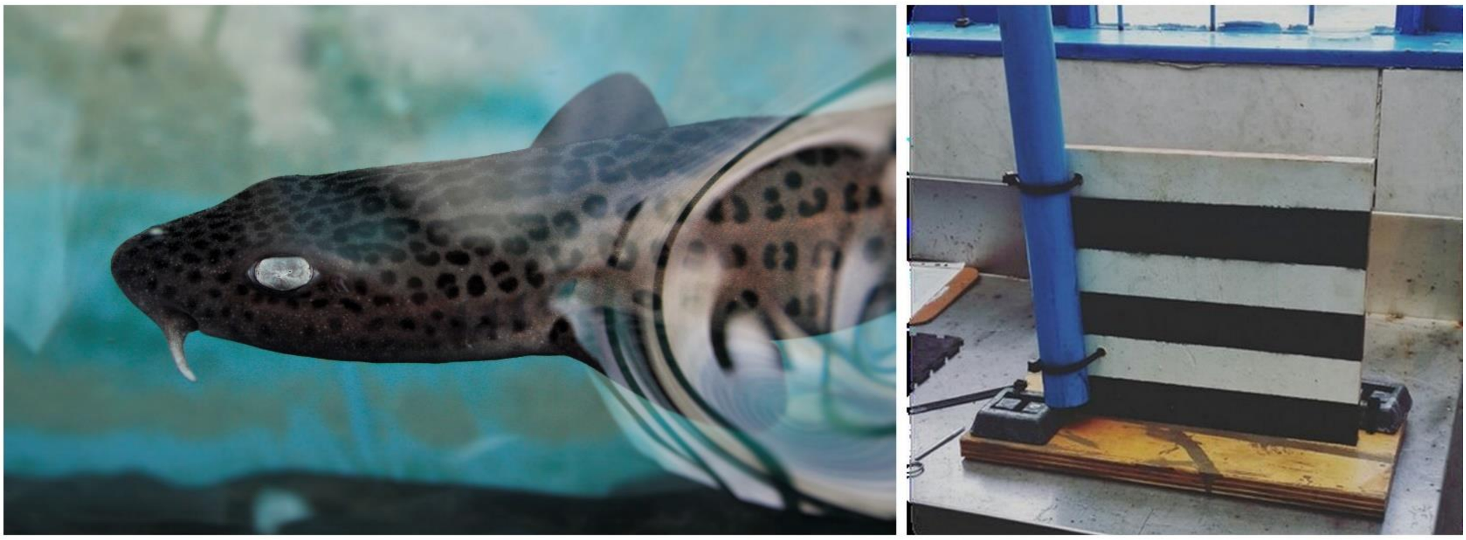
UNIVERSITY OF CAPE TOWN

Applied Marine Science

INSTRUMENTAL CONDITIONING AND LEARNING IN *PORODERMA PANTHERINUM*



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December 2016

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1. Plagiarism declaration

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2. Abstract

Recent research has shown that higher cognitive functions and learning occur in teleosts and elasmobranchs. Very little is known about the cognitive abilities of benthic sharks and no research has been published on the learning ability of the endemic Leopard catshark (*Poroderma pantherinum*) species of South Africa. This species is listed as data deficient on the IUCN red list and known threats due to anthropogenic impacts include fatalities because of bycatch and depredation in the small-scale commercial fishing industry. It has been suggested that sharks can be attracted to fishing boats through the sound of outboard motors and an association can be formed between the sound and easy prey on the hooks of fisherman. This pilot study examined the learning capacity of Leopard catsharks in Hermanus, South Africa, by using a series of food-reward tests based on instrumental conditioning. A target with black and white stripes was used as the discriminative stimulus, while an auditory cue acted as a bridging stimulus for the food-reward task. Sharks were collected by hand whilst diving and shore-angling in Hermanus from August 2015 to November 2015 and acclimatised before the onset of experiments. Four juvenile sharks were trained through operant conditioning using visual and auditory stimuli for ten days each, consisting of six trials per day. A fifth juvenile shark, acting as the control, was trained through the same method without the auditory stimulus to test the influence thereof on the learning rates of Leopard catsharks. This study showed that Leopard catsharks have the ability to associate a visual stimulus with a food reward through the aid of an auditory cue. The auditory cue was also shown to increase learning rates significantly as an association was formed between the presence of food in front of the target and the auditory signal. The individual sharks in this study displayed differing levels of stress and learning rates. It is suggested that even though Leopard catsharks show high diversity in learning rates and adaptation to stress, they possess the ability to learn and adapt rapidly to changing environments. The results possibly have important implications for the understanding of learning and conditioning in Leopard catsharks and the likely anthropogenic threats caused through learned behaviour in benthic sharks.

3. Introduction

For many years it was believed that only land vertebrates such as mammals and birds are capable of learning associations between different stimuli (Schluessel, 2015). These animals have a neocortex which plays a role in the control of higher perceptual processes, cognitive functions and intelligent behaviour (Bayer & Altman, 1991), and includes processes such as perception, recognition, judging and reasoning (Guttridge et al., 2009).

Over the last 60 years, research has shown that higher cognitive functions and learning, occurs not only in terrestrial vertebrates, but also in teleosts and elasmobranchs (Brown & Laland, 2003). The majority of studies on the learning ability of aquatic vertebrates are centred around fish with commercial importance such as salmon (Salmonidae) and cod (Gadidae) (Brown & Laland, 2003). Little research has focused on the learning abilities of elasmobranchs and the understanding of the basic principles of learning is therefore lacking (Kimber et al., 2014).

Elasmobranchs evolved independently of bony fishes (Osteichthyes) to form a diverse group known as the cartilaginous fishes (Chondrichthyes) (Pough et al., 1999). They occur in highly dynamic environments and have evolved different levels of phenotypic plasticity which can accommodate adaptations in morphology and behaviour (Guttridge et al., 2009). Coastal environments are particularly dynamic due to freshwater influxes, atmospheric influences and anthropogenic impacts (Dill, 1983). Recent studies show that relative brain size is linked to the ability to adapt and survive in changing environments (Reader & MacDonald, 2003; Sol et al., 2008). Elasmobranchs living in highly dynamic coastal regions would therefore require significant cognitive functions to optimise behaviours such as navigating, locating food, avoiding predators and acquiring appropriate mates for reproduction (Kimber et al., 2014).

The ability to learn a specific reactive behaviour associated with particular stimuli is highly advantageous for an animal's survival and could result in the animal responding more effectively and accurately to biologically significant stimulatory events. For example, associating a stimulus (such as a smell with a visual observation of a predator), could lead to increased alertness and preparedness to flee (Guttridge & Brown, 2014).

Certain species of elasmobranch are known to migrate over vast distances (thousands of kilometres) for several months, while others undergo daily migrations over short distances (few metres) to retrieve food (Guttridge et al., 2009). Whether the migrations are monthly or daily endeavours, learning and spatial memory are very advantageous in these circumstances.

Understanding the topographical relationship between changing environmental signals and storing that as a cognitive spatial map can increase chances of survival (Schleussel & Bleckmann, 2005).

3.1 Shark sensory systems

An understanding of the sensory systems of elasmobranchs is one of the most important starting points in evaluating their behaviour. It is well known that sharks possess an impressive array of highly specialised sensory systems. These sensory systems allow them to respond to biotic and abiotic changes in the environment. Elasmobranchs have different eye structures depending on whether they occur in deep, dark or shallow brightly-lit habitats. In shallow waters sharks often have larger eyes that provide a detailed image of the environment (Tester, 1963). The high concentration of rods in elasmobranch eyes, suggests high sensitivity to light and the ability to adapt to low-light habitats. Certain species have a high abundance of retinal cones, which indicates toward the possibility of colour vision. Previous studies concerning elasmobranch behaviour have shown that they can differentiate between contrasting colours (Greenberg et al., 1981).

With paired nasal organs as olfactory receptors sharks are capable of detecting small differences in the concentration of odours allowing orientation in the direction of the source of smell (Tester, 1963). Previous research showed that lemon sharks (*Negaprion brevirostris)* and nurse sharks (*Ginglymostoma cirratum)* are capable of detecting amino acids, amines and electrolytes at concentrations below 1 mg/l (Hodgson & Mathewson, 1978).

Hearing also plays an important role in the behaviour of sharks. The inner ear of elasmobranchs is similar to that of bony fish, however the macula neglecta is enlarged (Myberg, 1978). Skates, rays and benthic sharks that feed on invertebrates have a smaller macula neglecta, unlike pelagic sharks that feed through raptorial predation (Corwin, 1991). The enlarged macula neglecta in pelagic sharks is suggested to be an adaptation for the localization of distant sound sources. The ability to detect low frequency vibrations allows sharks to successfully hone in on distressed fish (Myberg, 1978).

Like other fishes, elasmobranchs have a mechanosensory lateral line system. Much less is known about the functionality and biological role thereof in sharks, although it is known to respond to hydrodynamic motion (Gardiner & Atema, 2014). It has been shown by Gardiner (2012) that when a shark's vision and lateral line system is removed, the amount of wall collisions in captivity increased. This suggests that the lateral line system assists in obstacle detection especially when vision is lacking. Hydrodynamic imagery is achieved through using the flow field around the body of the shark and as water is displaced around the head. When in close proximity to an obstacle, the flow field becomes distorted and the lateral line system detects the presence of the obstacle (Gardiner and Atema, 2014). The lateral line system also facilitates prey detection, predator avoidance, conspecific interaction and detection of water movement through electroreception. This sense is the last to be used by a shark in the process of foraging or hunting as it assists in the detection of the precise location of prey and shark's jaw orientation. Electroreception is specifically crucial for the success of benthic shark species due to the inconspicuous nature of their prey (Kimber et al., 2014).

The shark brain is relatively larger in volume compared to the brain size of birds and mammals (Bres, 1993). Controlled environment studies have shown that sharks are able to learn associations as fast as land vertebrates and are capable of retaining memory of previously learnt tasks. It is suggested that learning improves the animal's ability to search for prey, find appropriate mates, and navigate and most importantly increasing the individual's chances of survival and reproductive success (Guttridge et al., 2009). Current research suggests that the relative brain size in vertebrates correlate to the ability to adapt to changing environments. It is known that coastal regions are highly variable due to terrestrial, atmospheric and anthropogenic influences and organisms inhabiting these regions should therefore theoretically display behavioural flexibility in order to survive and reproduce (Guttridge et al., 2009). The ability to learn is essential in assisting adaptation to such changing environments.

3.2 Types of learning

The skill of learning is associated with a change in behaviour as a result of experience. External information is processed and assessed to adjust response behaviours to change. It excludes permanent behaviour changes due to maturation or instinctive behavioural changes, which is mostly due to physiological variables (Guttridge et al., 2009). There are two distinct categories of learning namely associative and non-associative learning where the categories are distinguished based on whether the learning requires an association between two stimuli or between a stimulus and a response (Kandel et al., 1983).

Non-associative learning consists of habituation and sensitization. Habituation is a process where the exposure to repetition of a stimulus leads to a decrease in the response of the animal. It could be said that the animal becomes habituated to that stimulus. Sensitization occurs when the repetition of a stimulus causes an increase in the response of the animal (McGreevy, 2014).

Associative learning occurs when an association between two events is established and it consists of three concepts namely classical conditioning, operant conditioning and observational learning (Guttridge et al., 2009). Classical conditioning is probably the most studied form of associative learning in vertebrates, first discovered by Ivan Pavlov during experimentation with dogs. Pavlov conditioned dogs by exposing them to a neutral stimulus (conditioned stimulus) such as a bell, paired with food as an unconditioned stimulus which caused the dogs to salivate (involuntary bodily response). The dogs were therefore able to learn that the neutral stimulus indicates towards a food reward they are about to receive (Skinner, 1938). Classic conditioning therefore occurs when a neutral stimulus (conditioned stimulus) evokes a conditioned response and the strengthening between these two factors could indicate towards learning. (Schunk, 2012).

Operant conditioning is a method used where rewards become associated with the responses of the individual. Positive and negative reinforcement is suggested to change the rate of occurrence of response behaviours by increasing or decreasing the probability of the behaviour occurring (Schunk, 2012).

3.3 An overview of research illustrating learning in elasmobranchs

Sharks belong to the class Chondrichthyes and evolved separately from teleosts, hence research on the cognitive ability of teleosts cannot be used as a proxy for understanding shark cognitive abilities (Sims, 2003). Different shark species live in a large variety of habitats throughout the oceans, leading to adaptations of diverse reproductive strategies, life expectancies and brain complexities. It is important to recognise too that generalization of results based on the cognitive ability of a specific shark species, in a particular habitat, is therefore not considered scientifically acceptable (Guttridge et al., 2009).

The very first evidence of learning ability in sharks was collected by Eugenie Clark (1959), who trained two adult lemon sharks (*Negaprion brevirostris*) in captivity via the method of operant conditioning. These sharks were trained to make physical contact with an underwater target to which food was attached for six weeks. In each case, once the sharks were feeding a submerged bell was rung. After the six weeks of training was completed, the trial began where the target was lowered into the water without food and only after making contact with the target did the sharks receive a food reward. This experiment showed that, conditioning of these lemon sharks took place in accordance with positive reinforcement and that the sharks retained the conditioned behaviour even after a period of ten weeks of inactivity (Clark, 1959). In a similar study (Wright & Jackson, 1964) using five juvenile lemon sharks and three bull sharks (*Carcharhinus leucas)* results suggested that the learning rates for conspecifics were similar, but differed between species (Wright & Jackson, 1964). Even though this particular study had a low sample size, it was a breakthrough in the understanding of shark cognition. Several other studies followed on from these initial experiments based on operant conditioning methods and revealed comparable learning rates of sharks to land vertebrates (Aronson et al., 1967).

In 1967 Aronson et al. developed discriminative tasks to study the abilities of mice, teleosts and sharks to select a white target from black and white targets. The results of this study indicated that sharks were able to learn and complete discriminative tasks as quickly as other vertebrates in the study (Aronson et al., 1967). These initial studies on shark cognition inspired further investigation via more controlled, classical conditioning methods.

Sharks possess eyelids referred to as nictitating membranes. They are similar to that of the transparent nictitating membrane found in amphibians, birds and mammals, however in sharks they are opaque and covered in placoid scales. These membranes are well developed in Carcharhinidae and thought to act as protection for the cornea against mechanical damage (Gruber, 1977). Gruber and Schneiderman (1975) tested the conditioning of the nictitating membrane response in twenty juvenile lemon sharks (*Negaprion brevirostris*). The sharks were restrained and exposed to a flashing light (conditioning stimulus), along with an electric shock (unconditioned stimulus), which produced an eye-blink reaction. The results of this study concluded that, conditioning of the nictitating membrane response occured in these particular sharks and that learning rates were similar to that of mammals (Gruber & Schneiderman, 1975).

Until 2005 little information was available on the memory functions of elasmobranchs in general, while the spatial learning systems of mammals, birds, reptiles and even insects had already been studied. Schluessel and Bleckmann (2005) set out to investigate whether juvenile freshwater stingrays (*Potamotrygon motoro*) could solve spatial tasks by creating a cognitive map of the environment. The rays were trained to locate a food reward in a maze using visual spatial stimuli. The starting position of the rays within the environment changed while the location of the food reward remained constant. Results showed that all rays were able to solve the task and locate the food reward. It was concluded that these freshwater stingrays used visual cognitive maps of the environment to orientate themselves and furthermore that spatial memory functions are a general feature of the vertebrate brain (Schluessel and Bleckmann, 2005). Schleussel and Bleckmann (2012) aimed to investigate the spatial learning system of sharks.

They studied nine juvenile grey bamboo sharks (*Chiloscyllium griseum)* that were trained in numerous spatial tasks and tested after ranging periods of inactivity to test memory retention. Results indicated that these sharks could retain spatially acquired information for up to six weeks without reinforcement (Schleussel and Bleckmann, 2012).

The ability of *Scyliorhinus canicula* (small-spotted catshark) to improve foraging efficiency and memory retention thereof was investigated by Kimber et al. (2014). Positive reinforcement was used through operant conditioning to study the catshark foraging behaviour towards artificial (prey-like) electric fields. The sharks received a food reward when they responded accordingly. The experiments were repeated after a period of three weeks of inactivity and the sharks that previously received a food reward for responding, did not receive a reward in the repeat experiment. The results revealed that rewarded sharks showed more interest in the electrical stimulus than unrewarded sharks. The sharks were also found to improve their foraging efficiency over time through learning to locate electrodes in order to receive a food reward. The unrewarded sharks became habituated and their responses decreased over time until it completely ceased. The sequences of each sharks' search path showed that habituation and learning occurred after only a few foraging events (Kimber et al., 2014).

