CRANFIELD UNIVERSITY

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ELASMOBRANCH ELECTRORECEPTIVE FORAGING BEHAVIOUR: MALE-FEMALE INTERACTIONS, CHOICE AND COGNITIVE ABILITY

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Elasmobranch electroreceptive foraging behaviour: male-female interactions, choice and cognitive ability

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GENERAL ABSTRACT

Aspects of electroreceptive foraging behaviour were investigated in a benthic elasmobranch, *Scyliorhinus canicula* (small-spotted catshark). The findings build on current knowledge of sexual conflict in this species and provide novel information concerning differentiation ability, choice and cognition relating to elasmobranch electroreceptive foraging behaviour. Hierarchical catshark behaviours towards artificial, prey-type electric fields (E fields) following stimulation by food-derived scent were recorded under laboratory conditions.

Experiment 1: Male-female interactions

Foraging behaviour of single- and mixed-sex catshark groups were investigated using electroreception as a proxy for feeding levels. Results indicated significant reductions in foraging levels of being grouped with the opposite sex, in addition to higher responsiveness in females. These attributes are most likely consequences of differing reproductive strategies and resultant sexual conflict.

Experiment 2: Choice

Catsharks were trained to swim through narrow tunnels and upon exit were presented with two differing E fields simultaneously. Choices were recorded and analysed, and thereby their ability to distinguish between and/or show preferences for fields was determined. Differentiation ability was demonstrated by preferences for stronger rather than weaker direct current fields, and alternating rather than direct current fields. The fish were either unable to distinguish or showed no preference for artificial (electrodes) and natural fields (crabs).

Experiment 3: Cognitive ability

Response levels and changes in response levels over time of catsharks rewarded for responding to E fields were compared to those of catsharks that were not rewarded. Results demonstrated learning and habituation behaviour improving foraging efficiency over short time scales according to profitability of fields. Failure to retain altered behaviour after an interval indicated short memory windows. These attributes would prove beneficial in a variable environment.

Given many elasmobranchs' continuing population declines and increasing potential interactions with anthropogenic E fields, such information will hopefully benefit both fisheries managers and offshore renewable energy developers.

For Mum & Dad

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1. GENERAL INTRODUCTION

1.1. Introduction

Energy is crucial to living organisms. It is required for fundamental biological processes ranging from metabolism and life maintenance to development, growth and reproduction. Animals acquire energy by consuming and digesting food and the trade-offs involved in obtaining it contributes greatly to their Darwinian fitness via contribution to somatic and gonadal growth. Foraging for food is therefore vitally important and consequently animals devote significant time and effort to the behaviour (Elton, 1927; Stephens & Krebs, 1986). Compared to herbivores, predatory carnivores, in particular, are faced with a great challenge when locating and capturing their prey. In turn, the prey must strive to avoid detection and capture. In addition to the need to locate mates, to navigate and to identify suitable habitats, this evolutionary arms race between predator and prey has resulted in highly tuned senses to detect signals such as scent, noise, movement, shape, colour, pressure and heat.

Signals emitted by prey in aquatic environments (including oceans, lakes and rivers) often differ to signals emitted by terrestrial prey due to the vastly contrasting properties of water and air such as density, viscosity, pressure, temperature and diffusion rates (Denny, 1993; Tait & Dipper, 1994). For example, certain signals attenuate in water more rapidly than in air (e.g. light via absorption and scattering) whereas others attenuate less rapidly in water (e.g. sound via propagation of sound waves and impedance). Consequently, aquatic and terrestrial predators have often evolved to possess differing sensory adaptations and acuteness.

One sense is only observed in aquatic environments owing to the conductivity of water. Known as electroreception, it is the detection of electric fields and represents a key factor in certain animals' foraging behaviour and therefore highly important for their individual success (Tricas & Sisneros, 2004).

The following study investigated a number of important aspects of foraging behaviour exhibited by an electroreceptive elasmobranch (arguably the most electrosensitive taxon), the often studied, benthic, marine predator; the small-spotted catshark (*Scyliorhinus canicula).*

1.2. Electroreceptive organisms

Electroreception is the biological ability to receive and make use of electrical impulses. There are two varieties of the sense; passive and active. Passive electroreception involves the detection of the weak bioelectric fields emitted by aquatic animals (and possibly the Earth's electromagnetic fields). Active electroreception consists of the generation of electric fields and subsequent detection of distortions in the fields by the local environment (including aquatic animals) and can also be used as a means of communication.

There are a few mammals and amphibians that exhibit electroreception when foraging in water including the star-nosed mole, *Condylura cristata*; Gould *et al.*, 1993), the monotremes (echidnas and the platypus, *Ornithorhynchus anatinus*; Scheich *et al.*, 1986) and salamanders (Fritzsch, 1981). Otherwise the sense is thought to be exclusive to fish. Those possessing active electroreceptive capabilities are predominantly teleosts (bony fishes) such as synodontid catfishes (Hagedorn *et al.*, 1990) gymnotiform (the electric eel, *Electrophorus electricus*, and knife fishes; Nelson

& Maciver, 1999) and mormyriform fishes (elephant fish, *Gnathonemus petersii*; von der Emde, 1990). These are thought to have evolved from passive electroreceptive fish which are primarily primitive groups including lampreys (Bullock *et al.*, 1983), bichirs (Jorgensen, 1982), lungfish (Northcutt, 1986; Watt *et al.*, 1999), coelacanths (Bemis & Hetherington, 1982), chondrosteans (sturgeons and paddlefish, Teeter *et al.*, 1980; Northcutt, 1986) and the chondrichthyans (sharks, skates, rays & chimeras; Bullock *et al.*, 1983; Bodznick & Boord, 1986; Fields *et al.*, 1993). A sub-class of the latter provide the focus of this study; the elasmobranchs (sharks, skates and rays).

1.3. Elasmobranch electroreception

1.3.1. Early investigation

Electro-sensitivity in fish was discovered in the early twentieth century in the catfish, *Amiurus nebulosus* (Parker & van Heusen, 1917). However, it was not until Lissmann's research on weak electric fish and his suggestion that their abilities evolved from more primitive fish without electric organs, but sensitive to electric fields, that work on elasmobranchs began (Lissman, 1958). Evidence of electro-sensitivity in the latter began to surface in 1935 when Disgraaf and his student, Kalmijn, noticed that blindfolded *S. canicula* responded to rusty steel wires but not to glass rods. They suggested the sharks were sensitive to electric fields generated by the wire (Disgraaf & Kalmijn, 1962). In the 1960's Murray went further by demonstrating that the Ampullae of Lorenzini (named after the biologist who first described the organ in the late 17th century) were responsive to electrical stimulation (Murray, 1962). Kalmijn went further still by severing the organ's nerves, disabling the shark's ability to detect electric currents thereby proving the organ's electroreceptive function (Kalmijn, 1966).

Kalmijn proceeded to demonstrate the use of the sense during prey detection in a series of classic behavioural, laboratory experiments (Kalmijn, 1971) which can be summarised as follows:

a) Recorded *S. canicula* (small-spotted catshark) and *Raja clavata* (thornback ray) successfully searching for, locating and consuming live plaice (*Pleuronectes platessa*) entirely buried in sand.

b) Demonstrated *S. canicula* and *R. clavata* were still successful at locating and responding to buried plaice when the latter is positioned under an agar chamber (designed such that it transmits bioelectric fields but not scent).

c) The catsharks and rays were, however, unsuccessful in locating or responding to pieces of whiting (*Merlangius merlangus*) buried under the sand and placed under the chamber.

d) The elasmobranchs were also unsuccessful at locating or responding to live plaice under the chamber when the chamber was covered with thin plastic film (to block electric field transmission without inhibiting mechanical cue transmission).

e) The elasmobranchs were able to detect and attempted to bite buried electrodes emitting a small, artificial electric field.

f) The elasmobranchs responded to buried electrodes rather than exposed whiting pieces when the stimuli were placed in close proximity.

By a process of elimination he was therefore able to conclude that the elasmobranchs were using a sense other than sight (a), scent (b & c), and mechanical (d), that they were able to detect weak, artificial, electric fields and responded to them as if they were prey (e), and that the electric sense seemed more important than vision and olfaction when within close proximity to a stimulus (f). A decade later he confirmed that free ranging sharks (dusky smooth hound, *Mustelus canis* and blue shark, *Prionace glauca*) attracted by prey scent would, within short distances, respond to electrodes simulating prey electric fields and would do so preferentially over scent (Heyer *et al.*, 1981; Kalmijn, 1982).

1.3.2. Morphology and physiology

In the three decades that have followed a great deal of work has been undertaken to understand the morphology of the Ampullae of Lorenzini and the physiology behind the sense. A brief summary follows hereafter.

The ampullae consist of a network of clustered, small, continuous, alveolar sacs, each of which is connected to an open pore on the skin via a single canal that extends from the subdermal ampulla and through the dermis (Waltman, 1966; Tricas & Sisneros, 2004; Figure 1). Clusters sometimes number up to 400 in marine elasmobranchs (Chu & Wen, 1979).

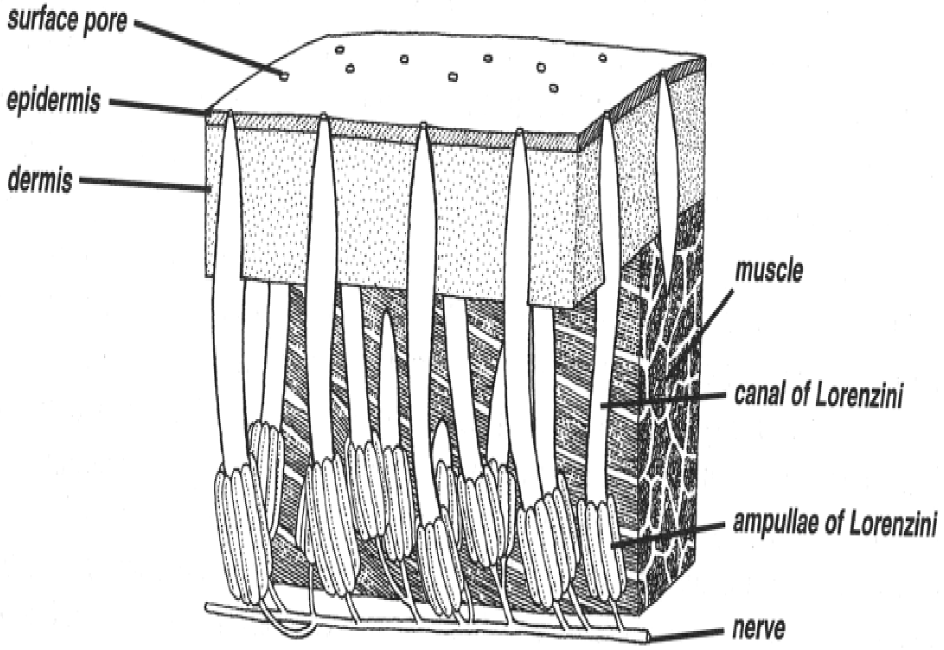


Figure 1. Diagram illustrating the morphology of the electroreceptive apparatus, the Ampullae of Lorenzini (Compagno *et al.*, 2005).

The pores are distributed over much of the head, particularly around the snout and mouth (Figure 2), and in the case of skates and rays extend on to the wing-like pectoral fins (Murray, 1960; Raschi, 1986; Tricas, 2001). Species that feed primarily on benthic prey tend to possess higher pore densities ventrally than those that feed primarily on pelagic prey (Raschi, 1986).

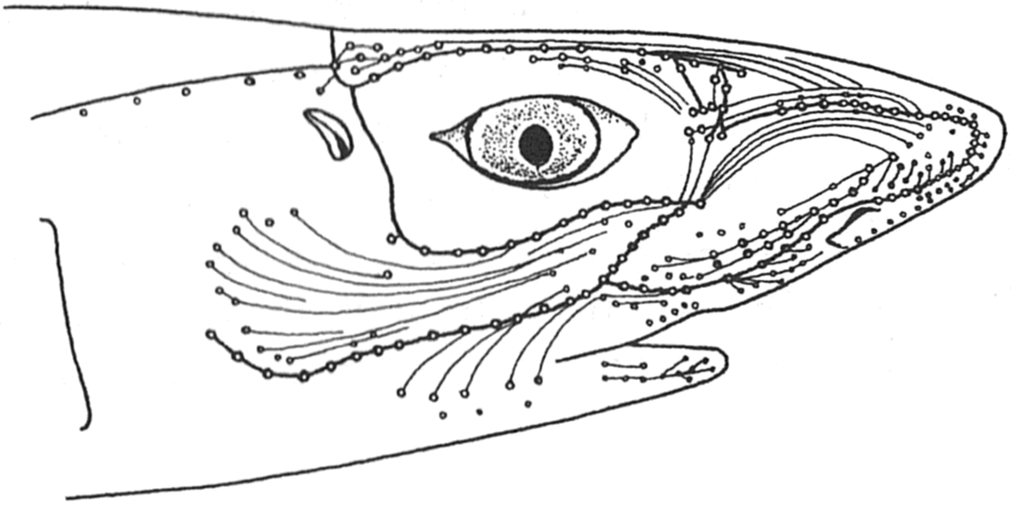


Figure 2. Diagram illustrating ampullary canal and pore distribution on a squaloid shark (Compagno *et al.*, 2005).

The canals' dimensions vary both within and between species (shorter in freshwater species; Zakon, 1986 & 1988) and even within each individual but can measure up to 200 mm in length and approximately 1 mm width (Murray, 1974; Whitehead, 2002). They radiate in all directions from the ampullary clusters, which is thought to allow directional sampling of electric fields surrounding the animal (Kalmijn, 1971; Murray, 1974). They are lined with thick, highly electrically insulating walls (Waltman, 1966). Both the sacs and canals are filled with a low resistivity, muccopolysacharride gel (Murray & Potts, 1961; Waltman, 1966). The ampullary sac walls consist of sensory epithelium (receptor and support cells joined by tight junctions, Tricas, 2001).

The receptor cells are stimulated by minute voltage changes between their basal surface and the water at the skin surface (conveyed along the canals) causing increasing (negative potential) or decreasing (positive potential) transmitter release and discharge rates onto primary afferent nerves leading to the brain (Montgomery, 1984; Tricas & New, 1998; Tricas, 2001). A sophisticated set of filter mechanisms in the brain then extract the weak electro-sensory signals from the strong background noise, the latter of which is largely created by the animal's own movement (Montgomery & Bodznick, 1999).

The numbers of nerves associated with the sense rival those associated with optical, auditory and mechanical senses underlining the relative importance of electroreception to the animals that possess it (Murray, 1974). In fact, it is thought to be one of the most acute senses known throughout the animal kingdom. Murray (1962) reported neural responses of skate electrosensory primary afferents to a voltage gradient of approximately 1 micro-volt per cm. This equates to being able to detect a 1.5 V gradient (akin to the battery power of a small torch) over 1600 km (Kalmijn 1971). More recent experiments have extended this sensitivity to below 20 nano-volts per centimetre (round stingray, *Urobatis halleri*; Tricas & New, 1998).

1.3.3. Behaviour

Despite the extensive literature on morphology and physiology, there has been a relative dearth of studies undertaken on electroreceptive behaviour (Bres, 1993; Tricas and Sisneros, 2004). Past work has often concentrated on proving the existence or descriptions of species' electroreceptive response. Of the nine extant orders within the subclass, Elasmobranchii, only species from four have had electroreceptive behaviour documented, two of which are represented by just one species (Table 1).

Table 1. Elasmobranchs for which electroreceptive behaviour has been identified and described

Order	Species	Author/s
Rajiformes	Clearnose skate (*Raja eglanteria*)	Sisneros *et al.*, 1998
	Little skate (*Raja erinacea*)	Bratton & Ayers, 1987
	Winter skate (*Leucoraja ocellata*)	Bratton & Ayers, 1987
	Thornback ray (*Raja clavata*)	Kalmijn, 1971
	Atlantic stingray (*Dasyatis Sabina*)	Kalmijn & Kalmijn, 1981; Blonder & Alvezion, 1988; Haine *et al.*, 2001
	Pacific electric ray (*Torpedo californica*)	Lowe *et al.* 1994
	Freshwater rays (*Potamotrygon* genus)	Szabo *et al.*, 1972
Orectolobiformes (Carpet Sharks)	Nurse shark (*Ginglymostoma cirratum*)	Johnson *et al.*, 1984
Lamniformes (Mackerel sharks)	Great white shark (*Carcharodon carcharias*)	Tricas & McCosker, 1984
Carcharhiniformes (Ground sharks)	Small-spotted catshark (*Scyliorhinus canicula*)	Kalmijn, 1971; Pals *et al.*, 1982a; Filer *et al.*, 2008
	Blue Shark (*Prionace glauca*)	Heyer *et al.*, 1981; Ryan, 1981; Kalmijn, 1982
	Bonnethead shark (*Sphyrna tiburo*)	Kaijura, 2003
	Scalloped hammerhead shark (*Sphyrna lewini*)	Kaijura & Holland, 2002
	Swell shark (*Cephaloscyllium ventriosum*)	Tricas, 1982,
	Blacktip reef shark (*Carcharhinus melanopterus*)	Haine *et al.*, 2001
	Sandbar shark (*Carcharhinus plumbeus*)	Kaijura & Holland, 2002
	Smooth dogfish (*Mustelus canis*)	Dawson *et al.*, 1980; Kalmijn, 1982

There have been even fewer rigorous studies of elasmobranchs' electroreceptive behavioural parameters and ecology. Examples include Dawson *et al.* (1980) and Kalmijn (1982) who compared the responses of smaller, younger *Mustelus canis* with larger, adult specimens to electric fields simulating both small and large prey. They found that their sensitivities to the electric fields and preference for size of "prey" differed. Blonder and Alevizon (1988) found that stingray were unable to discriminate between prey and non-prey invertebrates using electroreception alone. Kaijura & Holland (2002) worked on approach patterns of carcharhinid and sphyrid sharks to prey-simulating electric stimuli. They suggested that whilst the hammerhead head morphology does not confer greater electrical sensitivity it does permit larger search areas and increased manoeuvrability, both of which would improve prey capture success. Filer *et al.* (2008) investigated the electroreceptive foraging behaviour of *S. canicula* upon different substrates. They concluded that the fish responded significantly less over pebbles and rocks than over sand or no substrate and that responses were significantly reduced at substratum depths of more than 1 cm, with no bites occurring below 3 cm. Gill, A. B., Kimber, J. A. & Bellamy, P. H. (unpublished data) studied intraspecific variation in the electroreceptive foraging behaviour of small groups of the same species. They found significant variation both within and between groups.

1.3.4. Ecology and electrical environment

Elasmobranchs have evolved a hierarchical array of highly tuned senses over the past 450 million years. Electroreception is the last to be used over short distances of up to approximately 40 cm (Kalmijn, 1982; Haine *et al.*, 2001), although less for smaller, benthic species (Kalmijn, 1966, 1971; personal observation). The sense is crucial when accurately locating prey and orienting jaws moments before biting (Kalmijn, 1971;

Tricas, 1982; Blonder & Alevison, 1988; Haine *et al.*, 2001 to name but a few). It is also used during the location of and communication with conspecifics by those species that bury themselves in sediment (Bratton & Ayers, 1987; Tricas *et al.*, 1995; Sisneros, *et al.*, 1998), for the detection of predators, particularly by embryos, hatchlings and juveniles (Peters & Evers, 1985; Sisneros *et al.*, 1998), and is thought to aid navigation as the fish pass through the earth's natural electromagnetic fields (Kalmijn, 1974 & 1984, Pals *et al.*, 1982b; Paulin, 1995; Montgomery & Walker, 2001).

There are numerous sources of electric field (E field) that an elasmobranch may encounter in the marine environment. Measuring such (often minute) fields in sea water is very difficult owing to the variable nature of the fields in a complex, three-dimensional medium with variable salinity, conduction, dissipation and water and ion movement (Kalmijn, 1971, 1972; Kraus & Fleisch, 1999), not to mention the inhospitable and problematic working conditions. There are, however, a few references in the literature, a brief review of which follows.

Aquatic animals emit weak E fields of three types; those associated with (a) high frequency alternating currents caused by muscle action potentials (including heart, gill and motor function muscles), (b) direct currents associated with the difference in potential arising from membranous and epithelial proximity to water in body cavities (mouth, respiratory and anal), and (c) low frequency alternating currents caused by the alternating expansions and contractions of body cavities modulating the direct currents. The extent and strength of these E fields varies significantly among different taxa (Table 2) and in general each species' fields increase in strength with increasing specimen size.

Table 2. Measurements of the bioelectric fields emitted by marine organisms in the literature

Organism	Direct current E field	Alternating current E field	Author/s
Teleosts (small)	20 – 24 mV 10 – 100 µV To 500 µV	n/a 1.5 Hz, voltage not specified Low freq. (<20 Hz) to 500 µV, high freq. (>20 Hz) to 10 µV	Potts & Hedges, 1991 Haine *et al.*, 2001 Kalmijn, 1972
Chondrichthyes (small)	To 50 µV	Low freq. (<20 Hz) to 50 µV, high freq. (>20 Hz) to 10 µV	Kalmijn, 1972
Tunicata	To over 100µV	~ 130 µV, very low freq.	Kalmijn, 1972
Echinodermata	Mostly 10 µV (some species to 80)	None	Kalmijn, 1972
Crustacea	10 - 100 µV To 50 µV 0.1 – 1.0 mV (copepods)	None or negligible Some minimal low freq. 8 – 10 Hz, 0.1 – 1.0 mV	Haine *et al.*, 2001 Kalmijn, 1972 Wilkens *et al.*, 1997
Mollusca (small)	To 100 µV (gastropods) & 1 - 10 µV (other groups) 10 µV (bivalvia)	Very low freq. to 100 µV None	Kalmijn, 1972 Haine *et al.*, 2001
Annelids & Cnidaria	To 10 µV	None	Kalmijn, 1972
Spongiae	Negligible	None	Kalmijn, 1972

V = volts (voltage); Hz = hertz (frequency); n/a = no information available

These measurements were, however, often based upon just a few species and specimens and seem fairly variable, possibly owing to the difficulty of measurement methods. Nevertheless, the figures do provide the best gauge upon which to base assumptions about relatively small elasmobranch prey and conspecifics. Conversely, measurements of the E fields associated with larger predatory organisms are lacking. Sisneros *et al.* (1998) and Ball (2007) demonstrated embryonic thornback rays ceasing body movement that facilitates critical ventilatory movement of water upon sensing artificial E fields. This suggested the rays were employing detection minimisation behaviour as the E fields were similar to those of predatory animals (small, adult elasmobranchs, teleosts and molluscs). But there are no data in the literature concerning the bioelectric fields of larger predators capable of catching and consuming even small, adult elasmobranchs (such as large elasmobranchs, teleosts, cephalopods or sea mammals). It is clear that further research is required to develop a more complete picture of the E fields emitted by marine organisms.

Elasmobranchs also encounter uniform E fields due to the movement of sea water through the Earth’s geomagnetic field in the region of 0.05 to 0.50 µV/cm for ocean currents (von Arx, 1962; Kalmijn 1974, 1984) and 8 µV/m for tidal currents (Pals, 1982b). Many species exhibit migratory behaviour over wide ranging temporal and spatial scales often swimming along remarkably straight lines (Carey *et al.*, 1990; Klimley, 1993; Holland, *et al.*, 1999; Boustany *et al.*, 2002; Gore *et al.*, 2008). Kalmijn has long postulated that elasmobranchs may utilise electroreception to sense the geomagnetic field; passively by detecting fields induced by ocean currents and actively by detecting gradients produced by their own movement through the water. He demonstrated that stingrays could detect and orientate relative to the field (Kalmijn, 1978a, 1978b; Kalmijn & Kalmijn, 1981). Pals *et al.* (1982a) demonstrated *S. canicula* orienting to local E fields. A more recent investigation demonstrated sandbar and scalloped hammerhead sharks responding directly to changes in magnetic fields (Meyer *et al.*, 2004) yet it still remains unclear whether magnetoreception, electroreception, or both are involved.

Artificial sources of E fields also exist in the marine environment, significant examples of which are those associated with offshore renewable energy developments (ORED). Recent modelling (CMACS, 2003; Gill *et al.*, 2005) reported that offshore wind farm cable shielding successfully prevented direct leakage of E fields. However, shielding failed to prevent magnetic field leakage which induced alternating current E fields in the water close to the cable. The fields were estimated to be within the range detectable by electrically sensitive fish (from approximately 0.9 µV/cm at cable surfaces to 0.1 µV/cm eight metres from cables). Larger sharks may also be exposed to strong E fields associated with devices used by divers and surfers to prevent potentially dangerous shark encounters such as the SharkPOD or Shark Shield. The equipment utilise approximately 80 volts and 100 amps (Natal Sharks Board, 2002, personal communication; Shark Shield Pty Ltd., 2008) which elicit avoidance responses even in large, predatory sharks.

1.4. Requirement for further research

Throughout the world’s oceans, many species of elasmobranch are facing dramatic population declines. In 2003, of the species that had been assessed (less than a third) for the World Conservation Union (IUCN)), 21% were identified as Threatened with Extinction and 24% as Near Threatened (Compagno *et al.*, 2005). Another 24%

were deemed so data deficient as to be impossible to assess. These declines are primarily due to human overexploitation and poor fisheries management. 700,000 to 800,000 tonnes (or 70 to 100 million animals) are caught worldwide per annum, many solely for their fins. Moreover, this figure is probably grossly underestimated due to unreported bycatch, discards, recreational and illegal fishing. These catch rates are simply unsustainable (Dulvy *et al*., 2008). The situation is exacerbated by the slow life histories and therefore slow population recovery rates these fish exhibit (Smith *et al.*, 1999; Baum *et al.*, 2003; Frisk *et al.*, 2005; Dulvy *et al.*, 2008). Furthermore, many species have been demonstrated to segregate by sex (Springer, 1967; Klimley, 1987; Sims, 2005) which may cause additional complications in fisheries management. When one also considers the importance of these top predators in oceanic food webs (Stevens *et al.*, 2000; Schindler *et al.*, 2002) and the fragility of ecosystems to reduced species' abundances and extinctions (Chaplin *et al.*, 2000), it becomes clear that elasmobranch fisheries management and conservation demand immediate attention (Helfman, 2007; Dulvy *et al.*, 2008).

Moreover, there is growing concern that these vulnerable fish may be affected by increasing occurrences of anthropogenic E fields in many of the world's coastal, benthic habitats (Gill *et al.*, 2005; Gill & Kimber, 2005; Sutherland *et al.*, 2008). Historically, the possibility of electric fields emitted by, for example, sub-sea cables or cathodic protection of coastal structures and vessels affecting electrically sensitive fish has been overlooked. With the threats of rising carbon emissions and dwindling finite resources looming, deployment of offshore renewable energy developments (ORED) is increasing (The Crown Estate, 2008) with subsequent proliferation of sub-sea cabling. The developments often occur in shallow, coastal areas similar to those that support many elasmobranchs (Rogers *et al.*, 1998; Rogers & Ellis, 2000). The E fields induced in the proximity of the cables (see previous section) are within the range detectable by and attractive to elasmobranchs (Kalmijn, 1971; Gill *et al.*, 2005). Damage to sonar arrays in the late seventies was attributed to sharks via bite patterns (Johnson, 1978), and Kalmijn (1978b) suggested such attacks most likely arose due to E field emission resembling those of natural prey. Similarly, Marra (1989) recorded details of four power transmission failures in an AT & T transatlantic fibre-optic cable in the mid eighties. Upon raising the cable for repairs, bite marks and embedded teeth were found at the damaged sections. Further investigation revealed the damage was attributable to shark bites in all four instances. Attraction to electromagnetic fields emanating from the cable was considered the most likely reason for shark responses. There was no consideration of whether such incidences have potential to damage the fish physically or neurophysiologically. High frequency alternating current E fields have been shown to cause avoidance behaviour in a number of species (Yano *et al*., 2000). Despite these concerns Environmental Impact Assessments, designed to identify and predict possible effects of developments on the surrounding area, have largely overlooked possible interactions between elasmobranchs (or any other electroreceptive animals) and ORED (Gill, 2005; Sutherland *et al*., 2008). Many have focused instead upon effects on birds, sea mammals and the value of ocean front property.

Given the significance of electroreception in the final moments of foraging events, in addition to its use whilst searching for mates, avoiding predators and possibly navigating between habitats, it is clear how important the sense is for contributing to elasmobranch fishes' individual success (Tricas & Sisneros, 2004) and thus to their Darwinian fitness. Along with the possibility that these processes may be affected by increasing anthropogenic electric sources, our lack of knowledge with respect to the behaviour and ecology associated with this important and remarkably acute sense evidently needs to be addressed. Considering many areas of elasmobranch behaviour remain poorly understood, increased research of a broad range of such areas, including those addressed in this study (see Chapter 6.4), will improve our baseline knowledge of these predators' ecology and potentially contribute to better management of their populations.

1.5. Study objectives

The study documented hereafter therefore aims to address aspects of our poor understanding of elasmobranch electroreceptive behaviour and ecology.

1.5.1. Sexual conflict

Differences between the sexes' senses are common throughout the animal kingdom (e.g. Searcy, 1990; Murphy *et al.*, 2001; Arikawa *et al.*, 2005). The phenomenon has been relatively overlooked in elasmobranchs bar a few exceptions (Barber *et al.*, 1985; Carrier *et al.*, 1994; Sisneros & Tricas, 2000; Myrberg Jr., 2001). The sexes of many species of elasmobranch possess similar diet composition (Lyle, 1983; Matallanus *et al.*, 1993; Morato *et al.*, 2003) thus one may not expect differences in foraging behaviour or electroreceptive response between the males and females. However, *S. canicula* show markedly different behavioural strategies resulting in fine-scale sexual segregation (Sims *et al.*, 2001) thought to be driven primarily by aggressive courtship and mating behaviour of males towards females causing refuging behaviour in the latter (Sims, 2005; Sims *et al.*, 2005). Whilst the effects of similar strategies among poeciliid fish on their foraging have been studied (Magurran & Seghers, 1994; Griffiths, 1996; Agrillo *et al.*, 2006), the topic has not been considered for any elasmobranch. Electroreception is an important sensory mode during foraging in elasmobranchs and results in behavioural responses to artificial prey that are routinely repeatable and quantifiable. The following hypothesis was therefore formulated and is addressed in Chapter 3:

- H_1: There are significant differences between the electroreceptive foraging behaviour of mixed- and single-sex groups of *S. canicula* towards artificially created prey-type electric fields.

