.1681). There was likewise no significant difference in the number of “Echo still” behaviours between the control and the fish (Z=0.7322; df=2; p=0.4640).

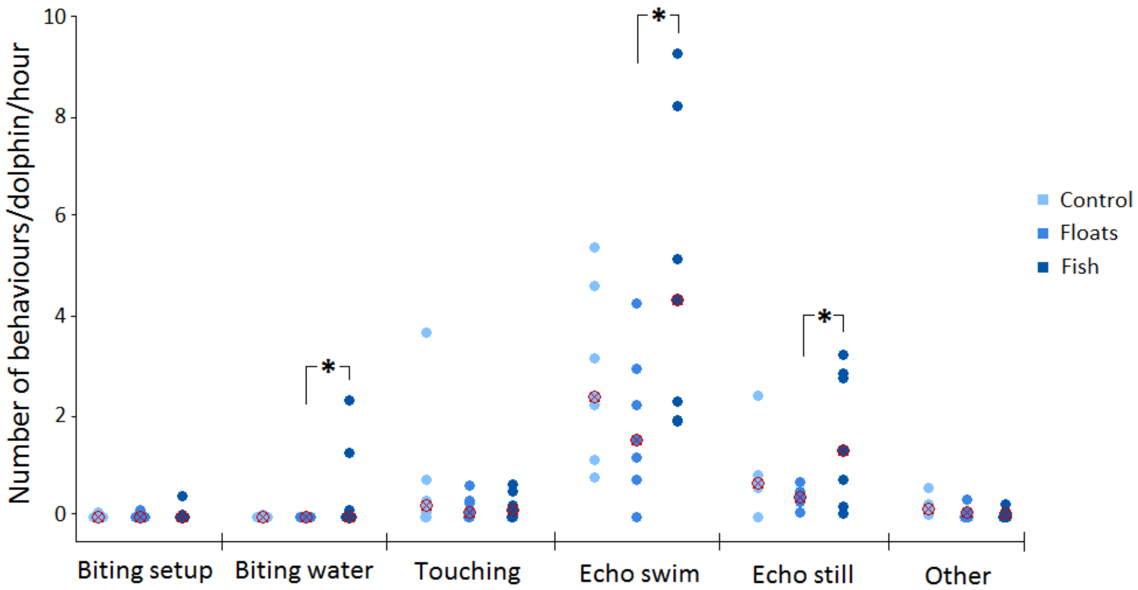

*Figure 9. The number of behaviours one dolphin performed per hour, from the behavioural observation. Red circle marks the median. *= p<0.05.*

4.2.2 Habituation

The number of “Echo swim” and “Echo still” behaviour events performed per dolphin and hour in each session can be seen in figure 10 and 11. When investigating the possible habituation effect on the number of “Echo swim” behaviours per dolphin and hour (figure 10) a reduction in the behaviour frequency could be seen in all of the test scenarios, although there is a big variation between sessions, rendering the regression rather weak. When performing a Regression Fitted Line Plot on the number of “Echo swim” a significant habituation effect could not be found in any of the test scenarios.

There was no significant difference in the number of “Echo swim” between any of the test sessions for the control (R^2=0.0343; S=1.8447; F=0.18; p=0.691), float (R^2=0.0633; S=1.5291; F=0.34; p=0.586) or fish scenarios (R^2=0.0508; S=3.2247; F=0.27; p=0.627).

When investigating the possible habituation effect on the number of “Echo still” behaviours per dolphin and hour (figure 11) a very slow reduction in the behaviour frequency could be seen in the fish scenario, whereas there was a slight increase in the control and the floats scenarios. No significant habituation effects were found in the control (R^2=0.2631; S=0.7090; F=1.78; p=0.239), float (R^2=0.5494; S=0.1628; F=6.10; p=0.057) or fish scenarios (R^2=0.0002; S=1.4704; F=0.0; p=0.979).