Training and conditioning of elasmobranchs has also become an important tool in aquaria and zoological institutions throughout the world. These programs mostly consist of conditioning through positive reinforcement. Training of animals in captivity has many benefits including mental stimulation, control during feeding sessions, reduced stress during veterinary examinations and enhanced educational presentations (Sabalones et al., 2004). At the Living Seas Pavilion (Orlando, Florida) a Bonnethead shark (*Spyrna tiburo*) and Blacknose shark (*Carcharhinus acronotus*) were successfully trained to move to specific feeding stations at feeding time. The target was made of PVC pipe painted with vertical black and white stripes. These sharks were trained to move towards the target in quarantine and retained the response to the target after being moved to the new environment. It was suggested that any type of target could work for this purpose (Sabalones et al., 2004). A research team at the Pacific Rim Centre for elasmobranch studies set out to train an immature female sand tiger shark (*Carcharias taurus*) to make contact with a target. A total of 38 training sessions over a period of three months were conducted with little success. The use of only a primary enforcer such as the target proved inadequate stimulation for learning. An auditory cue was added to the experiment in the form of a 6 Volt door-bell that was rung every time food was present in front of the target. The addition of a secondary stimulus (bridging stimulus) proved much more successful and the sand tiger shark had 100% successes at making contact with the target after a month of training. The shark was also able to retain the learnt behaviour for several weeks (Sabalones et al., 2004).

Even though research on learning in fishes is widespread due to the implications for behaviour and fisheries (Brown et al., 2011), very little is known about the fundamental learning abilities of demersal sharks (Malyukova et al., 1983) and to our knowledge no research is published on that of the endemic scyliorhinidae species of South Africa. More research is therefore needed to understand the behaviour and cognitive abilities of these sharks.

3.4 Anthropogenic impacts on benthic sharks

Throughout history sharks have made minor contributions to global commercial catches due to the high urea content in their meat (Cunningham-Day, 2001). Human-shark interactions have increased over the last two decades (Guttridge et al., 2009), where commercial fisheries and eco-tourism practices have allowed for the increased exposure of sharks to humans.

Over the last few years the demand for shark fin has increased tremendously and more than 150 countries now trade shark fins (Musick et al., 2000). The finning industry does not pose a major threat to, for example endemic catshark species in South Africa however, the large amount of catshark bycatch in non-directed fisheries does pose a major concern (Myers & Worm, 2003). Between the years 2000 and 2004 bycatch data were collected on board demersal pelagic longline vessels in South Africa (Basson et al., 2007). Of the total 11698 sharks counted as bycatch, 2733 were scyliorhinidae sharks, of which 222 were leopard catsharks (*Poroderma pantherinum*) and 1 was a pyjama catshark (*Poroderma afrricanum*) (Basson et al., 2007).

In the small-scale commercial line fishing industry, depredation (partial or complete removal of the hooked fish from the line) has become a source of lost revenue and threat to benthic and semi-pelagic shark species (Labinjoh, 2014). The increase of shark activity along the KwaZulu-Natal coastline in South Africa, has led to a 50% to 80% loss of catch due to shark depredation (Mike Milton, personal communication, 2013). A study along the coastline of KwaZulu-Natal reported shark activity in 62% of fishing trips and 44% of the target fish species was lost due to shark depredation (Dudley, 2003). A high number of shark species (51) are found off the coast in KwaZulu-Natal, hence shark depredation in the extensive fishing industry there is to be expected (Compagno, 2012). Many long-line fisherman have also reported loss of bait or half eaten target species, which creates the potential for learning (Rosa and Secchi, 2007).

As it has been shown that classical and operant conditioning do occur in sharks, it is possible and quite likely that sharks could learn to associate the sound of boats with the easy acquisition of prey. It has been suggested that localised shark populations may display behavioural adaptations through the association of easily acquired food with the sound of outboard engines (Wetherbee et al., 2010; Heithaus & Vaudo, 2012). It has also been shown that Reef sharks learnt to associate the noise of a boat and the sound of divers entering the water with food (Sabalones et al., 2004).

The smaller benthic sharks, such as shy sharks and catsharks, that are caught by fishermen in South Africa have no monetary value and are seen as pests. These sharks are most often killed to avoid further depredation (Phillip Colyn, personal communication, 2016). The role of learning in fisheries has been identified as an important factor that needs to be incorporated into ecosystem-based fisheries management (Ferno et al., 2006).

Human interaction during diving activities, capture of sharks, transportation of sharks and any form of captivity are potential stressors that could alter the behaviour of sharks (Smith, 1992). The swimming behaviour of sharks indicate stress levels when introduced to a new environment and should be monitored regularly in a captive environment (Charbeneau, 2004). Swimming behaviours indicative of stress include constant, rapid swimming, quick, jerky movements or having the head raised above the horizontal plane of the tail. Lifting the head above the surface of the water (spy hopping) also indicates increased stress levels (Charbeneau, 2004).

3.5 Leopard catsharks as experimental species

Catsharks are one of the most diverse chondrichthyan families consisting of 150 species. The number is expected to increase as more species are described (Ebert et al., 2006).

These sharks are relatively slow moving and non-migratory occurring world-wide through temperate and tropical regions. Catsharks are generally small (<1.6 m) and are harmless to humans. They play an important role in the ecosystem as they are consistent predators on squid, cephalopods, crustaceans and small fish. Catsharks are prey to larger sharks and fish (Helfman et al., 1997). The Leopard catshark, as seen in figure 3.5.1 (Rowlinson, 2016) is commonly found in kelp forests and reefs up to 50m deep (Fig. 3.5.2). These sharks are mostly nocturnal and hunt small fish and cephalopods (Smale et al., 2001). They do however, periodically abandon their nocturnal habits to hunt chokka squid during mass spawning events during day time (Smale et al., 2001).

Figure 3.5.1: Leopard catshark (*Poroderma pantherinun)* captured in Gansbaai, South Africa (Rowlinson, 2016).

Leopard catsharks are endemic to South Africa and very little is known about their behaviour. They can grow to a maximum total length (TL) of 84 cm with males considered juvenile at 10.0 – 51.9 cm TL, adolescent at 46.9 – 67.2 cm TL and mature at 60.7 -77.4 cm TL. Females are considered juvenile at 9.4 – 48.5 cm TL, adolescent at 43.0 – 64.1 cm TL and mature at 50.7 – 66.6 cm TL (Human, 2009).

Figure 3.5.2.: A map of southern Africa displaying the distribution of Leopard catsharks (IUCN, 2009).

Leopard catsharks have a single oviparous reproductive strategy, laying two egg cases at a time. The reproductive season is unknown and they are believed to be able to live for at least 15 years. Seeing that the habitat of the Leopard catshark is mostly coastal, they are heavily impacted by anthropogenic activities such as recreational diving, sport and commercial fishing, beach utilization and extensive pollution. These sharks are frequently caught by recreational anglers and commercial line fishermen who regard them as pests, often resulting in the killing of these sharks. There is also a high demand for this species in the aquarium trade seeing that they adapt well to captivity and are aesthetically appealing. The number of animals caught, however is unknown (Human, 2009). According to IUCN this species is classified as data deficient and further research on the abundance and distribution is therefore needed to establish whether the species is threatened or not.

3.6 Aim and hypotheses

The aim of this research is to test the learning capacity of Leopard catsharks (*Poroderma pantherinum*) when completing food-reward tasks through instrumental conditioning.

Hypothesis 1: Leopard catsharks can learn to associate a food reward with combined auditory and visual stimuli.

Hypothesis 2: Leopard catsharks will show improved efficiency in completing a food reward task as training days increase.

Hypothesis 3: Leopard catsharks can become conditioned to the presence of a target and show decreased stress-related behaviours over time during the ten-day training period.

Hypothesis 4: Individual Leopard catsharks demonstrate the same learning rates when associating visual and auditory stimuli with food rewards.

Results from this study will likely have important implications for understanding learning in catsharks, understanding of behavioural processes and the likely anthropogenic threats caused through learned behaviour by benthic sharks.

4. Materials and Methods

4.1 Study location and experimental sharks

This study was conducted at the South African Shark Conservancy located in Walker Bay, Hermanus (34°25’15.2” S, 19°14’36.6” E). Leopard catsharks were chosen as the study species due to their abundance in Walker Bay and hardiness in a captive environment. A total of five (50.22 cm average total length) Leopard catsharks were caught by hand (scuba diving and snorkelling) and through shore angling between August and November in 2015 (Figure 4.1.1). One male shark was used as the control, while the other male and three female sharks acted as the experiment. The sharks were caught in front of the research facility in Old Harbour and in New Harbour, Hermanus which is a ten-minute drive from the conservancy. In Old Harbour the sharks were caught by hand and immediately taken to the holding tank inside the building. Using this method, the shark is out of the water for no more than one minute. Sharks caught in New Harbour were transported in a large tub filled with sea water by car to the research facility in Old Harbour. Care was taken to keep the distress of sharks low by quick transportation to the holding tank and the minimum handling.

Table 4.1.1.: Biological data of Leopard catsharks used for an instrumental conditioning experiment in Hermanus, South Africa (2015).

Shark	**Sex**	**Weight (g)**	**Total length (cm)**	**Food/day (g)**
A	Male	550	44,6	8,25
B	Female	520	45,5	7,8
C	Female	530	45,8	7,9
D	Female	910	55,3	13,6
CONTROL	Male	1140	59,9	17,1

The pre-caudal length (PCL), inter-dorsal length (IDL) and total length (TL) of each shark was measured (cm) on a measuring mat (Fig. 4.1.1) where after they were weighed in a sling (Fig. 4.1.2). Each shark also received a unique triangular fin clip on a pectoral or pelvic fin for ease of identification.

Figure 4.1.1: Measuring of a Leopard catshark (*Poroderma pantherinum)* in Hermanus, South Africa (2015).

Figure 4.1.2: Weighing of a Leopard catshark (*Poroderma pantherinum)* in Hermanus, South Africa (2015).

The sharks were housed in a circular 3000 L indoor flow-through holding tank (Fig. 4.1.3). While in the holding tank each shark was fed squid or sardine on a daily basis using a feeding stick. They received 1.5% of their total body weight per day in order to ensure that the sharks retain motivation for a food reward task, based on previous studies focussed on instrumental conditioning of scyliorhinidae sharks conducted at the South African Shark Conservancy. Each shark was allowed to acclimatise in the holding tank for seven days before they were moved to the experimental tank for training. All sharks were moved to the experimental tank by hand. No sharks died during this study and all sharks were released at the site of capture after being tagged and weighed. The South African Shark Conservancy holds a Cape Nature permit (Res2015/44) that allows the capture, holding and experimentation on *Poroderma pantherinum.*

Figure 4.1.3: Indoor flow–through holding tank used to house Leopard catsharks (*Poroderma pantherinum) in Walker Bay, Hermanus, 2015.*

4.2 Experimental set up

The target comprised a 30 x 35 cm wooden board with black and white stripes (Fig. 4.2.3). Elasmobranchs are known to see contrasting colours (Greenberg, et al. 1981) and targets with similar dimensions and patters have been used in previous research on the learning abilities of elasmobranchs (Guttridge et al., 2012; Sabalones, 2004). Two weights were attached to the bottom of the target in order to stabilise it on the bottom of the tank. A pipe that extends through the surface of the water attached to the front of the target. The pipe acted as a conductor for the auditory cue. The cover comprised a black rubber square that was connected to a pulley system in the roof of the building for ease of lifting and lowering.

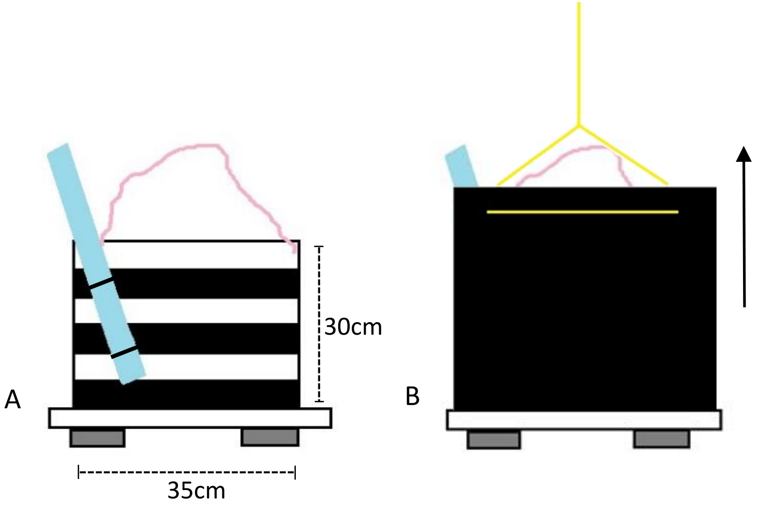

Figure 4.2.3: The target (A) and target cover (B) used in the instrumental conditioning of Leopard catsharks (*Poroderma pantherinum)* in Walker Bay, Hermanus in 2015.

A flow-through seawater system was used for the holding and experimental tanks. The exchange rate of seawater through the tanks was two hours. The temperature and visibility in the tanks were therefore dependent on the inflow from the sea. The holding tank was a 3000 L circular indoor tank with a depth of 0.7 m. The experimental tank was a 2000 L circular indoor tank with a diameter of 2.6 m and depth of 0.7 m (Fig. 4.2.1). Previous studies concerning conditioning of sharks by Guttridge et al. (2012) and Guttridge and Brown (2014) were conducted in experimental tanks or holding pens 2.5 m in length. Both tanks were indoors and received diffused natural light during daylight hours. The brightness of sunlight was equal throughout the tank. The holding tank contained sand and rock caves representing the natural habitat. The experimental tank, however was kept empty with only a half-pipe shelter that was placed in the tank overnight.

During the experimental phase the shark was moved to the experimental tank where it acclimatised and food was withheld for three days before the trials began. The experimentation phase for each shark comprised ten days of six trials per day. Each trial was limited to a maximum of ten minutes. The shark's daily feed (1.5% of the body weight), made up of chokka squid, was divided into six equal pieces to act as a food reward for each trial. Four hours before the trials started each day the target and cover were lowered into the experimental tank. For 15 minutes before and after the target was introduced to the tank the shark's behaviour was monitored. The resting and circling time was recorded. Special behaviours such as spy hopping, head shaking and rolling were also noted. These observations give an indication of the stress levels of the shark (Charbeneau, 2004). It also indicates when the shark becomes conditioned to the presence of the target.

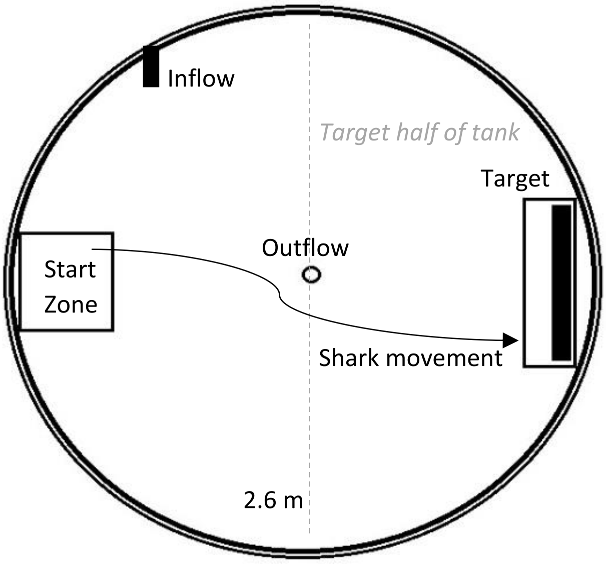

Figure 4.2.1: Experimental tank set-up

Table 4.2.1: Description of experimental steps for the instrumental conditioning of Leopard catsharks (*Poroderma pantherinum)* in Hermanus, South Africa (2015).

Task	**Description**
Shark capture	Sharks captured diving, snorkelling or shore angling, processed and introduced to holding tank
Acclimatization in holding tank	Sharks allowed to acclimatize for at least one week
Acclimatization in experimental tank	Experimental shark allowed to acclimatize for 3 days (shark is starved)
Training	Training resumes for 10 days (six trials per day)
Release	Sharks are weighed, tagged and released at site of capture

4.3 Experimental protocol

Ten days of experimenting were divided into four days of ‘active training’ and six days of ‘passive training’. This method would increase the probability of learning occurring in a shorter time period. A similar method was used in a previous unpublished study done on the learning ability of catsharks at the South African Conservancy (Lober, 2014). The active and passive training phases were used to train the control shark without the auditory cue.