1.5.2. Choice

Sensory preference has been demonstrated in a wide range of animals, no more so than for bees' preferences for certain flower colours and sizes (Dukas & Real, 1993; Dyer *et al.*, 2006; Ishii, 2006; Goulson *et al.*, 2007), female birds' preferences for different male songs (Collins, 1999; O'Loghlen & Rothstein, 2003; Swadle & Page, 2007) and stickleback fishes' preferences for colours and ultra violet (McKinnon, 1995; Smith *et al.*, 2002; Modarressie & Bakker, 2006). Study of elasmobranch sensory preferences has been lacking although a few have been identified (vision: Clarke, 1961; Strong, 1996, temperature: Wearmouth, 2006; Wallman & Bennett, 2006). Again, electroreceptive work in this area is slight (Dawson *et al.*, 1980; Kalmijn, 1982). The ability to differentiate between two or more stimuli is important to all animals, especially for opportunistic predators such as coastal elasmobranchs that inhabit variable environments and feed on diverse prey. It permits preference for certain stimuli and allows informed choices to be taken based upon prior experience. Whether elasmobranchs are able to detect differences and make choices between different prey and predators, sizes of prey and predators and suitability of mates and habitats will ultimately contribute to their individual success. Recent concerns surrounding interactions between these fish and electric fields associated with ORED also raise the question of whether they can differentiate between natural and anthropogenic E fields. The following hypothesis was therefore formulated and is addressed in Chapter 4:

- H_2: *S. canicula* can distinguish and show preferences between:
    - Artificially created E fields associated with 9 µA and 90 µA direct currents.
    - Artificially created E fields associated with 90 µA direct and 90 µA alternating currents.
    - Artificially created E fields associated with 16 µA direct currents and similar naturally occurring bioelectric fields emitted by a typical prey; the shore crab (*Carcinus maenas*).

1.5.3. Cognitive ability

The intelligence (in so far as one can define it) of teleost fish has recently received a great deal of attention. The general consensus is that many possess significantly superior cognitive ability than commonly believed (Huntingford, 2003). For example, learned foraging skills have been demonstrated in stickleback fishes (Croy & Hughes, 1991a; Girvan & Braithwaite, 1998; Odling-Smee & Braithwaite, 2003a), salmon, *Salmo salar* (Brown *et al.*, 2003) and mosquito fish, *Brachyraphis episcopi* (Brown & Braithwaite, 2005). Learned escape responses have been demonstrated in zebra fish, *Danio rerio* (Arthur & Levin, 2001) and haddock, *Melanogrammus aeglefinus* (Ozbligin & Glass, 2004). Memory windows of varying length have been demonstrated in a number of species (three hours in paradise fish, *Macropodus opercularis*: Csanyi *et al.*, 1989, three months in cod, *Gadus morhua*: Nilsson *et al.*, 2008). The cognitive ability of elasmobranchs, however, has received much less attention. In general, the few examples of investigations in the field are relatively old (Clarke 1959; Wright & Jackson, 1964; Aronson *et al.*, 1967; Graeber & Ebbesson, 1972; Schluessel & Bleckmann, 2005). Many coastal sharks, skates and rays are opportunistic predators and as such must adapt their foraging behaviour to suit a variable environment and a range of prey. Those that are able to successfully learn and remember to do so will benefit via improved individual success and Darwinian fitness. Recent concerns surrounding interactions between these fish and E fields associated with ORED also raise the question of whether they might be able to learn to ignore such stimuli. The following hypotheses were therefore formulated and are addressed in Chapter 5:

- H_3: *S. canicula* are able to learn to alter their electroreceptive foraging behaviour dependent on the success of foraging events towards artificially created prey type E fields.
- H_4: *S. canicula* are able to remember learned alterations in electroreceptive foraging behaviour after an interval.

2. GENERAL METHODOLOGY

2.1. Experimental animal

Scyliorhinus canicula (the small-spotted catshark, formerly known as the lesser spotted dogfish; Figure 3) is a benthic, marine elasmobranch found in the north-eastern Atlantic, distributed from Norway and the British Isles in the north to Senegal and the Ivory Coast in the south, including the Mediterranean Sea (Campagno *et al.*, 2005).

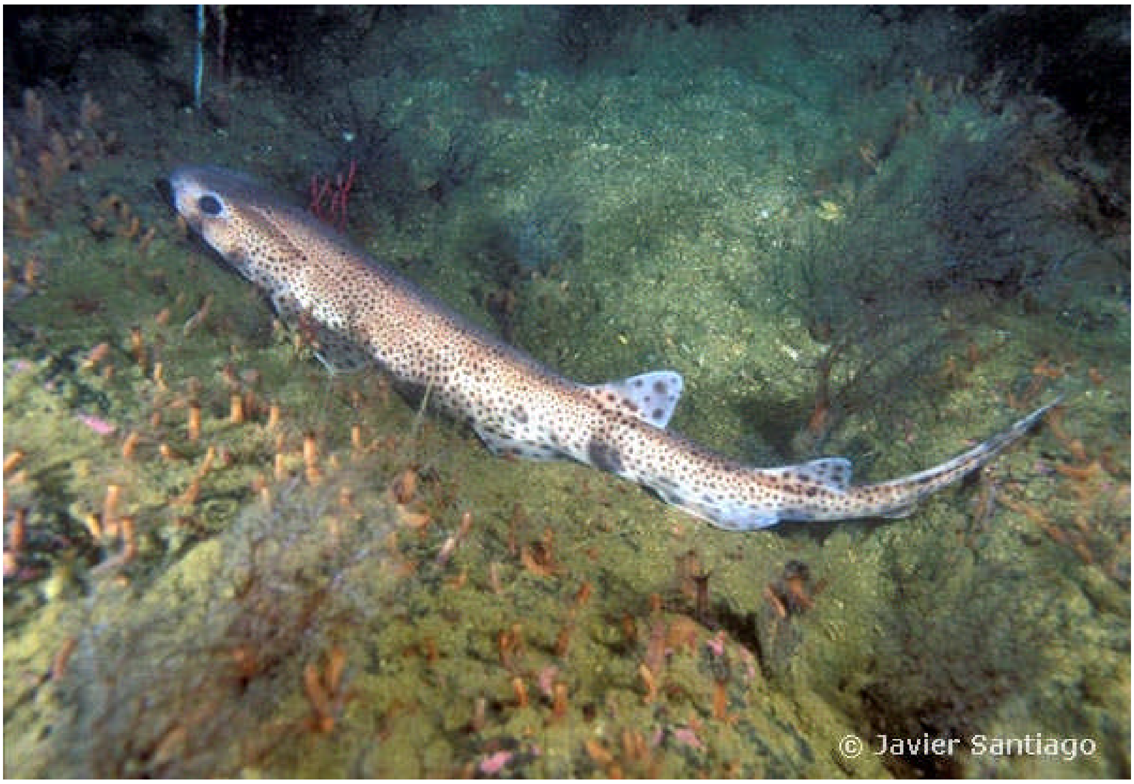


Figure 3. Photograph of experimental animal, *Scyliorhinus canicula*, in its natural environment, at rest on seabed (© Javier Santiago, www.mer-littoral.org/34/Scyliorhinus-canicula.php).

It inhabits continental shelves and upper slopes on various sediments to depths of 110 m and exceptionally 400 m. It is a monomorphic species (with respect to size) that matures at approximately 50 - 60 cm total length and is thought to grow to no more than 100 cm (although less in the Mediterranean). This oviparous species has an extended, if not continuous egg-laying season (Ford, 1921; Metten, 1939; Sumpter and Dodd, 1979; Henderson and Casey, 2001) and owing to different reproductive strategies, the sexes are known to periodically segregate into single-sex groups (Sims *et al.*, 2001; Sims, 2005; Wearmouth & Sims, 2008). They prey and scavenge on small benthic invertebrates (crustaceans, gastropods, cephalopods, polychaete worms) and bony fish (Ford, 1921; Lyle, 1983; Rodriguez-Cabello *et al.*, 2007).

Although not evaluated on the IUCN Red List, *S. canicula* remains fairly abundant with some populations stable or increasing (Compagno *et al.*, 2005). It is taken in many fisheries and when retained can be utilized fresh or dried-salted for human consumption and also for oil or fishmeal. When discarded the fish shows high survival rates. The species is also amenable to captivity and is commonly displayed in aquariums.

The species' small size, abundance and ease of capture have made it an important model for elasmobranch physiological studies (e.g. Scanes *et al.*, 1972; Bromm *et al.*, 1975; Peters & Evers, 1985; Masseck & Hoffmann, 2008). Its hardiness and amenability to captivity also render it an ideal model for laboratory behavioural studies (e.g. Kalmijn, 1971; Pals *et al.*, 1982a; Southall & Sims, 2003; Filer *et al.*, 2008; Wearmouth, 2008).

2.2. Laboratory facilities and animal husbandry

Fieldwork was undertaken at the Marine Biological Association of the United Kingdom (MBA), Plymouth. The catsharks were caught via demersal otter trawling at depths of 50 – 70 m off Plymouth (station L4: 50º15'N, 4º13'W) on an MBA research vessel, the *Quest*, during routine, bimonthly, benthic sampling. Only mature specimens were brought to the laboratory for experimentation (520 mm for males and 550 mm for females; Ellis & Shakley, 1997). They were transported to shore in small numbers within large tubs shaded from sunlight and with clean seawater pumped through constantly.

On arrival at the MBA the fish were weighed and measured and immediately transferred to aerated, 2242 litre capacity, circular holding aquaria (1.83 m diameter by 0.43 m depth). The aquaria were constantly fed by a flow and return system from a 300,000 litre reservoir that was part exchanged with clean sea water three times per week. Water quality was routinely and frequently checked by MBA staff. The photoperiod and water temperature matched natural conditions for the time of year. Prior to any experimental work, catsharks were allowed to acclimatise for approximately three weeks during which time behaviour and health were closely monitored by the author and a Named Animal Care and Welfare Officer (NACWO). Seven aerated, 792 litre capacity, rectangular arenas (1.65 m L x 0.80 W x 0.60 D) in isolated rooms and supplied with filtered water were available for experimental work. An acclimatisation period of 60 hours was imposed after transferring fish to these arenas and before experimental treatments commenced.

The catsharks were fed a mixture of squid (*Loligo forbesi*), whiting (*Merlanguis merlangus*) and marine pellets with liposome spray (New Era Aquaculture Ltd., Thorne, UK). Whilst in holding aquaria (both prior to and after experimentation) 20 g rations per fish were offered twice weekly (equivalent to approximately 3 % wet body mass per feed; Sims & Davies 1994). During experiments minimum rations of 13 g a week (approximately 2 % wet body mass per week: Sims & Davies, 1994) were used to ensure fish were motivated to feed throughout a week.

Two types of identification tag were used during the project. For individuals that needed to be identified within an experimental group a Petersen-type disk (2-cm diameter Floy Tag ® laminated discs; FLOY TAG Inc., Seattle, Washington, USA) was attached to the pectoral fins so as to enable identification directly and on video recordings of subsequent trials. Individuals that just needed to be identified whilst in holding aquaria had T-bar anchor tags (FLOY TAG Inc., as before) attached just below the base of the dorsal fins but above the lateral line. Skin was treated with iodine immediately after tagging.

2.3. Experimental apparatus & methodology

The basic experimental methodology used throughout this project was based on tried and tested techniques. These techniques involve observing elasmobranch responses to artificially created electric fields that mimic bioelectric fields emitted by their prey (Kalmijn, 1971; Heyer *et al.*, 1981; Blonder & Alevizon, 1988; Kaijura & Holland, 2002; Filer *et al.*, 2008). Stimulation by food-derived scent is utilitised to invoke foraging behaviour and attract the fish towards E field sources.

The apparatus used to produce the electric fields in this study were as follows (Figure 4); insulated wires fed through an upright, rigid, white, PVC pipe (20-mm bore), connected to a 90° bend and a short horizontal pipe, terminating with gold plated banana plugs inserted and sealed into separate, open-ended, rigid aquarium tubing (8-mm bore). The open ends of the tubing were held 1.5 cm apart and formed the dipole. The apparatus were attached to a transparent acrylic base with 20- and 5-cm radius circles centred on the dipole marked. These circles defined an Activity and Electrode Zone respectively. In this way, a 1.5-cm dipole salt-bridge was sunk to the base of an arena with the upright pipe leading the wires out up the side of the arena where they were connected to a power supply. Using different resistances electric fields of various voltages were then produced at the base of an arena. 2-mm bore transparent, flexible, aquarium tubing was fed down the PVC tubing ending 7.5 cm before the dipole. Food-derived scent (sieved whiting and squid mixed with seawater) was then introduced into the arena using a syringe. The scent was required to induce experimental catsharks to begin foraging behaviour, thereby increasing their swimming activity and passes over the Activity Zone, and attracting them to close proximity of the dipole (over the Electrode Zone) to elicit electroreceptive responses. The equipment was easily transferred to different ends of an arena or between the seven experimental arenas.

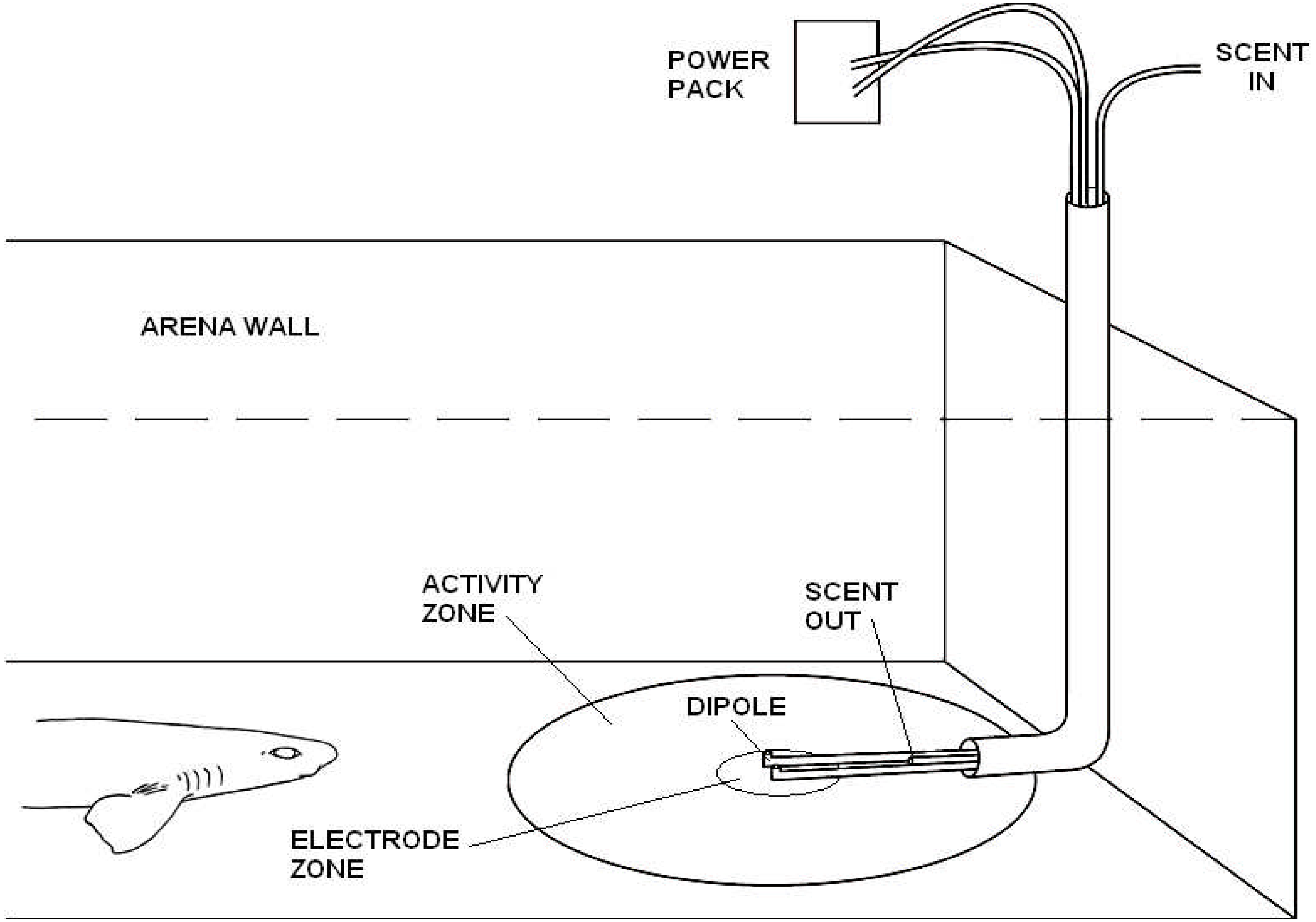


Figure 4. Schematic diagram of experimental apparatus showing basic design and implementation of a salt-bridge circuit and electrode plate in an arena (not to scale).

Behavioural observations were made directly by the author or using video replay of footage recorded by overhead cameras (Sanyo VCC 6572P and Panasonic WV-BP140 1/3” CCD). Activity levels were recorded by counting the frequency that each catshark swam over the Activity Zone (20-cm radius circle). Movement within close proximity of the dipoles could be recorded by counting the frequency the catsharks passed over the Electrode Zone (5-cm radius circle). An elasmobranch’s electroreceptive response towards an E field closely resembles an attack on a prey animal. When a catshark swims near the Electrode Zone and if it detects the prey-type E field emanating from the dipole and decides to attack, it will make an abrupt alteration to its swimming path and a sudden turn towards the perceived prey. It may also bite once, or repeatedly, upon the electrodes. Electroreceptive foraging behaviour was therefore recorded using the frequency and times of turns towards and bites on the electrodes. Only those turns and bites that were distinct, unambiguous and unmistakably directed towards the electrodes were recorded. For relatively small, benthic elasmobranchs, such as *S. canicula*, these behaviours predominantly occur within approximately 5 cm to 15 cm of stimuli (Kalmijn, 1966; Kalmijn, 1971; personal observation) compared with 25 to 40 cm for larger, pelagic species (Kalmijn, 1982; Haine *et al.*, 2001).

2.4. Ethical considerations

All techniques used in this study were approved by the MBA Ethical Review Committee and with reference to Stamp Dawkins & Gosling (1994). The author successfully completed a program of training in the Animals (Scientific Procedures)

Act, 1986 (Module 3: Fish). Home Office approval was not required as no regulated procedures were used.

2.5. Release of experimental animals

When catsharks' experimental trials had finished they were transferred back to the holding aquaria. Their tags were removed and attachment points treated. They were fed a normal ration and observed by the author and NACWO for at least two weeks to ensure health and normal behaviour. They were then certified for release to sea off Plymouth.

2.6. Notes on data analyses

Throughout this study, various types of generalized linear modelling were utilised with Genstat software to analyse data sets for the following reasons: (a) in many cases dependent data was distributed non-normally (e.g. count and binomial data), (b) the uncontrollable nature of the behaviour of catsharks and the fact that individuals were often utilised repeatedly over time required random effects to be accounted for, and (c) the complexity of the data to be analysed (e.g. categorical predictors included E field, week and sex group in Chapter 3) required powerful and flexible methods.

Specific types of distributions were chosen depending upon the type of dependent data (e.g. Poisson for count data, binomial for binomial data or normal for time (continuous) data). Appropriate link functions were then chosen to transform the parameters of these distributions to continuous distributions which therefore enabled regressions to be performed. Generalized linear modelling was performed with reference to McConway *et al*. (1999).

Catshark responses were often plotted using statistical effect sizes from Genstat outputs. Using the size and direction of an effect was a more accurate means of displaying responses than using means from the original data due to non-normal distributions of responses and the complex experimental set up.

3. THE EFFECTS OF MALE-FEMALE INTERACTIONS UPON ELECTRORECEPTIVE FORAGING BEHAVIOUR

3.1. Abstract

To examine potential effects of male-female interactions on the sexes’ foraging behaviour equal sized, single- and mixed-sex groups of a benthic elasmobranch, the small-spotted catshark (*Scyliorhinus canicula*), were studied in response to electric stimuli. A total of 88 size-matched, adult individuals were repeatedly presented with a range of artificial, prey-type electric fields (E fields) under laboratory conditions in groups of (i) four males, (ii) four females, and (iii) two of each sex. Females in single-sex groups were more responsive towards E fields than both single- and mixed-sex group males, despite exhibiting similar activity levels. Equally, females in mixed-sex groups were more responsive than males in mixed groups, but showed similar activity levels. Furthermore, females in mixed-sex groups were less responsive to E fields than females from single-sex groups, despite showing similar activity levels. Males in mixed-sex groups were less responsive than males in single-sex groups, but were more active. These results indicate more intense foraging behaviour among females than males and significant reductions in foraging behaviour when grouped with the opposite sex. Both findings are likely associated with consequences of differing reproductive strategies and resultant sexual conflict (specifically male harassment and female avoidance).

3.2. Introduction

Sexual differences in the foraging behaviour of elasmobranchs are perhaps not expected given that stomach analyses suggest that the sexes of many species seem to have similar diet composition (e.g. narrow mouthed catshark, Matallanus *et al.*, 1993; tiger shark, Simpfendorfer *et al.*, 2001; porbeagle shark, Joyce *et al.*, 2002; thornback ray and tope shark, Morato *et al.*, 2003). Nevertheless, although male and female small-spotted catsharks (*Scyliorhinus canicula*) also show evidence of similar diets (Lyle, 1983), this species' sexes show markedly different behavioural strategies resulting in year-round fine-scale sexual segregation (Sims *et al.*, 2001). At some sites, males occupy deeper water areas during the day and forage at night in warmer, shallow water, whereas females aggregate in shallow water in daytime and conduct nocturnal foraging in deep water (Sims *et al.*, 2006).

Segregation among *S. canicula* (a monomorphic species: Ford, 1921; Henderson & Casey, 2001) has been proposed to be partly driven by aggressive courtship and mating behaviour of males towards females, resulting in refuging behaviour of females in single-sex groups (Sims *et al.*, 2001; Sims, 2005; Sims *et al.*, 2005). Male catsharks are thought to mate throughout the year (Sims, 2005). Females, however, can store sperm for long periods of time (Metten, 1939), have a very extended, if not continuous, egg-laying season (Ford, 1921; Metten, 1939; Sumpter & Dodd, 1979; Henderson & Casey, 2001) and can lay eggs regularly (Harris, 1952). Therefore, it seems likely that females have the potential to exert considerable choice over copulation partners and may do so in part to avoid energetically costly courtship with multiple aggressive males since it may result in injury and/or energy loss through sustained high activity (Pratt, 1979; Carrier *et al.*, 1994; Sims *et al.*, 2006). As with many other animals (including insects, Parker, 1979; guppies, Magurran, 2001; lizards, Schutz *et al.*, 2007; sea lions, Chilvers *et al.*, 2005) these different mating strategies generate strong sexual conflict.

Male harassment of females could also cause decreased female foraging efficiency, as has been observed in female poeciliid fish as a consequence of male aggression (Magurran & Seghers, 1994; Griffiths, 1996; Plath *et al.*, 2003; Agrillo *et al.*, 2006). This may be why female *S. canicula* have been shown to refuge in female-only aggregations in shallow, labyrinthine caves, and conduct foraging in different habitat to that of males (Sims, 2005). However, whether the presence of males affects foraging behaviour of female catsharks has not been tested directly principally because of the logistical difficulties of recording free-ranging behaviours of multiple individuals across the spatio-temporal scales over which foraging takes place.

Electroreception is the last sense to be used in the hierarchical array of senses utilised by elasmobranchs when foraging, has been shown to override all other senses when in close proximity of a stimulus, and is crucial for predatory and reproductive success among benthic species that search for prey and mates buried beneath the substratum (see Chapter 1). Despite its importance, and notwithstanding substantial physiological investigation of the sense, knowledge concerning electroreceptive behaviour remains poorly described (see Chapter 1; Tricas & Sisneros, 2004). Past work has often concentrated on demonstrating and/or describing species' electroreceptive responses rather than focusing on quantifying the extent and variability in response to significant behavioural influences, such as individual variability (Gill *et al.*, unpublished data), male-female differences and interactions (see below), choice (Chapter 4) or profitability of stimulus (Chapter 5). Different prey species and sizes emit different bioelectric E fields (Kalmijn, 1972; Haine *et al.*, 2001; Chapter 1). One would therefore expect variability in electro-sensitivity between electroreceptive fish with differing diets. Although stomach content analyses suggest that the sexes of many elasmobranchs, including *S. canicula*, possess similar diets (Lyle, 1983), it is important to carefully investigate possible variability in electro-sensitivity when studying male and female electroreceptive foraging behaviour. This seems especially important when one considers stomach content may be more a result of prey availability than predator preference or sensory capability.

Intra-specific variability in senses between the sexes is common throughout nature. Differences in olfaction and/or dimorphism in chemosensory tissue have been observed in many organisms from crustaceans (Weissburg *et al.*, 1996; Bauer & Caskey, 2006) and amphibians (Woodley, 2007) to reptiles (Thorpe, 1989; Murphy *et al.*, 2001) and mammals (Garcia-Falgueras *et al.*, 2006; Murias *et al.*, 2007; Palagi & Dapporto, 2007). Acoustic differences and/or dimorphism in ears have been reported in amphibians (Narins & Capranica, 1974; Vassilakis *et al.*, 2004), birds (Searcy & Brenowitz, 1988; Searcy, 1990) and mammals (McFadden *et al.*, 1999; Smith *et al.*, 2007). Visual differences and/or dimorphism in eyes occur, for example, in crustaceans (Ohtsuka & Huys, 2001) and insects (Arikawa *et al.*, 2005; Lau *et al.*, 2007).

Among elasmobranchs, sex differences in auditory apparatus have been found (Barber *et al.*, 1985) and differences in hearing abilities suggested (Carrier *et al.*, 1994; Myrberg, 2001). Olfaction is thought to be important for males locating potential mates in many elasmobranchs (Myrberg & Gruber, 1974; Johnson & Nelson, 1978; Carrier *et al.*, 1994) and although female pheromone release and subsequent male chemo-reception has been suggested (Gordon, 1993; Kaijura *et al.*, 2000; Pratt & Carrier, 2001), sex differences in sensitivity are yet to be investigated. Species that periodically bury themselves in substrate have been shown to utilise electroreception to locate conspecifics (stingrays: Tricas *et al.*, 1995; Sisneros & Tricas, 2000, skates: Bratton & Ayers, 1987; New, 1994; Sisneros *et al.*, 1998). Gonadal androgens have been shown to induce neurophysiological changes in the electro-sense of male Atlantic stingrays (Sisneros & Tricas, 2000). However, there is no available evidence of sex differences in electroreceptive behaviour in these, or any other elasmobranchs. A recent investigation of the effects of substratum type and depth on *S. canicula* electric field detection indicated no apparent sex differences (Filer *et al.*, 2008). However, a comprehensive investigation of whether male and female *S. canicula* possess differing sensitivities to different E fields (which may relate to different prey animals and sizes) has not been undertaken.

3.2.1. Objectives

The effects of male-female interactions on the foraging behaviour of sharks, skates and rays have not been the subject of quantitative studies. Electroreception is an important sensory mode during foraging in elasmobranchs and results in behavioural responses to artificial prey that are routinely repeatable and quantifiable. To date, laboratory studies focused on male and female shark responses to prey-simulating electric fields as a measure of sex related feeding behaviour have not been attempted. Therefore the aim of the present study was to use electroreceptive responsiveness as a behavioural proxy for feeding to examine whether there were differences between male and female *S. canicula* foraging behaviour. The hypothesis was tested that the proximity of the opposite sex may affect electroreceptive foraging behaviour of male and female *S. canicula*. The behaviour of equal-sized, single- and mixed-sex groups of *S. canicula* towards artificial, prey-type electrical fields was examined under laboratory conditions.

A range of E fields was used in order to confirm whether or not the sexes possess differing sensitivities to different E field intensities.

3.3. Methodology

3.3.1. Animals and apparatus

Eighty eight size-matched, adult small-spotted catsharks (*S. canicula*) were caught on a Marine Biological Association of the U.K. (MBA) research vessel off Plymouth, southwest England (station L4: 50º15’N, 4º13’W), between May and September 2005. The 44 male and 44 female fish averaged 66.45 ± 3.29 cm and 59.66 ± 2.67 cm total length (±SD), respectively. Catsharks were maintained as described in Chapter 2.

Four catsharks were transferred into each behavioural arena (see Chapter 2) in early July 2005 with three arenas containing two of each sex, two arenas containing four males, and two arenas containing four females. Catsharks were tagged with a Petersen-type disk through the pectoral fin (2-cm diameter Floy Tag ® laminated discs; FLOY TAG Inc., Seattle, Washington, USA) according to licensed Home Office animal welfare regulations. Tagging of different pectoral fins and use of black and white disks enabled each of four individuals per arena to be identified directly and on video recordings of subsequent trials.

Salt-bridge apparatus were used to present the catsharks with biologically relevant electric fields (E fields) produced by 0.9 µA, 9.0 µA and 90.0 µA currents in addition to control treatments (0.0 µA) during which apparatus set up remained the same except that the power was disconnected (see Chapter 2; specifically Figure 4). The E fields in question (approximately 0.095, 0.95 and 9.5 µV/cm at the dipole, respectively) were within the range previously shown to be associated with the bioelectric fields of prey and attractive to elasmobranchs (Kalmijn, 1972, 1982; Chapter 1).

3.3.2. Experimental procedure

The catsharks in each arena were presented with one different E field per day over four days at random ends of the arenas, with fields assigned using stratified, random numbers. The catsharks were fed to satiation immediately following the experiment on the fourth day. A four day period was termed an experimental week. There was a three day gap before another four day experimental week to ensure the catsharks were motivated to forage upon introduction of food-derived scent. In order to account for individual variation the experimental week was repeated five times such that each catshark was presented with each of the four E fields (and control) a total of five times. Three similar phases of experiments were undertaken using different groups of catsharks to increase statistical power whilst reducing the confounding effects of using the same catsharks over protracted time periods. The data from each of the 88 catsharks was recorded separately to enable individual ID use as a random factor in statistical analysis. Once each group’s experiments were concluded they were moved to holding aquaria, and after a short period of observation were certified for release to the sea off Plymouth.

Prior to any particular treatment a salt-bridge apparatus was introduced into a randomly chosen arena, and at a randomly chosen end, with the power switched off to allow the catsharks to settle down. Water flow was stopped and the temperature of the water was measured. After 20 minutes the power was switched on, except during controls, and 20 ml of food-derived scent (sieved squid and whiting added to water) was introduced into the arena and behavioural observations commenced. Treatments lasted for 20 minutes during which time direct observations were made together with video recordings of the following hierarchical foraging behaviours of each catshark: (a) Reaction time to scent, (b) number of times the Activity Zone was entered (an indicator of movement around an arena), (c) time latency of turns towards electrodes (scent time from turn times), (d) frequency of turns towards electrodes, (e) proportion of times each catshark turned towards electrodes having entered the Electrode Zone, and (f) frequency of bites upon electrodes. Only those turns and bites that were distinct, unambiguous and clearly directed towards the electrodes were recorded.

3.3.3. Data analysis

Correlation analysis was used to test for relationships between temperature change over time and behaviour for the whole study period. Similarly, any effect of body size on behavioural responses was also examined.

Each catshark had a unique identifier. In addition, for each catshark, its ‘sex group’ was identified (Figure 5); i.e. a male in an all male arena (M), a female in an all female arena (F), a male in a mixed-sex arena (M_{+F}) and a female in a mixed-sex arena (F_{+M}).