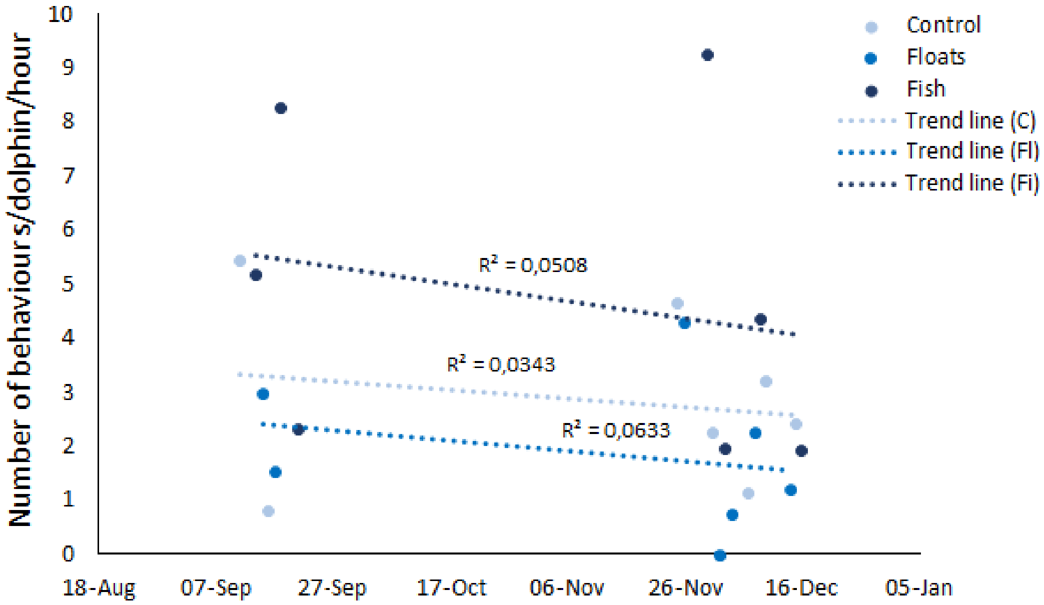

Figure 10. The number of “Echo swim” behavioural events performed per dolphin and hour during 7 days in the control (C), floats (Fl) and fish (Fi) scenarios. Dotted lines show the trend line for each test scenario.