Active training

During the first four days of training the cover was lifted off the target before the first trial. The cover remained lifted throughout the entire training session to give the shark maximum exposure to the visual stimulus. As the shark swam over the start zone, the trial began and the shark was led to the target with a feeding stick for each trial. As the shark makes physical contact with the target (with the rostrum) a food reward was released, allowing the shark to consume the reward. As the reward was taken by the shark an auditory cue (secondary stimulus) was given by tapping the flat end of a knife against the top opening of the pipe. The same knife was always used and the force applied to the pipe was kept as constant as possible to insure a consistent auditory cue (Table 4.3.1).. The auditory cue was a bridging stimulus and the target the discriminative stimulus. This had previously been found to enhance the learning ability of sharks in similar tasks (Sabalones, 2004).

Passive training

From the fifth to the tenth training day the shark was not led to the target with a feeding stick, but rather allowed to swim towards the target, make physical contact using its rostrum and consume the reward. As the shark swam over the start zone, the trial started and the cover was lifted. At the same time, the auditory cue was given, by tapping the flat end of the knife on the top opening of the PVC pipe, and a food reward placed in front of the target using a feeding stick. As the reward was consumed, the auditory cue is given again to reinforce the association between the sound signal and the food reward and the trial is concluded (Table 4.3.1).

Table 4.3.1: Description of a trial for the instrumental conditioning experiments of Leopard catsharks (*Poroderma pantherinum)* in Hermanus, South Africa (2015).

Active training steps	Passive training steps
1. Target cover is lifted	1. Target cover is lifted
2. Shark swims over start zone (trial begins)	2. Shark swims over start zone (trial begins)
3. Auditory cue is given	3. Auditory cue is given
4. Shark is led to the target with feeding stick	4. Food reward is placed in front of the target
5. Shark makes contact with the target	5. Shark makes contact with the target
6. Shark consumes reward	6. Shark consumes reward
7. Auditory cue is given	7. Auditory cue is given
8. Trial ends	8. Trial ends

Figure 4.2.2: A screen capture of video footage recording an instrumental conditioning trial of a Leopard catshark (*Poroderma pantherinum) in Hermanus, South Africa (2015).*

4.4 Data collection

The behaviour of each shark was monitored for 15 minutes before the target was inserted into the tank (covered) and for 15 minutes immediately after the insertion. The resting time, circling time, special behaviours such as rolling, head shaking and the number of spy hops were noted (Table 4.4.1). All data were recorded manually on a standardized data sheet. These measurements give an indication of the stress that the introduction of the target causes the shark due to the alteration of the environment and measures the conditioning of the shark to the presence of the target (Table 4.4.2).

Table 4.4.1: Behaviour codes used for assessing Leopard catsharks (*Poroderma pantherinum)* during instrumental conditioning in Hermanus, South Africa (2015).

Behaviour codes	**Behaviour**	**Definition**
R	Resting	Shark is relatively motionless on the bottom of the tank. Readjustment of body can occur but no swimming for more than 2 seconds
D	Doughnut	Shark curls up into doughnut shape with its tail wrapped around its head
CIR	Circling	Circular swimming around the tank
SPY	Spy hopping	Shark breaks the surface with rostrum through a vertical movement
RO	Rolling	Shark tilts sideways; no longer 180° in relation to substrate
HS	Head shaking	Rapid shaking of the head back and forth

During experiments the duration of trials were measured manually by means of a stopwatch. Physical contact with the target and consumption of the food reward was assessed visually. During trials a GoPro Hero 2 was used to record the experiment from above. Video footage was used to measure the duration of time the shark spent in the target half of the tank between trials and the number of approaches to the target between trials (where no food was present). The target half of the tank is defined in figure 4.2.1. Seeing that the sharks normally circle the tanks while swimming, the aim of this measurement is to determine if the sharks spend more than 50% of the time in the target half, indicating a significant interest in the target. The behaviour of the sharks were considered to be an approach to target when the shark swam directly towards the target swam across the feeding platform of the target or made physical contact with the target. These measurements indicate the ability of the shark to associate the target with a food reward and the auditory cue with a food reward.

The water temperature in the experimental tank was measured for the first experimental shark with the aim to assess the variable. Due to equipment failure, the temperature could not be measured for the rest of the experimental phase.

Table 4.4.2: Ethogram of behaviours measured during instrumental conditioning of Leopard catsharks (*Poroderma pantherinum)* in Hermanus, South Africa (2015).

Behaviour	**Measure**
Resting time	Duration(min:sec)
Number of resting events	Count
Circling time	Duration(min:sec)
Number of circling events	Count
Doughnut time	Duration(min:sec)
Spy hops	Count
Trial duration	Duration(min:sec)
Contact with target	Contact target with rostrum (Y/N)
Food reward consumed	Completely consume food (Y/N)

4.5 Data analysis

All data was analysed in Excel and XLSTAT.

4.5.1 Observations of stress-related behaviours before and after the insertion of the target

The circling durations for all experimental sharks were pooled per training day. The mean circling durations were calculated for all sharks before and after insertion of the target. Regression analyses were run separately for mean circling durations before and after insertion of the target over training days. Further statistical analyses were run to compare results before and after the insertion of the target. A two-way ANOVA with replicates tested the similarity of variances of the two databases.

The number of spy hops for all experimental sharks were pooled per training day. The mean number of spy hops were calculated for all sharks before and after insertion of the target. To assess the change over time, regression analyses were run separately for the mean number of spy hops before and after the target was inserted over training days. A two-way ANOVA with replicates tested similarity in variances of the number of spy hops before and after insertion of the target into the experimental tank.

4.5.2 Efficiency of Leopard catsharks completing a food-reward task in a captive environment

The trial duration for all experimental sharks were pooled per training day. The median trial durations for all sharks were calculated due to outliers and a regression analysis was calculated to show the trend over training days. Linear regressions were also calculated for the passive and active training phase separately. The median trial duration for all experimental sharks was calculated per trial for all training days. A regression analysis tested the trend over the six trials for all ten training days.

The number of target contacts and rewards consumed during trials were converted to a percentage indicating success per training day for each shark. The mean percentage of target contacts and rewards consumed was calculated for all experimental sharks per training day. Linear regressions were calculated separately for the average percentage of target contacts and rewards consumed for all sharks and combined over training days. Statistical analysis included a Levene's test for homogeneity of variances, followed by three one-way ANOVA tests of the percentage of target contacts, food rewards consumed and the combined percentage of success in target contacts and rewards consumed during the active and passive training period.

4.5.3 Behavioural observations and interactions with the target between trials

The behavioural observations between trials were measured through analysing video footage of the trials. The percentage of time that the shark spent in the target half of the tank between trials was calculated (Eq. 1), seeing that time between trials were not consistent. The percentages were pooled for all experimental sharks and a linear regression was calculated over training days.

$$Time\ in\ target\ half\ between\ trials\ (\%) = \frac{Time\ in\ target\ half\ between\ trials (min)}{Total\ time\ between\ trials\ (min)} \quad \ldots \text{Eq. 1}$$

The number of approaches made to the target between trials was calculated per minute to eliminate bias due to the time between trials being inconsistent (Eq. 2). The number of approaches to the target per minute was pooled for all experimental sharks and a linear regression calculated over training days.

$$Approaches\ to\ target\ per\ minute = \left(\frac{Number\ of\ approaches\ to\ target}{Total\ time\ between\ trials\ (s)}\right) * 60 \qquad \ldots\text{Eq. 2}$$

4.5.4 Comparative analysis of individual sharks

To assess the individual performance of sharks, the trial durations and stress-related behaviours were analysed for the individuals and compared.

Box plots were created to show the distributions of trial durations for the experimental sharks and the control shark. A Levene's test for homogeneity of variances concluded that a non-parametric test should be done to test the individual trial durations for individual sharks. Further statistical analysis included a Kruskal Wallis H test for the variances of the trial durations of individual sharks. A multiple pairwise comparison using Dunn's procedure tested the similarity of individual shark trial durations.

A two-way ANOVA with replicates was calculated to assess the similarity of variances of the circling durations for individual sharks and also the number of spy hops for individual sharks.

5. Results

5.1 Observations of stress-related behaviours of Leopard catsharks

5.1.1 Circling duration

For all sharks the duration of circling time before insertion of the target was measured to establish a baseline for stress-related behaviour in a captive environment. A simple linear regression was calculated to predict the mean circling duration before the target was inserted into the tank, based on the training day. A significant regression equation was found $F(1,8) = 5.886$, $\rho < 0.05$ with $R^2 = 0.424$. The mean observed circling times before the insertion of the target into the tank as shown in figure 5.1.1.1 decreased significantly over training days.

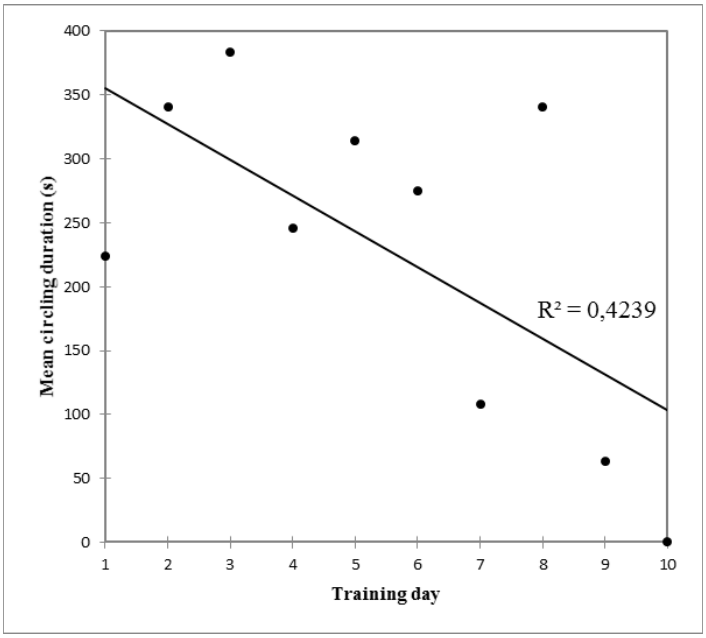

Figure 5.1.1.1: Mean circling duration of all four experimental Leopard catsharks measured for 15 minutes each training day before the target was inserted into the tank in Hermanus, South Africa (2015).

For all sharks the duration of circling time after insertion of the target was measured to establish resulting stress levels due to insertion of the target and also to test whether conditioning to the presence of the target took place. A linear regression was calculated to predict the mean circling duration after the target was inserted into the tank, based on the training day with a statistically insignificant result, $F(1,8) = 1.510$, $\rho > 0.05$ and $R^2 = 0.159$ (Appendix, Figure A).

A two-way analysis of variance tested the circling duration within 15 minutes before the insertion of the target and 15 minutes after the insertion of the target into the experimental tank. The results of the test indicated a significant difference between the circling durations before and the circling durations after insertion of the target over ten training days, *F*(1,72) = 58,007, ρ < 0.05 (Appendix, Table 1). The average circling duration for all sharks over the training period before insertion of the target is 229.48 seconds and the average circling time after insertion of the target 636.13 seconds. The average circling duration for all sharks is significantly higher after the target is inserted than before.

5.1.2 Number of spy hops

For all sharks the number of spy hops was measured before the target was inserted into the experimental tank. This measurement establishes a baseline for stress-related behaviour in a captive environment. The mean number of spy hops over ten training days before insertion of the target for all experimental sharks was calculated through a simple linear regression with a statistically insignificant result, *F*(1,8) = 0.689, ρ > 0.05 with $R^2 = 0.079$ (Appendix, Figure B).

The number of spy hops for all sharks was measured after insertion of the target into the experimental tank on each training day. The number of spy hops after inserting the target will indicate whether the sharks have increased stress levels due to the insertion of the target and also whether the sharks become conditioned to the presence of the target in a captive environment. The simple linear regression, seen in figure 5.1.2.1, was calculated to predict the mean number of spy hops based on training days. A significant regression equation was found *F*(1,8) = 6.057, ρ < 0.05 with $R^2 = 0.431$. The mean number of spy hops decrease significantly over the ten training days after the insertion of the target.

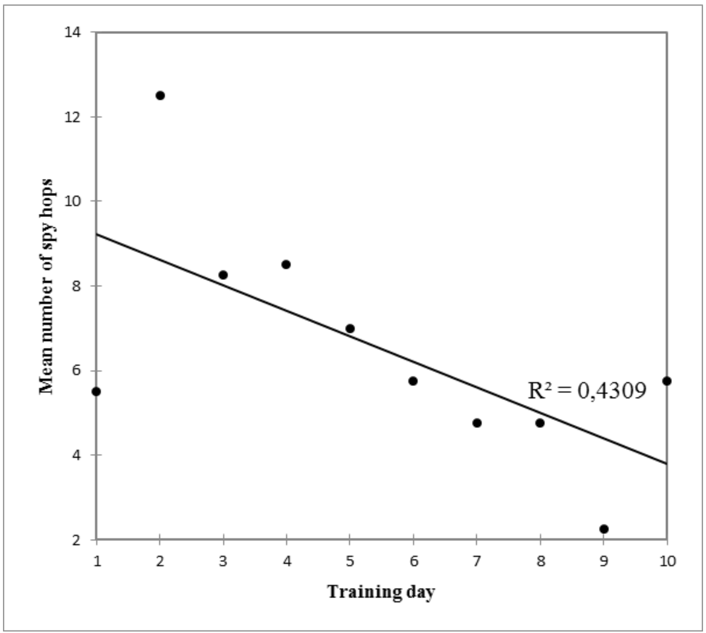

Figure 5.1.2.1: Mean number of spy hops of all four experimental Leopard catsharks measured for 15 minutes each day after the insertion of the target into the tank in Hermanus, South Africa (2015).

A two-way analysis of variance tested the number of spy hops within 15 minutes before the insertion of the target and 15 minutes after the insertion of the target into the experimental tank. The results of the test indicated toward a significant difference between the number of spy hops before and after insertion of the target for all sharks over ten training days, $F(1,72) = 53,089$, $\rho < 0.05$ (see Appendix, Table 2). The average number of spy hops for all experimental sharks over the training period before insertion of the target is 0.675 and 6.575 after the insertion of the target. The number of spy hops for all experimental sharks is significantly higher after the target is inserted into the tank than before it is inserted.

5.2 Efficiency of Leopard catsharks completing a food-reward task in a captive environment

5.2.1 Trial duration

The trial durations for all sharks over the ten-day training period is used as a measure of efficiency in completing the food-reward task. A simple linear regression was calculated to predict the median trial duration based on the training day. A significant regression equation was found $F(1,8) = 14.628$, $\rho < 0.05$ with an $R^2 = 0.647$. A statistically significant increase in median trial duration over training days is shown in figure 5.2.1.1.

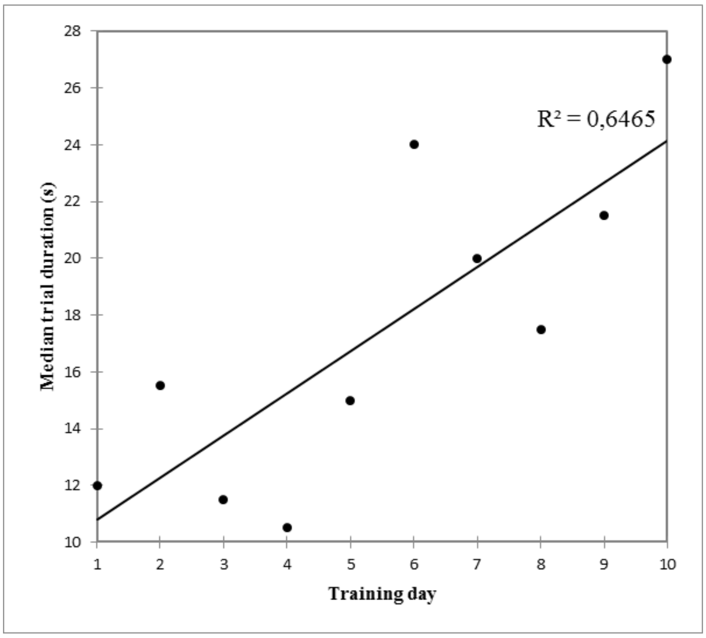

Figure 5.2.1.1: Median trial duration for all experimental Leopard catsharks over the training period in Hermanus, South Africa (2015).