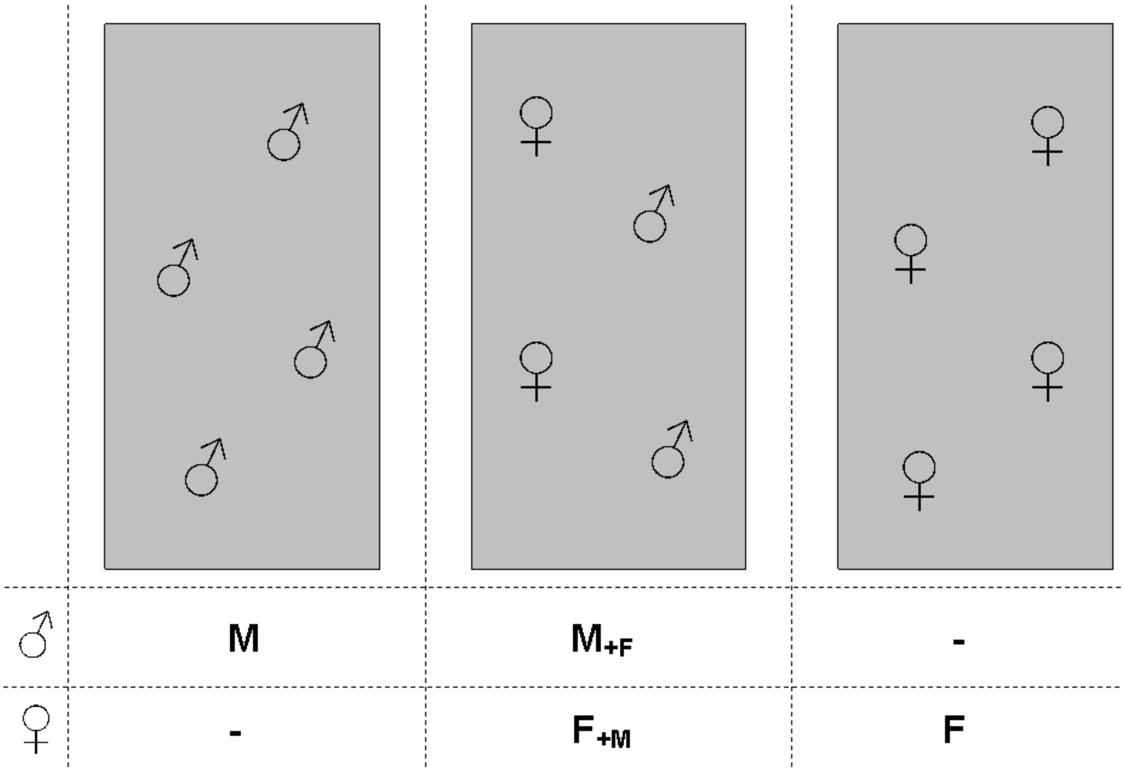


Figure 5. Diagram illustrating different sex group configurations in arenas and highlighting identifiers allotted to catsharks of each sex within those groups (♂ = male; ♀ = female).

Hierarchical generalized linear mixed modelling (HGZMM) repeated measures were used to evaluate the differences in electroreceptive foraging responses (a) to (f) with respect to sex groups. For count data, the HGZMM used a Poisson distribution owing to the large number of zero and low integer values. A logarithm link function then transformed this to a normal distribution. For continuous data, a normal distribution was used with an identity link function (which simply multiplies the distribution by 1 and thus does not transform it). For proportion data, a binomial distribution was used which was then transformed to continuous logit curves using a logit link function. In all three cases, fixed effects of E field, week and sex group were used such that each data point for each catshark was utilised only once during each analysis whilst ensuring the unbalanced design attributed to single- and mixed-sex groups was accounted for. Random effects of individual by week were used to account for replication.

For all HGZMM results reported, the distribution of the residuals was plotted and found to be close to normal so the model could be assumed to be satisfactory. It was not possible to use scaled deviances to estimate goodness of fit owing to the inclusion of a random effect in the model. Residual degrees of freedom were between approximately 500 and 1600 for all analyses (except turn times which was 2079). All statistical analyses were performed using Statistica 7.0 and Genstat 9.0 software.

Statistical sizes of effect from Genstat outputs were used to plot responses of catsharks compared between the sex groups. Throughout, the F group (females in single-sex arenas) was the reference value (zero) to which the other three groups were compared. The effects shown in the graphs indicate the relative size and direction of any differences. Significant differences between groups' response levels are annotated using differences in lower case, italic letters. For example, in Figure 6 the F, F+M, M and M+F groups are labelled *ab*, *ab*, *a* and *b* respectively which indicates that the final two groups are significantly different to each other (M and M+F) but that all other groups are similar to each other.

3.4. Results

The food-derived scent introduced to arenas initiated catshark movement in 93.5 % of the trials. The average time for catsharks to respond to the scent by swimming movement was 1.97 min ± 2.28 (SD; N = 88). Following stimulation by the scent a total of 2472 turn responses and 1093 bite responses to E fields were recorded. On average 44.0 % ± 14.9 (SD; N = 88) of turns resulted in a bite.

Over the course of the experimental period there was a gradual linear decrease in water temperature with time (slope = -0.15, $N = 20$, $P < 0.0001$ for arenas 1-4, mean water temperature 15.2 ºC ± 1.09 SD; slope = -0.62, $N = 10$, $P < 0.0001$ for arenas 5-7, mean water temperature 15.8 ºC ± 1.93 SD). There was no correlation between temperature and catshark activity or electroreceptive responsiveness (Spearman's rank correlation: R range, -0.067 - 0.067, $N = 809$, P range, 0.06 - 0.72, for male responses; R range, -0.050 - 0.013, $N = 836$, P range, 0.15 - 0.81, for female responses). Similarly, there was no correlation between catshark body length and movement within arenas (Spearman's rank correlation: $R = -0.088$, $N = 88$, $P = 0.42$). There were also no correlations between catshark body length and responsiveness to E fields (Male turns, $R = -0.065$, $N = 44$, $P = 0.68$; male bites, $R = -0.145$, $N = 44$, $P = 0.35$; female turns, $R = -0.139$, $N = 44$, $P = 0.37$; female bites, $R = -0.142$, $N = 44$, $P = 0.36$).

HGZMM analysis showed turn and bite responses were significantly less for control experiments than those with applied E fields ($P < 0.01$). There were no differences between the turn and bite responses towards 0.950 and 9.500 μV fields, but responses to 0.095 μV fields were significantly less than 0.950 and 9.500 μV fields ($P < 0.01$). This pattern of responses to different E fields was observed within all sex groups (Kendall's coefficient of concordance $W = 0.91$, $N = 4$, $k = 8$, critical value at $P = 0.05$ is 0.376).

Mixed group males (M_{+F}) entered the Activity Zone significantly more than males in male-only groups (M) ($P < 0.05$, Figure 6). The other sex groups' activity levels were not different (Figure 6).

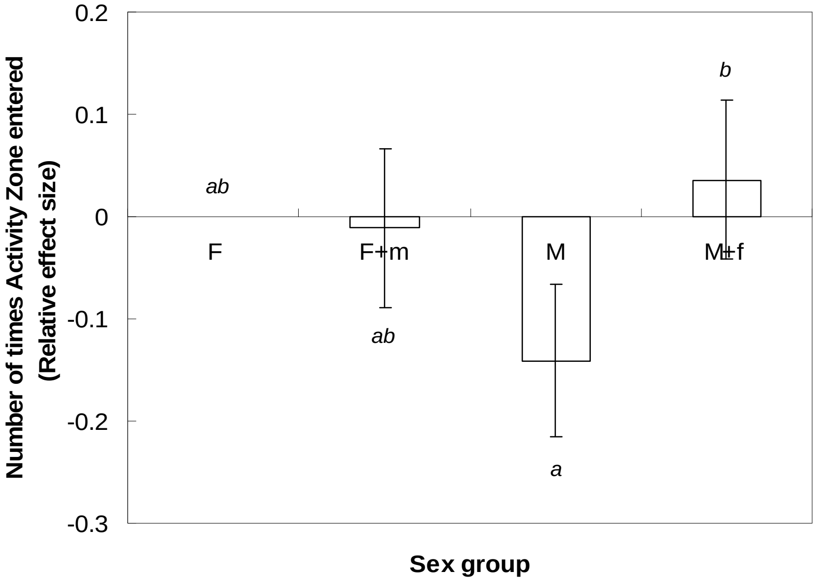


Figure 6. Relative number of times Activity Zone entered by different sex groups with F as the zero reference (+/- standard error; Y axis plotted using statistical effect size; differences in lower case, italic letters denote significant differences between groups)

The latency in time between food scent stimulus and turn response to E fields was similar between the sexes, but those in mixed arenas turned significantly later than those in single-sex arenas (P < 0.01, Figure 7). When only the first turn made by each catshark in each treatment was considered a similar pattern was found (mixed groups responding later than single-sex groups), though differences were not significant probably owing to the reduced sample size.

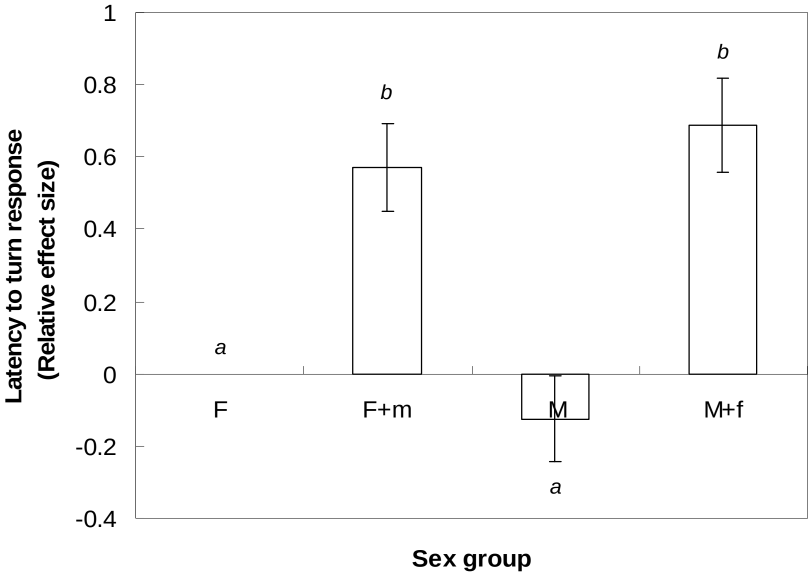


Figure 7. Relative time latency between stimulation by scent and turn response toward E field of different sex groups with F as zero reference. (± standard error; Y axis plotted using statistical effect size; differences in lower case, italic letters denote significant differences between groups.)

F females turned towards the electrodes more than all other sex groups ($P < 0.01$, Figure 8a). M_{+F} males turned less than M males and F_{+M} females ($P < 0.05$, Figure 8a). The proportion of times F females turned when passing over the Electrode Zone was significantly higher than all other sex groups ($P < 0.02$, Figure 8b) and the proportion for M_{+F} males was lower than for M males ($P < 0.05$, Figure 8b). Females (F and F_{+M}) made bites at the electrodes significantly more than M males ($P > 0.01$) and M_{+F} males ($P > 0.01$ for F and $P > 0.02$ for F_{+M}, Figure 8c).

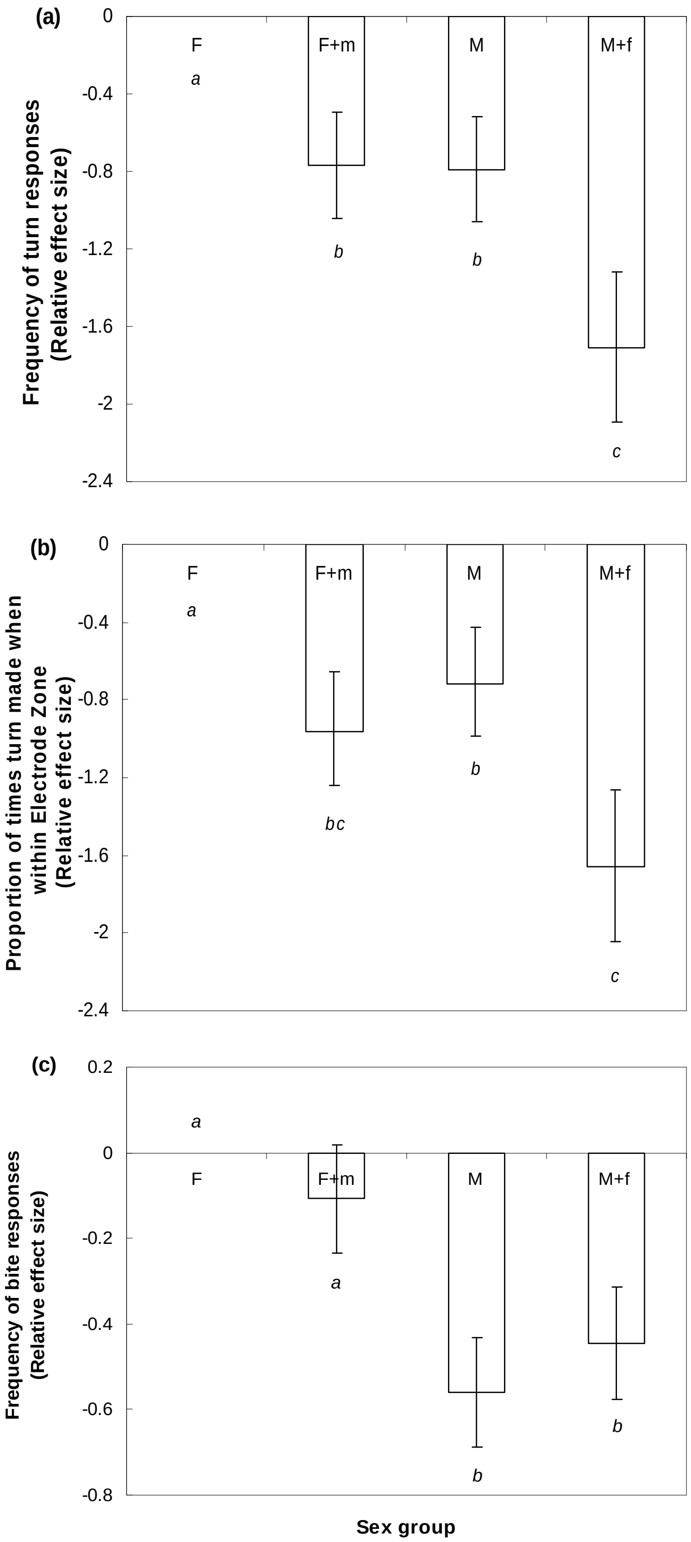


Figure 8. Relative **(a)** frequency of turns towards electrodes, **(b)** proportion of times turns made when within Electrode Zone, and **(c)** frequency of bites on electrodes by different sex groups with F as zero reference. (± standard error; Y axis plotted using statistical effect size; differences in lower case, italic letters denote significant differences between groups.)

3.5. Discussion

The results demonstrate higher foraging responsiveness in females than males, independent of activity level. F females turned towards and made bites on electrodes more often than M and M_{+F} males even though they displayed similar activity levels. Equally, F_{+M} females appeared more responsive to the electrodes than M_{+F} males, with more turns towards and bites on electrodes despite similar activity levels. Furthermore, the results provide evidence that the presence or absence of the opposite sex had striking effects on activity levels and foraging responsiveness. F females were more responsive than F_{+M} females in that they exhibited more turns towards the electrodes and higher proportions of turns having entered the Electrode Zone despite similar activity levels. Equally, M males were more responsive than M_{+F} males in that they exhibited more turns and higher proportions of turns having entered the Electrode Zone despite lower activity.

The study was designed to minimise factors other than sex affecting electroreceptive foraging behaviour. Temperature has been shown to affect the sensitivity of fish electro-receptive apparatus (Henzel, 1955; Akeov *et al.*, 1980; Akeov, 1990). However in this study no correlation was found of electroreceptive responsiveness with temperature. Responsiveness was not correlated with catshark body size either. The catsharks were size matched as far as possible to minimise any age related effect and were all sexually mature. Also, catsharks are generalist feeders (Ford, 1921; Lyle, 1983) and one would therefore expect them to be less dependent on prior experience than specialists (Hirvonen *et al.*, 1999). The catsharks used in the study were from the same geographic location thereby reducing any influence of local effects on experience even further. They were also allowed to acclimatise for three weeks in a homogenous environment with a regulated feeding regime to help equalise in so far as is possible their feeding motivation. Furthermore, the diet composition of male and female catsharks has been found to be similar (Lyle, 1983; Sims, 2005; Rodriguez-Cabello *et al.*, 2007). The sexes' sensitivities to prey E fields would therefore be expected to be similar and thus not likely to cause differences in the sexes' responsiveness to such fields. The data from this study confirm this by demonstrating a similar pattern of responses to the four prey-type E fields in all four sex groups. Equally, although electroreception is important for elasmobranchs that search for conspecifics buried under substrate (stingrays: Tricas *et al.*, 1995; Sisneros & Tricas, 2000, skates: Bratton & Ayers, 1987; New, 1994; Sisneros *et al.*, 1998), *S. canicula* tend to rest on, rather than under, the substrate (Kalmijn, 1971; Froese & Pauly, 2008). Differential electrical sensitivities and responsiveness are therefore not expected to be linked to searching for mates in this species (olfaction is likely more important: Kleerekoper, 1978; Pratt & Carrier, 2001). Again, the findings from this study confirm no sex-specific electrical sensitivity differences.

Although catshark behaviour was not analysed over time, no change in behaviour was found with temperature, which decreased over time. Equally, noteworthy changes in behaviour were not noted by the observer during the five week experimental periods. Thus the effects of male-female interactions observed during this study cannot simply be considered as initial anomalies caused by naturally segregating sexes being confined together in experimental arenas.

Taking into account the factors above, an intriguing question arose of why female foraging levels were higher than those of males. Gonadal androgens have been shown to induce neurophysiological changes in the electro-sense of male Atlantic stingrays (Sisneros & Tricas 2000), thought to aid mate detection by honing electro-sensitivity to female bioelectric fields. However, Sisneros and Tricas (2000) found no effect upon foraging electroreception. One possible explanation for higher responsiveness by females is that they may possess higher energy requirements for egg production (fish, Hendry & Berg, 1999; Trudel *et al.*, 2000; Whiteman & Cote, 2002; reptiles, Kwan, 1994; Nunez *et al.*, 1997; birds, Moore *et al.*, 2000) so may need to forage more intensively than males. They have been shown to undertake shorter foraging excursions than males from their refuges which was suggested to reduce foraging times and overlap with males that remain active throughout the diel cycle (Sims *et al.,* 2001). Thus, there may be pressure for female excursions to be more intense and successful in an attempt to consume as much prey as possible, as rapidly as possible. In the present study, this apparent intraspecific, sex-based competitive release effect may be the reason why we observed similar activity levels between females in single- and mixed-sex groups that confound assumptions that the latter would be more active in an attempt to avoid males.

Male and female foraging levels were markedly reduced when in the presence of the opposite sex. The most plausible explanation for this finding is between-sex inhibition arising from male harassment of females (note that female avoidance of males may also be considered as a form of harassment with the potential to affect male foraging). Whilst no observations of overt, physical interactions between the sexes were observed during experiments, it is likely that such interactions did occur at some point during the catsharks’ time spent in the arenas when not under observation (approximately 14 hours per week compared to 154 hours per week respectively). Moreover, subtle but powerful and long-lasting effects of the risk of interaction are sufficient to induce marked reactionary behaviour (Smith 1997; Scott *et al*. 2005; V.J. Wearmouth & D.W. Sims unpublished data). For many female elasmobranchs, courtship with multiple, aggressive males are costly since they may result in injury and/or energy loss through sustained activity (Pratt & Carrier 2001). This is evidenced by females having evolved vigorous evasive behaviours to avoid males (Klimely 1980; Castro *et al*. 1988) and thicker skin as a defence mechanism against males' bites (up to three times; blue shark, Pratt & Carrier 2001).

In this study, males and females isolated in single-sex groups showed more numerous, rapid and probable foraging responses to electric stimuli when within the Electrode Zone. In mixed-sex groups the males' higher activity levels but lower responsiveness to E fields are most likely related to primary responses towards female presence rather than to the presence of prey-simulating dipoles. Equally, the females' reduced responsiveness in mixed-sex groups is most likely linked to female avoidance of males. Whilst the precise mechanism underlying these behaviours is unknown, male aggressive courtship behaviour, and the risk of such behaviour (possibly involving pheromones and olfaction, see Johnson & Nelson 1978; Pratt & Carrier 2001) are the most probable causes. In the context of the behavioural arenas used in this study, the reduced foraging activity of females may reflect a refuging behaviour in response to heightened male reproductive activity, a combination of behaviours that are generally consistent with field observations of male and female *S. canicula* interactions (Wearmouth & Sims, 2008).

Reduced female foraging behaviour due to male harassment has also been observed in poeciliid fish (Magurran & Seghers, 1994; Plath *et al.*, 2003; Agrillo *et al.*, 2006). Furthermore, as in this study, Griffiths (1996) demonstrated decreased foraging levels in both female and male poeciliid fish in mixed-sex groups relative to single-sex groups. Griffiths (1996) suggested that males make feeding and mating choices based upon hunger, whereas female feeding is constrained by sexual harassment. A similar constraint is apparent with catsharks. Indeed, the inhibition of female catshark feeding may be costly enough to combine with factors such as energy loss and injury in having directed the evolution of sexual segregation in this, and species exhibiting similar strategies (e.g. Port Jackson sharks, *H. portuskacksoni*, in which females refuge in reef caves; McLaughlin & O'Gower 1971). Furthermore, such inhibition may contribute to increased pressure upon female feeding which is manifested in higher foraging levels among females than males.

It is important to note that subtle male-female interactions other than harassment and avoidance may have also, in part, influenced catshark foraging behaviours. For example, male competition for mates (Lucifora *et al*. 2002) could contribute to decreased foraging levels in both sexes (and may also be linked to harassment). Disparity in intra-specific competitiveness between males and females may occur. Due to a lack of research in these, and many other areas of elasmobranch behaviour, it is unclear whether such factors may have any influence. Equally, whilst pheromones and steroids can affect food conversion efficiency in teleost fish (Mandiki *et al*. 2004), the physiological effect is unknown in elasmobranchs.

The results from this study significantly enhance understanding of foraging behaviour and sexual segregation among male and female elasmobranchs, and how interactions between the sexes may shape these behaviours. Such information may benefit the management of species in which male-female interactions have implications for population dynamics (Chapters 1 & 6).

4. ABILITY TO DISCRIMINATE BETWEEN DIFFERENT ELECTRIC FIELDS: ELECTRORECEPTIVE PREFERENCES

4.1. Abstract

The ability of a benthic elasmobranch to distinguish between different prey-type electric fields (E fields) was investigated. To address this objective, small-spotted catshark (*Scyliorhinus canicula*) preferences were studied by behaviourally conditioning the fish to swim through narrow tunnels, and simultaneously presenting them with two different E fields upon exit. Their subsequent choices were recorded, given the following options of E field pairs;

a) E fields with differing strength direct current (D.C.)

b) E fields with similar strength direct (D.C.) and alternating current (A.C.)

c) E fields with similar strength natural and artificial direct current (D.C.)

Analysis showed a highly significant preference for the stronger D.C. electric field and a less pronounced, but still significant, preference for A.C. electric fields rather than D.C. electric fields. No preference was demonstrated between artificial and natural D.C. electric fields. The findings are discussed in relation to the animal's diet and ecology and with regard to anthropogenic sources of E fields within their habitat.

4.2. Introduction

At their basic level foraging models predict that organisms will attempt to maximise gains and minimise costs to optimise somatic growth and reproduction (Optimal Diet Theory: Charnov, 1976). In the case of fish, this process may be affected by a number of factors (Kaiser & Hughes, 1993) including physiological state (Croy & Hughes, 1991b; Gill & Hart, 1998), experience (Croy & Hughes, 1991a; Warburton, 2003), competition (Thompson & Jones, 1983; Gill & Hart, 1996), and predator-prey interactions (Hart & Hamrin, 1990; Huang & Sih, 1991; Kaiser *et al.*, 1992a). However, two of the most fundamental considerations for all predators are prey choice and exploitation of prey within patches given the direct nature of their effect on energy intake (Stephens & Krebs, 1986). For instance, certain prey may be easier to catch and consume than another, although more difficult prey may possess higher nutritional value and might therefore be worth the extra effort (Gill & Hart, 1994). Equally, certain locations might provide high quality foraging potential with high density of suitable prey, but even better opportunities elsewhere may be lost by focusing entirely on the former (Stephens & Krebs, 1986). Decisions made between prey and patch options may arise directly through individuals making active choices or indirectly as phenotypic attributes arising via natural selection. Regardless of which scenario, these decisions are crucial to an individual’s success at obtaining food resources required for somatic and gonadal growth and therefore potentially influence Darwinian fitness.

The ability to differentiate between two or more stimuli is fundamental for animal behavioural choice scenarios. It enables preference for certain stimuli and therefore the opportunity for informed choices to be taken based upon prior experience. Sensory preference has been demonstrated throughout the animal kingdom. There is a large amount of literature regarding bees' preferences for certain flower colours and sizes (Dukas & Real, 1993; Dyer *et al.*, 2006; Ishii, 2006; Goulson *et al.*, 2007), female birds' preferences for different male songs (Collins, 1999; O'Loghlen & Rothstein, 2003; Swadle & Page, 2007), frog and toads' preferences for auditory cues (Jennions *et al.*, 1995; Bodnar, 1996; Schwartz *et al.*, 2001; Endepols *et al.*, 2003; Castellano *et al.*, 2004) and stickleback fishes' preferences for colours and ultra violet (Baube *et al.*., 1995; McKinnon, 1995; Smith *et al.*, 2004; Modarressie *et al.*, 2006; Rick *et al.*, 2006). Other examples of work on preferences range widely from taste in fruit flies (Gordesky-Gold *et al.*, 2008) and colour and movement in lizards (LeBas & Marshall, 2000) to light levels in cattle (Baldwin & Start, 1981) and vision and touch in pinnipeds (Busch & Duecker, 1987; Dehnhardt, 1994 respectively).

There are few studies that have investigated elasmobranch preferences for different stimuli relating to the same sense. Clarke (1961) demonstrated that adult lemon sharks (*Negaprion brevirpstris*) were able to discriminate targets differing in shape and pattern. Aronson *et al.* (1967) demonstrated light-dark discrimination in juvenile nurse sharks (*Ginglymostoma cirratum*). Strong (1996) demonstrated great white sharks' (*Carcharadon carcharias*) preference for seal-shaped visual targets rather than square targets. Wallman and Bennett (2006) observed preferences for cooler water among fed stingrays (*Dasyatis sabina*) and warmer water among pregnant female stingrays possibly owing to increased nutrient uptake and reduced gestation periods respectively. Following observations of sexual segregation in the wild (Sims, 2001; Sims, 2005), Wearmouth & Sims (2008) used laboratory studies to demonstrate that female small-spotted catsharks preferred warmer temperatures, despite higher energetic costs, when refuging from males. Gardiner *et al.* (2007) found preferences for scented and turbulent plumes over unscented and oozing plumes in the smooth dogfish (*Mustelus canis*). This final example, however, is limited in its context as the sharks were, in effect, offered a choice between the presence and absence of the stimuli rather than differing levels of the stimuli.

Weakly electric teleost fish have been shown to be able to discriminate and choose between objects differing in their electrical characteristics using active electrolocation (von der Emde, 1990). However, preferences for electric fields (E fields) using passive electroreception in elasmobranchs have received little attention. Blonder & Alevizon (1988) attempted to demonstrate whether stingrays were able to differentiate between prey and non-prey invertebrates using electroreception alone. Their findings suggested the stingrays were unable to do so, however they added that the findings were not conclusive; possible reasons for which included inability of stingray to identify a non-prey item, experimenters' uncertainty of the difference between prey and non-prey bioelectric fields and overly hungry stingrays feeding in a non-discriminatory manner, i.e. showing no preference. Indeed, this raises an important consideration when questioning whether elasmobranchs show preferences for certain types of E fields; whether the fish are actually able to differentiate between them. Behavioural studies have demonstrated various species to be attracted to a range of fields (as low as 5 nV/cm; Kalmijn, 1982; upper limits less certain but avoidance shown at higher voltages > ~ 1000 nV/m; Dijkgraaf & Kalmijn, 1962; 0.2 to 10 V; Yano *et al.*, 2000). It is also possible to measure the sensitivity range of the electroreceptive apparatus, Ampullae of Lorenzini, neuro-physiologically (to 20 nV/cm, Tricas & New, 1998; D.C. to approx. 10 Hz A.C., Montgomery 1984; Peters & Evers, 1985; Sisneros & Tricas, 2000). However, how the brain processes these inputs and the fish translate the information remains uncertain. It is not possible to determine whether the fish are capable of differentiation rather than simply detection of the presence of fields (more akin to an “on - off” switch) without observing behavioural responses to simultaneously presented E fields.

Electroreception has been demonstrated to be crucial in the final moments of elasmobranch feeding and is also used during the location of and communication with conspecifics, the detection of predators and possibly in aiding navigation (see chapter 1). The ability of fish to successfully detect differences and make choices between different types and sizes of prey, predators and symbionts and suitability of mates, habitats, swimming paths and navigation routes will ultimately contribute to individual success and Darwinian fitness.

4.2.1. Objectives

Given the importance of the electro-sense to elasmobranchs, but the lack of knowledge regarding their detection abilities of and preferences for different E fields, the aim of this study was therefore to determine whether a benthic elasmobranch, the small-spotted catshark (*Scyliorhinus canicula*), shows preferences for certain prey-type electric fields. In doing so the ability of the catsharks to differentiate between the fields, rather than merely detect the presence of fields, would also be ascertained. In the wild, prey and prey patches can be encountered sequentially or simultaneously (Stephens & Krebs, 1986), but in order to comprehensively address these objectives individual catsharks were simultaneously presented with sets of differing pairs of electric fields under laboratory conditions. Their subsequent choices were recorded and analysed.

4.3. Methodology

4.3.1. Experimental animals

Sixty size-matched small-spotted catsharks (*S. canicula*; hereafter referred to as ‘catsharks’) were caught on a Marine Biological Association of the UK (MBA) research vessel off Plymouth, southwest England (station L4: 50°15’N, 4°13’W), in late 2006 and early 2007. The fish averaged 62.0 ± 3.4 cm total length (±SD). Catsharks were maintained as described in chapter 2.

4.3.2. Experimental apparatus

Behavioural arenas, used previously (see Chapters 2, 3), were adapted for use during this study. Barriers, held in place by brackets on the arena walls, spanned the width of each arena such that the only access between a larger (100-cm length) and smaller (45-cm length) section was through a small and narrow tunnel (20.0 cm long x 10.0 wide x 8.5 high; Figure 9). A barrier consisted of two 3-mm thick, upright, acrylic sheets held 20 cm apart by two supporting plastic tubes at the top and an oven-folded 5-mm thick acrylic sheet at the base (Figure 9). The folded acrylic sheet formed the tunnel and was attached to the upright sheets using nylon screws. Rocks and sections of drainage pipe were added to the large sections of each arena to increase environment heterogeneity and thus provide a more inviting habitat for catsharks placed in the arenas. The upright acrylic sheets facing the large sections were opaque white (to imitate an arena wall from the point of view of the catsharks) whereas those facing the small sections and those forming tunnels were transparent (to allow the observer to see catsharks entering tunnels).