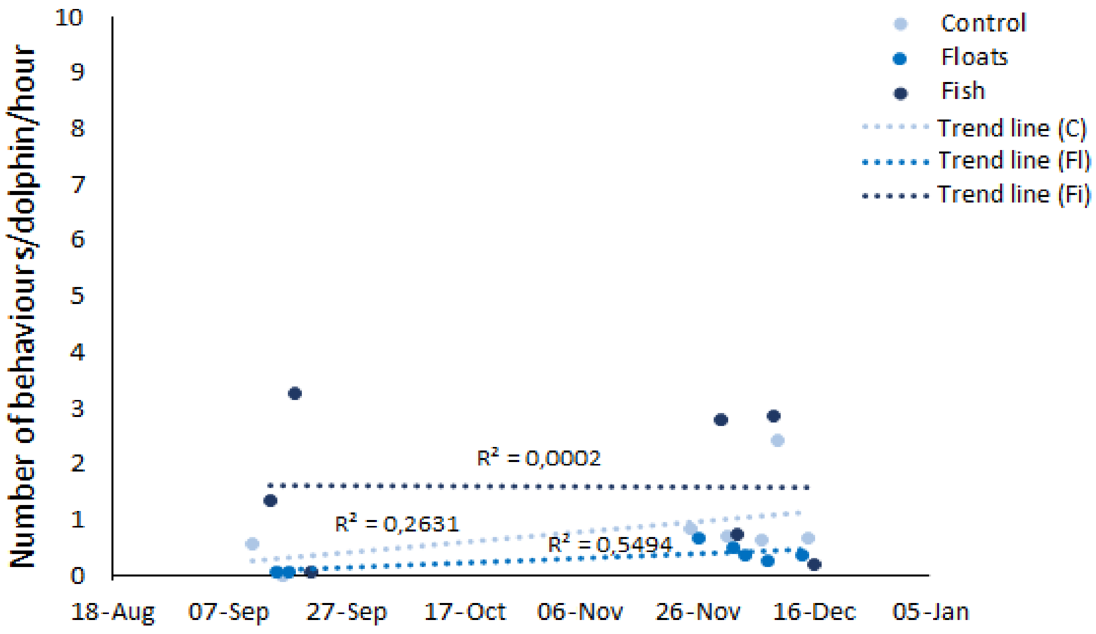

Figure 11. The number of "Echo still" behavioural events performed per dolphin and hour during the 7 control (C), floats (Fl) and fish (Fi) scenario sessions. Dotted lines show the trend line for each test scenario.

4.2.3 Duration

The duration each dolphin seemed to echolocate towards the setups, based on the behavioural observations, was also investigated. In figure 12 the duration of echolocation while swimming ("Echo swim") per dolphin and hour in the test scenarios can be seen. There was a significant difference between all three test scenarios (Chi2=86.90; df=2; p<0.000). Further analysis showed that the duration of "Echo swim" was significantly higher in the fish scenario than in the control (Z=5.0790; df=2; p<0.0000) and the floats (Z=5.0790; df=2; p<0.0000). Further the duration of "Echo swim" in the control scenario was also significantly higher than in the floats scenario (Z=4.1804; df=2; p<0.0000).

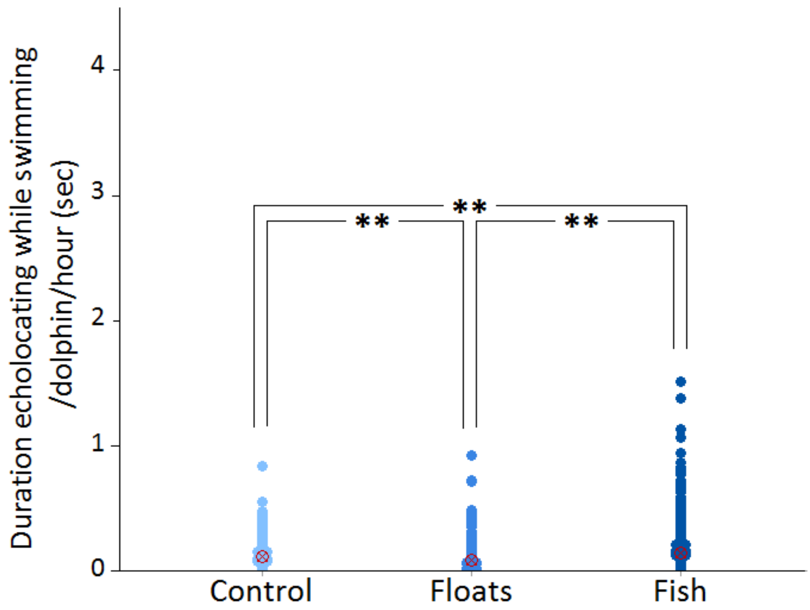

*Figure 12. The duration (sec) of echolocation while swimming ("Echo swim") per dolphin and hour towards the control, floats and fish setup. Red circle marks the median. **=p<0.0001.*

The duration of echolocation while still ("Echo still") per dolphin and hour is shown in figure 13, and a significant difference (Chi2=22.07; df=2; p<0.000) was found between all three test scenarios. There was a significantly higher duration of "Echo still" in the fish scenario than in the control (Z=3.3144; df=2; p=0.0009) and the floats scenario (Z=3.3144; df=2; p<0.0000), but no significant difference between the control and the floats (Z=1.8538; df=2; p=0.0638).