The training process that lasted ten days consisted of two methods, namely the active training period and the passive training period, the median trial durations for these periods were also analysed separately. The resulting regression for the active training period (day 1 to 4) yielded a statistically insignificant regression, $F(1,2) = 0.683$, $\rho > 0.05$ with $R^2 = 0.255$ (Appendix, Figure C). The regression analysis for the passive training period (day 5 to 10) also showed a statistically insignificant result, $F(1,4) = 2.437$, $\rho > 0.05$ with $R^2 = 0.379$ (Appendix, Figure D). The average trial duration was 17.46 s for the active training period and 29.03 s for the passive training period

The median trial duration was also analysed per trial for all training days and all experimental sharks. The regression analyses yielded a statistically insignificant result, $F(1,4) = 0.199$, $\rho > 0.05$ with $R^2 = 0.048$ (Appendix, Figure E).

5.2.2 Number of target contacts and food rewards consumed

The success of completion of the food-reward task was measured by the number of target contacts and food rewards consumed during trials. The average percentage of successes per day for the reward consumed, target contacts and reward consumed and target contact combined was calculated per day for all experimental sharks as shown in table 5.2.2.1.

Table 5.2.2.1: The average percentage of successful rewards consumed (RC), target contacts (TC) and combined rewards consumed and target contacts (RC&TC) per day for all experimental Leopard catsharks in Hermanus, South Africa (2015).

DAY	RC	TC	RC&TC
1	100	87,5	93,75
2	91,7	87,5	89,6
3	83,3	91,7	87,5
4	91,7	79,1	85,4
5	91,7	95,8	93,75
6	87,5	95,8	91,65
7	95,8	91,7	93,75
8	95,8	95,8	95,8
9	87,5	87,5	87,5
10	95,8	95,8	95,8

Linear regressions were calculated independently for each variable over the ten training days. A statistically insignificant result was found for number of rewards consumed over training days, $F(1,8) = 0.001$, $\rho = 0.973$, $R^2 = 0.0002$ (Appendix, Figure F), the number of target contacts over training days, $F(1,8) = 1.971$, $\rho = 0.198$, $R^2 = 0.198$ (Appendix, Figure G) and the combined number of rewards consumed and target contacts over training days, $F(1,8) = 1.017$, $\rho = 0.343$, $R^2 = 0.113$ (Appendix, Figure H).

A Levene's test for homogeneity of variances showed that there is no significant difference in the variances of the three variables and it was therefore decided to conduct a one-way analysis of variance test to determine whether there is a significant difference in the means of the active (day 1-4) and passive (day 5-10) training periods. A one-way ANOVA testing the percentage of rewards consumed during the active training period and passive training period showed a statistically insignificant result, $F(1,8) = 0.039$, $\rho = 0.848$ (Appendix, Table 3). There is no significant difference in the means of percentage rewards consumed during the active and passive training period.

A one-way ANOVA testing the percentage of target contacts during the active and passive training period showed a statistically significant difference in means $F(1,8) = 7.080$, $\rho = 0.029$ (Appendix, Table 4). The percentage of target contacts during the active training period is significantly different to the percentage of target contacts during the passive training period contacts. With an average of 86.45% during the active training period and 93.73% during the passive training period, the number of target contacts is higher during the passive training period than the active training period.

A one-way ANOVA testing the percentage of the combined rewards consumed and target contacts for the active and passive training period yielded statistically insignificant results $F(1,8) = 3.495$, $\rho = 0.098$ (Appendix, Table 5).

5.3 Behavioural observations and interactions of Leopard catsharks with the target between trials

Behavioural observations were made between trials (5 intervals) to establish behavioural patterns over the training period with no food present in front of the target through video analysis.

5.3.1 Percentage of time spent in the target half of the tank between trials

The video recordings of the trials were analysed to determine the duration of time the sharks spent in the target half of the tank between trials with no food present in front of the target. A simple linear regression was calculated to predict the percentage of time spent in the target half of the tank between trials over the ten-day training period. A significant regression equation was found $F(1,36) = 1.765$, $\rho = 0.02$ with an $R^2 = 0.138$. A statistically significant increase in percentage of time spent in the target half of the tank between trials over training days is shown in figure 5.3.1.1.

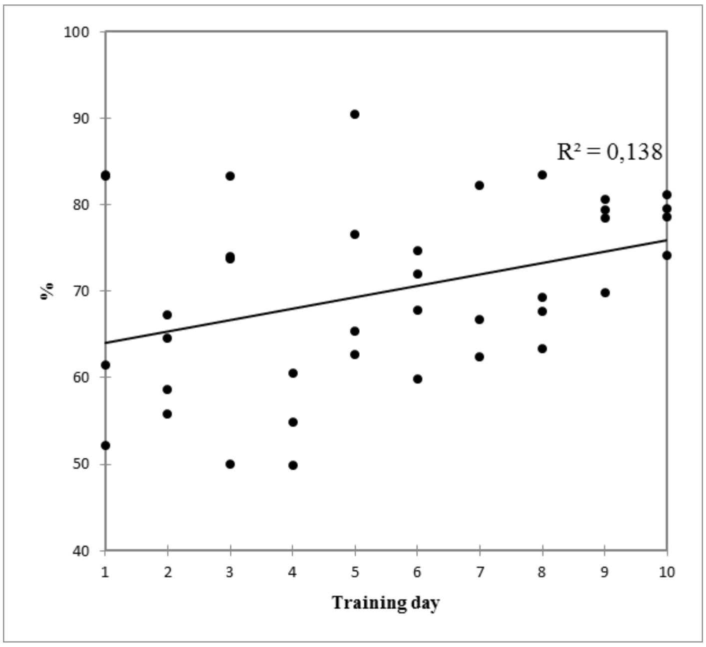

Figure 5.3.1.1: The percentage of time all experimental Leopard catsharks spent in the target half of the tank between trials over ten training days in Hermanus, South Africa (2015).

5.3.2 Number of approaches to the target between trials

The number of approaches that each shark made towards the target between trials with no food reward present, was calculated during video analysis. A linear regression was calculated to determine the number of approaches to the target over training days for all sharks. In figure 5.1.3.2 a statistically significant decrease in the number of approaches to the target per minute over the ten training days is shown, *F*(1,36) = 9.464, ρ = 0.004 and $R^2 = 0.208$.

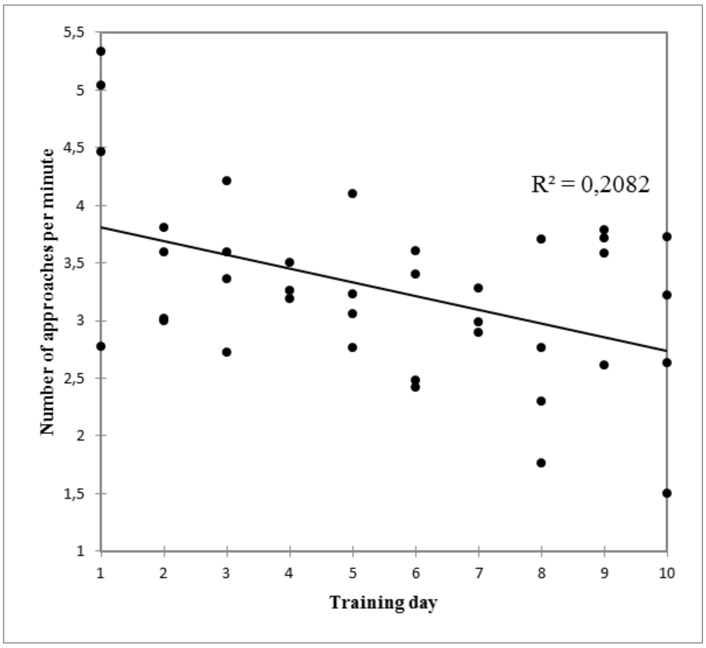

Figure 5.3.1.2: The number of approaches experimental Leopard catsharks made to the target per minute between trials over ten training days in Hermanus, South Africa (2015).

5.4 Comparative analysis of individual Leopard catsharks

5.4.1 Comparison of trial durations of individual sharks

A box plot was created as seen in figure 5.4.1.1 to visually describe the comparison of trial durations of the four experimental sharks and the control shark. From the plot it is clear that the trial durations of the control shark had a much wider range than that of all the experimental sharks. A Kruskal Wallis H test showed a statistically significant difference in the trial duration of the different sharks, $X^2(4) = 9.488$; $\rho < 0.05$, with a mean trial duration of 16.5 for shark A, 32.0 for shark B, 28.8 for shark C, 20.3 for shark D and 235.3 for the control.

A multiple pairwise comparison using Dunn's procedure showed that trial durations of shark A and D were grouped as similar, while shark B, C and D were grouped as similar and the control separate from all the other sharks (Appendix, Table 8).

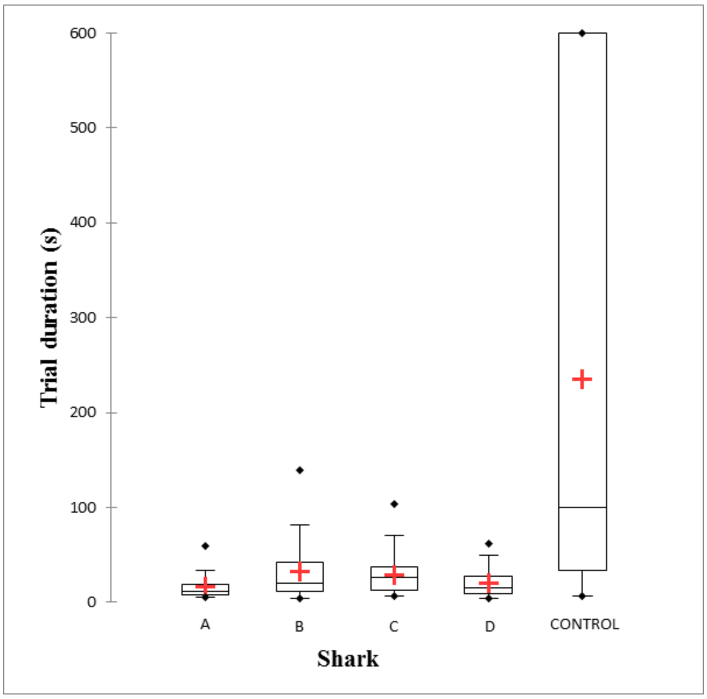

Figure 5.4.1.1 A boxplot presenting the trial duration distribution of individual Leopard catsharks in Hermanus, South Africa (2015).

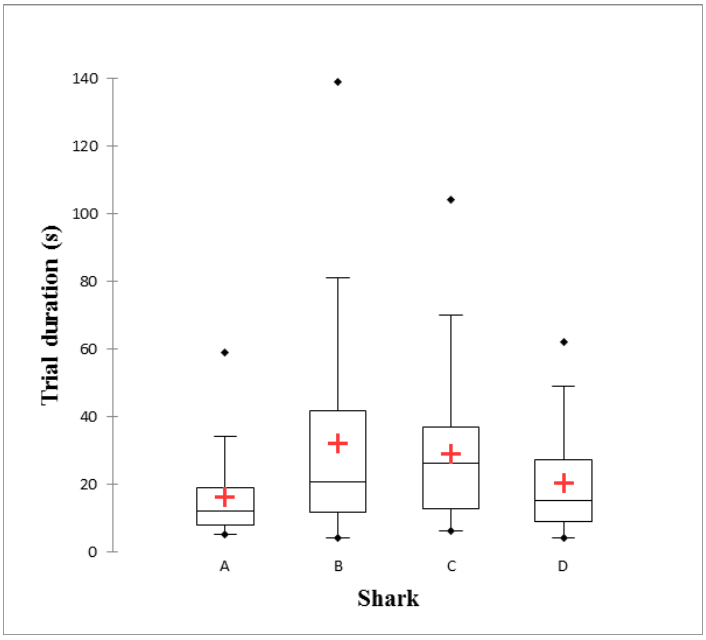

Figure 5.4.1.2 A boxplot presenting the trial duration distribution of individual experimental Leopard catsharks in Hermanus, South Africa (2015).

5.4.2 Comparison of stress-related behavioural observations for individual sharks

A two-way analysis of variance tested the circling duration of individual sharks within 15 minutes before insertion of the target and 15 minutes after insertion of the target into the experimental tank. The results of the test indicated a significant difference between the circling durations of individual sharks over ten training days, $F(4,90) = 19.703$, $\rho < 0.05$ (Appendix, Table 6).The total average circling duration for shark A is 432.5 s, 482.05 for shark B, 292.05 for shark C, 524.6 for shark D and 1.1 for the control shark.

To test the similarity of variances of the number of spy hops before and after insertion of the target for individual sharks, a two-way analysis of variance was calculated. The result showed a significant difference in the number of spy hops of individual sharks over the ten training days, $F(4,90) = 13.640$, $\rho < 0.05$ (Appendix, Table 7). The total average number of spy hops was 2 for shark A, 4.1 for shark B, 1.5 for shark C, 6.9 for shark D and 0 for the control shark.

6. Discussion

6.1 Stress-related behaviours

In this study all experimental sharks were found to have increased stress-related behaviours after the target was inserted into the experimental tank. The increase in activity of the sharks, measured as circling duration and number of spy hops, has been used in previous studies to assess stress in elasmobranchs where a change has been made to the captive environment (Charbeneau, 2004; Lober, 2014). The increase in stress after the target was inserted is to be expected as a change occurred in the captive environment.

It is interesting to note the deviation from the trend in stress-related behaviours on day two and eight. On those two days the average circling durations after insertion of the target and number of spy hops before insertion of the target peak. The mean circling duration before insertion of the target show a peak on day three and day eight, and the number of spy hops after insertion of the target peak on day two. There are many variables that can contribute to increased stress levels. Seeing that the sharks were starved for the three day acclimatisation period before day one of training, it is possible that stress-related behaviours are low on day one due to decreased energy levels. The peak in stress-related behaviours on day two or three (circling duration before insertion of the target) could be due to the increase in energy after the shark has eaten. The reason for the peak in stress levels on day eight is unclear. Stress is a reaction to any variable that causes imbalance to the internal environment (homoeostasis) of an organism and can be caused by many biotic and abiotic variables, such as variability in water temperature and oxygen levels, physical injury, modification of the external environment or mechanical vibrations (Smith, 1992). Seeing that all experimental sharks had increased stress levels on their eighth training day, even though it was on a different day for each shark, further research is needed to determine the reason for the peak in stress-related behaviours at that stage of instrumental conditioning.

The decrease in average circling duration before insertion of the target could indicate that habituation to the experimental tank occurred. The number of spy hops before insertion of the target however, showed no significant change over time. If the outlier on day eight had to be removed from the time-series, the number of spy hops would have shown a significant decreasing trend over training days before insertion of the target. Even though the average number of spy hops are higher after the target was inserted, it showed a decreasing trend over training days, indicating decreased stress over time. It is possible that habituation to the presence of the target was occurring as training days progressed.

6.2 Efficiency in completing food-reward tasks

The trial durations for all experimental sharks increased significantly over ten training days. The average trial duration for the active training phase (day 1-4) is lower than the average trial duration or the passive training phase (day 5-10). This is to be expected as the sharks were not lead to the target during the passive training phase, but rather allowed to approach the target voluntarily. Neither the active nor passive training phases showed a significant trend over training days and this could be due to the low number of days in each phase.

The increase in median trial duration could possibly be affected by satiation. Even though sharks were fed only 1.5% of their body weight per day to ensure the need to feed on the next training day, it is possible that the sharks became satiated after consecutive days of feeding and motivation to complete trials in short time frames decreased.

It is important to note the increase in trial duration on day two. As mentioned in section 6.1, the stress-related behaviours of all sharks peaked on day two. It is possible that the increased stress levels on the second training day could have contributed to increased trial durations on that day for all sharks.

The number of target contacts and rewards consumed showed no significant trends over the ten training days. It is interesting however, that the number of target contacts was significantly higher in the passive training phase than in the active training phase. This implies that the sharks became conditioned to the target, which acted as the visual stimulus, and therefore made physical contact with the target more often when retrieving the food reward as training progressed.