Salt-bridge electrode circuits and plates similar to those used previously (see Chapters 2, 3) were used to present catsharks with prey-type electric fields (E fields). The apparatus were modified to enable two E fields to be presented simultaneously to a catshark exiting a tunnel into the small section of an arena. The alterations were such that when the apparatus were placed in the small section the two dipoles were positioned at equal distances from the centre of the exit of a tunnel (11 cm; Figure 10). The dipole pairs were both aligned in the same direction as the tunnel so that the catsharks approached the E fields along their most intense gradients (along, rather than perpendicular to axes; Figure 10). This also prevented the formation of quadropoles (whereby the positive and negative components of one dipole interact with the opposite components of the other dipole to effectively form four dipoles). Using plots of decreasing electrical potential with increasing distance (calculated with reference to Kraus & Fleisch, 1999), the dipoles were located (a) far enough apart to ensure that (i) any interference and/or additive effect between E fields were minimised and kept below the emission intensity at the dipoles, and that (ii) stronger E fields would not swamp weaker fields, but (b) close enough together so that both E fields were simultaneously detectable by catsharks exiting tunnels (based on *S. canicula* sensitivity documented in Kalmijn, 1982). The optimum electrode placement, estimated by balancing these conditions, was achieved by forming an isosceles triangle between the centre of a tunnel exit and the two dipoles measuring 15 x 11 x 11 cm (Figure 10).

The narrow tunnels restricted the movement of catsharks enough to keep their bodies straight, whilst not preventing swimming action, and in effect acted as funnels to ensure the catsharks always approached the dipole pairs from a uniform direction and that their snouts always entered the two E fields at the same location. On exit from the tunnels, the dipoles were positioned far enough away from the barriers such that the catsharks were able to either twist their bodies to turn toward and respond to one electrode or the other, or could ignore both and pass between them.

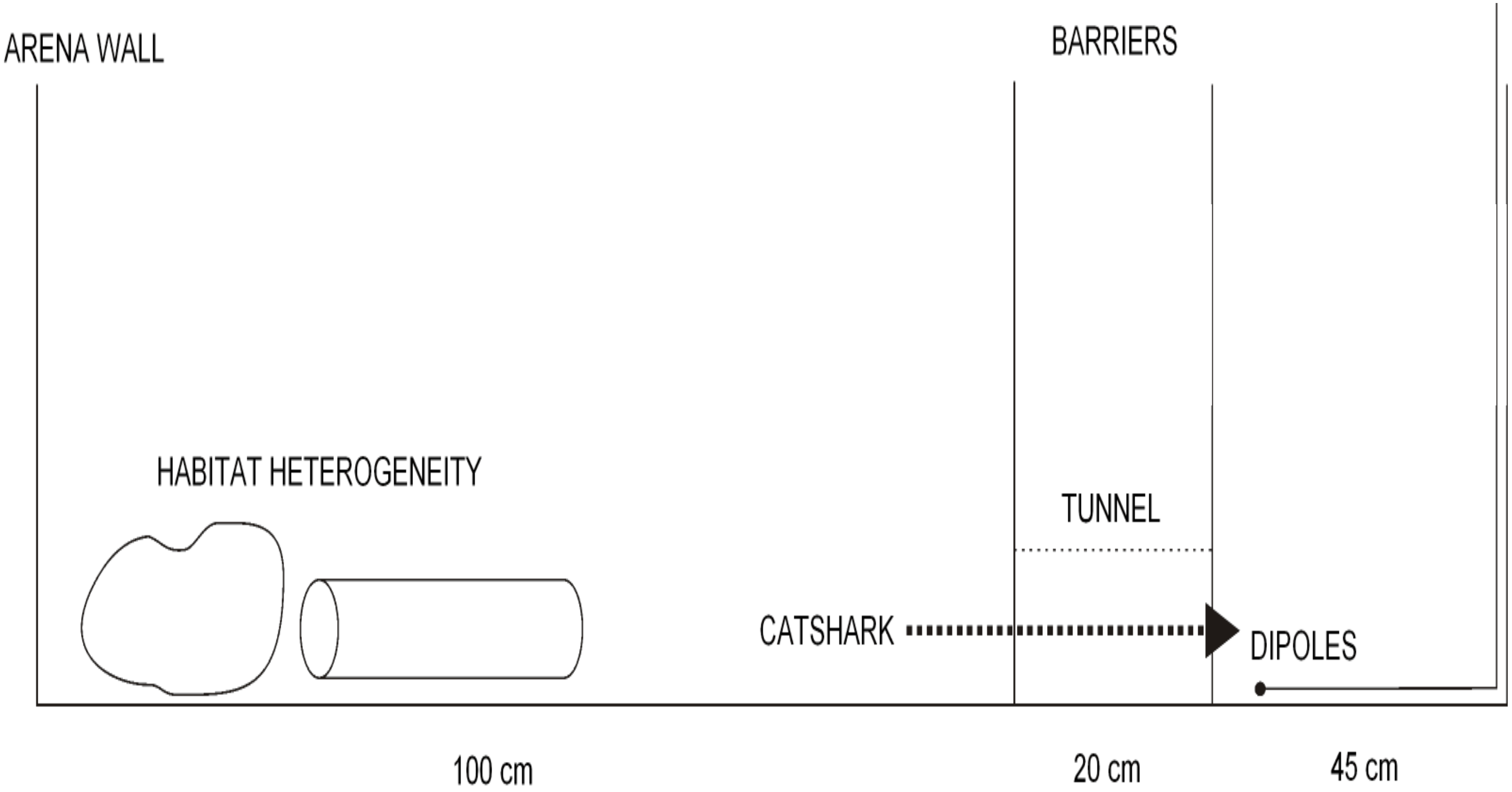


Figure 9. Schematic cross-sectional diagram of behavioural arena set-up showing large and small sections with tunnel and dipole position (not to scale).

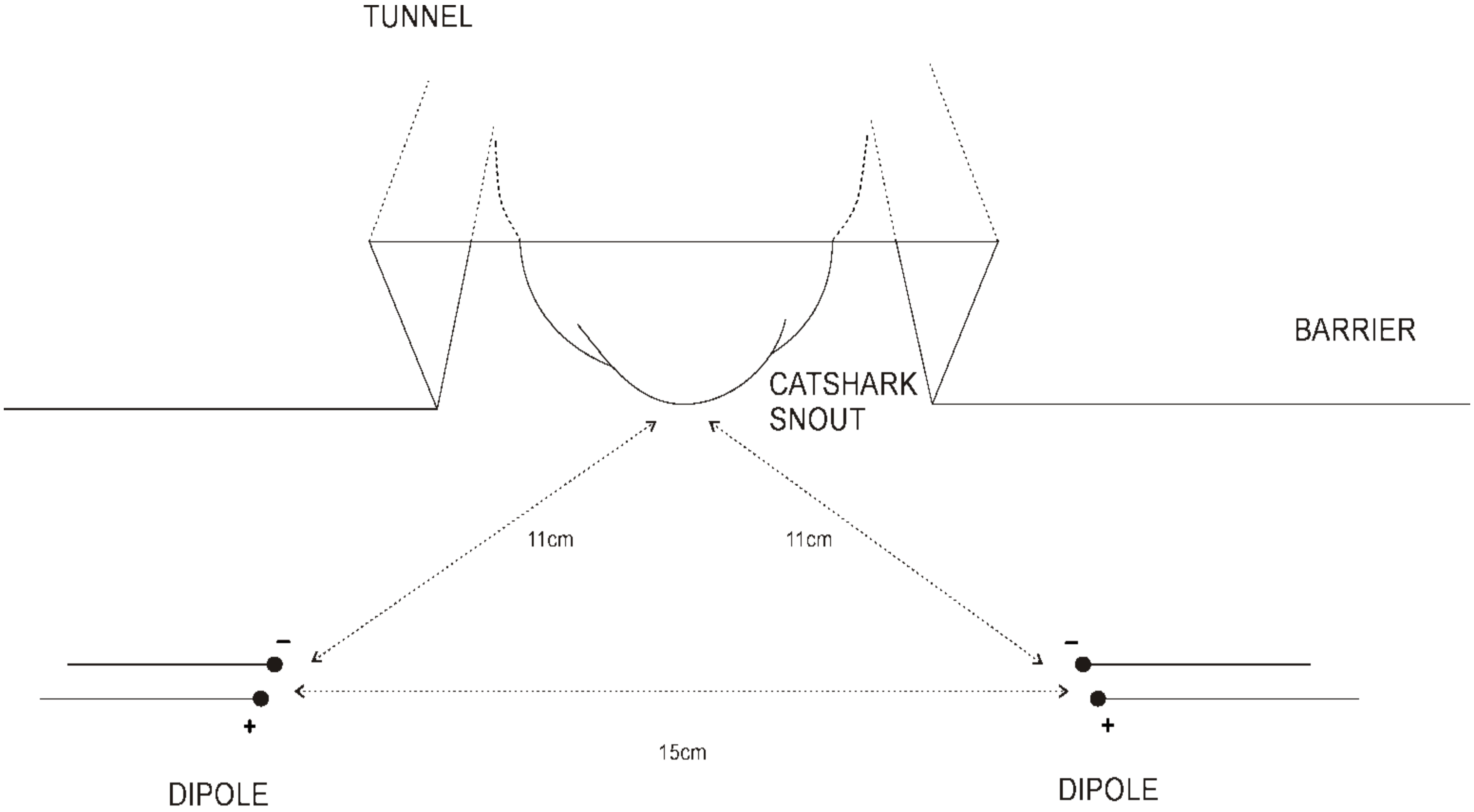


Figure 10. Schematic diagram illustrating the positions of the dipoles relative to the tunnel exit (not to scale).

4.3.3. Experimental procedure

Six catsharks were transferred from the holding aquaria into the larger sections of the six behavioural arenas (one per arena) and allowed to acclimatise for 60 hours. Prior to any particular treatment the salt-bridge apparatus was carefully placed in the small section of the arena. After approximately 2 minutes (to ensure the catshark was not disturbed) the two E fields were switched on and an experimental trial commenced.

Each trial consisted of inducing a catshark through the tunnel by remotely introducing 40 ml food-derived scent (sieved squid and whiting added to water) through flexible air tubing housed within a rigid plastic tube. Approximately half the mixture was injected from the small section through the tunnel to diffuse from the entrance into the large arena section. The remainder was used to leave a trail of scent leading back through the tunnel and beyond the dipoles in the small section. Catshark responses, having swum through and exited the tunnel, were recorded as either no response to electrodes, or a response (turn or bite; see Chapter 2) to one or the other electrode. Catsharks were then rewarded whilst still in the small arena section with approximately 1.3 g food regardless of their response. All six catsharks underwent the same experimental procedure and this procedure was repeated twice a day over five days (an experimental week: a total of ten trials). The two E fields were randomly assigned to left or right sides of the plate in each trial. The reward system and lowered feeding regime (approximately 2 % wet body mass per week: Sims & Davies, 1994; Chapter 2) ensured the catsharks were motivated to forage throughout a week and conditioned to swim through the tunnel throughout a week as consistently as possible. Those catsharks not passing through the tunnel every trial were fed the remainder of their 13.0 g weekly ration at the end of the experimental week. The original six catsharks were then swapped with six from the holding aquaria and the experimental week was repeated for all subsequent groups of six catsharks.

Three sets of experiments were undertaken to assess preference for three different pairs of E fields. The types and intensities of fields were chosen to reflect those that the fish are likely to encounter in their natural environment and that are known to be attractive to elasmobranchs (Kalmijn, 1971; Kalmijn, 1972; also see Chapter 1). The pairs of prey-type E fields were those associated with:

A) Artificial 9 µA and 90 µA direct currents (D.C.)

B) Artificial 90 µA D.C. and alternating currents (A.C. 2 Hz frequency)

C) Artificial and natural 16 µA D.C. (approximately)

The artificial D.C. fields in Experiment (A) were produced and monitored using 9 v power-packs and multi-meters, as were the D.C. fields in Experiments (B) and (C). The artificial A.C. fields used in Experiment (B) were produced and monitored using a function generator, inverting amplifier and oscilloscope. The natural D.C. fields in Experiment (C) were emitted by live shore crabs (*Carcinus maenas*). Those with approximately 4-cm carapace diameters were selected. Their D.C. bioelectric fields were estimated to be similar to those associated with 16 µA currents. Estimations were based upon measurements of a selection of reasonably small crabs' (*Portunus holsatus*, *Eupagurus prideauxi* and *Dardanus arrosor*) bioelectric fields made by Kalmijn (50 µV at carapace surface; 1972) and with reference to calculations in Kraus & Fleisch (1999) to adjust for size. Crabs were contained and concealed beneath re-mouldable, dome-shaped, 1.5-cm thick, 4 % agar chambers fastened to the plate using nylon screws (Figure 11).

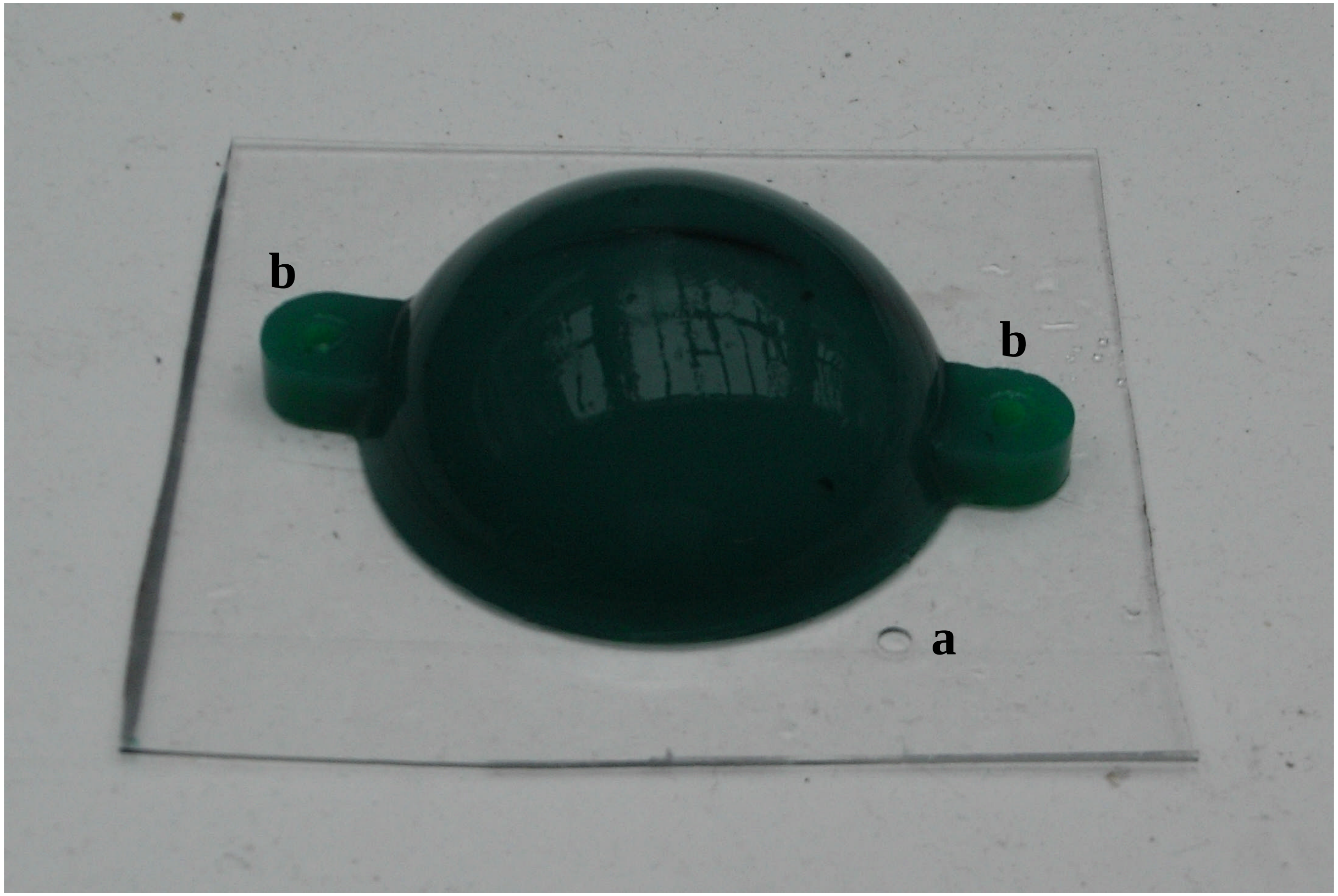


Figure 11. Photograph of an agar chamber on a small acrylic plate (not submerged) showing attachment points **(a)** and sockets for nylon screws **(b)**. Measurements: 11 & 4.5 cm external diameter & height; 8 & 3.0 cm internal diameter & height; 1.5 cm thickness. Note: the chamber is not screwed down, is flooded with sea water and contains a crab, yet the crab is not visible and there is no leakage of water.

The chamber methodology was adapted from pioneering work performed by Kalmijn (1971) and subsequent experiments by Tricas (1982) and Blonder & Alevizon (1988) and utilizes the screening of all but electrical stimuli. The chambers were designed and constructed such that crabs underneath them were not visible to catsharks exiting a tunnel (Figure 11). The chambers formed a seal with the plate and therefore omitted mechanical and chemical signal conduction. They did, however, permit electric fields to pass through uninterrupted (confirmed in pilot experiments using electrodes under chambers to induce unmistakeable electroreceptive responses in catsharks). During Experiment (C), one dipole was removed from each of the two salt-bridge apparatus, but from opposite sides. A crab could therefore be randomly presented on different sides of the tunnel exit throughout experiments simply by alternating the apparatus. Chambers were also placed over the remaining dipoles to ensure the catsharks encountered stimuli differing only in the E field they emitted.

Prior to experiments, power analysis was performed to determine sample sizes required to detect a range of possible ratios of choices made between a pair of E fields (Lenth, 2001; Lenth, 2006; Table 3).

Table 3. Sample sizes of choices recorded required to demonstrate different margins in ratio of choice between two E fields (significance 0.05; power 90 %)

Ratio	Choice Sample Size
0.9 : 0.1	12
0.8 : 0.2	24
0.7 : 0.3	63
0.6 : 0.4	264
0.55 : 0.45	1000

Previous work had shown catsharks to respond significantly less to controls (no current) and 0.9 µA D.C. E fields than 9.0 and 90.0 µA D.C. fields when encountering the fields sequentially (Chapter 3). There was no significant difference between the number of responses to the 9.0 and 90.0 µA fields, indicating the catsharks may choose both fields in similar amounts when encountered simultaneously. No previous work was available to consult regarding potential choice ratios between either 90 µA D.C. and A.C. fields or 16 µA natural and artificial D.C. fields. Due to the large number of specimens and replicates required to detect a ratio of 0.55 : 0.45 (1000; Table 3), especially considering time, apparatus and ethical constraints, and considering such a small ratio would be difficult to interpret as anything other than random choice, no preference for or inability to discriminate between fields, a ratio of 0.6 : 0.4 was chosen as the level to imply preference. Therefore, based on Table 3, a sample size of 240 (6 groups of 6 fish with 10 experimental treatments each) was decided upon for each paired E field experiment. However, sample size flexibility was incorporated into experimental design. Numbers of specimens and replicates could be adjusted during trials as follows: a) lowered, if a considerable preference (and therefore a large ratio) became apparent b) raised, should fish fail to pass through tunnels consistently and/or if a more narrow preference (and smaller ratio) became apparent.

4.3.4. Data analysis

Chi squared contingency analysis was used on the total number of choices made towards the two different E fields by all catsharks in each of the three experiments. Generalized linear model analysis (GZM) was then performed on the choice data to analyse whether all individuals and both sexes responded similarly to each other, and therefore whether the contingency analysis result was a reflection of the behaviour of all catsharks and both sexes or biased by a few individuals and/or one sex. Owing to the binomial distribution of the data (choice x or y), binomial models were used. Logit link functions were applied to transform the distribution onto continuous logit curves. Individual (catshark) and sex were used (separately) as categorical predictors and choice was used as the dependent variable. All statistical analyses were performed using Statistica 8.0 and Genstat 10.0 software.

4.4. Results

During Experiment (A) a total of 134 responses by 24 fish were recorded. The catsharks responded to the 90 µA D.C. electric field on significantly more occasions (91%) than the 9 µA D.C. electric field ($\chi^2 = 69.96$, d.f. = 1, $P << 0.0001$; Figure 12a).

During Experiment (B) 202 responses by 30 catsharks were recorded. The catsharks responded to the A.C. 90 µA electric field on significantly more occasions (61%) than the D.C. 90 µA electric field ($\chi^2 = 8.62$, d.f. = 1, $P = 0.003$; Figure 12b).

During Experiment (C) 679 responses by 36 catsharks were recorded. There was no significant difference between the number of occasions (52 & 48%) the catsharks responded to the artificial and natural 16 µA D.C. electric fields ($\chi^2 = 2.39$, d.f. = 1, $P = 0.12$; Figure 12c).

Table 4 shows the number of individual catsharks that exhibited different responses during each of the three experiments. For all three Experiments, general linear modelling determined that, on average, neither individuals nor sexes made significantly different choices to each other (Table 5). The results from the chi squared contingency analysis can therefore be assumed to be unbiased and represent all catsharks within a given Experiment.

Table 4. Numbers of catsharks exhibiting different responses during each experiment

Exp A		**Exp B**		**Exp C**	
Response	**Number of fish**	**Response**	**Number of fish**	**Response**	**Number of fish**
No choices	2	No choices	3	No choices	0
Equal choices to both	1	Equal choices to both	7	Equal choices to both	5
Only 90	9	Only A.C.	2	Only artificial	0
Only 9	0	Only D.C.	0	Only natural	0
90 > 9	12	A.C. > D.C.	15	Artificial > natural	17
9 > 90	0	D.C. > A.C.	3	Natural > artificial	14

Table 5. Results of GZM analysis of individual and sex effect upon response differences

	Exp A		**Exp B**		**Exp C**	
	Individual	**Sex**	**Individual**	**Sex**	**Individual**	**Sex**
Log likelihood	-41.91	-50.05	-121.68	-130.82	-443.48	-462.94
Degrees of freedom	13	1	25	1	35	1
***P* value**	0.21	0.83	0.83	0.95	0.28	0.46

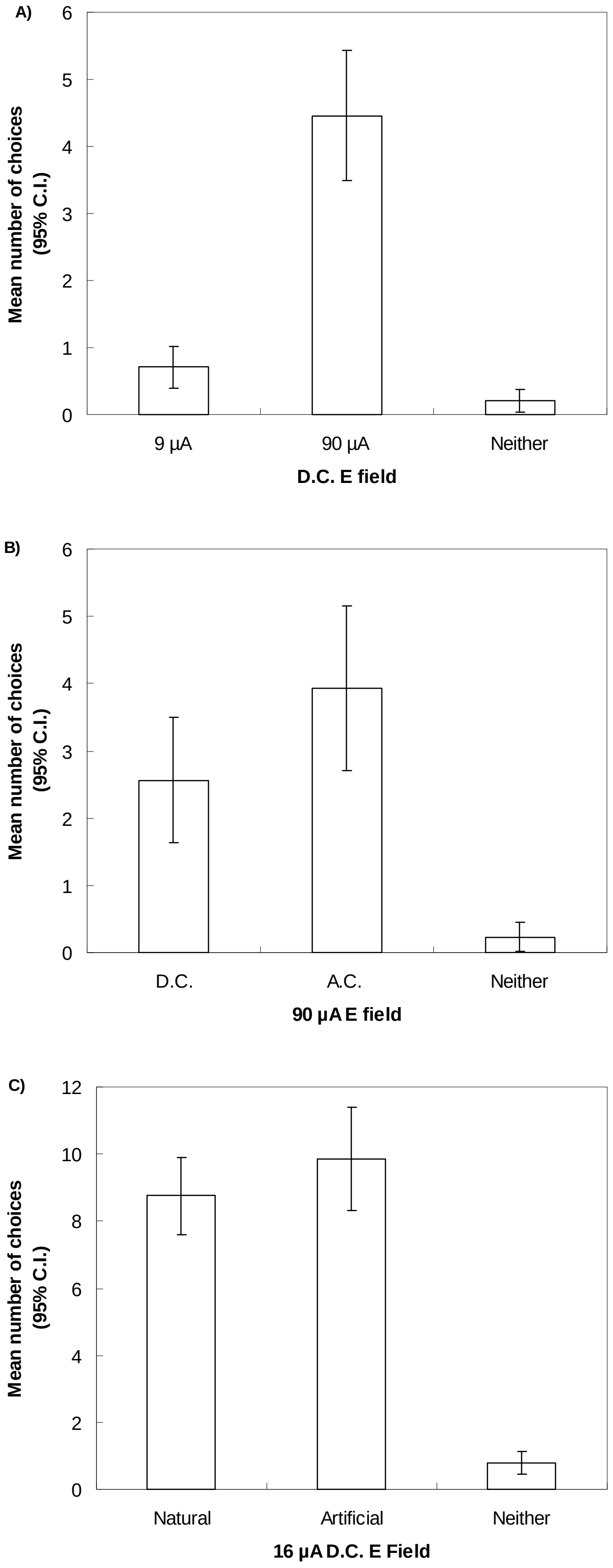


Figure 12. Mean number of choices made to different E fields during each experiment; **A)**, **B)** & **C)** (± 95% confidence limits).

4.5. Discussion

Previously it was unclear whether these fish merely detect the presence of electric fields or whether they are able to differentiate between them. By assessing their preferences through presentation of different E fields simultaneously, this study has demonstrated that they do possess the ability to distinguish some fields but are either unable to distinguish between, or at least show no preference for others.

Results from Experiment (A) show the catsharks possess a highly significant preference for the stronger direct current E field (Table 4 & Figure 12a). Overall, catsharks that swam through the tunnels and reached the dipoles directed responses to the 90 µA field rather than the 9 µA field on 91 % of occasions. Results from Experiment (B) reveal a less pronounced, but still significant, preference for alternating current E fields rather than direct current fields (Table 4 & Figure 12b). Overall, catsharks reaching the electrodes directed responses to the 90 µA alternating current field rather than the 90 µA direct current field on 61% of occasions. The results from experiment (C), however, show no preference between artificial and natural direct current E fields (Table 4 & Figure 12c). In general, the catsharks exiting the tunnel directed responses to the 16 µA artificial and natural direct fields in approximately equal proportions (52% and 48% respectively).

The original sample size of 240 (6 groups of 6 fish subjected to 10 trials) was not reached for Experiment (A) due to failures to respond to scent and swim through the tunnel. However, the resulting sample size of 134 proved powerful enough to detect the wide choice margin between 9 and 90 µA. The sample size was raised in Experiment (B) to account for failures to reach the electrodes and the narrower margin between 90 µA D.C. and A.C. E fields observed in early experimental weeks (18 fish subjected to

10 trials and 12 fish to 15 trials). The lack of difference between E field choice in Experiment (C), despite raising sample size and statistical power even further (12 fish subjected to 10 trials and 24 subjected to 30 trials), confirmed the lack of preference or inability to distinguish the artificial and natural 16 µA D.C. E fields.

Trials in which catsharks failed to respond to scent or failed to swim through tunnels (on average 36% of trials in each experiment) were not included in analyses. This study was simply interested in the choice made by catsharks between two electric fields and therefore analysis focused upon trials in which catsharks responded to scent and reached the tunnel exit. There is a possibility that those catsharks failing to reach the electrodes more than others (i.e. less bold) may have possessed differing preferences than other (more bold) catsharks. However, behavioural syndromes are unknown in elasmobranchs. Furthermore, the GLZ investigating potential bias in the data between individuals would have to some extent accounted for such an effect.

The pairs of E fields used in these experiments were chosen owing to their similarity to E field types and intensities that the species is likely to encounter in its natural environment and that are within the range attractive to elasmobranchs (Kalmijn, 1971; Kalmijn, 1972; Chapter 1). It is therefore possible to make some inferences about the results with respect to foraging behaviour in the wild.

The catsharks' clear preference for the larger D.C. current E fields (Exp A) may, for example, relate to preferences for larger prey (electroreception operates over such small distances that effects of distance on E field size, e.g. a smaller E field simply relating to a similar sized prey further away, would be minimal). A larger specimen of a species will provide a greater food energy source, and therefore more nutrients and energy, than a smaller specimen of the same species. Despite this preference, when the catsharks encounter the same, two E fields (9 & 90 µA) sequentially rather than simultaneously, they respond to both equally (Chapter 3). Responses to different fields therefore seem to be context specific (similar to differences in stickleback response owing to context of prey encounter; Gill & Hart, 1999). This makes sense for an opportunistic predator in a variable environment such as elasmobranchs in coastal habitats. After all, weaker D.C. fields may be associated with entirely different prey species of differing nutrient content and/or ease of capture and consumption, rather than, or in addition to, smaller specimens of the same prey species (Kaiser *et al.*, 1992b; Gill & Hart, 1994). Previous work has demonstrated catsharks to be less responsive to E fields associated with 0.9 µA currents compared to 9.0 and 90.0 µA currents in sequential encounters (Chapter 3). This supports the suggestion that these fish prefer stronger D.C. fields. However, as current increases much beyond 100 µA, and especially as E fields reach approximately 1000 nN/cm, catsharks would be expected to exhibit avoidance responses (Dijgraaf & Kalmijn, 1962; Yano *et al.*, 2000).

The preferences observed for A.C. rather than D.C. electric fields were less pronounced but still significant. Bioelectric A.C. fields are predominately associated with fish (Kalmijn, 1972; Chapter 1). Both teleosts and chondrychthians emit low and high frequency A.C. fields. This suggests that, given the choice, catsharks may choose to prey on fish rather than invertebrates, but this remains to be tested. Again, such decisions will be context specific and may be based upon nutrition and/or ease of capture and consumption. And similarly, the fact that the fish also often respond to the D.C. fields strengthens the suggestion that these opportunistic predators could optimise foraging by adapting their behaviour and responding to a variety of options sequentially over extended time periods (Dill, 1983). Stomach analyses show this species preys on a wide range of macrobenthic fauna (Lyle, 1983; Olaso *et al.*, 2005; Rodriquez-Cabello *et al.*, 2007) ranging from teleosts and crustaceans to molluscs and polychaetes.

Despite the ability of these fish to distinguish between the two pairs of artificial E fields it seems as though they are either unable to discriminate between or show no preference for similar strength, D.C., natural or artificial E fields. From this methodology alone it is not possible to discern which scenario is occurring. It appears that elasmobranchs might respond to both prey and artificial electric sources of similar strength should they encounter them either simultaneously or sequentially in the marine environment. This finding has important implications when considering possible interactions between electrically sensitive fish and anthropogenic E fields (Chapter 1) as it would appear that the fish may confuse prey bioelectric fields with artificial E fields whilst foraging (see Chapters 5 & 6).

5. LEARNING, HABITUATION AND MEMORY EFFECTS ON ELECTRORECEPTIVE FORAGING BEHAVIOUR

5.1. Abstract

Whether a benthic elasmobranch, *Scyliorhinus canicula* (small-spotted catshark), is able to learn to alter its electroreceptive foraging behaviour and remember learned changes was investigated. To address these objectives the behaviour of individual catsharks towards artificial, prey-type electric fields (E fields) following stimulation by food-derived scent was studied. Catsharks that were rewarded for responding to the E fields throughout a week were compared to those that were not. Trials were then repeated after a three week interval with previously rewarded catsharks receiving no rewards and vice versa.