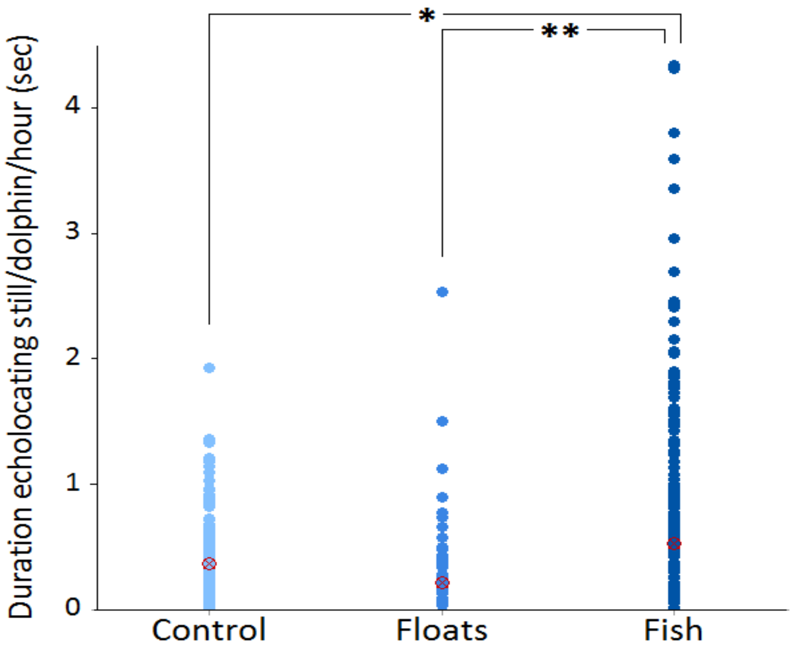

*Figure 13. The duration (sec) of echolocation while still ("Echo still") per dolphin and hour in the control, floats and fish setup sessions. Red circle marks the median. *= p<0.05; **=p<0.0001.*

5 Discussion

5.1 PCL Data

5.1.1 Clicks and click trains

Many parameters in the PCL data e.g. the total number of clicks and click trains and the number of clicks per click train, can be used to evaluate if the dolphins have a preference towards a particular type of enrichment, like the ones tested in this study. In table 3 a general picture of how the dolphins related to the test scenarios can be seen. Although no significant differences were found in the median values a significant difference was found in the total number of clicks and click trains and the total number of click trains per dolphin and hour. All these values were higher than the expected value in the fish scenario, with the total number of clicks even being twice as high as the expected value. The control was close to the expected values and the float values were below the expected values. These findings provide clear evidence that the dolphins aimed more echolocation clicks at the fish setup and spent more time investigating this setup. Interestingly the control had higher values than the float setup. This may be due to the location of the floats in the container re. to the

PCL hydrophone, as they were floating at the upper part of the setup, close to the floating platform, whereas the hydrophone was situated in the low end of the test setup, some 25cm below the underside of the platform. If the dolphins were inspecting the floats from close range, the sonar beam might have missed the hydrophone altogether or only occasionally hit it with beam periphery clicks. Another reason why the control setup had more clicks than the floats may be because the dolphins were playing and manipulating the control containers ("Touching", figure 9) more and while doing this aimed more clicks at the PCL. This will be discussed further in the section on the behavioural observation data below.

The median number of clicks per click train aimed by the dolphins at the setups was also compared (figure 4), as this contributes to an indication of the dolphins' interest in them. A higher number of clicks per click train means that the dolphins investigate the enrichment more closely and more focussed. In the present study it was found that the median number of clicks per click train was significantly higher towards the floats and the control than towards the fish. Although table 3 showed both more clicks and click trains towards the fish, according to the data in figure 4 the dolphins were producing lower median number of clicks in each click train towards the fish setup than towards the floats and the control. One possible explanation to this is that the dolphins were following the moving fish, and only hit the hydrophone when the fish was in line with it. However the number of dolphins aiming clicks at the test setups at the same time may have interfered with these results. If many dolphins echolocate towards the PCL at the same time it can be hard to determine how long the actual click train is and how many clicks each click train actually contains when compiling the data. E.g. one dolphin may start echolocating for a while, then another dolphin shows up from a distance with a higher ICI which in the data looks as an end to the click train, when the first dolphin is actually still generating clicks. However the same apply to all the test scenarios, but there were usually more dolphins echolocating at the same time in the fish scenario, in itself indicating a bigger interest in the fish. As mentioned before another reason may be that all of the clicks generated by the dolphins might not have been recorded by the PCL, e.g. the clicks were not directed at the PCL hydrophone but at its casing, the floats or the fish, when at a close distance. The floats were located at the top end of the plastic bags whereas the fish were moving around in them. Since the PCL hydrophone was located around 25cm from the top of the container, it is plausible that the PCL might not have recorded all the clicks when the dolphins were closely inspecting the floats and the fish.