The average percentage of rewards consumed for all sharks are high (above 80%) across training days. On the first training day 100% consumption of the reward is to be expected as the sharks were starved for the three day acclimatisation period before day one of training ensued. The decrease in consumption of the reward over day two and three of training could be due to the increase in stress because of the process of conditioning and training. From day four to ten the percentage of rewards consumed increase and could indicate a potential significant increasing trend if the training had to continue for more than ten days. An interesting pattern develops from day four to ten where alternate higher and lower percentage of rewards are consumed with two days higher and one day low followed by two days higher and the next day low again. It would be interesting to determine whether this is a pattern that would continue if training lasted more than ten days. It is possible that the alternating pattern could occur because of satiation and digestion. Menon and Kewalramani (1958) has shown that gastric digestion in the Grey bamboo shark (*Chiloscyllium* griseum) was complete within 24 hours of feeding and within 48 hours the food contents reached the rectal region. It was also found that after 72 hours of feeding the entire intestine became alkaline, so even though only 1.5% of their body weight is being fed to the sharks each day during training, it is possible that after two days of feeding, the intestines become highly acidic during digestion (Menon and Kewalramani, 1958) and a decrease in rewards consumed occurs on the third day. Another factor that could cause the decrease in rewards consumed on day three and day nine, is the increased stress level on day two and eight. It is possible that the increased stress caused the sharks to consume less food on the following day during trials. More research is needed to determine the feeding patterns of Leopard catsharks and whether this could influence training outcomes that are based on food-reward tasks.

Over the ten-day training period however, it is not possible to confirm that sharks learnt to complete the task, as the trial duration increased over training days and the number of target contacts and rewards consumed show no significant trend over time. The increased number of target contacts in the passive training phase however, point towards conditioning occurring within ten training days.

6.3 Behavioural observations and interactions with the target between trials

The measurements and behavioural observations made during the five intervals between trials on each day gave further insight into the rates of learning and conditioning. No food was present in the tank between trials with the target being the only stimulus during the interval.

All experimental sharks were found to spend more time in the target half of the tank as training days progressed. The normal behaviour of the sharks would be to circle the tank, but seeing that all sharks spent more than 50% of the time during the interval in the target half of the tank (except for one shark on day 3 and 4), and this percentage increased significantly with training days, it is possible that the sharks learnt to associate the target (visual stimulus) with the food reward. Increased amounts of time were spent in the target half of the tank as the sharks learnt that the food-reward would be presented in front of the target. It has been shown that goldfish and stingrays are capable of remembering feeding locations even with alternating starting positions (Rodriguez et al., 1994; Schluessel and Bleckmann, 2005) and the performance of the rays decreased significantly when the visual stimulus was removed, suggesting that the visual sense plays a primary role for rays (Rodriguez, et al., 1994).

It would be expected then that the shark would spend increasing amounts of time approaching the target directly, seeking out the food reward. The results show that the number of target approaches between trials decrease significantly over training days. It is very interesting that the sharks spent more time in the target half of the tank, but made less direct approaches to the target between trials. It could be suggested that the sharks made less approaches to the target over time as they learnt to wait for the auditory cue that announces the presence of the food reward in front of the target. It is possible that the sharks learnt to associate the auditory signal with the presence of the food reward. It has been suggested that sharks can learn to associate sound cues with food in their natural environment through anthropogenic impacts such as fishing (Wetherbee et al., 2010).

6.4 Assessment of individual shark behaviour

Each shark used in this study had significantly different trial durations and stress-related behaviours. The large range in trials durations for the control shark, compared to the experimental sharks could indicate that the addition of an auditory cue as a bridging stimulus during training is very effective and can hasten the process of conditioning for Leopard catsharks. It has been shown before with other elasmobranch species that a bridging stimulus such as an auditory cue can increase the frequency of correct responses when combined with the visual stimulus (Sabalones et al., 2004).

It is important to note that the experimental sharks consisted of three females and one male. Due to the skewed sex ratio and low sample size conclusions about sex having an influence on learning rates could not be made. It is interesting however, that even though the trial durations for all sharks were shown to be significantly different, the three female sharks were shown to have the most similar range in trial durations. Further investigation is necessary to determine whether sex plays a role in conditioning and learning rates.

All individual sharks showed different stress levels throughout the training process. This could mean that Leopard catshark individuals would react to stressors such as changing environments differently and adapt accordingly.

A study done by Wright and Jackson (1964) on the learning ability of Lemon sharks and Bull sharks showed that the Lemon sharks learnt faster than the Bull sharks and that conspecific learning rates were similar. The sharks in that study however, was not tested individually and facilitation of learning could have occurred between subjects. The finding of this study that Leopard catshark individuals show different learning rates and stress reactions, should be further investigated.

6.5 Limitations and recommendations for further studies

A limitation of this study was the six months' time frame that was determined by the structure of the coursework masters degree. It took longer than expected to capture Leopard catsharks for experiments. The pump that ensures a constant flow of sea water through the tanks also stopped working twice during the experimental period, and water quality meters for temperature and oxygen failed, hence delaying the experiments. The sharks had to be released during those events and all of the experiments restarted. This resulted in a low sample size, another major limitation in this study. The failure of equipment for water temperature and oxygen measures limited the data collection on environmental variables.

Recommendations for further studies on the conditioning and learning of benthic sharks should be conducted over longer time periods and with larger sample sizes. The addition of environmental data such as water temperature and oxygen levels would complete the experimental design. The rate of conditioning should also be tested during night time as Leopard catsharks are mostly nocturnal.

6.6 Conclusion

In conclusion, Leopard catsharks show the potential to learn to associate food rewards with a visual stimulus of a target in combination with an auditory cue. The auditory cue was shown to increase the learning rate and improve the efficiency of the conditioning process. Even though the associations between the visual and auditory stimuli and a food reward were formed, the efficiency for the completion of trials did not improve over ten days of training. The results indicate that stress-related behaviours could decrease over time as the sharks become conditioned, but further research is required to assess the effects of stress on conditioning in a captive environment through a lengthier conditioning process. Individual

Leopard catsharks exhibit dissimilar learning rates and adaptations to changes in the captive environment through stress-related behaviours. This could imply that individual Leopard catsharks might adapt to stressful situations and learn to associate sound cues with food at different rates in the wild. This has implications for understanding conspecific behaviour of Leopard catsharks and the ability of populations to withstand anthropogenic impacts through fishing and accidental bycatch. As it has been found for other reef shark species, it is suggested that Leopard catsharks have the ability to learn to associate the sound of outboard motors of a fishing boat with food through many events of depredation. Further research is required to assess the conditioning of Leopard catsharks in their natural environment as it could contribute to an increase of interactions with fishing lines for a food reward.

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8. Acknowledgements

I would like to give a very special thank you to my course convenor and supervisor, Cecile Reed for all her assistance and amazing ability to bring calm to a stressful situation – I could not have done this without her support! I would also like to thank The South African Shark Conservancy for providing me with facilities and insight for conducting the experiments for this project. To my family and friends, you formed the consistent support system that carried me through this process, thank you!

9. Appendix

9.1 Figures

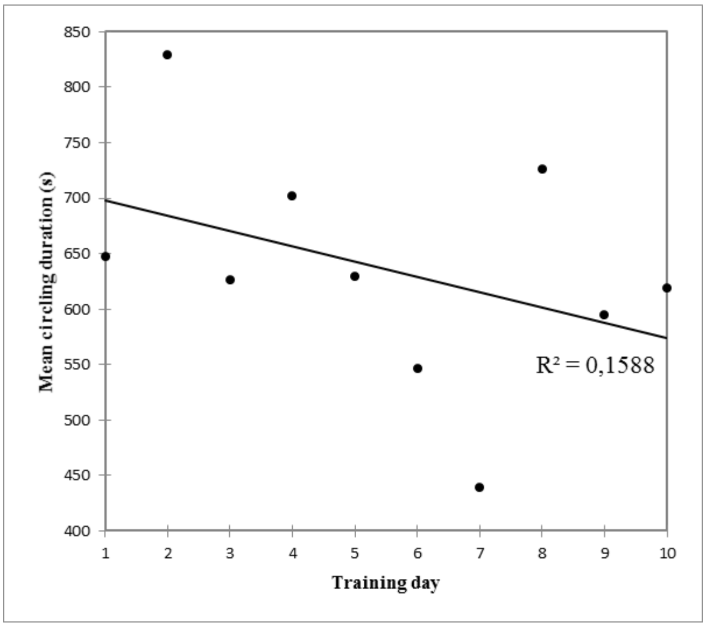

Figure A: Mean circling duration of all four experimental sharks measured for fifteen minutes each training day after insertion of the target in Hermanus, South Africa (2015).

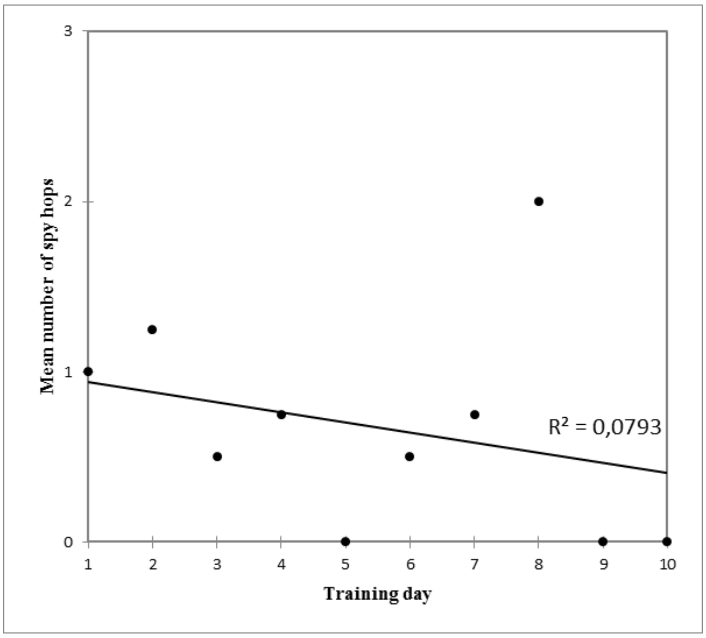

Figure B: Mean number of spy hops of all four experimental sharks measured for fifteen minutes each day before insertion of the target in Hermanus, South Africa (2015).

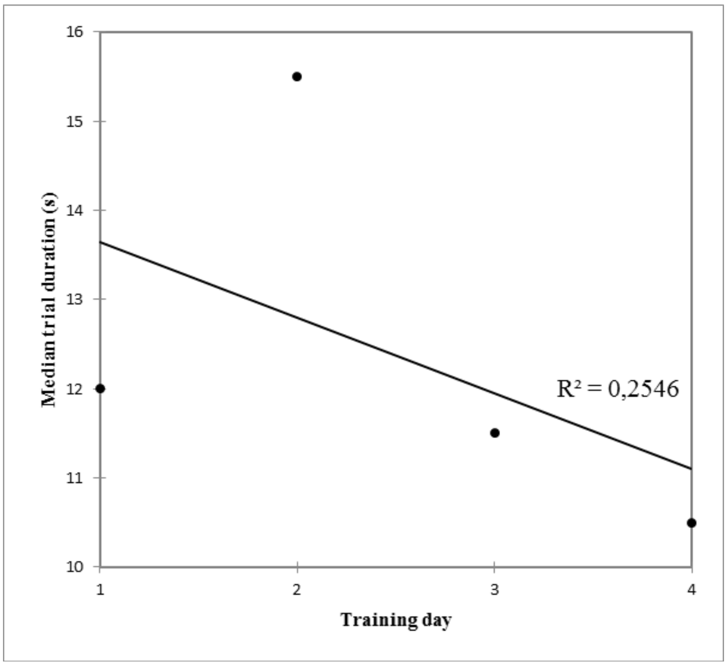

Figure C: Median trial duration for all experimental sharks over the active training period in Hermanus, South Africa (2015).

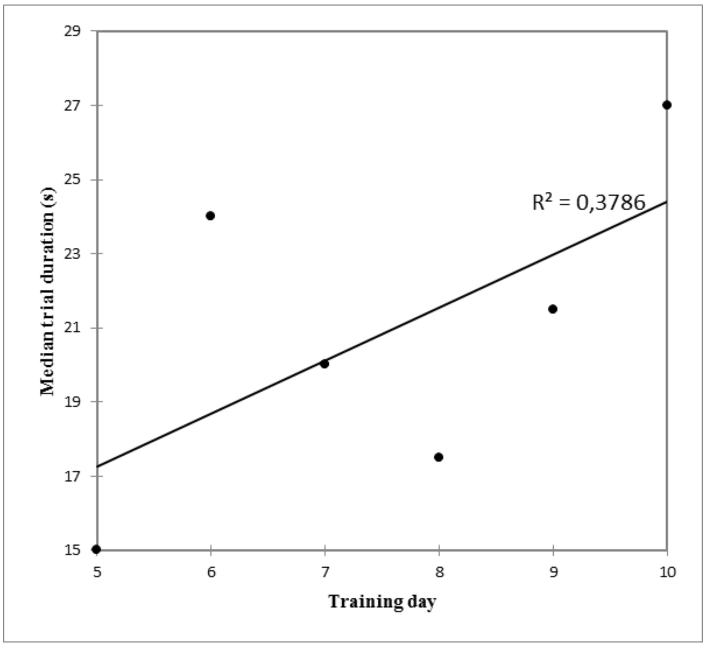

Figure D: Median trial duration for all experimental sharks over the passive training period in Hermanus, South Africa (2015).

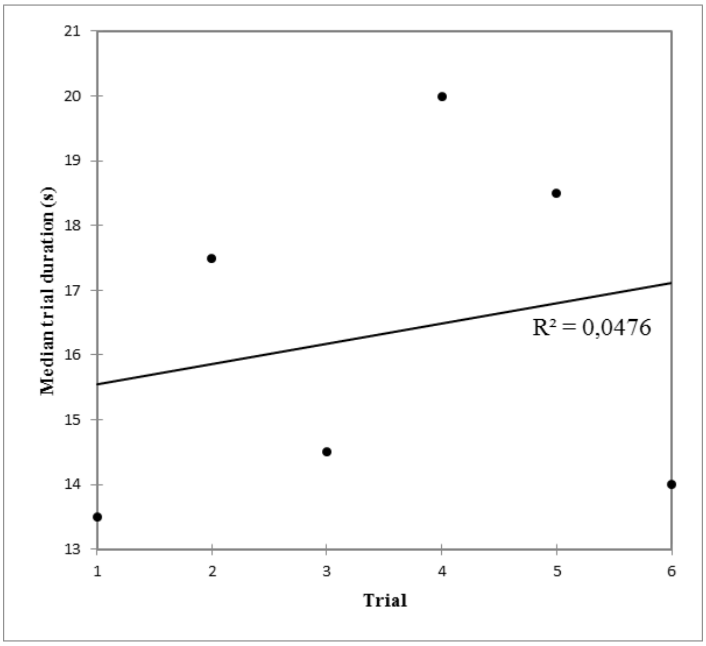

Figure E: Median trial duration per trial for all experimental sharks over the ten-day training period in Hermanus, South Africa (2015).

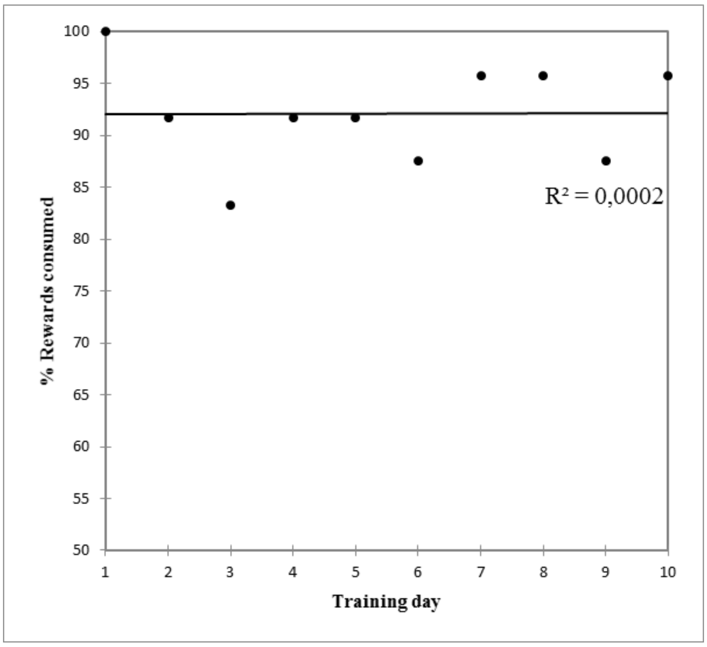

Figure F: Percentage of food rewards consumed by all experimental sharks over six trials per day for ten training days in Hermanus, South Africa (2015).