Analysis of the data demonstrated significant differences in the behaviour of rewarded and unrewarded fish. Rewarded catsharks exhibited more intense foraging behaviour towards E fields than unrewarded catsharks. Furthermore, rewarded catsharks improved their foraging efficiency throughout experimental weeks. Conversely, unrewarded catsharks reduced their interest in E fields throughout experimental weeks. These altered behaviours were not, however, continued after the experimental interval and electroreceptive foraging behaviour was independent of whether rewards were offered before or after the interval.

The findings demonstrated learning and habituation occurring after very few foraging events but suggested a memory window of less than three weeks. Some of the series of search paths taken to electrodes by individual fish are reproduced herein to clearly illustrate these behavioural adaptations. The findings are discussed in relation to

the animals’ diet, habitat and ecology and with regards to anthropogenic sources of E fields within their habitat.

5.2. Introduction

The attributes of the natural environment vary both spatially and temporally. Coastal environments are particularly changeable due to the convergence of dynamic marine, freshwater, terrestrial, atmospheric and anthropogenic inputs. Organisms that inhabit such an environment should theoretically exhibit behavioural flexibility to enable them to adapt to changing conditions and thereby maximize individual success (Dill, 1983). Though certain behaviours are innate and inflexible, others can be adjusted based upon experience (Kieffer & Colgan, 1992). Learning and memory are crucial means with which to facilitate such adaptation.

In general, the widely held view that fish (common inhabitants of shallow coastal seas) possess limited intelligence, memory and adaptive ability has been consigned to the past (Laland *et al.*, 2003). The current consensus is that many teleosts (bony fish) possess significantly superior cognitive ability than previously believed (Huntingford, 2003; Brown *et al.*, 2006). They have been observed to co-operate (Milinski *et al.*, 1990a), manipulate (Bshary & Wurth, 2001), use tools (Bshary *et al.*, 2002), build nests (Paxton & Eschmeyer, 1998), possess complex social relationships (McGregor, 1993) and exhibit cultural traditions (Helfman & Schultz, 1984).

Learning and memory are also well developed in teleosts. For example, learned foraging skills have been demonstrated in stickleback fishes (Croy & Hughes, 1991a; Girvan & Braithwaite, 1998; Odling-Smee & Braithwaite, 2003a), salmon, *Salmo salar* (Brown *et al.*, 2003) and mosquito fish, *Brachyraphis episcopi* (Brown & Braithwaite, 2005). Learned escape responses have been demonstrated in zebra fish, *Danio rerio* (Arthur & Levin, 2001) and haddock, *Melanogrammus aeglefinus* (Ozbligin & Glass, 2004). Varying memory windows have been demonstrated in rainbowfish

(*Melanotaenia duboulayi*) escape responses (Brown, 2001), trout (*Salvelinus fontinalis*) and stickleback fishes prey handling skills (Bryan & Larkin, 1972; Mackney & Hughes 1995 respectively), and paradise fish (*Macropodus opercularis*) predator recognition (Miklosi *et al.*, 1992).

This evidence presents a compelling case for teleosts having comparable cognitive abilities to many land vertebrates (Laland *et al.*, 2003). Conversely, chondrichthyans (cartilaginous fish) have received relatively little attention with respect to cognitive ability. This is surprising given that certain elasmobranchs (sharks, skates and rays) possess brain to body mass ratios that overlap those of mammals and birds (Demski & Northcutt, 1996). In general, the few examples of investigations in the field are relatively old. Clarke (1959) conditioned two adult lemon sharks to consistently press a target (associated with an acoustic stimulus) in order to receive food. The sharks retained this behaviour after a 10 week interval. She subsequently demonstrated that the lemon sharks could also discriminate targets differing in shape and pattern (Clarke, 1961). Wright & Jackson (1964) performed very similar experiments but used groups of immature lemon (*Negaprion brevirostris*) and bull (*Carcharhinus leucas*) sharks. The results suggested that younger sharks learn more quickly than adults and that lemon sharks are quicker than bull sharks. Aronson *et al.* (1967) demonstrated that young nurse sharks (*Ginglymostoma cirratum*) were able to be similarly conditioned, that their response latencies reduced over time and that they possessed similar learning curves for light-dark discrimination to teleosts and mice. Similar sharks were also shown to be able to discriminate both black-white and vertical-horizontal patterns (Graeber & Ebbesson, 1972; Graeber *et al.*, 1978). More recently, Schluessel & Bleckmann (2005) demonstrated spatial memory of environment in juvenile freshwater stingrays (*Potamotrygon motoro*).

Electroreception has been demonstrated to be crucial in the final moments of elasmobranch feeding and is also used during the location of and communication with conspecifics, the detection of predators and possibly in aiding navigation (see Chapter 1). Flexibility in their responses to electric stimuli within their environment via learning and memory would provide ecological benefits to these fishes' life processes. Recently, there has been growing concern over possible interactions between elasmobranchs and anthropogenic sources of electric fields (E fields) in the coastal environment (see Chapter 1). The research in Chapter 4 demonstrates that a benthic elasmobranch was able to discriminate between certain types of E fields by showing preferences when fields were presented simultaneously. However they were either unable to differentiate between or showed no preference for similar artificial (electrodes) and natural (crab) E fields. This raises the question of whether these fish would effectively waste time and energy "hunting" artificial E fields or whether they could learn to adapt their behaviour depending on the relative success of encounters with different electric stimuli. To date, there have been no investigations into either learning or memory associated with elasmobranch electroreceptive foraging.

5.2.1. Objectives

The aim of this study was therefore to investigate the ability of a model species of benthic elasmobranch, the small-spotted catshark (*Scyliorhinus canicula*), to learn to adapt its electroreceptive foraging behaviour and remember learned changes. Laboratory studies were therefore designed to make comparisons between fish rewarded for responding to E fields (R) and fish that were unrewarded (U). Experiments were developed to compare:

a) Behavioural response levels between R and U

b) Changes in behavioural responses through time between R and U

c) Both (a) and (b) for R and U before and after an interval comprised of normal feeding and no electric stimuli.

5.3. Methodology

5.3.1. Experimental animals

Twenty four size-matched small-spotted catsharks (*S. canicula*) were caught on a Marine Biological Association of the U.K. (MBA) research vessel off Plymouth, southwest England (station L4: 50°15’N, 4°13’W), in May 2006. The fish averaged 61.8 ± 4.8 cm total lengths (±SD). They were randomly assigned to one of four groups (1 to 4) and tagged with different coloured, individually numbered T-bar anchor tags (FLOY TAG Inc., Seattle, Washington USA) accordingly. Catsharks were maintained as per descriptions in Chapter 2.

5.3.2. Experimental apparatus

Salt-bridge electrode circuits and plates similar to those used previously (see Chapters 2 & 3) were used to present catsharks with prey-type electric fields. The apparatus were modified to allow rewards to be presented to catsharks after responding to an electric field. The plate that the dipole was attached to was made opaque white (to match arena base) and raised by 3 cm with gently inclining ramps added to each side. In this way a hidden trap-door mechanism and food compartment was attached under the plate whilst not hindering the catsharks’ movement over the plate (Figure 13).

The mechanism consisted of an acrylic panel with a sunken, circular food compartment (5 cm diameter x 1 cm depth) that could be pushed and pulled back and forth within rails on both sides, and blockers at both ends. As such, the compartment could be “opened” under a similar sized hole in the electrode plate when in the forward position, and then “closed” again when slid back to the original position (Figure 14).

The trap-door mechanism was constructed to minimise potential seepage of food scent from the compartment containing the food. A sealed, water-filled system of 1 mm and 20 mm polypropylene BD Luer Lok syringes, Nalgene 380 PVC 3.2 x 1.6 mm (bore x wall) tubing and an elastic band allowed the trap-door to be opened and closed remotely, smoothly, quickly and silently by the observer (Figure 14). This apparatus permitted presentation of rewards to catsharks in close proximity to the dipole, immediately after a response to an electric field, and with minimal disturbance.

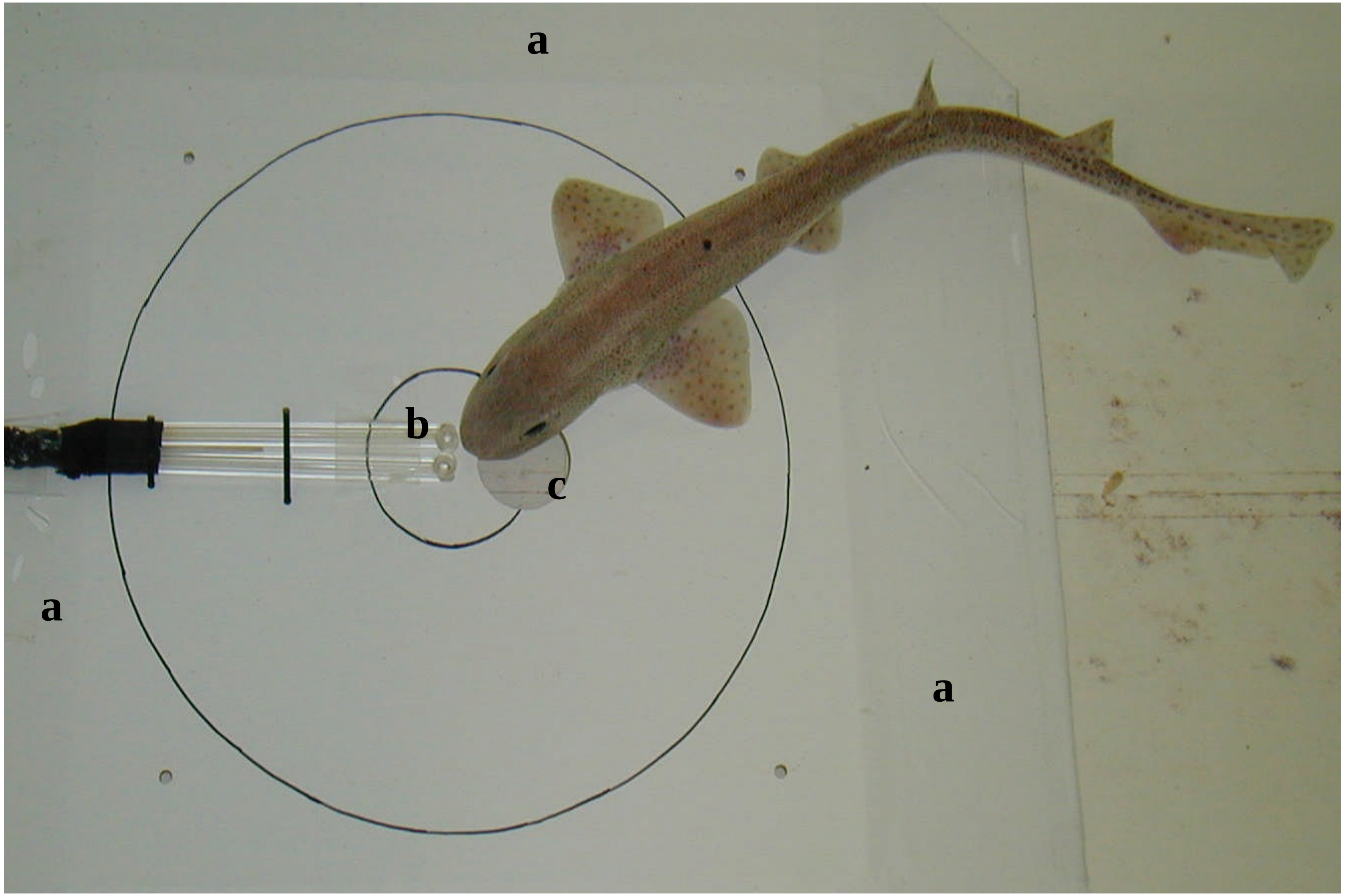


Figure 13. Photograph of modified salt-bridge apparatus, from above, showing the gentle ramps **(a)** and position of the dipole **(b)** next to the trap door **(c)**.

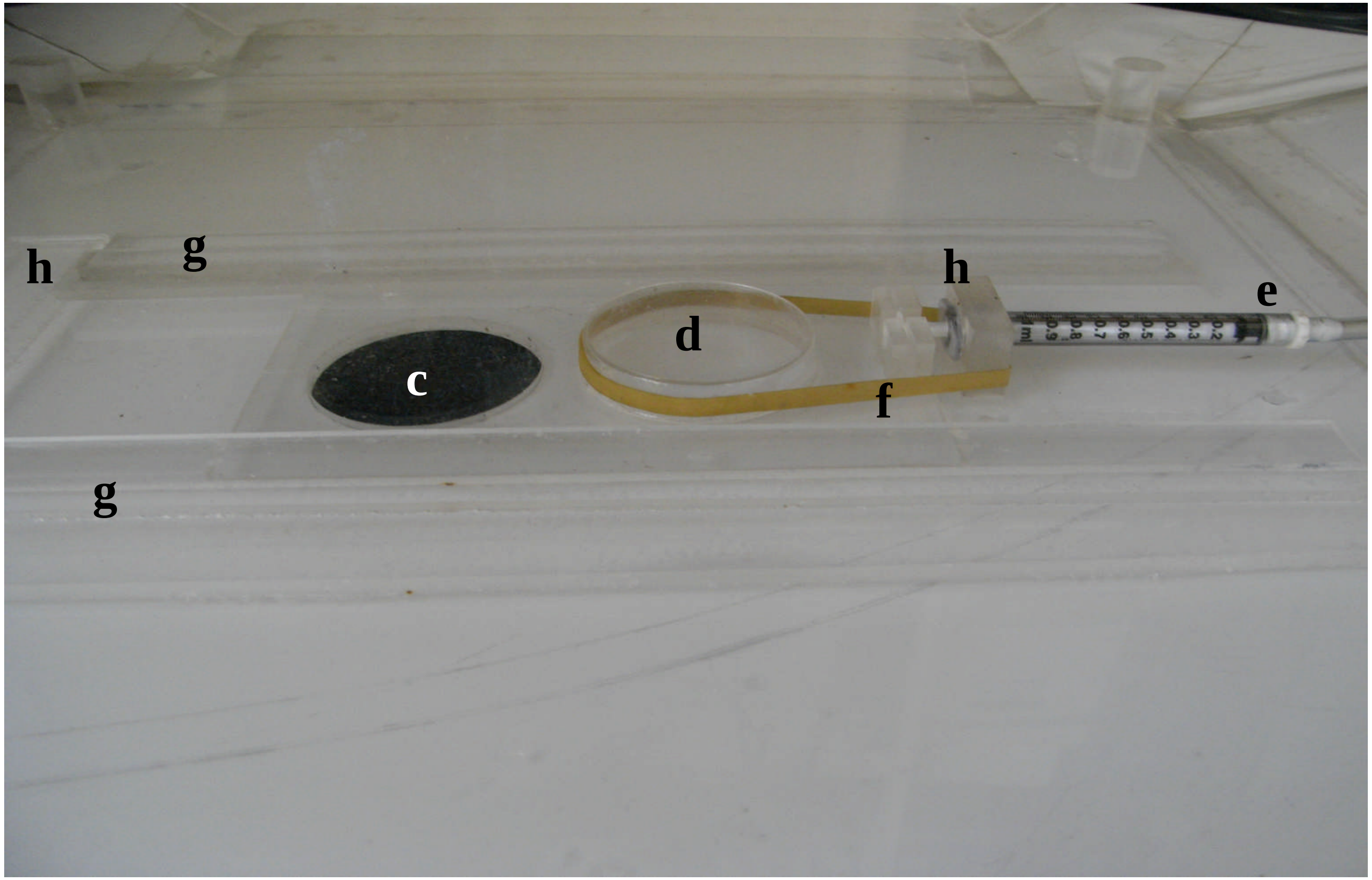


Figure 14. Photograph of the underside of the modified plate showing the trap door mechanism. Mechanism is shown in original, “closed” position. Compartment **(d)** is pushed forward (right to left) by hydraulic system **(e)** to “open” under trap door **(c)**. It can then be brought back to original position partly using hydraulic system but with aid of elasticity of band **(f)**. Rails **(g)** and blocks **(h)** are also shown.

5.3.3. Experimental procedure

The six catsharks from group 1 were transferred to randomly chosen behavioural arenas (one fish in each of six arenas) and allowed to acclimatise for 60 hours (see Chapter 2). The fish were then presented with an electric field produced by a 9 µA current twice per day (early morning & late afternoon) for five days (a total of 10 consistently spaced experiments: an experimental week). Three of the fish were rewarded with approximately 1.3 g food for their first response to the electrodes in each experiment. Fish not taking all of their rewards were fed the rest of their weekly 13.0 g ration at the end of the experimental week. This reduced feeding ration (approximately 2 % wet body mass per week: Sims & Davies, 1994; Chapter 2) ensured satiation was not reached during the week and that hunger and normal foraging behaviour persisted. The other three fish were not rewarded at all during experiments but were instead fed a 13.0 g ration at the end of the experimental week. On completion of the experimental week the group 1 fish were transferred back to the holding aquaria and replaced with the fish from group 2. After 60 hours acclimatisation group 2 then underwent a similar experimental week. This procedure was repeated for groups 3 and 4.

Once group 4’s experimental week was complete, each group (1 - 4 in turn) then underwent a second experimental week (with catsharks in different, randomly assigned arenas), similar except that those previously rewarded were not rewarded and vice versa. There was therefore a 3 week interval between each group’s two experimental weeks (Table 6).

Table 6. Sequence of experimental weeks for six catsharks in a group

Catshark	Week Before (10 experiments)	3 week interval	Week After (10 experiments)
1	R	Other Groups in use	U
2	U		R
3	R		U
4	U		R
5	R		U
6	U		R

R = rewarded; U = unrewarded

Prior to any particular experiment a salt-bridge apparatus was introduced into a randomly chosen arena, and at a randomly chosen end (to ensure catsharks were not simply learning the location of food source), with the power switched off and water flow stopped to allow the catsharks to settle down. After 20 minutes the power was switched on, and 20 ml of food-derived scent (sieved squid and whiting added to water) was introduced into the arena next to the dipole and behavioural observations commenced. Experiments lasted for no more than 15 minutes and were recorded using overhead cameras. The observer recorded (directly or later by reviewing videos) the following hierarchical response variables of each fish: (a) Reaction time to scent, (b) number of times Activity Zone entered (see Chapter 2 & Figure 4), (c) number of times Electrode Zone entered (see Chapter 2 & Figure 4), (d) time latency of turns and bites towards electrodes (scent time from response times), (e) frequency of turns towards and bites on electrodes, and (f) bite number and time when a reward was taken. Only those turns and bites that were distinct, unambiguous and unmistakably directed towards the electrodes were recorded.

The paths the catsharks took from their starting position (prior to scent detection) to their first response at the dipole were traced from video footage on a large monitor. These traces were then converted to JPEG files using an HP Designjet 815 mfp scanner and subsequently opened using ArcGIS software, converted to polyline shape files and geo-referenced to arena size. Both the distance from catshark starting position to dipole and the distance of their search paths to the dipole were measured using ArcGIS tools. The relationship between these two distances generated an index of the catsharks' path directness to the electrodes (catshark path ÷ original distance to electrodes = path directness: Figure 15).

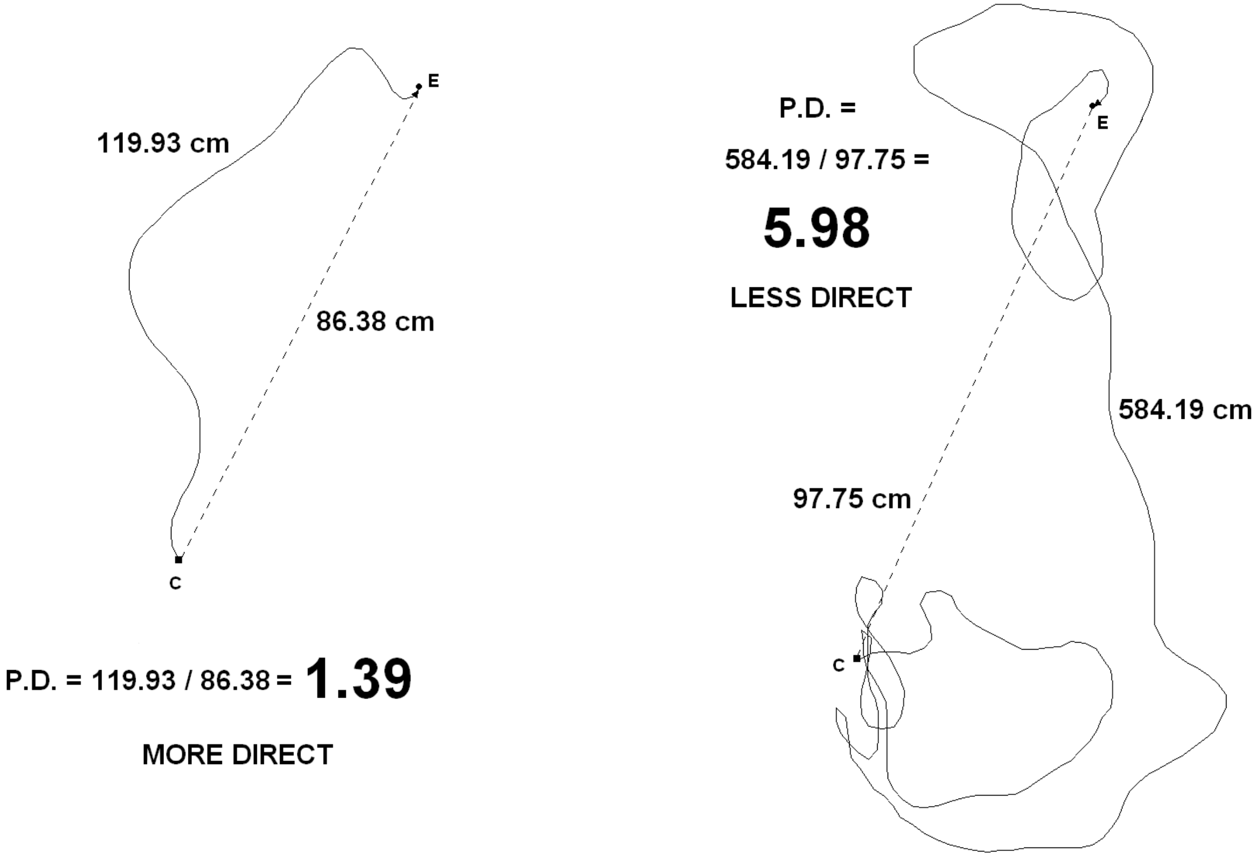


Figure 15. Two traces with similar original catshark and electrode positions but differing search paths illustrating how measurements of paths were taken and path directness calculated (C = catshark starting position; E = electrodes; P.D. = path directness (2 d.p.); unbroken line = catshark swimming path; hashed line = direct line from start position to electrode).

5.3.4. Data analysis

Hierarchical generalized linear mixed models (HGZLMM) on count and $\log_e$ transformed continuous (e.g. times) data, and general linear models (GLM) on path directness ratios were used to evaluate differences in the electroreceptive foraging behavioural parameters (a) to (f) and paths between rewarded catsharks and unrewarded catsharks, and between experimental weeks before and after the three week interval. The HGZLMM on the count data used a Poisson distribution owing to many zero and low integer values. A logarithm link function then transformed this to a normal distribution. The HGZLMM on continuous data used a normal distribution and an identity link function (which simply multiplies the data by 1 and thus does not transform the distribution). In both cases, a fixed effect of reward (yes or no) by week (before or after) and a random effect of individual (catshark) by experiment treatment number (1 to 10) were used. The GLM used individual, reward and week as categorical predictors related to $\log_e$ transformed path data. Transformations were made to satisfy the assumption of normality of residuals.

HGZLMM was also used to analyse changes in parameter levels (a) to (f) within experimental weeks, having split the data into rewarded and unrewarded sets. Again, for count data, a Poisson distribution and logarithm link function were used and for $\log_e$ transformed continuous data a normal distribution and identity link function were used. In both cases a fixed effect of experimental number (1-10) and a random effect of individual were used. Similar analysis of trends in path directness data was performed using GLM with individual as the categorical predictor and experimental number as the continuous predictor.

For all model results reported, the distribution of the residuals was plotted and found to be close to normal so the model could be assumed to be satisfactory unless stated otherwise. Residual degrees of freedom were between approximately 215 and 460 unless stated otherwise. All statistical analyses were performed using Statistica 8.0 and Genstat 10.0 software (see Chapter 2).

Statistical effect sizes with standard errors were used to plot responses of catsharks compared between rewarded and unrewarded catsharks, or weeks before and after the interval (e.g. Figure 16). One treatment is compared to the other, the latter of which is the reference value (zero). The effects shown in the graphs indicate the relative size and direction of any differences. Statistical effect sizes were also used to plot responses throughout an experimental week (e.g. Figure 17). Effects were calculated for each experiment number (1-10) using slope and constant parameters from Genstat outputs and then plotted as a continuous variable. Responses throughout weeks for different treatments could then be compared to each other. Time data were back-transformed and plotted on log scales, whereas original count data were plotted on normal scales.

5.4. Results

5.4.1. Learning and habituation

a) Response time to scent:

There was a suggestion that catsharks rewarded for electroreceptive responses reacted to the scent more quickly than those that were not, but the difference was not significant due to large variation (Wald = 1.08; P = 0.298; Figure 16). However, rewarded catsharks were slower to react to scent through the week (t = 2.79; P = 0.005) whereas unrewarded catsharks' times showed no change (t = 0.82; *P* = 0.412; Figure 17).

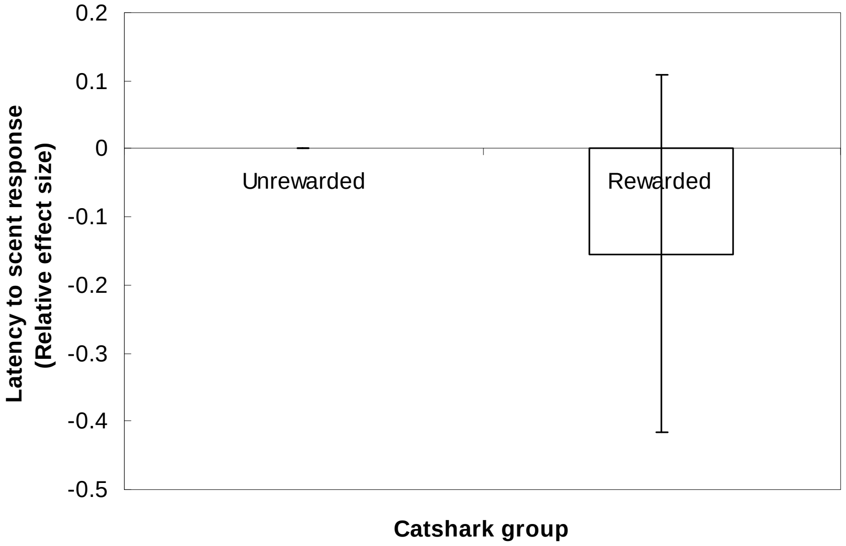


Figure 16. Relative average times to respond to scent by rewarded and unrewarded catsharks (± standard error. Note: Y axis plotted using statistical effect sizes. Unrewarded is zero reference).

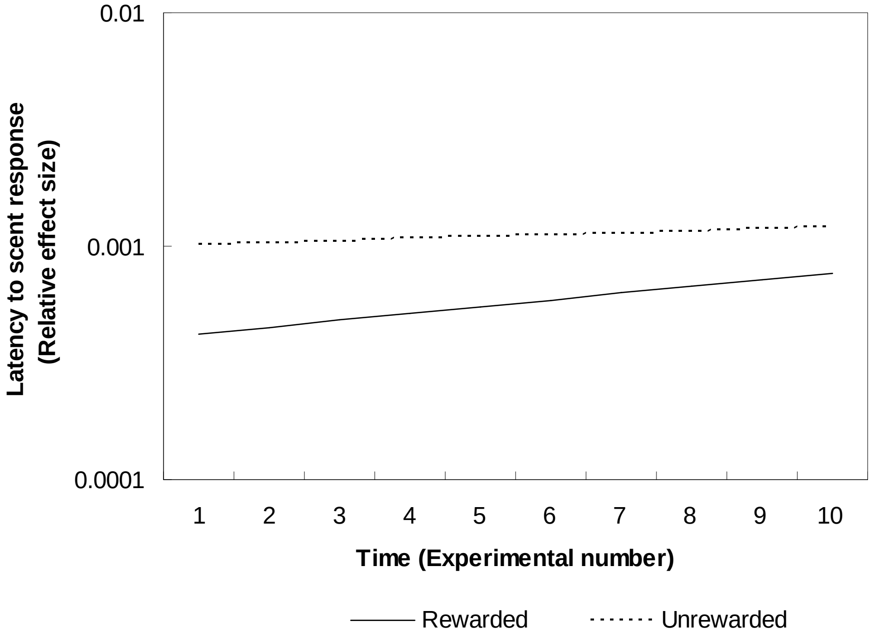


Figure 17. Relative changes in scent response times throughout experimental week (note: Y axis values are back transformations of statistical effect sizes plotted on log scale).

b) & c) Passes over Activity and Electrode Zones:

Before first contact with the electrodes rewarded catsharks swam round arenas (Activity Zone passes; Chapter 2) and passed near electrodes (Electrode Zone passes; Chapter 2) significantly more than unrewarded catsharks (t = 5.49 (Activity) & 8.61 (Electrode); both $P << 0.0001$; Figures 18a.i & b.i). Rewarded catshark activity levels were also higher than unrewarded catshark activity levels after first contact with the electrodes, but to a lesser extent (t = 3.24; $P = 0.001$; Figure 18a.ii). However, rewarded catsharks did not pass the electrodes more than unrewarded cathskarks after first contact (t = 1.26; $P = 0.208$; Figure 18b.ii).