The median inter-click interval (ICI) per click train was also investigated (figure 5), where a short interval means the dolphins were echolocating close to the object under investigation, since ICI depends on the time for the echo to return from a target. It was found that the median ICI per click train was significantly higher in the fish scenario than in both the control and the floats scenario. This indicates that the dolphins were generally further away when echolocating towards the fish setup (supporting this interpretation is the number of echolocation behaviours in figure 10 and 11; this is discussed further in the "Habituation" section). Shorter median ICI's in the floats and control setups might indicate that the dolphins found these setups more interesting than the fish. However, this interpretation is contradicted by the fact that the total number of clicks and total number of click trains (table 2) was significantly higher in the fish scenario. This seemingly puzzling findings may have been caused by the dolphins starting to echolocate towards the fish from a longer distance, indicating that they provided a more dynamic and naturalistic target.

Yet another reason could be that the PCL did not register all ICI because the clicks merged to long clicks due to much reverberation, which might have skewed the results. This is corroborated by the finding of abnormally large click lengths in the collected data (figure 6), with click lengths reaching 32000 µs. The click length is normally between 50-150 µs. However it is more likely that these very long clicks are artefacts, and after removing all these likely artefact CL's, setting the realistically maximum CL to 500 µs, the CL was found to be significantly longer in the fish and the control scenario than in the floats scenario. Longer CL in the fish scenario may be the result of reverberations caused by the echoes from many swim bladders from fish close to the hydrophone, merging with the direct click. This means that longer CL's do not necessarily indicate that the dolphins were more interested in the control and fish than in the floats, and it is hard to draw any conclusions from the CL data other than it may have affected the other click data.

5.1.2 Beam

We also investigated whether the dolphins aimed their beam core or beam periphery at each of the test setups, based on the ratio between the amplitude through the high and low filter (figure 7). A dominance of beam core clicks would indicate a specific interest in either an object in line with the PCL hydrophone or the hydrophone itself. However, no significant differences were found, in either the median number of beam core or beam periphery clicks per dolphin and hour, between the three scenarios. These results would indicate that there was no specific interest towards any of the setups and that they investigated the setups in a similar way. However as other PCL results (discussed above) point at a difference in interest, and in the number of investigation events in the three setups, a ratio >1, i.e. beam core clicks, may not be a good indicator. The reason why no significant differences could be found might be because of the small sample size (n=7) as well as the large standard error in the number of beam core clicks per dolphin and hour from day to day.

Interestingly there was no significant difference between the median number of beam core clicks compared to the median number of beam periphery clicks in the three test scenarios. It would be expected that there would be more beam core clicks aimed towards the setups as it was the setup which was investigated and not anything close to the setup. These results might indicate, as discussed above, that the PCL hydrophone was not placed as well as hoped. As mentioned above, the PCL was mounted with the hydrophone directed downward, approximately 25cm from the top of the plastic bags and the underside of the platform; all the floats were gathered at the upper part of the bags. Hence the results rather indicate that in this scenario the dolphins were more prone to investigate the floats than the PCL hydrophone. With the fish, which moved in the entire water volume, it makes sense that when, at close distance, the sonar beam core was locked on the fish, it would not hit the hydrophone unless the fish happened to be in line with it, producing shorter click trains if the fish swam around a lot. In the control scenario the dolphins preferred to manipulate and play with the bags, i.e. there was not anything else inside the bags which could capture the interest of the dolphins, and probably focused their beam more towards the ropes and the plastic bags and not directly at the hydrophone. The get a clearer result of search patterns it would have been better to have a multi-hydrophone array with a small distance between the hydrophones.