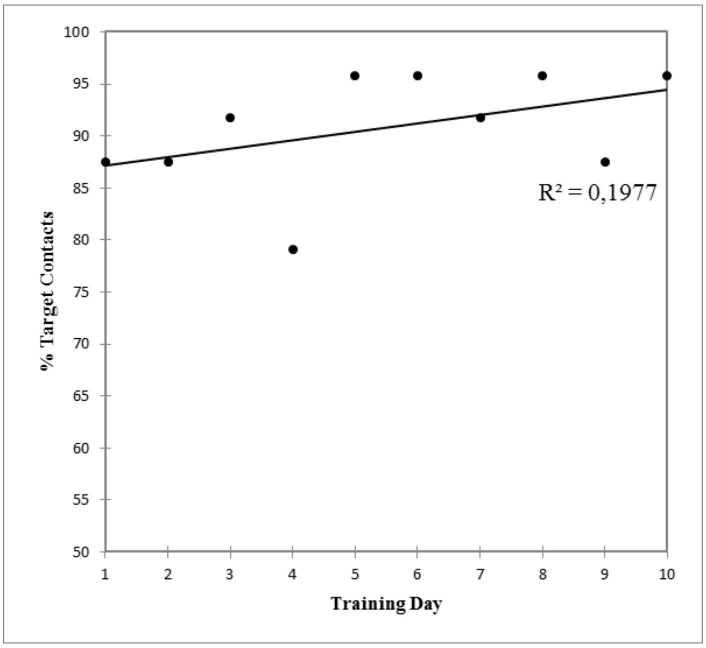

Figure G: Percentage of target contacts by all experimental sharks over six trials per day for ten training days in Hermanus, South Africa (2015).

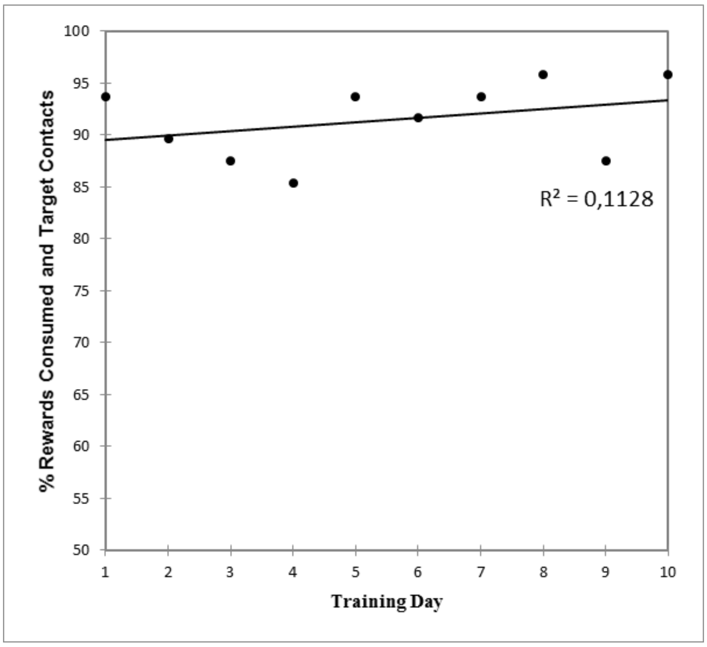

Figure H: Percentage of rewards consumed and target contacts by all experimental sharks over six trials per day for ten training days in Hermanus, South Africa (2015).

9.2 Tables

Table 1: Two-way ANOVA test with replication for the circling duration before and after the insertion of the target in Hermanus, South Africa (2015).

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	3307284	1	3307284,45	58,00693	7,96E-11	3,973897
Columns	613269,1	3	204423,0333	3,585404	0,01779	2,731807
Interaction	537611,1	3	179203,6833	3,143079	0,030353	2,731807
Within	4105104	72	57015,33611			
Total	8563269	79				

Table 2: Two-way ANOVA test with replication for the of number of spy hops before and after the insertion of the target in Hermanus, South Africa (2015).

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	696,2	1	696,2	53,08875	3,27E-10	3,973897
Columns	362,15	3	120,7167	9,205253	3,13E-05	2,731807
Interaction	156,2	3	52,06667	3,970345	0,011209	2,731807
Within	944,2	72	13,11389			
Total	2158,75	79				

Table 3: One-way ANOVA testing the percentage of rewards consumed for the active (day 1-4) and passive (day 5-10) training periods in Hermanus, South Africa (2015).

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1,0935	1	1,0935	0,039295	0,84781	5,3176551
Within Groups	222,6225	8	27,82781			
Total	223,716	9				

Table 4: One-way ANOVA testing the percentage of target contacts for the active (day 1-4) and passive (day 5-10) training periods in Hermanus, South Africa (2015).

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	127,3127	1	127,3127	7,0796	0,028772	5,317655
Within Groups	143,8633	8	17,98292			
Total	271,176	9				

Table 5: One-way ANOVA testing the percentage of combined rewards consumed and target contacts for the active (day 1-4) and passive (day 5-10) training periods in Hermanus, South Africa (2015).

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	38,00104	1	38,00104	3,494993	0,098484	5,317655
Within Groups	86,98396	8	10,87299			
Total	124,985	9				

Table 6: Two-way ANOVA test with replication for the circling duration of individual sharks before and after the insertion of the target in Hermanus, South Africa (2015).

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	3595107,34	4	898776,8	19,70263	1,12E-11	2,472927
Columns	2652989,44	1	2652989	58,15777	2,39E-11	3,946876
Interaction	1191930,26	4	297982,6	6,532255	0,000116	2,472927
Within	4105539,8	90	45617,11			
Total	11545566,84	99				

Table 7: Two-way ANOVA test with replication for the number of spy hops of individual sharks before and after the insertion of the target in Hermanus, South Africa (2015).

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	572,4	4	143,1	13,64011862	9,85303E-09	2,472927
Columns	556,96	1	556,96	53,08875238	1,17465E-10	3,946876
Interaction	295,44	4	73,86	7,040245711	5,60637E-05	2,472927
Within	944,2	90	10,49111			
Total	2369	99				

Table 8: A multiple pairwise comparison of trial durations using Dunn's procedure in Hermanus, South Africa (2015).

Sample	Frequency	Sum of ranks	Mean of ranks	Groups		
SHARK A	60	5835,000	97,250	A		
SHARK D	60	7130,000	118,833	A	B	
SHARK B	60	8963,000	149,383		B	
SHARK C	60	9366,500	156,108		B	
CONTROL	60	13855,500	230,925			C

Table A1: Experimental data sheet for instrumental conditioning of Leopard catshark (*Poroderma pantherinum)*
A on day 1 to 3 in Walker Bay, Hermanus

Key	
ATT	Approach to target
TITH	Time in target half of the tank between trials (no food present)
TBT	Total time between trials
% TITH	Percentage of time in target half of tank between trials

MARK	Outer left pec	**TAG #**	
		RECAP	N
Capture date	07/10/2015	**Total length(cm)**	44,6
Species	Poroderma panthera	**Weight(g)**	550
Sex	Male	**Food p/d (g)**	8,25

		R time (min:s)	**No of R events (#)**	**CIR time (min:s)**	**No of CIR events (#)**	**No of SPY (#)**	**D time (min:s)**	**Special behviours (RO, HS, BR)**	**Approaches to target (#)**	**COMMENTS**
Training day	**BEHAVIOUR**									
1	Before inserting target (15min)	07:21	5	07:43	4	0	0	0	0	
Date:	After inserting target(15min)	0	0	15:00	1	10	0	0	0	Anxious
10/11/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	15:04	15:05	15:06	15:08	15:09	15:10		ATT: 12	Lead in every trial
	Trial end time (hh:min:s)	15:04	15:05	15:07	15:08	15:09	15:10		TITH: 2:15	Not scared of target
	Trial duration (min:s)	00:33	00:27	00:20	00:07	00:10	00:09		TBT: 4:19	
	Target contact (Y/N)	Y	Y	Y	Y	Y	Y		% TITH: 52,1	
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			

		R time (min:s)	**No of R events (#)**	**CIR time (min:s)**	**No of CIR events (#)**	**No of SPY (#)**	**D time (min:s)**	**Special behviours (RO, HS, BR)**	**Approaches to target (#)**	**COMMENTS**
Training day	**BEHAVIOUR**									
2	Before inserting target (15min)	12:58	3	01:16	2	0	0	0	0	
Date:	After inserting target(15min)	02:22	2	12:39	2	3	0	0	0	
11/11/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	15:23	15:24	15:26	15:26	15:28	15:30		ATT: 19	Lead in all trials
	Trial end time (hh:min:s)	15:23	15:24	15:26	15:27	15:28	15:30		TITH: 3:41	sharp turns back onto target
	Trial duration (min:s)	00:08	00:09	00:08	00:14	00:10	00:10		TBT: 6:17	
	Target contact (Y/N)	Y	N	Y	Y	Y	Y		% TITH: 58,62	
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	N			

		R time (min:s)	**No of R events (#)**	**CIR time (min:s)**	**No of CIR events (#)**	**No of SPY (#)**	**D time (min:s)**	**Special behviours (RO, HS, BR)**	**Approaches to target (#)**	**COMMENTS**
Training day	**BEHAVIOUR**									
3	Before inserting target (15min)	03:10	6	11:11	6	2	0	0	0	
Date:	After inserting target(15min)	0	0	15:00	1	1	0	0	0	
12/11/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	15:13	15:14	15:15	15:16	15:17	15:18		ATT: 16	
	Trial end time (hh:min:s)	15:13	15:14	15:15	15:16	15:17	15:18		TITH: 2:10	Lead in all trials
	Trial duration (min:s)	00:07	00:09	00:06	00:06	00:13	00:09		TBT: 3:48	
	Target contact (Y/N)	Y	Y	Y	Y	Y	Y		% TITH: 83,33	
	Reward consumed (Y/N)	Y	Y	Y	Y	N	Y			

Table A2: Experimental data sheet for instrumental conditioning of Leopard catshark (*Poroderma pantherinum)* A on day 4 to 6 in Walker Bay, Hermanus

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
4	Before inserting target (15min)	07:40	7	06:49	8	0	0	0	0	
Date:	After inserting target(15min)	00:56	1	14:00	1	7	0	0	0	
13/11/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	14:09	14:11	14:12	14:13	14:14	14:15		ATT: 16	
	Trial end time (hh:min:s)	14:09	14:11	14:12	14:13	14:14	14:15		TITH: 2:46	
	Trial duration (min:s)	00:05	00:07	00:08	00:07	00:13	00:07		TBT: 4:34	
	Target contact (Y/N)	Y	Y	Y	Y	Y	Y		%TITH: 60,58	
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
5	Before inserting target (15min)	09:15	8	05:45	5	0	0	0	0	
Date:	After inserting target(15min)	03:23	4	11:36	6	3	0	0	0	
14/11/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	15:58	15:59	15:59	16:01	16:03	16:04		ATT: 12	
	Trial end time (hh:min:s)	15:58	15:59	16:00	16:01	16:03	16:05		TITH: 2:43	
	Trial duration (min:s)	00:05	00:14	00:08	00:14	00:12	00:46		TBT: 4:20	
	Target contact (Y/N)	Y	Y	Y	Y	N	Y		% TITH: 62,69	
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
6	Before inserting target (15min)	15:00	1	0	0	0	0	0	0	
Date:	After inserting target(15min)	06:20	5	08:37	7	4	0	0	0	
15/11/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		Video analysis	
	Trial start time (hh:min:s)	13:32	13:33	13:35	13:36	13:38	13:39		ATT: 16	
	Trial end time (hh:min:s)	13:32	13:34	13:35	13:37	13:38	13:40		TITH: 3:57	
	Trial duration (min:s)	00:07	00:57	00:09	00:45	00:31	00:33		TBT:6:36	
	Target contact (Y/N)	Y	Y	Y	Y	N	Y		% TITH: 59,84	
	Reward consumed (Y/N)	Y	Y	Y	N	Y	Y			

Table A3: Experimental data sheet for instrumental conditioning of Leopard catshark (*Poroderma pantherinum)* A on day 7 to 10 in Walker Bay, Hermanus (2015)

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
7	Before inserting target (15min)	15:00	1	0	0	1	0	0	0	
Date:	After inserting target(15min)	04:57	5	10:03	9	2	0	0	0	
16/11/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	14:50	14:51	14:53	14:54	14:55	14:57		ATT: 11	
	Trial end time (hh:min:s)	14:50	14:51	14:53	14:54	14:56	14:57		TITH: 2:14	
	Trial duration (min:s)	00:18	00:13	00:06	00:13	00:32	00:22		TBT: 3:21	
	Target contact (Y/N)	Y	Y	Y	Y	Y	Y		% TITH: 66,67	
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
8	Before inserting target (15min)	10:50	4	04:10	2	0	0	0	0	
Date:	After inserting target(15min)	04:30	3	10:30	6	3	0	0	0	
17/11/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		Video analysis	
	Trial start time (hh:min:s)	14:56	14:57	14:59	15:01	15:02	15:04		ATT: 10	
	Trial end time (hh:min:s)	14:56	14:57	14:59	15:01	15:03	15:04		TITH: 2:44	
	Trial duration (min:s)	00:09	00:08	00:18	00:09	00:59	00:13		TBT: 4:21	Very murky water
	Target contact (Y/N)	Y	Y	Y	Y	Y	Y		% TITH: 63,4	
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
9	Before inserting target (15min)	11:47	7	03:13	3	0	0	0	0	
Date:	After inserting target(15min)	09:21	3	05:40	2	2	0	0	0	
18/11/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	14:55	14:56	14:58	15:00	15:01	15:03		ATT: 14	
	Trial end time (hh:min:s)	14:56	14:56	14:58	15:00	15:02	15:03		TITH: 3:44	Long rest on target
	Trial duration (min:s)	00:09	00:07	00:30	00:12	00:29	00:08		TBT: 5:21	
	Target contact (Y/N)	Y	Y	Y	N	Y	Y		% TITH: 69,78	
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
10	Before inserting target (15min)	15:00	1	0	0	0	0	0	0	
Date:	After inserting target(15min)	08:48	8	06:12	6	2	0	0	0	
19/11/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	15:02	15:03	15:04	15:05	15:06	15:08		ATT: 7	
	Trial end time (hh:min:s)	15:02	15:04	15:04	15:05	15:07	15:08		TITH: 3:40	
	Trial duration (min:s)	00:34	00:22	00:13	00:27	00:17	00:18		TBT: 4:40	
	Target contact (Y/N)	Y	Y	Y	Y	Y	Y		% TITH: 78,59	
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			

Table B1: Experimental data sheet for instrumental conditioning of Leopard catshark (*Poroderma pantherinum*) B on day 1 to 3 in Walker Bay, Hermanus

Key	
ATT	Approach to target
TITH	Time in target half of the tank between trials (no food present)
TBT	Total time between trials
% TITH	Percentage of time in target half of tank between trials

	RECAP	N	
Capture date	27/08/2015	**Total length(cm)**	45,5
Species	Poroderma panthera	**Weight(g)**	520
Sex	Female	**Food p/d (g)**	7,8

		R time (min:s)	**No of R events (#)**	**CIR time (min:s)**	**No of CIR events (#)**	**No of SPY (#)**	**D time (min:s)**	**Special behviours (RO, HS, BR)**	**Approaches to target (#)**	**COMMENTS**
Training day	**BEHAVIOUR**									
1	Before inserting target (15min)	15:00	1	0	0	0	0	0	0	
Date:	After inserting target(15min)	03:21	2	11:45	3	0	0	0	0	
24/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	15:45	15:47	15:48	15:49	15:50	15:52		ATT: 16	Lead in all trials
	Trial end time (hh:min:s)	15:46	15:48	15:48	15:49	15:50	15:52		TITH: 2:30	Interested in target
	Trial duration (min:s)	00:13	00:15	00:09	00:21	00:12	00:09		TBT: 3:00	
	Target contact (Y/N)	Y	Y	Y	Y	Y	N		% TITH: 83,3	
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			
		R time (min:s)	**No of R events (#)**	**CIR time (min:s)**	**No of CIR events (#)**	**No of SPY (#)**	**D time (min:s)**	**Special behviours (RO, HS, BR)**	**Approaches to target (#)**	**COMMENTS**
Training day	**BEHAVIOUR**									
2	Before inserting target (15min)	00:11	1	14:45	3	3	0	0	0	
Date:	After inserting target(15min)	0	0	15:00	1	10	0	0	0	
25/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	14:30	14:31	14:32	14:32	14:33	14:35		ATT: 12	
	Trial end time (hh:min:s)	14:30	14:31	14:32	14:33	14:34	14:35		TITH: 2:02	Lead in all trials
	Trial duration (min:s)	00:19	00:14	00:15	00:10	00:20	00:07		TBT: 3:09	
	Target contact (Y/N)	Y	Y	Y	Y	Y	Y		% TITH: 64,55	
	Reward consumed (Y/N)	Y	Y	N	Y	Y	Y			
		R time (min:s)	**No of R events (#)**	**CIR time (min:s)**	**No of CIR events (#)**	**No of SPY (#)**	**D time (min:s)**	**Special behviours (RO, HS, BR)**	**Approaches to target (#)**	**COMMENTS**
Training day	**BEHAVIOUR**									
3	Before inserting target (15min)	13:58	5	01:02	4	0	0	0	0	
Date:	After inserting target(15min)	02:31	2	11:36	2	17	0	HS x 1	0	
26/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	15:05	15:06	15:09	15:10	15:11	15:12		ATT: 16	
	Trial end time (hh:min:s)	15:05	15:07	15:09	15:10	15:11	15:12		TITH: 3:30	
	Trial duration (min:s)	00:09	00:56	00:18	00:19	00:13	00:04		TBT: 4:45	
	Target contact (Y/N)	Y	Y	Y	Y	Y	Y		% TITH: 73,68	
	Reward consumed (Y/N)	Y	N	Y	Y	Y	Y			