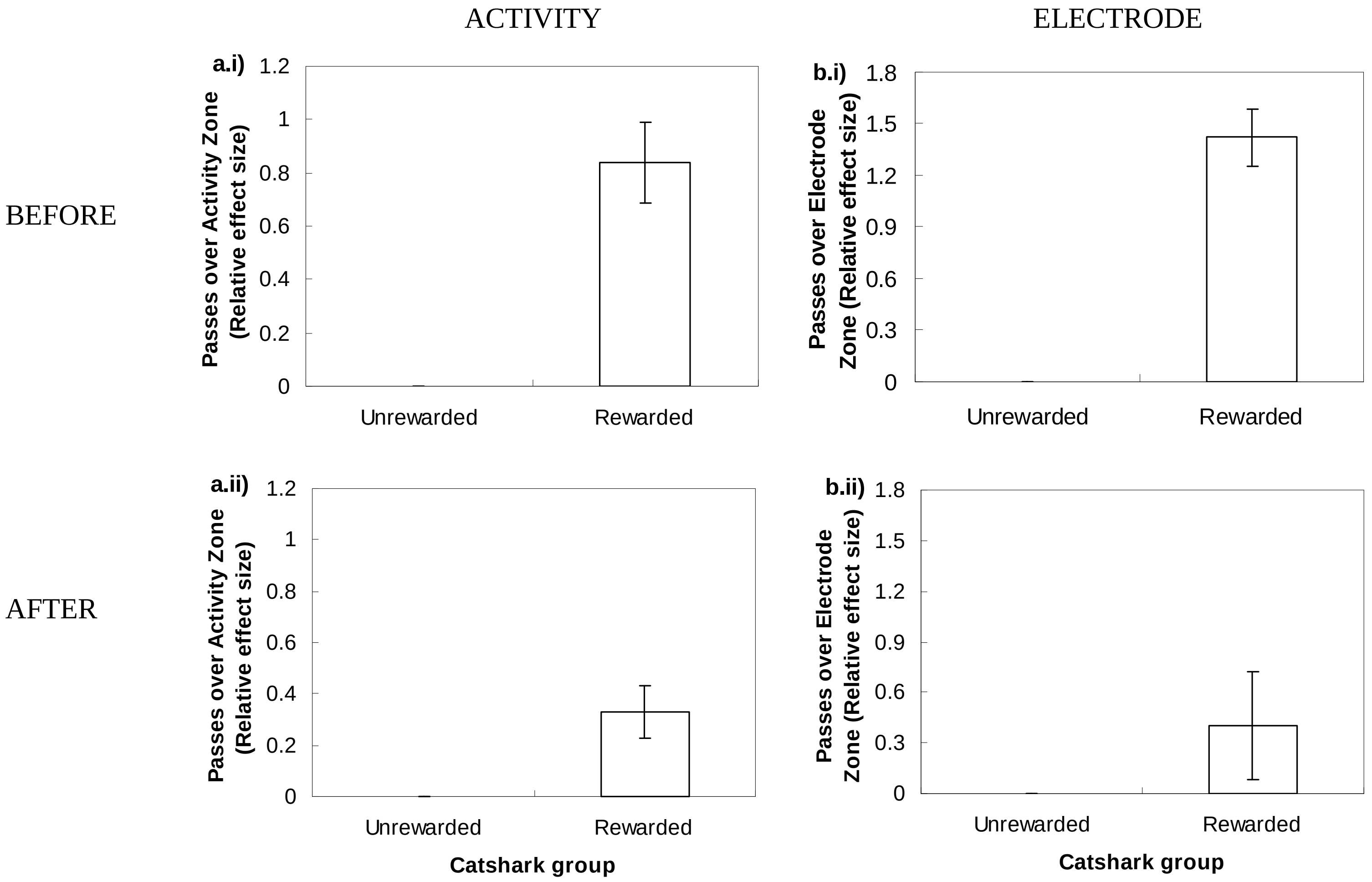


Figure 18. a) Relative activity levels and **b)** electrode passes **i)** before and **ii)** after first contact with the electrodes (± standard error. Note: Y axis plotted using statistical effect sizes. Unrewarded is zero reference).

Rewarded catsharks showed decreasing activity and electrode passes before first contact with electrodes within experimental weeks (t = -4.22 (Activity) & -4.59 (Electrode); both $P < 0.0001$; Figures 19a.i & b.i). A slight increase in activity level and no change in electrode passes were observed in unrewarded catsharks although the models did not fit well due to lack of data (dotted lines; Figures 19a.i & b.i). After first contact with electrodes, rewarded catsharks showed less marked decreases (t = -2.02 & -1.87; P = 0.044 & 0.062 respectively; Figures 19a.ii & b.ii). Contrastingly, unrewarded catsharks now showed more significant decreases (t = -1.94 & -3.84; P = 0.053 & 0.0001 respectively; Figures 19a.ii & b.ii).

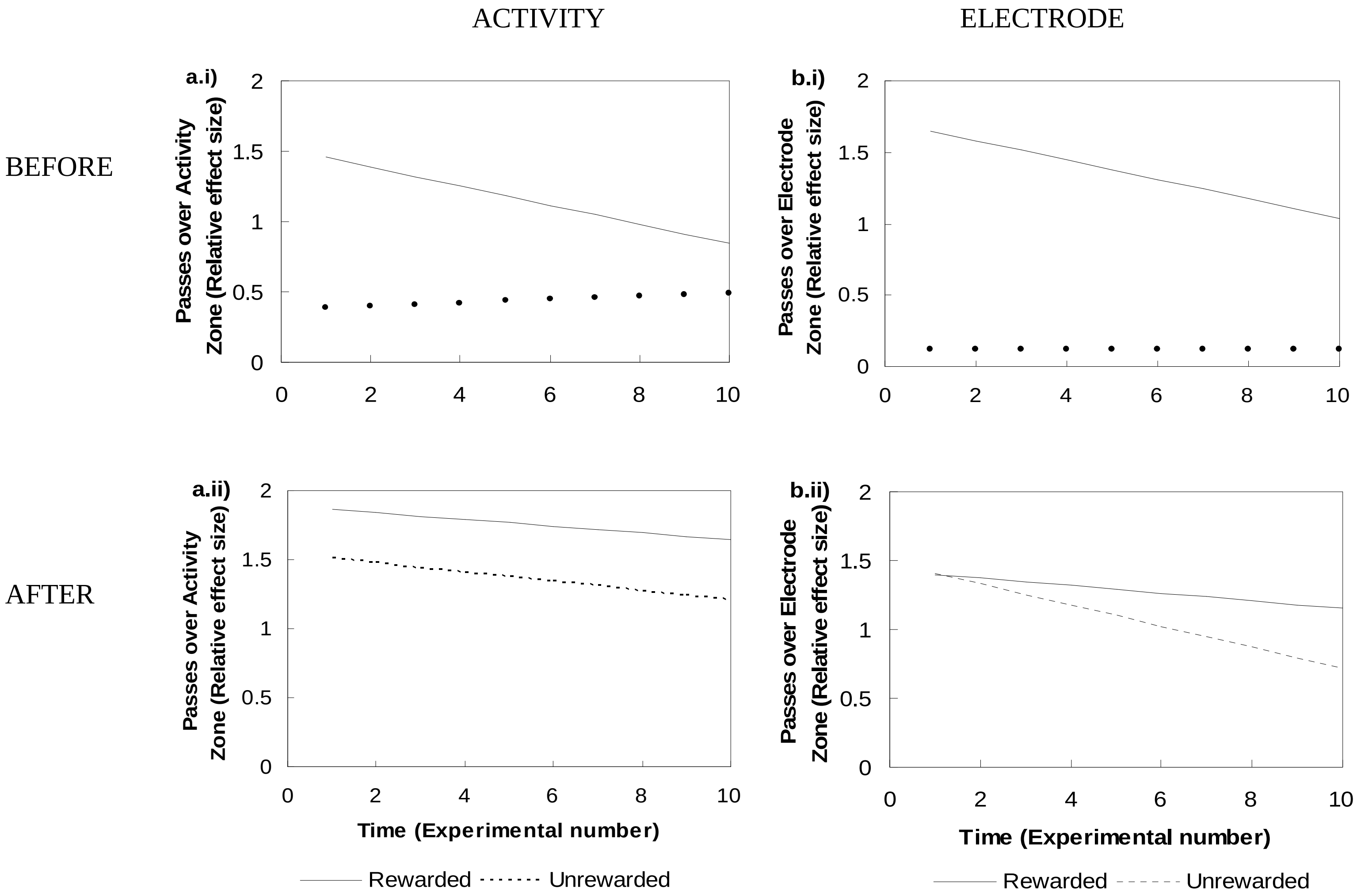


Figure 19. Relative changes in **a)** activity levels and **b)** electrode passes throughout experimental week **i)** before and **ii)** after first contact with electrodes (note: Y axis plotted using statistical effect sizes).

d) Turns & bites:

There were significantly more turns and bites made by those catsharks that were rewarded (t = 5.35 (turns) & 5.57 (bites); both *P* << 0.0001; Figures 20a & b). Both rewarded and unrewarded catsharks showed decreasing turns and bites throughout experimental weeks though the trend was more pronounced for unrewarded catsharks (rewarded: t = -6.70 (turns) & -8.27 (bites); unrewarded: t = -10.77 (turns) & -9.93 (bites); all *P* < 0.0001; Figures 21a & b). Indeed, on average, unrewarded catsharks stopped biting the electrodes altogether between six and seven treatments.

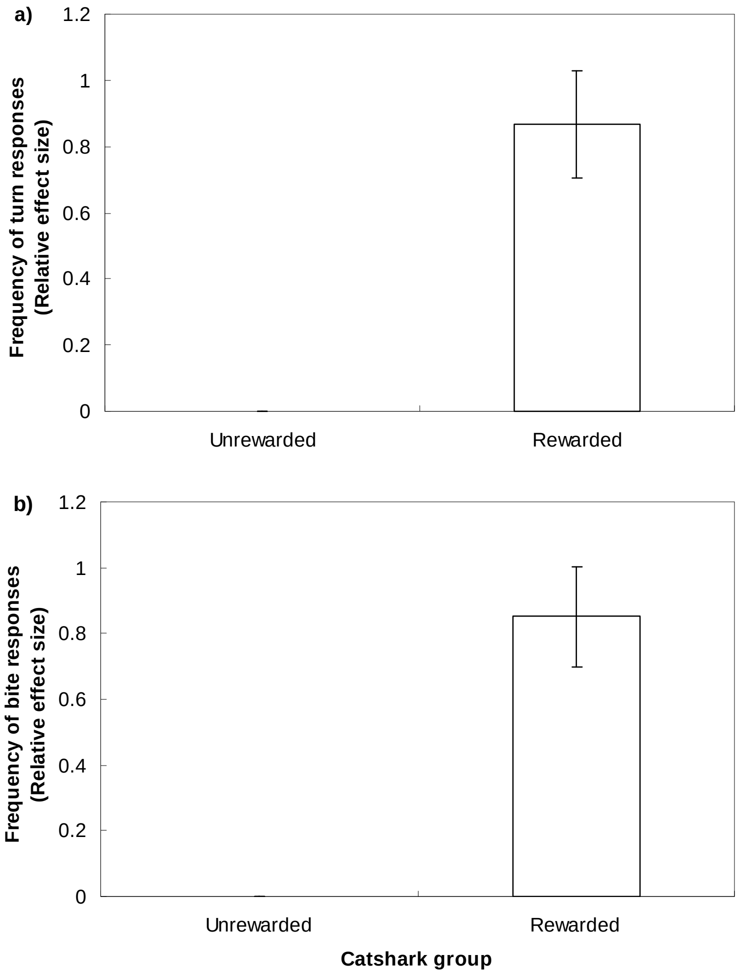


Figure 20. Relative **a)** turn and **b)** bite levels by rewarded and unrewarded catsharks (± standard error. Note: Y axis plotted using statistical effect sizes. Unrewarded is zero reference).

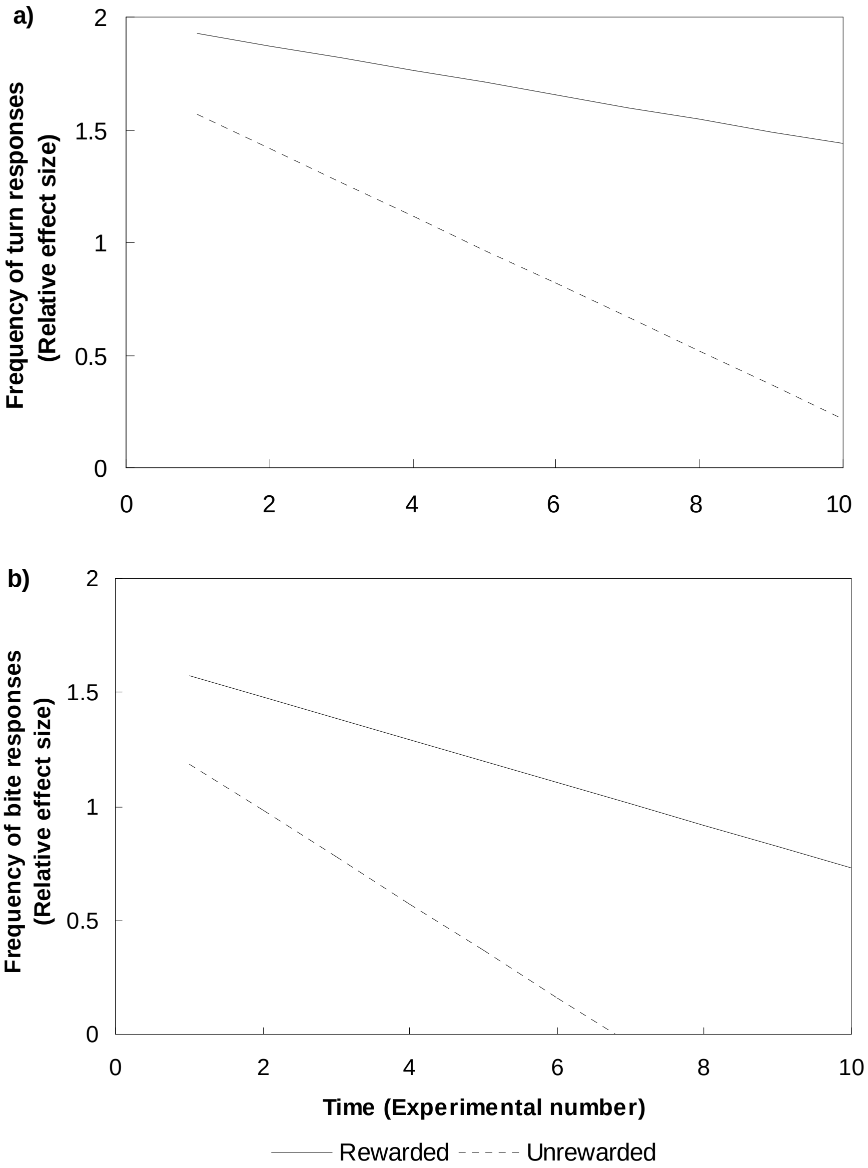


Figure 21. Relative changes in **a)** turn and **b)** bite levels throughout experimental week for rewarded and unrewarded catsharks (note: Y axis plotted using statistical effect sizes).

e) Turn & bite time latencies:

The larger number and therefore later average times for rewarded catsharks may have skewed the modelling of turn and bite time latencies. Therefore only time latencies of first turns and bites were analysed. Too few data were available to analyse bite times (model not well fitting), and although there was a suggestion that rewarded catsharks turn earlier the effect was not significant (t = -1.46; *P* = 0.145; Figure 22). However, within an experimental week rewarded catsharks showed decreasing turn times (t = -2.47; *P* = 0.014; Figure 23a). Again, although there was a suggestion bite times decreased too, the effect was not significant (t = -1.42; *P* = 0.156; Figure 23b). There were no trends for unrewarded catsharks (both t = -0.11; both *P* = 0.912; Figures 23a & b).

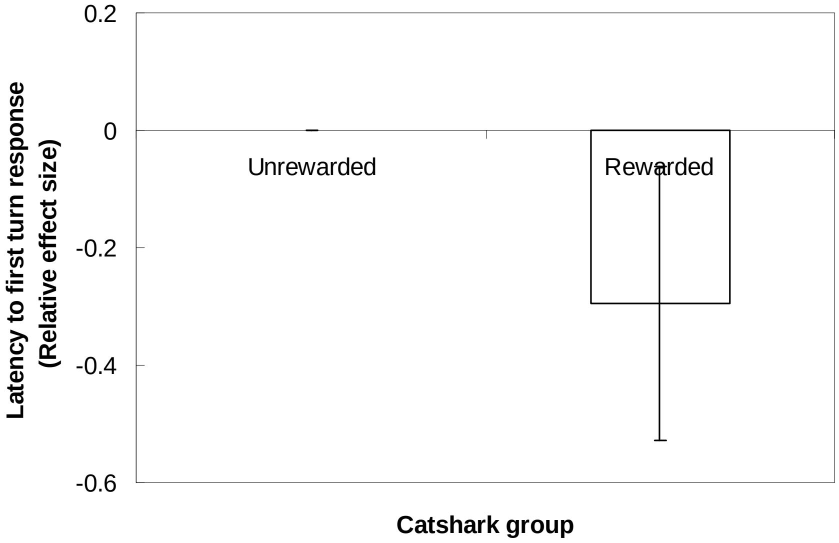


Figure 22. Relative first turn time latencies by rewarded and unrewarded catsharks (± standard error. Note: Y axis plotted using statistical effect sizes. Unrewarded is zero reference).

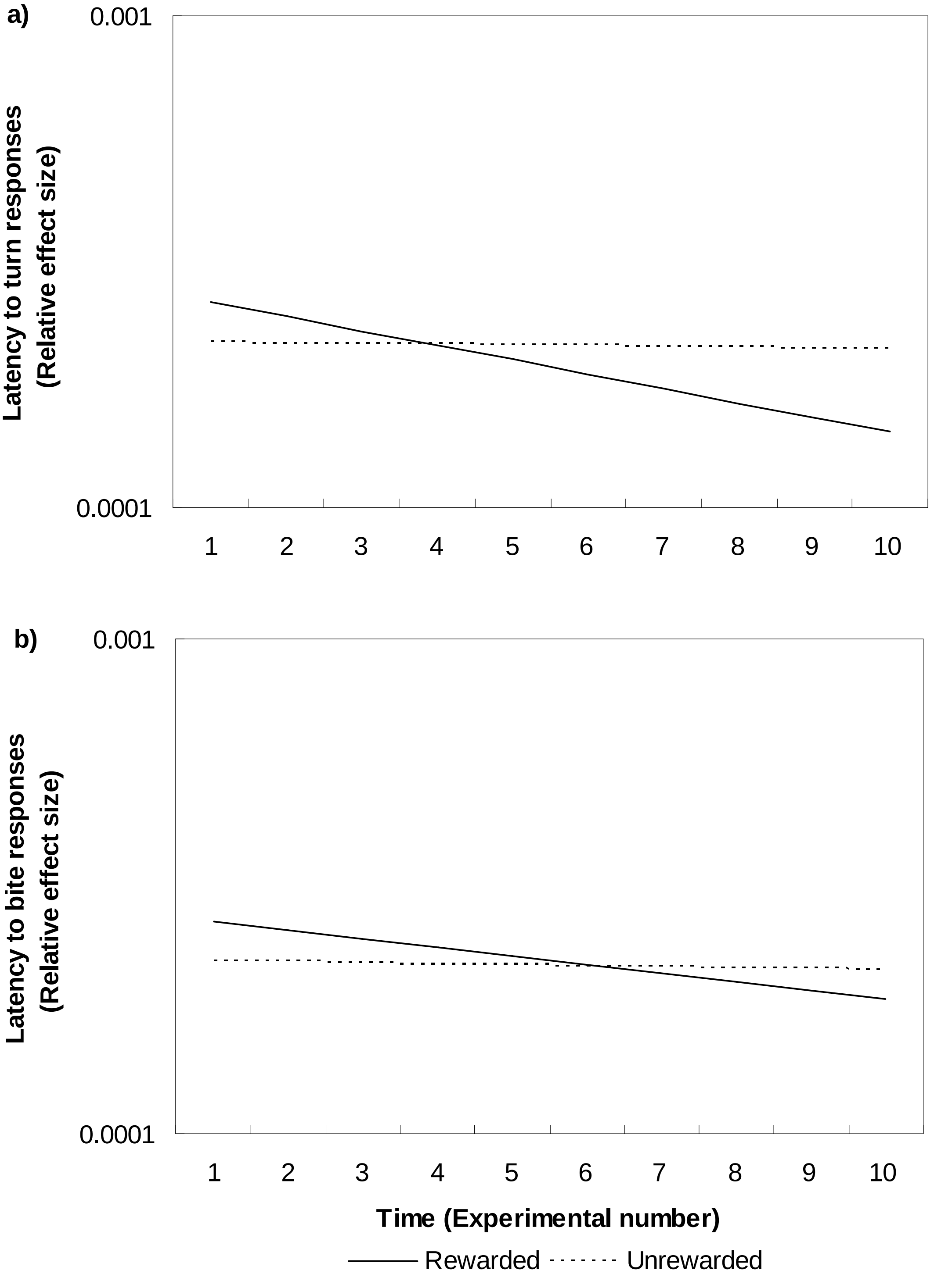


Figure 23. Relative changes in **a)** first turn and **b)** first bite time latencies throughout experimental week for rewarded and unrewarded catsharks (note: Y axis values are back transformations of statistical effect sizes plotted on log scale).

f) Bite number and time of bite at which reward acquired:

Among rewarded catsharks, there were significant decreasing trends observed for the time and bite number at which rewards were acquired throughout the experimental week (t = -2.87 (time) & -3.94 (bite); *P* = 0.004 & 0.0001; Figure 24a & b). These parameters were not applicable for unrewarded catsharks.

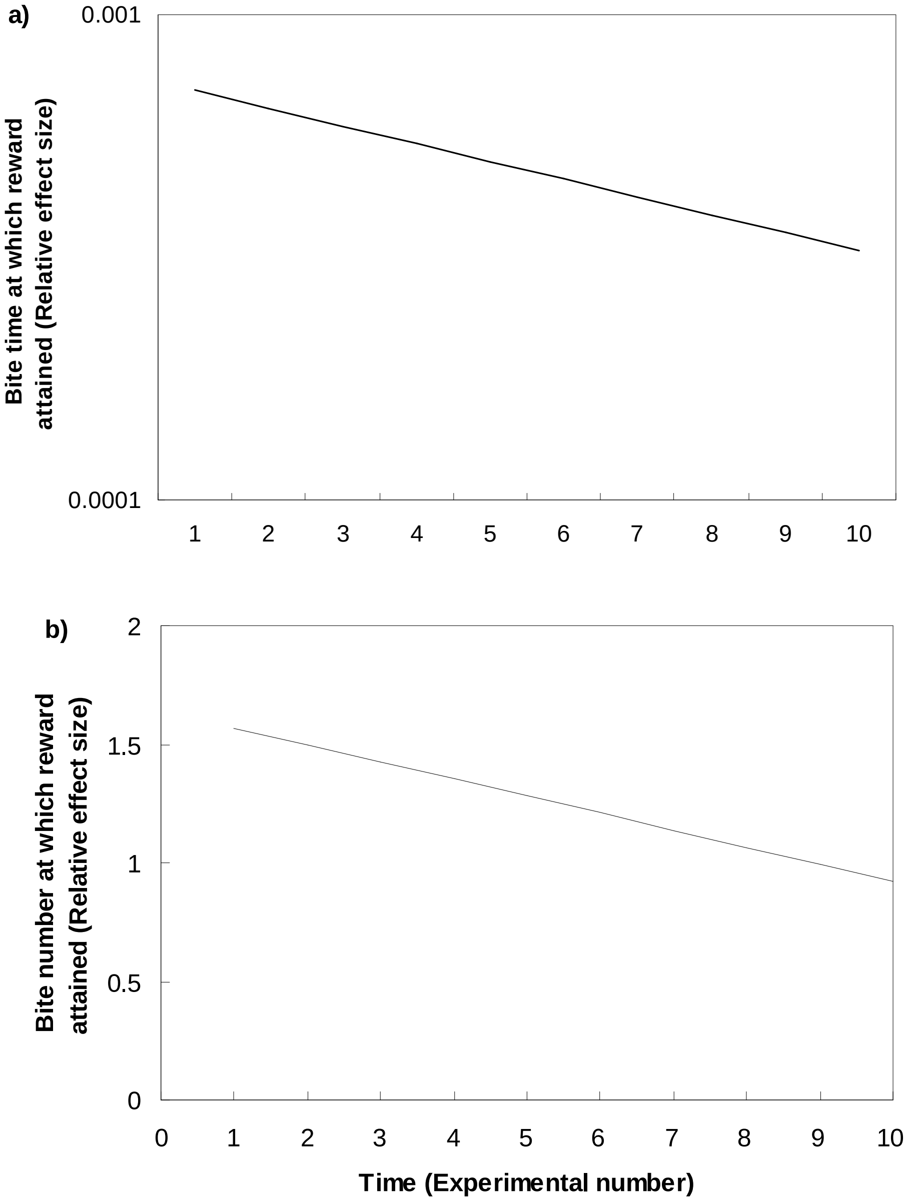


Figure 24. Changes in the **a)** time and **b)** bite number at which rewarded catsharks acquired food reward throughout experimental week (note: Y axis plotted using statistical effect sizes; back transformed and plotted on log scale for time).

Table 7 shows the number of rewarded individuals exhibiting different trends (from correlation and Kendal Tau analyses) in the bite number and time of bite at which they attained food rewards (76 and 70 % showed decreasing trends in time and bite respectively).

Table 7. Numbers of rewarded catsharks showing different trends in bite numbers and times of bites at which rewards were taken

Trend	Reward time	Reward bite
Decrease	13	12
No change	0	2
Increase	4	3

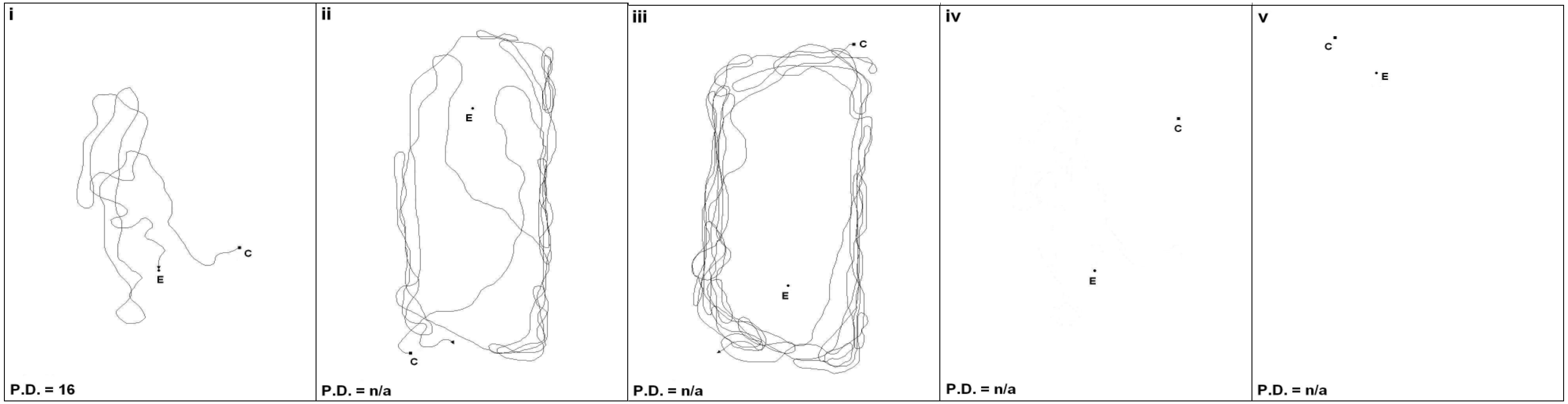
(Note: Seven catsharks not available - not enough data points for correlation and Kendal Tau analyses)

Search paths:

Visual comparisons highlight differences between individual catshark's search path trends in rewarded and unrewarded weeks (Figures 25 - 27). The diagrams also highlight the fact that learning and habituation effects begin within just a few experiments (within 3 -5). Individuals Blue1501 and Red1506 were unrewarded in week 1 and then rewarded in week 2. When unrewarded, Blue1501 responded to the electrode in the first experiment, but subsequently failed to respond despite long tracks and eventually began not even responding to the scent (Figure 25; top row). Red1506 began by finding and responding to the electrodes fairly quickly and directly, but after a few such responses started to undertake longer search paths and failed to respond to the E field (Figure 26; top row). However, when rewarded, Blue1501 initially embarked on very long tracks, which progressively became shorter and were eventually very direct (Figure 25; bottom row). Similarly, when rewarded, Red1506's tracks gradually became more consistently short and direct, having been higher initially (Figure 26; bottom row).

Individual Green230 was rewarded in week 1 and unrewarded in week 2. When rewarded, after a few very indirect and lengthy tracks, this catshark’s paths to the electrodes became consistently shorter and more direct (Figure 27; top row). When unrewarded, its paths showed no clear trend with some very direct, but also very long paths, and some occurrences of failure to respond to the scent or electrodes (Figure 27; bottom row).

Before interval: **UNREWARDED**



After interval: **REWARDED**

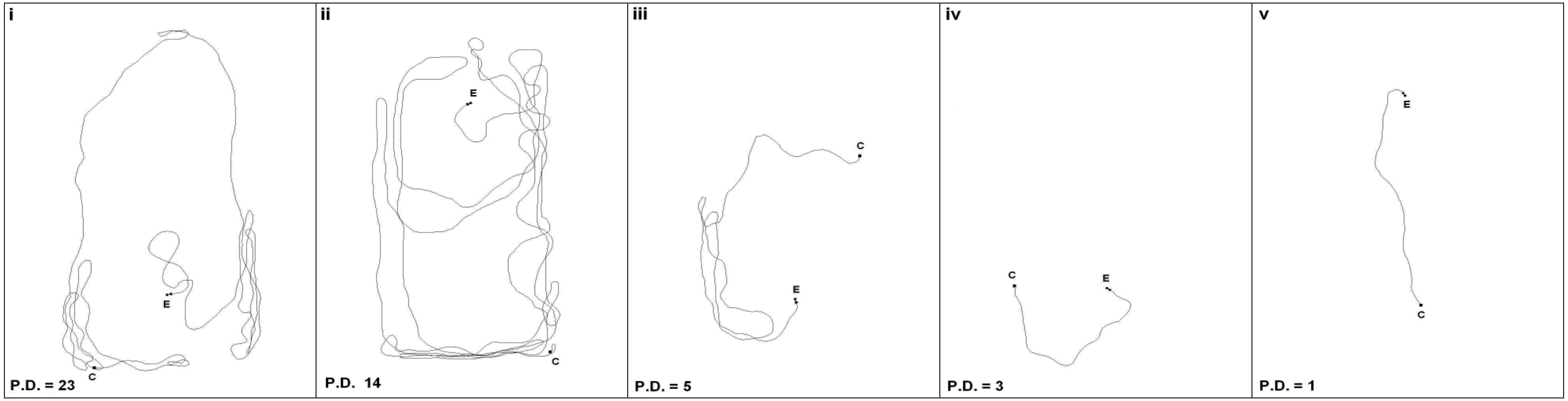
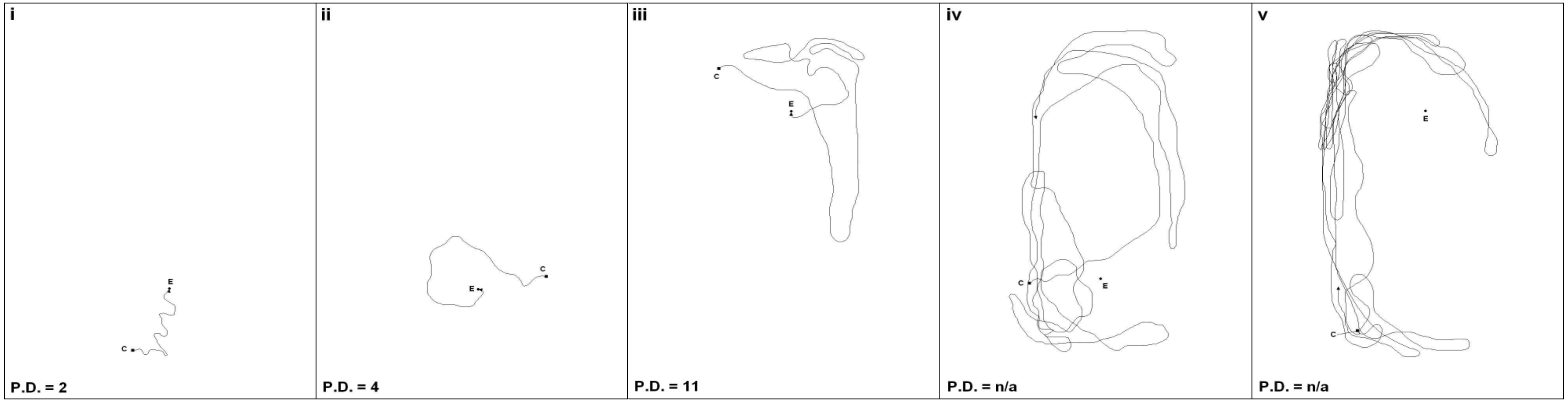


Figure 25. Search paths of an individual catshark, Blue1501, throughout week (i to v) when unrewarded (top) and rewarded (bottom) before and after experimental interval respectively. C = catshark start position; E = electrode position; P.D. = path directness (rounded figures). Note: where no path is shown, catshark did not even respond to scent.