We were also interested in investigating the correlation between number of buzzes (ICI<10ms) vs. regular click trains (ICI>10ms) and beam core clicks (ratio>1) vs. beam periphery clicks (ratio<1), expressed as the median number of clicks per dolphin and hour (figure 8). It was found that there were significantly more beam periphery clicks with >10ms inter-click intervals directed towards the fish setup than towards the floats. This is in accordance with previous results where the dolphins had a higher average ICI between the clicks directed towards the fish setup than towards the floats. This indicates that the dolphins inspected the fish setup from a longer distance, but did not aim the beam core towards the fish or that the fish were not often in line with the hydrophone. However no significant differences were seen between the three test scenarios in the median number of "buzz&beam core" clicks, indicating that the dolphins made as many close range inspections of all three setups.

5.2 Behavioural observation data

5.2.1 Number of behaviours

As mentioned above it was observed that the dolphins played with and manipulated the empty plastic bags in the control scenario more than in the fish and the floats scenarios. The behaviour data (figure 9) support that statement as more "Touching" behaviour was observed towards the control bags, even though it was not significant. Only "Biting water" was performed significantly more towards the fish setup than the floats other than the echolocation behaviours. It was almost exclusively performed towards the bags when there was fish in them (only performed during one day in the control and never in the floats scenario). Performing this behaviour might indicate that the dolphins wanted to eat the fish even though they never have been presented live fish before (except for one of the dolphins). This could mean that the dolphins either still have their hunting instincts or simply recognised the shape of the fish with the similar shape of the dead fish they are presented with during feeding. Another explanation could be that "Biting water" produced a water jet resulting in a pressure pulse hitting the bag, which the fish might have reacted to by moving or trying to escape as fish are very sensitive to variation in pressure.

The median number of investigatory behaviour potentially including sonar while swimming ("Echo swim") and being stationary ("Echo still") was significantly higher in the fish scenario than in the floats scenario. Both these behaviours together were also performed significantly more times in the fish setup. These are clear indications that the dolphins found the fish setup more interesting than the floats. Interestingly, there was no significant difference in the number of these behaviours between the control and fish scenarios. This could be because the dolphins played with and manipulated the control bags and while doing this also generated echolocation click trains (as supported by the total number of clicks in table 3).

Other behaviours which might have taken the attention away from the evaluated enrichments were listed under “Others”; these behaviours were mostly playing with balls. As it varied if there were any balls, and if so, how many and for how long, or other enrichment in the pool from day to day, not much can be said from this data. No significant differences could be seen between the three test scenarios in “Other” behaviours. It seemed, however, that playing with a ball was preferred over investigating any of the test setups. However this is not shown by the data.

5.2.2 Habituation

The dolphins did not show a significant habituation effect towards any of the test scenarios in the number of “Echo swim” (figure 10) and “Echo still” (figure 11) behaviours performed across the seven test sessions, even though the trend lines showed a decrease over time. These results should however be evaluated with caution due to problems with the technology which resulted in pauses in the data collection (a week or even a month until the next observation; see table 1). As data collection was not continuous the dolphins might have reacted to the setup as to a new enrichment; this is known to slow down habituation (Kuczaj *et al.*, 2002).

In figure 10 and 11 a large variation can be seen in the number of echolocation behaviours performed between each session, although not significant, which might be due to these pauses in the data collection. In table 1 the date for each data collection can be seen. The data from the three test setups were mostly collected close to each other in time, however at test day 3 the data from the fish scenario was collected a month earlier than the floats and the control. This can be an explanation to the peak in “Echo swim” behaviours at day 4 in the fish scenario (figure 10), which would correspond to the peak in the float and the control scenario at day 3; where there had been a pause in collection for approximately a month before each peak. To prevent a quick habituation effect to the enrichment it might be beneficial to have pauses between presenting the enrichment. Although it is difficult to say if those peaks in the number of “Echo swim” behaviours at day 3 and 4 was an effect of the pause or just a coincidence as there were not just one peak in figure 10 and 11.