Table B2: Experimental data sheet for instrumental conditioning of Leopard catshark (*Poroderma pantherinum)* B on day 4 to 6 in Walker Bay, Hermanus

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
4	Before inserting target (15min)	15:00	1	0	0	0	0	0	0	
Date:	After inserting target(15min)	0	0	15:00	1	12	0	0	0	
27/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	14:47	14:48	14:51	14:53	14:54	14:55		ATT: 17	
	Trial end time (hh:min:s)	14:48	14:50	14:52	14:53	14:54	14:56		TITH: 2:36	
	Trial duration (min:s)	00:51	01:09	00:33	00:10	00:24	00:33		TBT: 5:13	
	Target contact (Y/N)	N	N	Y	Y	Y	Y		% TITH: 49,84	
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
5	Before inserting target (15min)	15:00	1	0	0	0	0	0	0	
Date:	After inserting target(15min)	00:27	1	13:41	2	10	0	0	0	
28/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	15:02	15:03	15:04	15:06	15:08	15:10		ATT: 16	
	Trial end time (hh:min:s)	15:02	15:03	15:05	15:07	15:09	15:10		TITH: 2:59	
	Trial duration (min:s)	00:28	00:06	01:30	01:10	00:33	00:25		TBT: 3:54	
	Target contact (Y/N)	Y	Y	Y	Y	Y	Y		% TITH: 76,5	
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
6	Before inserting target (15min)	08:18	5	05:46	4	0	0	0	0	
Date:	After inserting target(15min)	0	0	15:00	1	12	0	0	0	
29/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	14:30	14:31	14:34	14:36	14:37	14:38		ATT: 17	
	Trial end time (hh:min:s)	14:30	14:32	14:35	14:36	14:37	14:38		TITH: 3:12	
	Trial duration (min:s)	00:10	01:01	01:21	00:09	00:27	00:06		TBT: 4:43	
	Target contact (Y/N)	Y	Y	Y	Y	Y	Y		% TITH: 67,84	
	Reward consumed (Y/N)	Y	Y	Y	N	Y	Y			

Table B3: Experimental data sheet for instrumental conditioning of Leopard catshark (*Poroderma pantherinum*) B on day 7 to 10 in Walker Bay, Hermanus

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
7	Before inserting target (15min)	15:00	1	0	0	0	0	0	0	
Date:	After inserting target(15min)	03:40	2	11:19	2	5	0	0	0	
30/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	15:02	15:03	15:05	15:07	15:09	15:12		ATT: 17	
	Trial end time (hh:min:s)	15:02	15:04	15:05	15:08	15:11	15:14		TITH: 3:33	
	Trial duration (min:s)	00:06	01:14	00:09	00:44	02:19	02:12		TBT: 5:41	
	Target contact (Y/N)	Y	Y	Y	Y	Y	Y		% TITH: 62,43	
	Reward consumed (Y/N)	Y	Y	N	Y	Y	Y			

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
8	Before inserting target (15min)	13:50	3	01:10	1	0	0	0	0	
Date:	After inserting target(15min)	02:15	3	13:43	4	6	0	0	0	
31/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	13:03	13:04	13:05	13:07	13:08	13:12		ATT: 28	
	Trial end time (hh:min:s)	13:03	13:04	13:06	13:07	13:08	13:13		TITH: 6:51	
	Trial duration (min:s)	00:27	00:15	01:09	00:08	00:32	00:29		TBT: 10:07	
	Target contact (Y/N)	Y	Y	Y	Y	Y	Y		% TITH: 67,71	
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
9	Before inserting target (15min)	15:00	1	0	0	0	0	0	0	
Date:	After inserting target(15min)	0	0	15:00	1	3	0	0	0	
01/11/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	14:02	14:03	14:06	14:07	14:08	14:09		ATT: 17	
	Trial end time (hh:min:s)	14:02	14:04	14:06	14:07	14:08	14:10		TITH: 3:43	
	Trial duration (min:s)	00:11	00:41	00:14	00:27	00:15	00:17		TBT: 4:44	
	Target contact (Y/N)	Y	Y	Y	Y	Y	Y		% TITH: 78,52	
	Reward consumed (Y/N)	Y	N	Y	Y	Y	Y			

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
10	Before inserting target (15min)	15:00	1	0	0	0	0	0	0	
Date:	After inserting target(15min)	0	0	15:00	1	4	0	0	0	
02/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	14:45	14:48	14:49	14:52	14:53	14:54		ATT: 18	
	Trial end time (hh:min:s)	14:47	14:48	14:50	14:52	14:53	14:54		TITH: 3:50	
	Trial duration (min:s)	01:30	00:13	01:02	00:26	00:22	00:49		TBT: 4:50	
	Target contact (Y/N)	Y	Y	Y	Y	Y	Y		% TITH: 79,5	
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			

Table C1: Experimental data sheet for instrumental conditioning of Leopard catshark (*Poroderma pantherinum*) C on day 1 to 3 in Walker Bay, Hermanus

Key	
ATT	Approach to target
TITH	Time in target half of the tank between trials (no food present)
TBT	Total time between trials
% TITH	Percentage of time in target half of tank between trials

MARK	Outer left pec	**TAG #**								
		RECAP	N							
	Capture date	27/08/2015	**Total length(cm)**	45,8						
	Species	Poroderma panthera	**Weight(g)**	530						
	Sex	Female	**Food p/d (g)**	7,95						
		R time (min:s)	**No of R events (#)**	**CIR time (min:s)**	**No of CIR events (#)**	**No of SPY (#)**	**D time (min:s)**	**Special behviours (RO, HS, BR)**	**Approaches to target (#)**	**COMMENTS**
Training day	**BEHAVIOUR**									
1	Before inserting target (15min)	12:52	4	01:57	3	0	0	0	0	Rest mostly opposite the target
Date:	After inserting target(15min)	13:34	5	01:27	5	0	0	0	0	
28/09/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	14:48	14:49	14:49	14:50	14:51	14:52		ATT: 14	Great response
	Trial end time (hh:min:s)	14:48	14:49	14:50	14:51	15:52	14:53		TITH: 2:37	No fear of target
	Trial duration (min:s)	00:11	00:11	00:08	00:15	00:12	00:11		TBT: 3:08	Lead in every trial
	Target contact (Y/N)	Y	Y	N	Y	Y	N		%TITH: 83,51	Many turns back on target
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			
		R time (min:s)	**No of R events (#)**	**CIR time (min:s)**	**No of CIR events (#)**	**No of SPY (#)**	**D time (min:s)**	**Special behviours (RO, HS, BR)**	**Approaches to target (#)**	**COMMENTS**
Training day	**BEHAVIOUR**									
2	Before inserting target (15min)	12:45	2	02:09	2	0	0	RO x 3	0	Very active
Date:	After inserting target(15min)	02:15	2	12:48	2	22	0	RO x 4	0	Curious about target
29/09/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	14:46	14:47	14:48	14:50	14:51	14:52		ATT: 13	Very active
	Trial end time (hh:min:s)	14:46	14:47	14:49	14:50	14:51	14:52		TITH: 2:55	Instantly react to feeding stick
	Trial duration (min:s)	00:20	00:37	00:41	00:10	00:16	00:20		TBT: 4:20	Lead in every trial
	Target contact (Y/N)	Y	Y	N	Y	Y	Y		%TITH: 67,31	
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			
		R time (min:s)	**No of R events (#)**	**CIR time (min:s)**	**No of CIR events (#)**	**No of SPY (#)**	**D time (min:s)**	**Special behviours (RO, HS, BR)**	**Approaches to target (#)**	**COMMENTS**
Training day	**BEHAVIOUR**									
3	Before inserting target (15min)	08:57	5	05:49	5	0	0	RO x1	0	
Date:	After inserting target(15min)	11:40	4	03:10	4	0	0	0	0	
30/09/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	14:40	14:41	14:42	14:43	14:44	14:46		ATT: 15	
	Trial end time (hh:min:s)	14:40	14:41	14:42	14:43	14:45	14:47		TITH: 3:05	Trial 1+3 food fell off stick
	Trial duration (min:s)	00:29	00:16	00:34	00:22	01:18	00:18		TBT: 4:10	
	Target contact (Y/N)	Y	Y	Y	Y	Y	Y		%TITH: 74	
	Reward consumed (Y/N)	N	Y	N	Y	Y	Y			

Table C2: Experimental data sheet for instrumental conditioning of Leopard catshark (*Poroderma pantherinum)* C on day 4 to 6 in Walker Bay, Hermanus

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
4	Before inserting target (15min)	14:31	2	00:26	1	0	0	0	0	
Date:	After inserting target(15min)	05:06	5	09:37	6	1	0	0	0	
01/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	13:10	13:11	13:11	13:12	13:13	13:14			
	Trial end time (hh:min:s)	13:10	13:11	13:12	13:12	13:14	13:15		Video error	Circling the target when no food present
	Trial duration (min:s)	00:08	00:27	00:11	00:10	00:34	00:13			
	Target contact (Y/N)	Y	Y	Y	Y	Y	N			
	Reward consumed (Y/N)	Y	Y	Y	Y	N	Y			

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
5	Before inserting target (15min)	14:49	2	00:09	1	0	0	0	0	
Date:	After inserting target(15min)	13:19	6	01:30	6	0	0	0	0	WHALE FESTIVAL
02/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	14:10	14:11	14:11	14:13	14:15	14:16		ATT: 16	
	Trial end time (hh:min:s)	14:10	14:11	14:13	14:15	14:16	14:16		TITH: 4:44	Trial 3 food fell off stick
	Trial duration (min:s)	00:13	00:06	00:54	00:39	00:54	00:10		TBT: 5:14	
	Target contact (Y/N)	Y	Y	Y	Y	Y	Y		%TITH: 90,45	
	Reward consumed (Y/N)	Y	Y	N	Y	Y	Y			

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
6	Before inserting target (15min)	09:41	6	05:00	6	0	0	RO x 1	0	WHALE FESTIVAL
Date:	After inserting target(15min)	09:18	5	05:30	4	0	0	0	0	
03/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	14:05	14:06	14:07	14:08	14:09	14:10		ATT: 10	
	Trial end time (hh:min:s)	14:05	14:07	14:08	14:09	14:10	14:10		TITH: 3:00	
	Trial duration (min:s)	00:07	00:37	00:36	00:28	00:17	00:21		TBT: 4:01	
	Target contact (Y/N)	Y	Y	Y	Y	Y	Y		%TITH: 74,68	
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			

Table C3: Experimental data sheet for instrumental conditioning of Leopard catshark (*Poroderma pantherinum)* C on day 7 to 10 in Walker Bay, Hermanus

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
7	Before inserting target (15min)	11:51	3	03:06	2	0	0	RO x 5	0	
Date:	After inserting target(15min)	15:00	1	0	0	0	0	0	0	
04/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	14:10	14:11	14:12	14:13	14:16	14:17		ATT: 16	Very interested in target
	Trial end time (hh:min:s)	14:10	14:11	14:12	14:15	14:16	14:17		TITH: 4:32	Many turning back onto target
	Trial duration (min:s)	00:12	00:28	00:14	01:27	00:26	00:12		TBT: 5:31	
	Target contact (Y/N)	Y	Y	N	Y	N	Y		% TITH: 82,17	WHALE FESTIVAL
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
8	Before inserting target (15min)	11:37	3	03:18	2	0	0	0	0	
Date:	After inserting target(15min)	01:35	1	13:22	2	0	0	0	0	
05/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video Analysis**	
	Trial start time (hh:min:s)	14:30	14:31	14:32	14:34	14:35	14:36		ATT: 25	Many turns in target half
	Trial end time (hh:min:s)	14:30	14:32	14:33	14:34	14:35	14:37		TITH: 5:38	
	Trial duration (min:s)	00:30	00:42	00:26	00:17	00:16	00:37		TBT: 6:45	
	Target contact (Y/N)	Y	Y	Y	N	Y	Y		% TITH: 83,45	
	Reward consumed (Y/N)	N	Y	N	Y	Y	Y			

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
9	Before inserting target (15min)	15:00	1	0	0	0	0	0	0	
Date:	After inserting target(15min)	00:11	1	14:45	2	3	0	RO	0	
06/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	14:20	14:22	14:23	14:26	14:27	14:29		ATT: 21	Interns making noise next to
	Trial end time (hh:min:s)	14:21	14:23	14:25	14:26	14:28	14:29		TITH: 4:29	tank
	Trial duration (min:s)	00:30	00:57	01:44	00:26	01:10	00:09		TBT: 5:39	
	Target contact (Y/N)	Y	Y	N	Y	N	Y		% TITH: 79,35	
	Reward consumed (Y/N)	Y	N	Y	N	Y	Y			

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
10	Before inserting target (15min)	15:00	1	0	0	0	0	0	0	
Date:	After inserting target(15min)	02:13	3	12:42	4	7	0	HS		
07/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	14:39	14:42	14:44	14:46	14:48	14:50		ATT: 20	
	Trial end time (hh:min:s)	14:40	14:43	14:44	14:47	14:49	14:51		TITH: 5:02	
	Trial duration (min:s)	00:48	00:59	00:29	00:39	00:48	00:28		TBT: 6:12	
	Target contact (Y/N)	Y	N	Y	Y	Y	Y		% TITH: 81,2	
	Reward consumed (Y/N)	Y	Y	Y	Y	N	Y			

Table D1: Experimental data sheet for instrumental conditioning of Leopard catshark (*Poroderma pantherinum)* D on day 1 to 3 in Walker Bay, Hermanus

Key	
ATT	Approach to target
TITH	Time in target half of the tank between trials (no food present)
TBT	Total time between trials
% TITH	Percentage of time in target half of tank between trials

MARK	Inner right pec	**TAG #**		
		RECAP	N	
	Capture date	27/08/2015	**Total length(cm)**	55,3
	Species	Poroderma panthera	**Weight(g)**	910
	Sex	Female	**Food p/d (g)**	13,65

		R time (min:s)	**No of R events (#)**	**CIR time (min:s)**	**No of CIR events (#)**	**No of SPY (#)**	**D time (min:s)**	**Special behviours (RO, HS, BR)**	**Approaches to target (#)**	**COMMENTS**
Training day	**BEHAVIOUR**									
1	Before inserting target (15min)	09:27	8	05:15	8	4	0	0	0	Accelerated swimming
Date:	After inserting target(15min)	0	0	15:00	1	12	0	0	0	Stressed
11/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	13:48	13:50	13:50	13:51	13:53	13:54		ATT: 19	
	Trial end time (hh:min:s)	13:49	13:50	13:51	13:52	13:53	13:54		TITH: 2:19	Lead in all trials
	Trial duration (min:s)	00:46	00:14	00:08	00:15	00:13	00:09		TBT: 3:46	
	Target contact (Y/N)	Y	Y	Y	Y	Y	Y		% TITH: 61,5	
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			

		R time (min:s)	**No of R events (#)**	**CIR time (min:s)**	**No of CIR events (#)**	**No of SPY (#)**	**D time (min:s)**	**Special behviours (RO, HS, BR)**	**Approaches to target (#)**	**COMMENTS**
Training day	**BEHAVIOUR**									
2	Before inserting target (15min)	11:20	5	03:47	4	2	0	0	0	Accelerated swimming
Date:	After inserting target(15min)	00:08	1	14:52	2	15	0	0	0	
12/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	15:10	15:11	15:12	15:14	15:16	15:17		ATT: 16	
	Trial end time (hh:min:s)	15:10	15:11	15:13	15:15	15:16	15:18		TITH: 2:29	Lead in all trials
	Trial duration (min:s)	00:16	00:20	00:56	00:42	00:19	00:10		TBT: 4:27	
	Target contact (Y/N)	Y	Y	N	Y	Y	Y		% TITH: 55,8	
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			

		R time (min:s)	**No of R events (#)**	**CIR time (min:s)**	**No of CIR events (#)**	**No of SPY (#)**	**D time (min:s)**	**Special behviours (RO, HS, BR)**	**Approaches to target (#)**	**COMMENTS**
Training day	**BEHAVIOUR**									
3	Before inserting target (15min)	07:19	4	07:33	3	0	0	0	0	
Date:	After inserting target(15min)	0	0	15:00	1	15	0	0	0	
13/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		Video analysis	
	Trial start time (hh:min:s)	14:49	15:00	15:01	15:02	15:03	15:04		ATT: 13	
	Trial end time (hh:min:s)	14:49	15:00	15:01	15:02	15:04	15:05		TITH: 2:23	Lead in all trials
	Trial duration (min:s)	00:07	00:31	00:07	00:10	00:06	00:07		TBT: 4:46	
	Target contact (Y/N)	Y	Y	Y	N	N	Y		% TITH: 50	
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			

Table D2: Experimental data sheet for instrumental conditioning of Leopard catshark (*Poroderma pantherinum)* D on day 4 to 6 in Hermanus, South Africa (2015).