Before interval: **UNREWARDED**



After interval: **REWARDED**

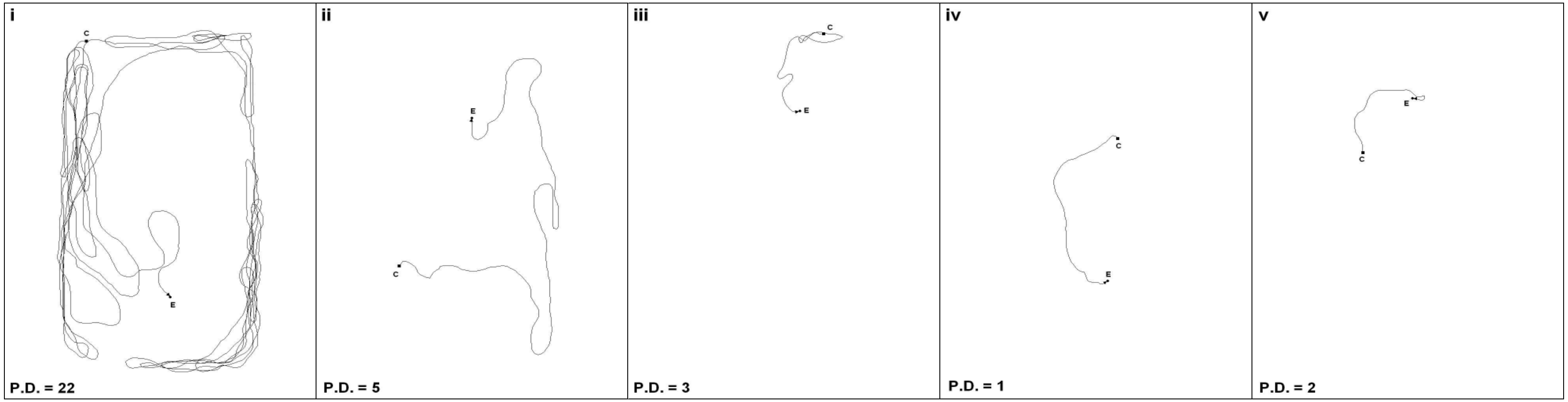
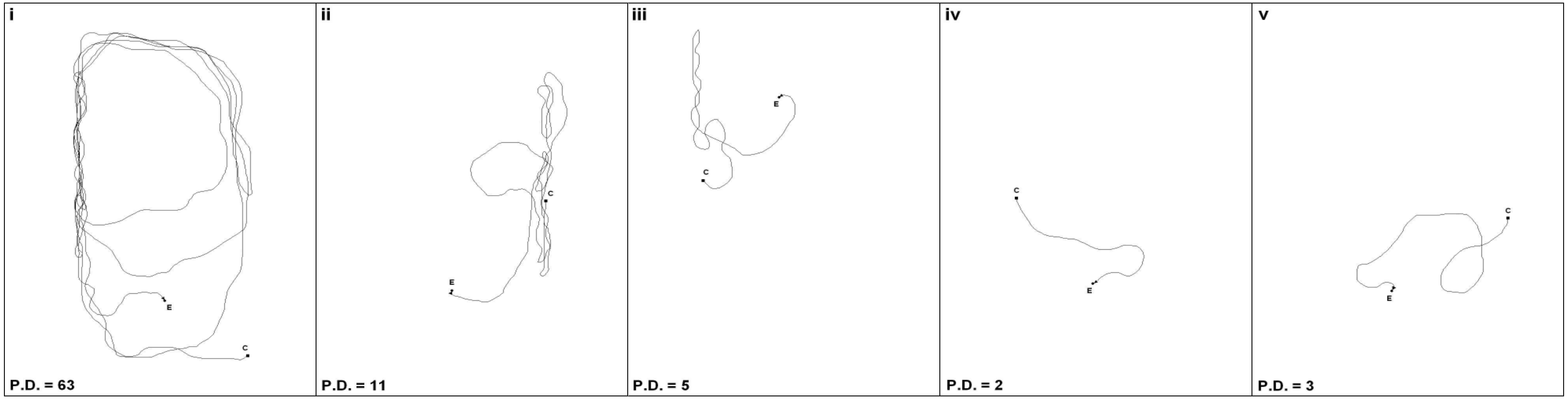


Figure 26. Search paths of an individual catshark, Red1506, throughout week (i to v) when unrewarded (top) and rewarded (bottom) before and after experimental interval respectively. C = catshark start position; E = electrode position; P.D. = path directness (rounded figures).

Before interval: **REWARDED**:



After interval: **UNREWARDED**:

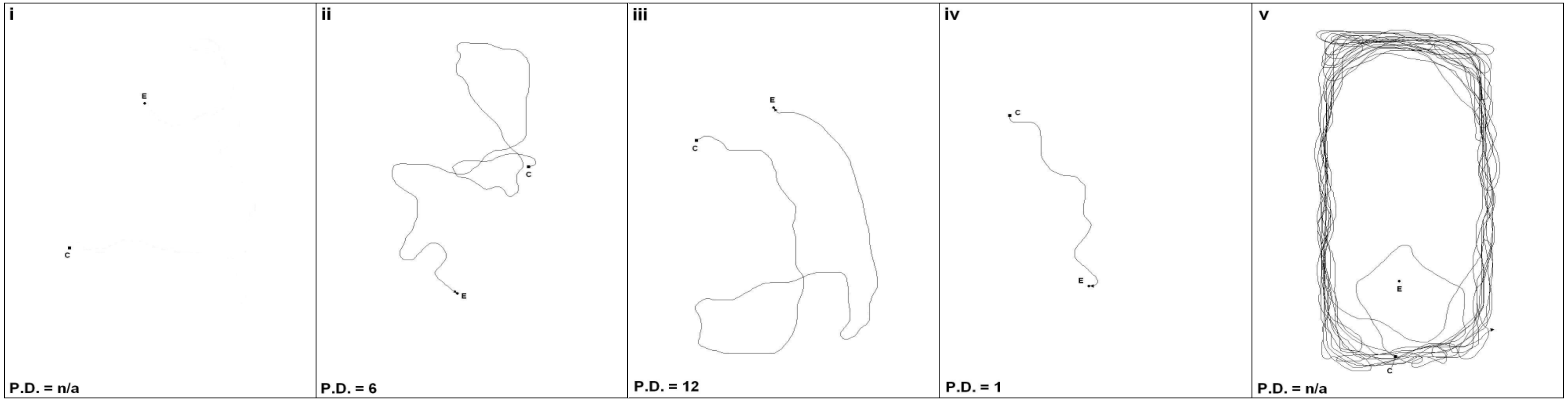


Figure 27. Search paths of an individual catshark, Green230, throughout week (i to v) when rewarded (top) and unrewarded (bottom) before and after experimental interval respectively. C = catshark start position; E = electrode position; P.D. = path directness (rounded figures). Note: where no path is shown, catshark did not even respond to scent.

Rewarded catsharks showed significant decreases in path directness (i.e. became more direct) through the experimental week (SS = 5.91; *P* = 0.019; Figure 28) whereas unrewarded catsharks showed no change (SS = 0.137; *P* = 0.693; Figure 28).

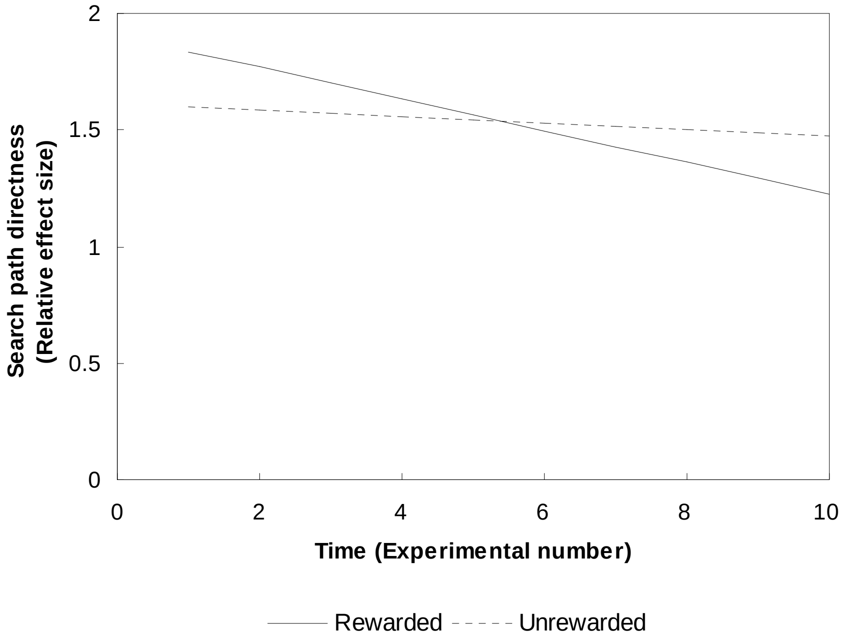


Figure 28. Relative changes in path directness to electrodes exhibited by rewarded and unrewarded catsharks throughout experimental weeks (note: Y axis plotted using statistical effect sizes).

Table 8 shows the different trends in path directness exhibited by individual catsharks. 83.3% exhibited trends that would be expected for learning (when rewarded) and habituation (when unrewarded). Only 17.7% exhibited unexpected trends.

Table 8. Numbers of catsharks exhibiting different trends in changes of path directness throughout experimental weeks (from correlation analyses)

Difference between paths	Number	Percentage
R down; U up, no change or n/a	11	83.3%
Both down but R more so	4	
R up; U down	3	17.7%

R = rewarded; U = unrewarded (note: Six catsharks not available - not enough data points)

5.4.2. Overview of rewarded catshark behaviour

There was a suggestion that rewarded catsharks responded more quickly to scent than their unrewarded counterparts. The rewarded catsharks became slower to react to scent throughout a week. They exhibited high levels of movement around the arenas and electrodes; more so before the first E field response than after. In both cases these levels of movement decreased throughout a week; again, more so before than after. They turned towards and bit the electrodes often, but these levels decreased markedly throughout a week. There is a suggestion that they were slightly quicker to respond to the E fields than unrewarded catsharks and these response times significantly decreased throughout a week. The time and bite at which they acquired the food reward and the directness of the search paths they employed all decreased significantly over time.

5.4.3. Overview of unrewarded catshark behaviour

Unrewarded catsharks exhibited no change in reaction time to scent. They showed very little movement around the arenas and electrodes prior to first response to the E fields and these levels remained similar throughout a week. However, movement around the arenas and, especially, the electrodes were considerably greater following first response, although these levels decreased markedly throughout a week. They responded to the E fields less frequently than their rewarded counterparts, and although they, too, showed decreasing response levels through time, the trend was more distinct than that of rewarded catsharks. There is a suggestion they may have responded later, but there was no change in these response times throughout a week. Nor was there any change in how direct their search paths towards the electrodes were.

5.3.4. Memory

There were no differences between experimental weeks before and after the interval for any of the hierarchical responses, except for first turn times (Table 9). In other words, when rewarded and unrewarded catshark data were grouped together and analysed by week, behavioural levels were, on average, similar before and after the three week interval. Neither were any interactions observed between reward (yes/no) and week (before/after) for any of the categories (Table 9). Therefore, behaviour was independent of whether a catshark was rewarded before the interval and unrewarded after the interval or vice versa. This suggests the altered behaviours, owing to the offer or denial of food reward, were not retained after a three week interval.

Table 9. *P* values generated by modelling when comparing hierarchical response parameters between week before and week after interval and interactions between rewarded/unrewarded and week before/after

Response Variable	Week before & Week after	Interaction
Scent time	0.236	0.602
Activity Zone	0.347	0.255
Electrode Zone	0.436	0.516
Turn frequency	0.112	0.881
Bite frequency	0.818	0.660
Latency to 1st turn	0.047*	0.134
Latency to 1st bite	0.424	0.358
Reward time $_{(d.f=139)}$	0.981	n/a
Reward bite$_{(d.f.=170)}$	0.749	n/a
Path directness	0.802	0.159

(* significant *P* value highlighted; n/a = not applicable)

5.4. Discussion

The objectives of this study were to investigate elasmobranch learning, habituation and memory, specifically with respect to electroreceptive foraging behaviour. Adjustments to foraging behaviour dependent on the relative success of responses to E fields (in terms of food gain) were clearly demonstrated within very few events (often as few as three). However these changes did not seem to be remembered after an interval with no experimental procedures and a normal feeding regime.

All fish were of similar size and maturity, were from the same geographic location, were acclimatized for equal time periods and fed on equal, minimum rations. Previous experience and initial motivation to feed could therefore be assumed to be as standardised as possible among experimental animals (possible effects of different phenotypes cannot obviously be controlled for; e.g. bold versus nervous fish). Despite becoming slightly slower to react to scent (Figure 17), rewarded catsharks consistently foraged and ingested rewards suggesting the small size of rewards prevented satiation and ensured continued motivation. Unrewarded catsharks showed consistent responses to introduced scent and a burst of interest in the E fields after first contact with them confirming continued, but not increasing motivation to feed (Figures 17 & 18).

Water temperature varied with natural conditions to some extent during experimental procedures (18.22 °C +/- 0.98 S.D.). However, this level of variation has previously been shown to have no impact on electroreceptive behaviour (Gill *et al.*, unpublished data; Chapter 3). All experimental animals were subjected to the same experimental conditions in stable, predator free environments and the large sample size and powerful modelling gives confidence that differences and trends observed are accountable to experience and behavioural flexibility.

Catsharks rewarded for electroreceptive responses exhibited more intense foraging behaviour in comparison to unrewarded catsharks: Higher levels of activity, more movement in close proximity to the electrodes and more frequent (and potentially quicker) turn and bite responses to them. Improvements in acquisition of rewards over time were also demonstrated suggesting the occurrence of learned behaviour: Activity levels and passes near the electrodes before first responding to the E field were significantly reduced; the number of responses to the electrodes also decreased markedly; furthermore, the time latency and bite number at which the catsharks acquired the food rewards also significantly declined; the search paths the fish employed to locate the electrodes became considerably more direct despite electrodes being placed at randomly assigned ends of arenas (see Figures 25 - 27). Combined, these behavioural alterations improved the catsharks' foraging efficiency over time and theoretically would have reduced energetic costs per unit food attained.

Conversely, catsharks that were not rewarded for electroreceptive responses, exhibited less intense foraging behaviour: Lower levels of activity, less movement near the electrodes and less frequent (and possibly slower) turn and bite responses to them. Changes in these catsharks' behaviour towards the electrodes over time were also demonstrated. However, these alterations differed to those of the rewarded catsharks and suggested habituation behaviour: Despite no clear trends in their low activity levels and electrode passes before first response to E fields (c.f. rewarded catsharks), the higher levels, and especially passes, after first response significantly reduced over time, even more than those of rewarded catsharks; the numbers of responses to E fields also declined over time and to a larger extent than did those of rewarded catsharks; their latencies to respond, however, showed no change; nor was there any change in the directness of the search paths they employed (see Figures 25 - 27). Although these behaviours would not improve the acquisition of food (c.f. rewarded catsharks), they could also be considered to reduce energetic costs over time by reducing wasted effort.

The fact that none of the learned and habituated behaviours described above were continued in the second experimental weeks and that behaviour levels were independent of whether rewards were offered before or after the experimental interval suggests that the memory window for these elasmobranchs is less than three weeks. Having returned to the experimental arenas after three week intervals the catsharks behaved as if they had not been subjected to the previous trials. They then began to learn or habituate again over the subsequent experimental weeks accordingly. For example, individual Green230 honed its search path to the electrodes when rewarded before the interval, but failed to exhibit the efficiency at the beginning of the subsequent week after the interval (Figure 27).

Like many other elasmobranchs, *S. canicula* inhabit highly unpredictable coastal waters (Compagno, 1984; Compagno *et al.*, 2005). In part owing to this fact, many are also opportunistic predators (Lyle, 1983; Ellis *et al.*, 1996; Laptikhovsky *et al.*, 2001). When considering populations of these elasmobranchs in their natural habitats, the cognitive abilities demonstrated here make ecological sense. In essence, the catsharks seem able to learn to improve their electroreceptive foraging efficiency towards rewarding stimuli over short temporal periods (and presumably within small spatial scales). Equally, within similar temporal and spatial limits they can habituate to (or learn to ignore) non-rewarding stimuli. This flexibility may therefore allow, for example, the predators to focus their efforts on easily caught, edible or nutritious prey (Dill, 1983; Stephens & Krebs, 1986; Kaiser *et al.*, 1992b; Gill & Hart, 1994).

Similarly, efforts towards inedible, nutrient deficient and difficult to catch prey could be reduced thereby permitting focus elsewhere and minimizing missed opportunities. These behaviours are generally consistent with those observed in other animals towards variable and non-variable foraging stimuli. For example, Micheli (1997) demonstrated greater modification of foraging behaviour based on experience in crabs encountering variable prey and prey patches than those within more stable environments. Similar differences were demonstrated between stickleback fishes inhabiting more and less stable habitats (Girvan & Braithwaite, 1998).

The coastal zone is especially variable due to both natural (Tait & Dipper, 1994; Lalli & Parsons, 1997; Barnes & Hughes, 1999) and anthropogenic factors (fishing: Blaber *et al.*, 1981; Frid *et al.*, 1999, land reclamation: McLusky *et al.*, 1992, eutrophication: Powers *et al.*, 2005, climate change: Rogers & McCarty, 2000, pollution: Matthiessen & Law, 2002). In such an environment, therefore, remembering foraging adaptations over longer temporal periods and larger spatial scales may not be of benefit (*sensu* Hirvonen *et al.*, 1999) considering prey diversity and distributions, in addition to physical habitat, may well change over relatively short time periods and over small distances.. Long memories have been demonstrated in fish inhabiting relatively stable environments (e.g. up to 6 months in trout: Bryan & Larkin, 1972; 3 months in cod: Nilsson *et al.*, 2008) and shorter memories demonstrated in those inhabiting more variable environments (e.g. 3 hours in paradise fish: Csanyi *et al.*, 1989; from minutes to days in sticklebacks: Mackney & Hughes, 1995; Hughes & Blight, 1999). It would be interesting to determine exactly where the memory window lies in *S. canicula*; seemingly somewhere between 12 to 24 hours (learned behaviour was obviously remembered between experimental treatments each day) and 3 weeks (the experimental interval period) and how it would relate to other teleost and chondrichthyian fishes. It is also interesting to note whether the catsharks were, in actual fact, “forgetting” or whether they were simply choosing to ignore previous experience and instead, choosing to thoroughly inspect all possible stimuli to track current prey and patch states and prevent missing any opportunities (Stephens & Krebs, 1986; Krebs & Inman, 1992; Dall *et al.*, 1999).

Recently, predation pressure was demonstrated to be an important influence upon three-spined stickleback (*Gasterosteus aculeatus*) learning and memory (Brydges *et al*., 2008). In fact, predation was observed to override the effect of habitat stability. Catsharks were studied in predator free environments during this study but it is important to note that, in the wild, in addition to habitat variability, predation pressure may also affect elasmobranch learning and memory. However, presumably the effect would be less pronounced on sharks, skates and rays compared with small teleosts owing to their comparatively larger size, their position further up the food chain and therefore their fewer numbers of natural predators.

The learning, habituation and memory skills associated with electroreceptive foraging demonstrated through this experiment represent valuable attributes in *S. canicula*’s capacity to maximise gains and minimize costs and thereby potentially optimise individual feeding success and improve Darwinian fitness. Such cognitive abilities may extend to other uses of the sense such as predator and symbiont detection, location of and communication with conspecifics and navigation, all of which would also have important implications on life processes. The abilities may also be important when considering how elasmobranchs may cope with being confronted with anthropogenic E fields that closely resemble their prey bioelectric fields (see Chapters 1, 4 & 6).

Cognitive abilities are likely to vary between elasmobranch species. For example, one may expect better memory windows in species inhabiting more stable habitats than more variable habitats (*sensu* Odling-Smee & Braithwaite, 2003a, b). Inter-specific differences in visual learning have previously been observed between lemon and nurse (Clarke, 1959) and lemon and bull sharks (Wright and Jackson, 1964).

Variation in brain to body mass ratios and in the relative mass of the major brain divisions can be used to postulate the capacities of different species' senses and cognition (Demski & Northcutt, 1996). *Scyliorhinus canicula* have average brain to body mass ratios (Ridet *et al.*, 1973) compared to higher and lower elasmobranchs such as scalloped hammerheads (*Sphyrna lewini*) and Greenland sharks (*Somniosus microcephalus*) respectively (Northcutt, 1978; Myagkov, 1991). They also possess generic relative brain division sizes (which can be used to estimate capabilities associated with different behaviours). For example, *Carcharodon carcharias* possess unparalleled olfactory bulb size suggesting that chemical stimuli may be important in guiding behaviour associated with, for example, feeding, mating and social interactions. Carcharhinids and Sphyrnids posses enlarged central nuclei which may be related to complex social and territorial behaviours and therefore learning of conspecific and habitat stimuli, exemplified by hammerheads observed aggregating in sub-divided segregations based upon social dominance, size and sex (Myrberg & Gruber, 1974; Klimley, 1987).

6. GENERAL DISCUSSION

Locating and consuming food are essential to animals (Elton, 1927; Stephens & Krebs, 1986), and among elasmobranch fish electroreception is arguably the most important sense utilised over short distances for prey detection and jaw orientation (Kalmijn, 1971; Tricas, 1982; Blonder & Alevison, 1988; Haine *et al.*, 2001). One would assume coastal benthic species that inhabit waters with lower light intensity and greater turbidity, in comparison to pelagic species, will be less likely to rely on vision and become more reliant on other senses such as electroreception (Raschi *et al.*, 2001; Collin & Whitehead, 2004; Linsey & Collin, 2007). Accordingly, species of skate inhabiting deeper, and therefore darker habitats, possess more and larger Ampullae of Lorenzini (the electroreceptive apparatus) than those in shallower habitats with higher light levels (Raschi & Adams, 1988). Coastal benthic species' diets often include prey that may be hidden from view; for example amongst marine algae or mangrove root systems, within coralline or rocky structures or actually buried beneath substrates such as sand and silt (Lyle, 1983; Matallanus *et al.*, 1993; Morato *et al.*, 2003). Electroreception significantly aids the location, manipulation and ingestion of prey otherwise less detectable by other sensory modalities (Raschi, 1986; Tricas, 2001). The sense also enables nocturnal predation (Tricas, 1982; Blonder & Alevizon, 1988).

Electroreception is therefore a decisive contributor to the acquisition of food among such species. Owing to the influence that obtaining food has upon survival and the maintenance of somatic and reproductive growth, the electro-sense can also be considered one of the key factors affecting Darwinian fitness. The fact that electroreception is also widely used in predator detection (Peters & Evers, 1985;

Sisneros *et al.*, 1998), conspecific location and communication (Bratton & Ayers, 1987; Tricas *et al.*, 1995; Sisneros, *et al.*, 1998) and possibly during navigation (Kalmijn, 1974 & 1984, Pals *et al.*, 1982b; Paulin, 1995; Montgomery & Walker, 2001) only strengthens the fundamental significance of the electro-sense to these animals and their life processes.

Despite electroreception's biological and ecological importance, and notwithstanding considerable physiological work on the apparatus facilitating electric field detection (the Ampullae of Lorenzini; e.g. Waltman, 1966; Raschi, 1986; Montgomery & Bodznick, 1999; Tricas, 2001; Chapter 1), the associated behaviour and ecological context of the sense has received little attention (Collin & Whitehead, 2004; Tricas & Sisneros, 2004). Considering the importance of elasmobranchs to ecosystems as apex predators (Stevens *et al.*, 2000, Schindler *et al.*, 2002), their vulnerable life histories (Smith *et al.*, 1999; Baum *et al.*, 2003; Frisk *et al.*, 2005; Dulvy *et al.*, 2008), their continuing population declines (Compagno *et al.*, 2005; Dulvy *et al.*, 2008) and ever increasing interactions with human activities and development (Stevens *et al.*, 2000; Gill, 2005; Dulvy *et al.*, 2008) the requirement to reduce such gaps in our understanding becomes ever more urgent. The behavioural research on *Scyliorhinus canicula* detailed within this thesis was formulated in order to address some of the poorly understood aspects of elasmobranch behaviour, especially those linked to electroreceptive foraging, that have hitherto remained poorly described. The topics covered related to important population and life processes and are summarised as follows:

1) Sexual conflict: the effects of male harassment and female avoidance upon elasmobranch feeding behaviour using electroreception as an indicator (Chapter 3).
2) Choice: the ability of elasmobranchs to discriminate between E fields and preferences they show for them (Chapter 4).
3) Cognitive ability: the ability of elasmobranchs to learn to alter their behaviour towards E fields of differing profitability and remember learned alterations. (Chapter 5).

6.1. Overview of results

There were clear findings from the research that not only advance understanding of the functionality of electroreception, but also shed light on some fundamental, and yet previously un-described, elasmobranch life processes.

The findings from Chapter 3 build on our current understanding of sexual conflict in elasmobranchs and its role in causing sexual segregation. A marked reduction in foraging efficiency among individuals in mixed-sex groups compared to same-sex groups was demonstrated. Mixed-sex groups responded to the electrodes less than single-sex groups despite exhibiting similar or higher activity levels. There was also evidence of higher responsiveness towards electric fields (E fields) by females compared to males, despite similar activity levels between the sexes. Furthermore, the sexes' sensitivities to different E fields were similar, supporting stomach analysis evidence that suggests male and female *S. canicula* possess similar diets.

Chapter 4 details one of the very few studies investigating the capacity of an elasmobranch to differentiate and choose between E fields (and one of only very few investigating choice in any of the elasmobranch senses). A considerable preference for stronger rather than weaker direct current (D.C.) fields was demonstrated. A less marked preference, though a significant one, was demonstrated for alternating current (A.C.) fields rather than D.C. fields. However, either an inability to discriminate between, or no preference for, similar artificial and natural fields was revealed.

The work documented in Chapter 5 provides demonstration of learning, habituation and memory capabilities associated with electroreceptive foraging behaviour (and one of only very few investigating cognitive abilities related to any elasmobranch behaviours). The catsharks were able to learn to improve their foraging efficiency towards rewarding E fields and habituate towards non-rewarding E fields. Behavioural adaptations were, however, not retained after a three week interval suggesting the catsharks' memory window lies somewhere between twelve to twenty four hours and three weeks.

6.2. Advantages of current study

6.2.1. Scope of investigation

When studying a specific aspect of an organism's behaviour it is crucial to be aware of the general biology and ecology of the species in question (Krebs & Davies, 1997). This enables appropriate explanations of findings and suggestions of plausible causes and effects to be made. Foraging behaviour, in particular, is a highly complex phenomenon which effects and is affected by many varied processes. The success of foraging will ultimately determine the amounts of energy available for partitioning into somatic and reproductive growth and therefore individual success and Darwinian fitness (Stephens & Krebs, 1986).

Foraging behaviour is, in turn, influenced by a number of intrinsic and extrinsic factors (boxes on Figure 29). Intrinsic factors are those influenced by the individual's biology and life processes (shaded boxes) whereas extrinsic factors are external influences including the biotic and abiotic environments (un-shaded boxes). A predatory fish's intrinsic factors may include physiological state (e.g. Griffiths, 1996; Gill & Hart, 1998; Gill, 2003), adaptive flexibility (e.g. Croy & Hughes, 1991a; Hughes & Blight, 1999) and sensory capability (e.g. Holmes & Gibson, 1986; Kaiser & Hughes, 1993). For example, foraging levels and efficiency are likely to increase in a hungry fish, with proficient learning, habituation and memory capabilities, and the ability to acutely detect and recognise prey. Alternatively, satiation, reproductive urge and poorer cognitive and sensory abilities are likely to have the opposite effect. External factors may include conspecifics (e.g. Magurran & Seghers, 1994; Gill & Hart, 1996), predators (e.g. Holbrook & Schmitt, 1988) and natural and anthropogenic environments (e.g. Girvan *et al.*, 1998; Odling-Smee & Braithwaite, 2003a; Filer *et al.*, 2008). For example fish foraging behaviour will likely be affected by inter-and intra-specific competition, mating, shoaling, predation risk (timing or location of feeding events and escape ability), habitat variability and human use of the coastal zone. Encounters with prey are also, obviously, crucial (point of thick arrow, Figure 29) with factors such as prey size, health, crypsis, handling time, armour, digestibility and escape capability potentially having significant effects on their predators' foraging success (e.g. Kislalioglu & Gibson, 1976; Kaiser *et al.*, 1992a, b; Gill & Hart, 1994). Given these numerous influences, the foraging process is therefore highly variable (as represented by the variable length of shaft of thick arrow, Figure 29).

In addition to directly affecting foraging behaviour, many of these factors will be interlinked and thus provide indirect influences on the behaviour (dotted arrows on Figure 29). For example the outcome of a prey encounter may have consequences for both hunger (physiological state) and learning; habitat variability (environment) may affect learning and memory window; sensory capability may influence both predation risk and the detection of mates (conspecifics); hunger may impinge on reproductive urge (both physiological states).

Note that the predator in question could also be considered as prey for a larger predator (predation risk box; Figure 29) and would thus represent a factor in that predator’s foraging processes. Equally the prey (point of thick arrow, prey encounter; Figure 29) may also prey on smaller organisms and would therefore represent a factor in that prey’s foraging processes. The diagram could consequently be repeated through different trophic levels.