Using a variety of objects in combination with occasional novel object to enrich the environment for animals kept in zoos, laboratories etc. is commonly used as animals may lose interest in some enrichments faster than in other (Kuczaj *et al*., 2002). This is why the fish setup might be more interesting for the dolphins as it is constantly changing when the fish are swimming around, and a bigger aquarium with more fish (and maybe also different types of fish) might make the enrichment even more interesting. Some zoos have enclosures where two or more species lives together, providing a social enrichment (as well as a more natural environment) for all of the animals (Carlstead and Shepherdson, 2000) which is constantly changing, e.g. Kolmården Wildlife Park where different savannah living animals such as grévy's zebras (*Equus grevyi*), some antelopes (e.g. sable antelope (*Hippotragus niger*)) and white rhinoceros (*Ceratotherium simum*) etc. are kept together. Kolmården Wildlife Park also has aquatic mammals such as harbour seals (*Phoca vitulina*), and fur seal (*Arctocephalus pusillus)* living together with bottlenose dolphins (*Tursiops truncatus)*.

Using live fish together with dolphins would require a biological life support system, and fish that would not be eaten by the dolphins. In a chlorinated system, an underwater aquarium with a separate biological water system for the fish would be a viable alternative. It is then important to choose a sound-transparent material for the aquarium walls.

5.2.3 Duration of the apparent sonar behaviours

In addition to counting the number of “Echo swim” and “Echo still” events, the duration of these behaviours was also recorded (figure 12 and 13). The results corresponded well to the number of these echolocating behaviours. The duration of “Echo swim” as well as “Echo still” per dolphin and hour was significantly higher in the fish scenario than in the control and the floats scenarios. “Echo swim” was also significantly higher in the control scenario than in the floats scenario. Between the floats and control scenarios, however, there was no significant difference in either of these two behaviours.

When comparing the number of echolocation behaviours (“Echo swim” and “Echo still”) with the duration the dolphins spent performing these behaviours it can be seen that while the dolphins performed a higher number of “Echo swim” than “Echo still” behaviours, the duration performing these behaviours was higher in the "Echo still" behaviour in all the test scenarios. This would indicate that the dolphins performed a higher number of approaches to the setups but stayed in front of it for close investigation for longer periods of time. From this we can draw the conclusion that the dolphins actually find the enrichments interesting enough to investigate them further, especially the fish setup (as discussed above).

5.3 PCL data versus behavioural observations

The PCL data had some disparities, with some parameters such as median number of clicks/click train and median ICI/click train indicating that the dolphins had a higher interest in investigating the float and control setups rather than the fish setup; however this may be also explained by the dolphins locking their beam on a moving fish, and hence sweeping by the hydrophone quicker, which would result in shorter click trains. Other parameters such as the total number of clicks, click trains and the total average number of click trains per dolphin and hour showed that the dolphins investigated the fish setup significantly more than expected and more than the control and the floats setups. The median click length was longer in the fish and the control scenarios than in the floats scenario, but this cannot be claimed to support that the fish and the control were investigated more than the floats. In all the PCL data a high variation can be observed.

The behavioural observation results showed the dolphins to perform both a higher number of echolocation behaviours ("Echo swim" and "Echo still") as well as for a longer period of time in the fish scenario compared to the floats scenario. Also the dolphins performed these echolocation behaviours for a longer period of time in the fish scenario than in the control scenario.

The PCL results together with the behavioural observations strongly support the prediction that the fish setup was the most interesting to the dolphins. These results also points to the floats being the least interesting to the dolphins. However this could be due to the floats being close to the top end of the setup, resulting in the beam not being recorded by the PCL hydrophone.