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
4	Before inserting target (15min)	05:47	2	09:06	3	3	0	0	0	
Date:	After inserting target(15min)	03:41	3	11:11	3	14	0	0	0	
14/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	16:10	16:11	16:13	16:14	16:15	16:15		ATT: 16	
	Trial end time (hh:min:s)	16:10	16:12	16:13	16:14	16:15	16:16		TITH: 2:45	
	Trial duration (min:s)	00:14	00:07	00:04	00:26	00:06	00:07		TBT: 4:20	
	Target contact (Y/N)	Y	Y	N	Y	N	Y		% TITH: 54,81	
	Reward consumed (Y/N)	N	Y	Y	Y	Y	Y			

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
5	Before inserting target (15min)	00:00	0	15:00	1	0	0	0	0	spy hops mostly by target
Date:	After inserting target(15min)	00:00	0	15:00	1	15	0	0	0	
15/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	12:39	12:41	12:34	12:44	12:45	12:46		ATT: 14	
	Trial end time (hh:min:s)	12:40	12:41	12:43	12:44	12:45	12:47		TITH: 2:50	
	Trial duration (min:s)	00:11	00:16	00:09	00:32	00:10	00:22		TBT: 4:20	
	Target contact (Y/N)	Y	Y	Y	Y	Y	Y		% TITH: 65,38	
	Reward consumed (Y/N)	Y	N	Y	Y	Y	Y			

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
6	Before inserting target (15min)	08:07	7	06:31	8	2	0	0	0	
Date:	After inserting target(15min)	07:26	3	07:08	3	7	0	0	0	
16/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	14:59	15:00	15:02	15:04	15:04	15:06		ATT: 19	
	Trial end time (hh:min:s)	14:59	15:01	15:03	15:04	15:05	15:06		TITH: 4:01	
	Trial duration (min:s)	00:07	00:28	01:02	00:07	00:18	00:09		TBT: 5:35	
	Target contact (Y/N)	Y	Y	Y	Y	Y	Y		% TITH: 71,94	
	Reward consumed (Y/N)	Y	N	Y	Y	Y	Y			

Table D3: Experimental data sheet for instrumental conditioning of Leopard catshark (*Poroderma pantherinum)* D on day 7 to 10 in Hermanus, South Africa (2015).

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
7	Before inserting target (15min)	10:42	6	04:06	5	2	0	0	0	
17/10/2015	After inserting target(15min)	07:18	2	07:54	2	12	0	0	0	Resting by target
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	13:28	13:30	13:32	13:33	13:34	13:35		video error	
	Trial end time (hh:min:s)	13:29	13:31	13:32	13:33	13:34	13:35			
	Trial duration (min:s)	00:37	00:55	00:18	00:41	00:08	00:15			
	Target contact (Y/N)	Y	Y	Y	Y	Y	Y			
	Reward consumed (Y/N)	Y	Y	Y	y	Y	Y			

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
8	Before inserting target (15min)	00:51	2	14:03	3	8	0	0	0	spy hops by target
Date:	After inserting target(15min)	04:10	4	10:49	4	10	0	0	0	resting by target
18/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	14:51	14:52	14:53	14:59	15:01	15:03		ATT: 17	
	Trial end time (hh:min:s)	14:51	14:52	14:53	15:00	15:02	15:03		TITH: 6:03	
	Trial duration (min:s)	00:16	00:14	00:10	00:46	00:07	00:22		TBT: 9:37	
	Target contact (Y/N)	Y	Y	Y	Y	Y	Y		% TITH: 69,3	
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
9	Before inserting target (15min)	14:00	3	00:59	2	0	0	0	0	Resting by the target
Date:	After inserting target(15min)	10:35	5	04:14	5	4	0	0	0	
19/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	15:49	15:51	15:51	15:52	15:54	15:56		ATT: 17	
	Trial end time (hh:min:s)	15:50	15:51	15:52	15:53	15:55	15:56		TITH: 3:37	
	Trial duration (min:s)	00:45	00:12	00:12	00:45	00:35	00:16		TBT: 4:29	
	Target contact (Y/N)	Y	Y	Y	Y	Y	Y		% TITH: 80,67	
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
10	Before inserting target (15min)	15:00	1	0	0	1	0	0	0	Resting by target
Date:	After inserting target(15min)	07:37	2	07:23	2	13	0	0	0	
20/10/2015										
	CONDITIONING	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**		**Video analysis**	
	Trial start time (hh:min:s)	14:30	14:31	14:32	14:33	14:35	14:36		ATT: 8	
	Trial end time (hh:min:s)	14:30	14:31	14:32	14:34	14:34	14:36		TITH: 2:15	
	Trial duration (min:s)	00:15	00:19	00:09	00:49	00:27	00:16		TBT: 3:02	
	Target contact (Y/N)	Y	Y	Y	Y	Y	Y		% TITH: 74,18	
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			

Table E1: Experimental data sheet for instrumental conditioning of the control Leopard catshark (*Poroderma pantherinum*) on day 1 to 3 in Hermanus, South Africa (2015).

Key	
ATT	Approach to target
TITH	Time in target half of the tank between trials (no food present)
TBT	Total time between trials
% TITH	Percentage of time in target half of tank between trials

	Capture date	27/08/2015	**Total length(cm)**	59.9						
	Species	Poroderma panthera	**Weight(g)**	1140						
	Sex	Male	**Food p/d (g)**	17.1						
Date moved to exp:										
3/9/2015		**R time (min:s)**	**No of R events (#)**	**CIR time (min:s)**	**No of CIR events (#)**	**No of SPY (#)**	**D time (min:s)**	**Special behviours (RO, HS, BR)**	**Approaches to target (#)**	**COMMENTS**
Training day	**BEHAVIOUR**									
1	Before inserting target (15min)	15	1	0	0	0	0	0	0	1 readjustment
Date:	After inserting target(15min)	14:09	5	0:22	4	0	0	0	2	Resting by target
7/9/2015										
Temp:	**CONDITIONING**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**			
15	Trial start time (hh:min:s)	14:59	15:04	15:20	15:24	15:27	15:31			Shark lead to target in
Oxygen:	Trial end time (hh:min:s)	15:00	15:14	15:21	15:25	15:29	15:41			all trials
	Trial duration (min:s)	1:18	10:00	1:27	0:47	1:53	10:00			
pH:	Target contact (Y/N)	N	N	N	Y	Y	N			
	Reward consumed (Y/N)	Y	N	Y	Y	Y	N			
		R time (min:s)	**No of R events (#)**	**CIR time (min:s)**	**No of CIR events (#)**	**No of SPY (#)**	**D time (min:s)**	**Special behviours (RO, HS, BR)**	**Approaches to target (#)**	**COMMENTS**
Training day	**BEHAVIOUR**									
2	Before inserting target (15min)	15	1	0	0	0	0	0	0	Bad visibility
Date:	After inserting target(15min)	15	1	0	0	0	0	0	0	
Temp:	**CONDITIONING**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**			
	Trial start time (hh:min:s)	14:56	15:08	15:26	15:39	15:51	16:05		trial 3: 5	Trial 1 + 2 lead
Oxygen:	Trial end time (hh:min:s)	15:06	15:18	15:36	15:49	16:01	16:15		Trial 4: 3	
	Trial duration (min:s)	10:00	10:00	10:00	10:00	10:00	10:00		Trial 5: 6	
pH:	Target contact (Y/N)	N	N	N	N	N	N		Trial 6 zero	
	Reward consumed (Y/N)	Y	N	N	N	N	N			
		R time (min:s)	**No of R events (#)**	**CIR time (min:s)**	**No of CIR events (#)**	**No of SPY (#)**	**D time (min:s)**	**Special behviours (RO, HS, BR)**	**Approaches to target (#)**	**COMMENTS**
Training day	**BEHAVIOUR**									
3	Before inserting target (15min)	15	1	0	0	0	0	0	0	
Date:	After inserting target(15min)	15	1	0	0	0	0	0	0	
Temp:	**CONDITIONING**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**			
	Trial start time (hh:min:s)	14:22	14:26	14:30	14:40	14:42	14:48			Shark lead in every trial
Oxygen:	Trial end time (hh:min:s)	14:42	14:27	14:31	14:41	14:43	14:49			
	Trial duration (min:s)	0:20	0:35	1:07	0:44	1:02	1:01			
pH:	Target contact (Y/N)	N	N	Y	Y	Y	Y			
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			

Table E2: Experimental data sheet for instrumental conditioning of the control Leopard catshark (*Poroderma pantherinum)* on day 4 to 6 in Hermanus, South Africa (2015).

		R time (min:s)	No of R events (#)	CIR time (min:s)	No of CIR events (#)	No of SPY (#)	D time (min:s)	Special behviours (RO, HS, BR)	Approaches to target (#)	COMMENTS
Training day	**BEHAVIOUR**									
4	Before inserting target (15min)	15	1	0	0	0	0	0	0	
Date:	After inserting target(15min)	15	1	0	0	0	0	0	0	
10/9/2015										
Temp:	**CONDITIONING**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**			
13.69	Trial start time (hh:min:s)	14:57	15:00	15:02	15:10	15:11	15:15			Trial 4 ate away from target by accid
Oxygen:	Trial end time (hh:min:s)	14:58	15:00	15:04	15:11	15:12	15:21			
104.5	Trial duration (min:s)	0:18	0:08	2:22	0:27	0:45	6:21			
pH:	Target contact (Y/N)	Y	Y	Y	Y	Y	N			Shark lead in all trials
7.89	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			
		R time (min:s)	**No of R events (#)**	**CIR time (min:s)**	**No of CIR events (#)**	**No of SPY (#)**	**D time (min:s)**	**Special behviours (RO, HS, BR)**	**Approaches to target (#)**	**COMMENTS**
Training day	**BEHAVIOUR**									
5	Before inserting target (15min)	15	1	0	0	0	0	0	0	0
Date:	After inserting target(15min)	15	1	0	0	0	0	0	0	0
11/9/2015										
Temp:	**CONDITIONING**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**			
15.57	Trial start time (hh:min:s)	13:53	13:54	13:57	14:01	0				
Oxygen:	Trial end time (hh:min:s)	13:53	13:55	13:59	14:01	14:04	14:08			Great response to food + approach to t
106.3	Trial duration (min:s)	0:24	0:47	1:28	0:23	0:13	3:05			
pH:	Target contact (Y/N)	Y	Y	Y	Y	N	Y			
7.88	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			
		R time (min:s)	**No of R events (#)**	**CIR time (min:s)**	**No of CIR events (#)**	**No of SPY (#)**	**D time (min:s)**	**Special behviours (RO, HS, BR)**	**Approaches to target (#)**	**COMMENTS**
Training day	**BEHAVIOUR**									
6	Before inserting target (15min)	15	1	0	0	0	0	0	0	
Date:	After inserting target(15min)	15	1	0	0	0	0	0	0	
12/9/2015										
Temp:	**CONDITIONING**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**			
	Trial start time (hh:min:s)	14:33		14:36	14:41	14:46	14:47			
Oxygen:	Trial end time (hh:min:s)	14:33		14:37	14:45	14:46	14:47			
	Trial duration (min:s)	0:24	10:00	0:59	3:21	0:07	0:09			
pH:	Target contact (Y/N)	Y	N	N	Y	Y	Y			
	Reward consumed (Y/N)	Y	N	Y	Y	Y	Y			

Table E3: Experimental data sheet for instrumental conditioning of the control Leopard catshark (*Poroderma pantherinum*) on day 7 to 10 in Hermanus, South Africa (2015).

		R time (min:s)	**No of R events (#)**	**CIR time (min:s)**	**No of CIR events (#)**	**No of SPY (#)**	**D time (min:s)**	**Special behviours (RO, HS, BR)**	**Approaches to target (#)**	**COMMENTS**
Training day	**BEHAVIOUR**									
7	Before inserting target (15min)	15	1	0	0	0	0	0	0	
Date:	After inserting target(15min)	15	1	0	0	0	0	0	0	Moved behind target
17/09/2015										
Temp:	**CONDITIONING**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**			
13.43	Trial start time (hh:min:s)	15:34							EXP DONE BY INTERNS	
Oxygen:	Trial end time (hh:min:s)	15:45								
1041	Trial duration (min:s)	10:20								No response, old food used or techr
pH:	Target contact (Y/N)	Y								
7.69	Reward consumed (Y/N)	Y								
		R time (min:s)	**No of R events (#)**	**CIR time (min:s)**	**No of CIR events (#)**	**No of SPY (#)**	**D time (min:s)**	**Special behviours (RO, HS, BR)**	**Approaches to target (#)**	**COMMENTS**
Training day	**BEHAVIOUR**									
8	Before inserting target (15min)	15	1	0	0	0	0	0	0	
Date:	After inserting target(15min)	15	1	0	0	0	0	0	0	
18/09/2015										
Temp:	**CONDITIONING**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**			
12.8	Trial start time (hh:min:s)	15:07	15:09	15:12	15:13	15:19	15:26			
Oxygen:	Trial end time (hh:min:s)	15:08	15:11	15:13	15:18	15:24	15:29			
100.5	Trial duration (min:s)	0:59	2:36	0:10	4:32	5:02	3:01			shark seems relaxed
pH:	Target contact (Y/N)	N	Y	Y	N	N	Y			
7.61	Reward consumed (Y/N)	Y	Y	Y	Y	Y	Y			
		R time (min:s)	**No of R events (#)**	**CIR time (min:s)**	**No of CIR events (#)**	**No of SPY (#)**	**D time (min:s)**	**Special behviours (RO, HS, BR)**	**Approaches to target (#)**	**COMMENTS**
Training day	**BEHAVIOUR**									
9	Before inserting target (15min)	15	1	0	0	0	0	0	0	No reaction
Date:	After inserting target(15min)	15	1	0	0	0	0	0	0	
19/09/2015										
Temp:	**CONDITIONING**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**			
13.7	Trial start time (hh:min:s)	14:16	14:21	14:23	14:24	14:26	14:31			
Oxygen:	Trial end time (hh:min:s)	14:19	14:22	14:24	14:24	14:30	14:41			
110.9	Trial duration (min:s)	3:01	0:30	0:07	0:23	3:44	10:00			
pH:	Target contact (Y/N)	N	Y	Y	y	Y	N			
7.5	Reward consumed (Y/N)	Y	Y	Y	Y	Y	N			
		R time (min:s)	**No of R events (#)**	**CIR time (min:s)**	**No of CIR events (#)**	**No of SPY (#)**	**D time (min:s)**	**Special behviours (RO, HS, BR)**	**Approaches to target (#)**	**COMMENTS**
Training day	**BEHAVIOUR**									
10	Before inserting target (15min)	15	1	0	0	0	0	0	0	
Date:	After inserting target(15min)	15	1	0	0	0	0	0	0	
20/09/2015										
Temp:	**CONDITIONING**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**			
	Trial start time (hh:min:s)	14:30	14:33	14:35	14:37	14:38	14:42			
Oxygen:	Trial end time (hh:min:s)	14:32	14:34	14:36	14:38	14:41	14:53			
	Trial duration (min:s)	2:05	0:41	0:10	1:10	2:15	10:00			
pH:	Target contact (Y/N)	Y	Y	Y	Y	Y	N			
	Reward consumed (Y/N)	Y	Y	Y	Y	Y	N			