5.4 Ethical aspects

Using fish as an environmental enrichment may raise not only questions of welfare and ethics for the fish but also for the dolphins. The goldfish used during the present should not be exposed to a higher stress impact than when humans are standing close to an aquarium, possibly with the exception of the effect of the “Biting water” behaviour. Doing this may have generated a squirt of water, hitting the bag as a pressure pulse. This may have affected the fish, which are sensitive to such pressure variations. In Popper *et al.* (2004) it is described that some clupeid fish such as blueback herring (*Alosa aestivalis*), American shad (*Alosa sapidissima*) and gulf menhaden (Brevoortia patronus) can detect ultrasonic sounds up to 180 kHz. These fish are thought to have evolved their utricle region in their inner ear to be able to detect ultrasonic sounds to avoid echolocating predators, such as the bottlenose dolphin (Myrberg Jr., 1997; Popper *et al*., 2004). If these fish would be used in a study like the present one they would probably be extra stressed by the dolphins directing their sonar towards them and not be able to avoid it; however it is rather unlikely that the goldfish has evolved this mechanism as they have not been subjected to dolphin predation during their evolution. However if a dolphin produced very strong clicks containing >153 dB re 1µPa at 8-15 kHz close to a goldfish, it would be able to detect them (Nedwell *et al.*, 2004); however a dolphin does not produce such strong clicks at a close distance; the source level of buzz clicks usually are 10-20dB below that of clicks generated during the approach phase (Kloepper *et al.*, 2014), and at 8-10kHz these clicks would be below the hearing threshold of the goldfish.

If deciding to use an underwater aquarium with fish as enrichment for dolphins it would be beneficial to choose fish which are bred to do well in an aquarium such as goldfish or other aquarium fishes, or having a very large aquarium if marine fish would be preferred. Goldfish and other common aquarium fishes are usually rather small, with a small swim bladder, which do not provide the dolphins with as good sonar target as a bigger fish with a bigger swim bladder. As mentioned before there are both positive and negative effects of using either a bigger or a smaller fish. The most important choice is however to choose a fish species that cannot detect ultrasonic sounds. A variety in the types of fish would also be beneficial to provide the dolphins with different types of sonar targets.

The effects on the dolphins when providing them with fish would need to be investigated further. Dolphins could possibly become frustrated by not being able to get to the fish. However as only one of the dolphins at Kolmården Wildlife Park has actually been in contact with live fish before and no frustrated behaviours towards the setup were observed during the study it might not be a problem. Some dolphins did perform biting behaviour towards the fish setup; however there were only a few of these behaviours observed (figure 9). The period when the fish setup was in the pool was not that long, and if there would be fish in an aquarium near or in the pool all the time they might make the dolphins frustrated or they would learn that they could not get to them and loose interest after a while. As there was variation in the amount of behaviours (“Echo swim” and “Echo still”) from session to session (figure 10 and 11) it would probably take a while for the dolphins to lose interest as the fish are always moving and changing places and may also elicit predation behaviours in the dolphins even though they have not seen live fish before.

If providing the dolphins with a permanent installation with this type of enrichment it would be good to observe and record the dolphin’s behaviours 24/7 after introduction for at least a week to observe any possible negative effects (e.g. stress) on the dolphins as well as the fish.

5.5 Conclusions

All the data from the behavioural observations show that the fish setup was investigated the most by the dolphins, and probably was the most interesting for them. The control was next, and the floats were the least interesting. Most of the PCL data supported and strengthened the behavioural observations; some of the data (median number of clicks/click train and median ICI/click train) was seemingly contradictory indicating that the floats and control setups were the most interesting than the fish, but alternative interpretations are possible.

From the present study the main conclusion is that live fish is good echolocation enrichment for bottlenose dolphins and possibly for other zoo-living Odontocetes. However before installing such enrichment an extended behavioural study investigating the pros and cons of a bigger installation should be carried out.

6 Acknowledgement

I would like to thank my supervisor Mats Amundin for his idea of doing this project as well as his guidance and help throughout the whole thesis. I would also like to thank the trainers at the dolphinarium at Kolmården Wildlife Park with help implementing and designing the setup so it would be safe for the dolphins, as well as letting me using their dolphins and the dolphinarium for my study. I would also like to thank Leif Larsson for helping me take care of the fish used in the present study after the study was finished. I would also like to thank Louise Brodd and Márton Kuti and my examiner Carlos Guerrero-Bosagna for good feedback on improving my thesis.

7 References

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