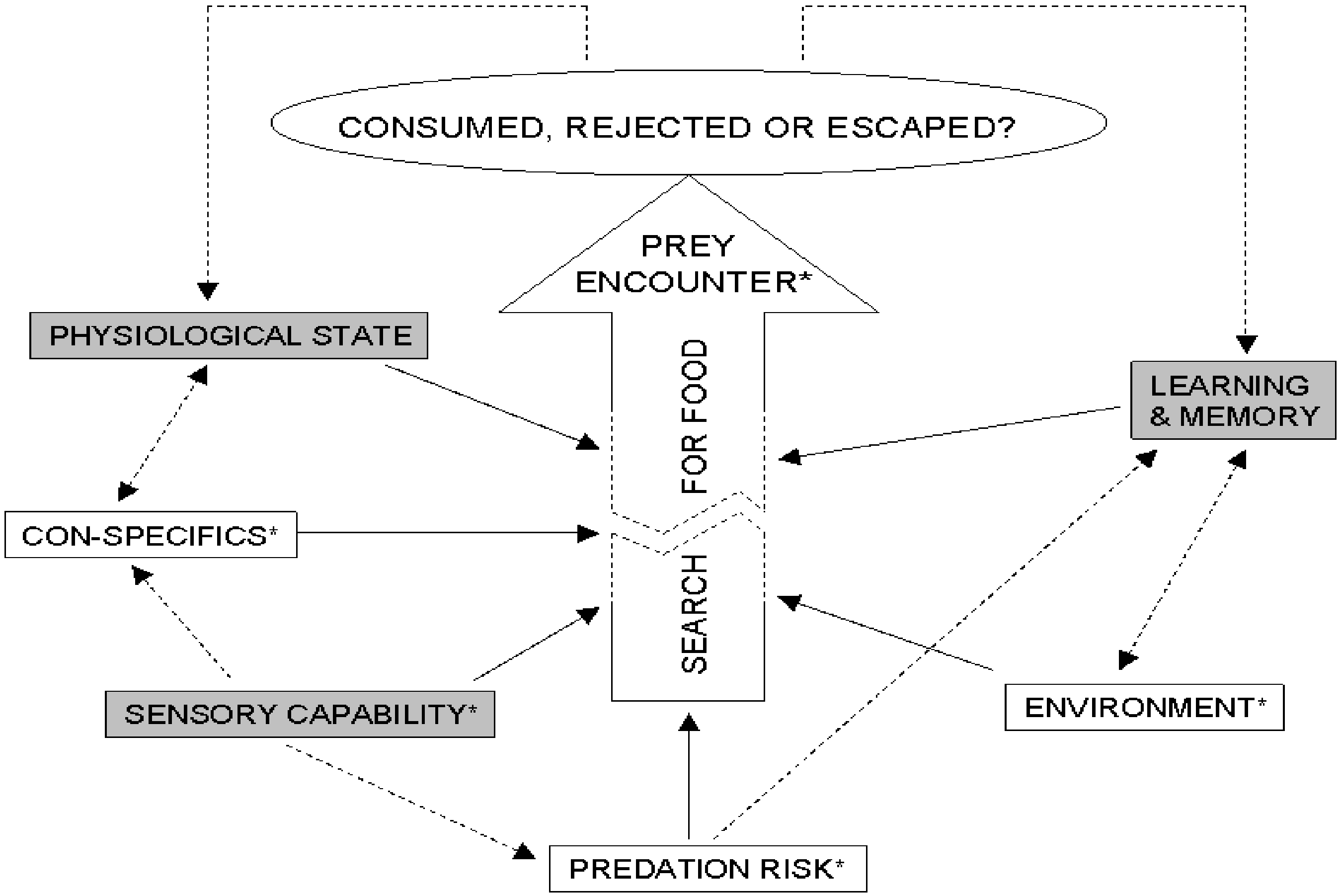


Figure 29. Diagram illustrating major factors affecting a predatory fish's foraging behaviour (shaded boxes = intrinsic factors; un-shaded boxes = extrinsic factors), important interrelations between them (solid arrows = direct effect; broken arrow = indirect effect), and points at which electroreception is a significant consideration (asterisks).

The current study’s three research topics, though all related to electroreception, encompass a broad spectrum of the factors and linkages influencing foraging and illustrated in Figure 29. Chapter 3’s content (male-female interactions) relates to both physiological state and conspecifics via hunger and reproductive urge. Chapter 4’s content (ability to distinguish and preference) is relevant when considering both prey encounters and environment (anthropogenic versus natural stimuli). Chapter 5’s content (cognitive ability) concerns learning, memory and environment (variability). Furthermore, electroreception considered generally, can obviously be applicable in many areas including sensory capability, prey encounter, the detection of conspecifics and predators and the environment (see asterisks, Figure 29). Therefore, contrary to its apparent focus on a specialised and unusual sense, the results and findings generated by the research in this thesis fit well with more general aspects of foraging behaviours in these fish.

6.2.2. Experimental sample sizes and repeated measures

In order to achieve optimum statistical power, a large data set was used throughout this study whilst bearing in mind time and facility constraints. Studies utilised large sample sizes of catsharks compared with many other elasmobranch behavioural investigations (88 specimens during the sexual conflict experiments for example; Chapter 3), aided by the ability to release animals following experimentation. This enabled data analyses to cope with inherent, natural, individual variability (Magurran, 1986), increased the likelihood of distinguishing subtle behavioural differences and reduced the confounding effects of using the same fish over protracted time periods. Furthermore, the ability to run treatments across seven, similar experimental tanks, whilst acclimatising fish for subsequent trials in adjacent holding tanks permitted an intense regime of experimentation to maximise use of limited time. In turn, this allowed experimental treatments to be repeated a number of times on the same specimens which not only increased the ability to reduce variance in the data but enabled investigation of temporal changes in behaviours. Studies observing individuals on relatively few occasions preclude temporal effects and are therefore momentary representations of behaviour (Hughes *et al.*, 1992). A more dynamic approach involves considering more variables over time (Houston *et al.*, 1988; Hughes, 1990). Obviously, a trade-off results in which one must balance observing individuals on enough occasions to obtain a representation of a behaviour and its variation over time, with using individuals for too long and possibly recording erroneous results (such as seasonal effects, habituation to experimental procedures and the erosion of natural behaviour and/or health in prolonged captivity) .

The work in Chapter 3 (sexual conflict) illustrates the importance of designing experiments with large sample sizes and repeated measures in order to address subtle aspects of a species' behaviour. Differences in electroreceptive behaviour between the sexes were previously unsupported (Gill *et al.*, unpublished data; Filer *et al.*, 2008). However, with eighty eight specimens subjected to twenty five treatments each, resulting in a total of over two thousand turn and one thousand bite responses recorded, this study distinguished subtle, but important, differences between the foraging behaviour of different sex groups. Similarly, the vast majority of previous work on the electro-sense has focused on describing and/or demonstrating electroreceptive foraging behaviour whereas the work in Chapter 5 illustrates the importance of considering how the behaviour may change over time as a result of intrinsic and extrinsic factors.

6.3. Ecology

Sharks, skates and rays have been major predators in marine ecosystems for millions of years, but the biological functions and selective pressures that shape the evolution of their ampullary electro-sensory system are poorly known (Tricas & Sisneros, 2004). How does the current study help to address some of the shortcomings in current understanding of elasmobranch electroreceptive foraging behaviour?

6.3.1. Sexual conflict

Results from chapter 3 corroborate the hypothesis from previous studies that *S. canicula* sexual segregation is caused by male harassment and female avoidance (Sims, 2001; Sims, 2005; Wearmouth & Sims, 2008). It goes further by suggesting that, in addition to injury caused and energy spent during aggressive courtship by males (Pratt 1979; Carrier *et al.*, 1994; Sims *et al.*, 2006), the subsequent significant reduction in female foraging efficiency may also be an important contributing factor in driving female avoidance behaviour. Males also showed reduced foraging efficiency in mixed-sex groups but this would likely be a result of choice rather than a constraint. Male feeding and mating decisions seem dependent on hunger whereas female foraging is more constrained by harassment (*sensu* Griffiths, 1996), although male mating will also be dependent on female avoidance to some extent (and avoidance can be thought of as a form of female harassment of reproductively active males). Harassment therefore has a true cost in terms of lost feeding opportunities for females under conditions where trade-offs concerning mating and feeding have to be made simultaneously (*sensu* Griffiths, 1996). Similar reductions in foraging efficiency (sometimes halved) caused by male aggression have been demonstrated in poeciliid fishes (Agrillo *et al.*, 2005; Magurran & Seghers, 1994a, 1994b; Griffiths, 1996; Plath *et al.*, 2003). Females of these teleost fishes subsequently varied shoaling behaviour when harassed by 1) approaching other males to promote male-male interactions, and 2) reducing distance to other females to dilute male disturbance. Female catsharks may refuge in all female aggregations (Sims, 2005; Wearmouth & Sims, 2008) for similar reasons.

Increased electroreceptive foraging levels by females compared to males demonstrated in this study also suggest that when not in the company of males, female *S. canicula* may undertake more intensive feeding forays. This makes sense given the short time periods in which females are able to leave their refuges to forage (Sims *et al.*, 2001), combined with possible higher energy requirement for egg production. This may be the first demonstration of sex-based, intra-specific competitive release as opposed to the more commonly discussed inter-specific (Holbrook & Schmidt, 1995; Persson & Hansson 1999) and ontogenic (Persson & Broenmark, 2002) forms. One might assume elasmobranch species exhibiting similar reproductive strategies (e.g. female Port Jackson sharks, *H. portuskacksoni*, refuging in reef caves off Australia; McLaughlin & O'Gower, 1971) may exhibit similar interactive effects.

Despite the difference in electroreceptive behaviour discussed above, both sexes showed similar preferences for different strength, direct current E fields presented sequentially (Chapter 3). Preferences for three different pairs of E fields presented simultaneously also showed no variation by sex (Chapter 4). When considered alongside the similar diets observed in stomach analyses (Lyle, 1983; Rodriguez-Cabello *et al.*, 2007), these similar electro-sensitivities support the general view that this species shows no difference in dietary preference by sex.

6.3.2. Choice

Early methods of discovering fishes' prey preferences involved dietary analysis by recording stomach content. This is a rather limited means of acquiring such information as contents may well be determined by prey distribution, availability, ease of capture and digestibility rather than preference *per se*. Hence observed predatory diets in the field are rarely random samples of available prey (Stephens & Krebs, 1986; Hughes, 1988). Food is not homogeneously distributed; prey behaviour, habitat association and other factors lead to heterogeneous patches of prey. Within these patches prey will vary by species, size and abundance and thus predators have a number of options available to them. A predator's discriminatory ability and preference will significantly shape the types, sizes and amounts of prey consumed (Kaiser & Hughes, 1993). Foraging theory was developed to predict what animals will ultimately choose in their diets to maximize net yield of energy per unit foraging time (Charnov, 1976; Stephens & Krebs, 1986). Sensory ability, including the electro-sense, will be a crucial component of diet choice through enabling recognition and/or discrimination of different prey to permit choice (Hughes, 1979).

Von der Emde (1990) demonstrated the weakly electric teleost fish, *Gnathonemus petersii* was able to distinguish objects of different complex electrical impedances. Similarly, Graff *et al.* (2004) demonstrated that *Gnathonemus petersii* & *Sternopygus macrurus*, which possess no vibrissae and limited vision, were able to use electro-location to distinguish horizontal conductance zones (akin to vertical plant shoots) from horizontal resistance zones (akin to floating or sunken twigs or rocks). Their results demonstrated the ability to recognize spatial patterns and object characteristics independently of optical and mechanical stimuli. The ability of passive electric fishes to distinguish between objects has, however, not been demonstrated (Blonder & Alevizon, 1988). The work detailed in Chapter 4 demonstrates that passive electroreception may be an important means of facilitating recognition and choice in elasmobranch feeding. *S. canicula* was able to distinguish between differing types and strengths of E fields.

Bioelectric fields will vary with organism size and type (see Chapter 1) and therefore, contrary to Blonder and Alevizon's findings (1988; stingray were unable to discriminate prey species using electroreception alone, although results were inconclusive) the results from Chapter 4 suggest that sharks may be able to differentiate between different sizes and species of prey using electroreception. This would be especially beneficial for benthic elasmobranchs whose prey is often varied and may be conspicuous owing to burial (under sandy or silty substrates for example), refuging (amongst algal fronds, mangrove roots or rocky/coralline caves for example) or crypsis. Their individual ability to discriminate and choose successfully would ultimately affect their Darwinian fitnes by affecting somatic and gonadal growth. The ability appears especially important when one considers they may also be able to recognise a number of other important signals: the risk associated with differing species and sizes of potential predators; different conspecifics (possible rivals or suitable mates); optimum navigation paths (to reach feeding grounds, mating areas or refuges); and differing symbionts such as mutualistic cleaning fish and shrimps as opposed to parasitic copepods and mimic cleaner fish.

Whether a fish chooses to attack a prey item will depend on a number of intrinsic and extrinsic factors (Kaiser & Hughes, 1993; Figure 29, section 6.2.1.). However, sensory capability is an important intrinsic factor (e.g. Holmes & Gibson, 1986; Kaiser & Hughes, 1993; Figure 29) and electroreception has been shown to effectively override all other senses when elasmobranchs are within close proximity of a prey stimulus (Kalmijn, 1971, 1982; Heyer *et al.*, 1981; personal observation). The electro-sense is therefore crucial in the final moments of a feeding event for these fish. By using an E field created by dipoles (a stationary and small target) with isolated and continually motivated catsharks, the simplified methodology utilised in this study effectively controlled for all other factors, allowing choices demonstrated between different E fields to be attributed to an individual's preference or sensory ability.

6.3.3. Cognitive ability

Foraging and feeding behaviour in teleost fishes are energetically costly (Lucas *et al.*, 1991) and there is pressure for them to acquire the most energy possible whilst ensuring least energy is expended (e.g. eels; Helfman & Winkelman, 1991; sunfish; Stein *et al.*, 1984). Foraging theory predicts that predators will maximise net energy intake through learning and habituation (Hughes, 1979). Differences in search behaviour and capture techniques, reinforced by learning and habituation, can lead to individual variations in food specialization (Bryan and Larkin, 1972) and therefore variations in energy acquisition and growth allocation. This in turn may lead to disparity in individual success which may also influence Darwinian fitness. The effect will be magnified among those species that inhabit variable environments in which adaptive flexibility is even more vital (Dill, 1983). In fact, among fishes, there seems to be little relationship between learning ability and taxonomy (Hughes *et al.*, 1992); instead, it seems correlated more with environment (Girvan & Braithwaite, 1998; Odling-Smee & Braithwaite, 2003a, b). Predation may also be important (Brydges *et al.*, 2008) but will depend on the species of fish.

One may assume that benthic elasmobranchs occupying variable habitat (Compagno, 1984), and with few natural predators, will possess well developed learning and habituation capabilities to cope with unpredictable prey availability, distribution and diversity. Despite this, following a brief surge involving visual conditioning nearly half a century ago (initiated by Clarke, 1959, 1961), this topic has since received little attention with respect to sharks, skates and rays. Chapter 5 documents the first demonstration of learning and habituation in an elasmobranch's electroreceptive foraging behaviour. *S. canicula* were shown to improve their foraging success and minimise wasted effort by learning and habituation respectively when repeatedly presented with E fields differing in their profitability (in terms of food gain). Furthermore, they did so within a reasonably short time frame; usually within as few as 3 to 5 encounters.

Owing to these E fields' similarity to prey bioelectric fields, it seems, therefore, that these elasmobranchs might be able to effectively and rapidly adapt their foraging efficiency by reducing responses to less rewarding prey and increasing their responses and speed of response to more rewarding prey. When one considers these elasmobranchs may also be able to recognise different prey (Chapter 4) the benefits of such processes would be even further enhanced. The catsharks also showed an aptitude for being trained to swim through a narrow tunnel in order to be fed (Chapter 4). Whilst considerable variation was apparent (some individuals were slow to learn the task, a few proved completely unwilling or unable to do so), most individuals eventually learned to negotiate the tunnel. Indeed, some were extremely quick to learn the task, swam through the tunnel in all trials and occasionally even stretched the observer's reaction times by exiting the tunnel and responding to an E field a few seconds after the scent had been introduced into the arena. Combined with the previously mentioned visual conditioning studies, the work from these studies presents a compelling case for the existence of well developed adaptive flexibility across elasmobranch sensory behaviour. However, certain associations are likely to be learned more readily than others (*sensu* Garcia & Koelling, 1966; Goldsmith *et al.*, 1981; Cole *et al.*, 1982) which will depend on relative development of brain areas (Demski & Northcutt, 1996) which will, in turn, have evolved as a consequence of ecology. For example learned foraging is likely to be influenced more by electroreception in benthic elasmobranchs but more by vision or olfaction in pelagic species.

Despite well developed capacities to learn and habituate, *S. canicula* failed to remember (or at least ignored) foraging adaptations after 3 weeks. Precisely how long the memory window is remains unknown but from this research it can be assumed to be between 12 to 24 hours (behaviour was obviously remembered between treatments each day) and 3 weeks (the experimental interval length). Without a more specific measurement of the window it is difficult to discuss the ecological significance of *S. canicula*'s memory. However, one can suggest that environment may play an important role considering the substantial observations of significant inter-specific and inter-population differences correlated with ecology among teleost fishes (Warburton, 2003). For example, paradise fish remember conspecifics for only one week, but inter-specifics for at least three months (Miklosi, 1992) thought to be due to breeding territories being reorganised every week, but predators constantly being important throughout life history. Also, freshwater, anadromous and marine sticklebacks possess foraging memory windows of 25, 10 and 8 days respectively as a result of the stability of their particular habitats (Mackney & Hughes, 1995).

In a variable habitat it would make sense to forget learned behaviour considering conditions are susceptible to significant and frequent change. As such, over larger temporal and spatial scales, previous experience is rendered less important. This promotes the investigation of as many stimuli as possible to track current status which will minimise missed opportunities that may, otherwise, have arisen by focusing on fewer stimuli (Stephens & Krebs, 1986; Krebs & Inman, 1992; Dall *et al.*., 1999). In a more stable environment (such as lakes or the abyssal oceanic zone), an animal would probably benefit most from remembering learned foraging behaviour as conditions would remain similar temporally and spatially (*sensu* Girvan & Braithwaite, 1998; Odling-Smee & Braithwaite, 2003).

Again, memory has received very little attention among elasmobranchs. Clarke (1959) demonstrated lemon sharks retaining learned visual conditioning for a period of ten weeks. Although considered a coastal species, lemon sharks have been observed migrating across Open Ocean (Froese & Pauly, 2008) and have proven homing ability (Edrem & Gruber, 2005) and may possess a reasonably longer memory window than would normally be expected**.** Significant spatial memories have been suggested in the Port Jackson shark, *Heterodontus portusjacksoni,* (O’Gower, 1995) and freshwater stingray, *Potamotrgon motoro*, (Schluessel & Bleckmann, 2005) but with no window lengths reported.

The findings from Chapter 5 support the notion that elasmobranchs are well adapted to their heterogeneous environment. At small scales variation in food distribution encourages rapid learning but over large scales habitat variability necessitates rapid forgetting (Hughes *et al.*, 1992).

6.4. Application of study

Whilst there is a significant biological focus to this research, the findings have a wider relevance to the coastal environment that many elasmobranchs inhabit and have potential important coastal management applications.

6.4.1. Sexual segregation and fisheries

Many elasmobranch species are currently undergoing substantial population declines due to a combination of over exploitation by humans and the slow life history traits they exhibit (Vas, 1994; Baum *et al.*, 2003; Dulvy *et al.*, 2008). When one also considers the importance of these top predators in oceanic food webs (Stevens *et al.*, 2000, Schindler *et al.*, 2002) and the fragility of ecosystems to reduced species' abundances and extinctions (Chaplin *et al.*, 2000) it becomes clear that elasmobranch fisheries management and conservation demand immediate attention (Helfman, 2007; Dulvy *et al.*, 2008). The importance of animal behaviour to the development of conservation strategies has received increased recognition in recent years (Rubin & Bleich, 2005). It is now accepted that animal behaviour plays a large role in ecological processes and therefore has great implications for conservation (Gosling & Sutherland, 2000).

Whilst *S. canicula* is not currently considered at risk (Compagno *et al.*, 2005) the reason it is utilised so frequently in research is that it represents a useful model for other, more vulnerable elasmobranch species with similar life history and ecology (Sims *et al.*, 2001). There is also the possibility that, with decreasing stocks of familiar fishes such as cod, plaice and skate (Cook, 1997; Dulvy & Reynolds, 2002; Helfman, 2007; van Keeken *et al.*, 2007), landings of *S. canicula* (commonly referred to as rock salmon or rock, along with *Squalus acanthias* and *Mustelus mustelus*) may increase and consequently apply enhanced pressure on their populations.

Sexual segregation can have significant repercussions on conservation (Rubin & Bleich, 2005) as temporal and spatial groupings of males and females have implications for habitat management, population monitoring, research and management. A good example of the concept comes from the protection of bighorn sheep (*Ovis canadensis*) solely on upper slopes being deemed ineffective owing to the tendency of males to occupy the lower slopes (Bleich *et al.*, 1997). But this segregation only occurred during a certain period of the year (December to July, Festa-Bianchet, 1988; Bleich *et al.*, 1997) and thus most effective and least costly protection measures would result from considering both spatial and temporal factors. Segregation was found to be caused by differing reproductive strategies; males increased fitness by inhabiting lower, gentler slopes offering superior nutrition, though increased predator risk, whereas females (and their offspring) inhabited higher, steeper slopes in order to decrease predation risk, whilst incurring poorer nutrition (Bleich *et al.*, 1997).

It is not currently known whether spatially focused fisheries exacerbate population declines by differential exploitation of a particular sex compared to the other within a species. However, anecdotal evidence suggests human exploitation of sexually segregated sharks may lead to dramatic population declines (Wearmouth & Sims, 2008). The aggregating behaviour of mature, female piked dogfish (*Squalus acanthias*, called spurdog locally) into unisexual schools, and their dominance in landings in southwest England during the early 20th century (Wearmouth & Sims, 2008) were probably major factors causing the collapse of the English Channel spurdog fishery between 1928 and 1931 (2710.3 tons to 802.4 tons; Steven, 1933). Similarly, an apparent local basking shark (*Cetorhinus maximus*) population collapse was thought to be partly due to large female-biased catches in the harpoon fisheries off west Ireland and northwest Scotland (McNally, 1976; Anderson, 1990).

Determining sex-based differences in behavioural strategies of wild fishes is important to marine fisheries management as any differences will provide information on spatio-temporal dynamics of male-female distribution and abundance, and thus their availability and catch rates (Sims *et al.*, 2001, 2005). Should *S. canicula* (or any species exhibiting similar segregation; for example the Port Jackson shark, *H. portuskacksoni*, Mclaughlin & O'Gower, 1971) face population declines it would be prudent for fisheries managers to consider their sex-specific behaviours and reasons for them. *S. canicula* segregation has been suggested to be caused by male harassment and female refuging (Sims *et al.*, 2001, 2005) possibly driven partly by decreased female foraging efficiency (Chapter 3). Males may prove more susceptible to fishing pressure considering they inhabit open water throughout the day and night rather than seeking refuge in rocky caves (Sims *et al.*, 2001, 2005). Equally, it is important to understand whether one sex is more vulnerable than the other due to foraging restricted to differing depths (males, shallower; females, deeper, Sims *et al.*, 2001, 2005). Furthermore, one would assume it is especially crucial to prevent disruption to the briefer, more intense forays undertaken by females (Chapter 3; Sims *et al.*, 2001, 2005). The research in Chapter 3 builds on current knowledge of sexual segregation among elasmobranchs by demonstrating the implications of male harassment, female refuging and associated intra-specific competitive release on foraging efficiency.

6.4.2. Possible interactions between fish & anthropogenic E fields

With the threat of rising carbon emissions and dwindling finite resources looming, deployment of offshore renewable energy developments (ORED) around the world, and in particular the United Kingdom's coast, is increasing (The Crown Estate, 2008). Wind farms are currently the most commonly implemented method, as other technologies (such as wave and tidal) are still being developed. Arrays of cables are used to transmit electricity between wind turbines and from turbine grids to the shore. These cables are usually buried at shallow depths beneath the substrate to avoid physical damage to them (by trawling for example), and are covered with protective shielding which prevents direct emission of electric fields. However, recent modelling (CMACS, 2003; Gill *et al.*, 2005) demonstrated that despite insulation, alternating current E fields are induced in the surrounding water owing to magnetic field leakage. The fields were estimated to range from approximately 0.9 µV/cm at cable surfaces to 0.1 µV/cm eight metres from cables. The strengths of these artificial fields are similar to many bioelectric fields produced by small marine animals (see Chapter 1) and are within the range detectable by and attractive to elasmobranchs (Kalmijn, 1971; Tricas & Sisneros, 2004). The presence of these fields has recently been confirmed (Gill, personal communication).

There are nearly two dozen species of sharks and a similar number of rajiformes that are either resident in or regular visitors to U.K. waters (Compagno, 1984; Vas, 1994; Compagno *et al.*, 2005). The majority of these are benthic or near benthic species, and many inhabit shallow sandy areas (Rogers *et al.*, 1998; Rogers & Ellis, 2000) which also happens to be the preferred substrate type for offshore wind farm installations. Unsurprisingly, there is therefore mounting concern over potential interactions between

ORED and elasmobranchs (Gill, 2005; Gill *et al.*, 2005; Sutherland *et al.*, 2008). Indeed, even as long ago as the late seventies, damage to sonar arrays was attributed to sharks via bite patterns (Johnson, 1978), and Kalmijn (1978) suggested such attacks most likely arose due to E field emission resembling those of natural prey.

Whilst increasing our knowledge of the behaviour of a model, benthic, predatory elasmobranch towards prey-type E fields, the findings from this study also allow improved understanding of how elasmobranchs might respond to anthropogenic sources of E field, such as those associated with ORED. The research detailed in Chapter 4 describes experiments in which elasmobranchs are offered a simultaneous choice between bioelectric and anthropogenic E fields. The results demonstrate that *S. canicula* are either unable to decipher between or show no preference for similar strength, natural and artificial direct current E fields. It would therefore appear that, for whichever reason, wild elasmobranchs are likely to respond equally to natural and artificial E fields upon first encounter. In turn, this raises the question of whether these predators might effectively waste time and energy “hunting” E fields associated with ORED whilst searching for bioelectric fields associated with their prey. Over time, such a consequence would ultimately increase effort expenditure whilst decreasing energy gain. Subsequent reductions in somatic and gonadal growth, and therefore survival and populations may potentially occur. Figure 30 explicitly illustrates how physical aspects and environmental effects of cabling associated with ORED (1 & 2) may affect individual elasmobranch life processes (3), which in turn may affect elasmobranch populations (4).

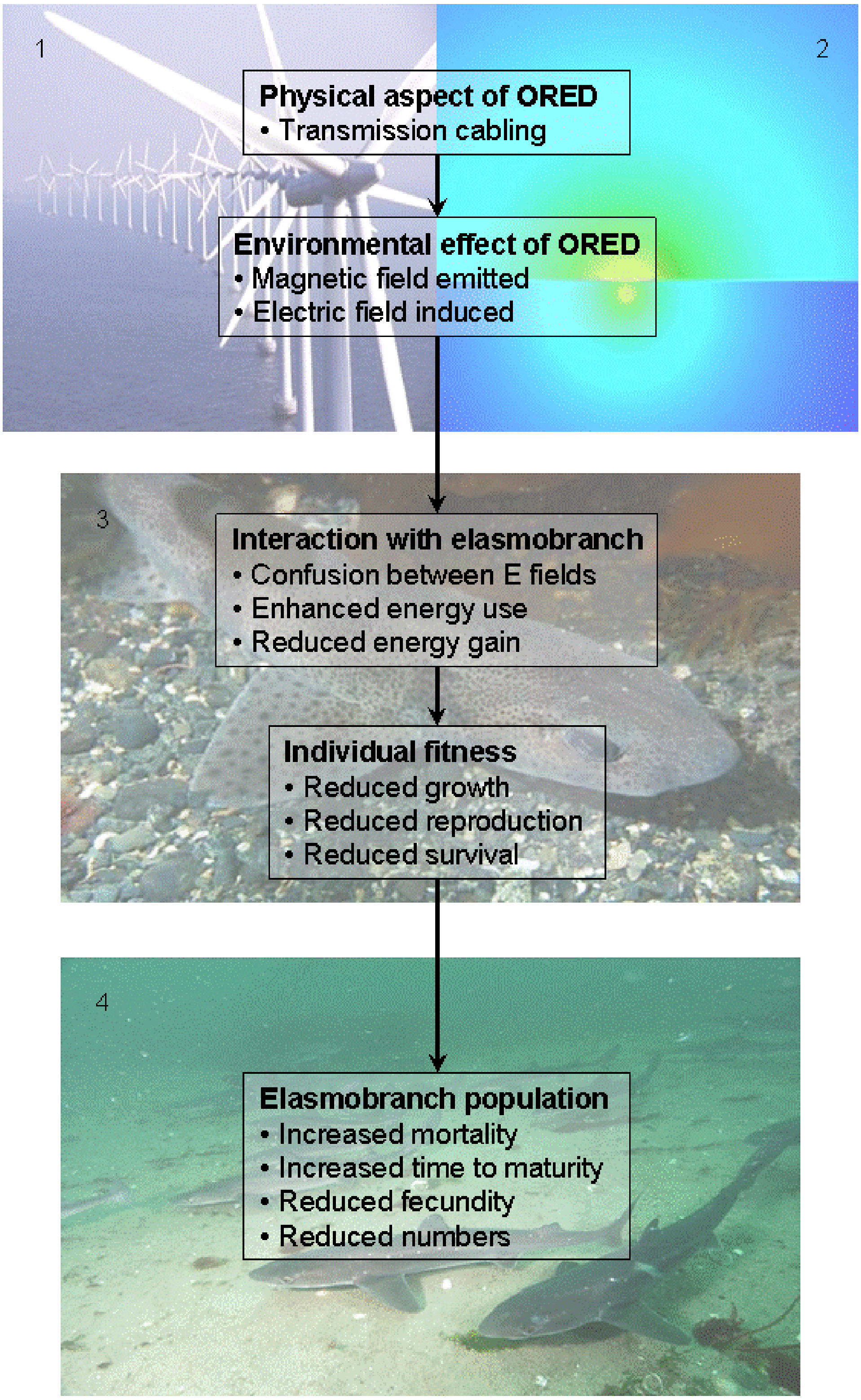


Figure 30. Flow diagram illustrating how ORED could potentially affect elasmobranchs through confusion of E fields associated with ORED with prey bioelectric fields (adapted from Fox *et al.*, 2006). (1 = wind farm photograph, Vestas Wind Systems A/S, from www.owen.eru.rl.ac.uk/; 2 = cross-sectional modelling of induced E field surrounding buried cable, adapted from CMACS, 2003; 3 & 4 = individual *S. canicula* and school of *Squalus acanthias* photographs, © Andy Murch, from www.elasmodiver.com)

However, it is important to consider if elasmobranchs may be able to adapt their electroreceptive behaviour when repeatedly encountering an E field, dependent on the field's apparent profitability; i.e. whether food is gained from attacking an E field or not. Chapter 5 details an investigation into whether elasmobranchs possess adaptive flexibility, via cognition, with respect to electroreceptive foraging behaviour. *S. canicula* rewarded for responding to E fields exhibited more intense foraging behaviour towards E fields than those that were unrewarded. Furthermore, rewarded catsharks rapidly improved their foraging efficiency towards E fields, whereas unrewarded catsharks' interest in E fields swiftly decreased. These findings suggest that, in the short term (within 3 to 5 encounters) elasmobranchs would be capable of habituating and thereby avoiding E fields associated with ORED and learning to focus more efficiently upon actual prey bioelectric fields. In doing so they would be effectively maximising net energy gain. This scenario would depend on their capacity to discriminate the E fields present. If the equal number of responses to artificial and natural fields observed during choice experiments (Chapter 4) arose because catsharks were able to distinguish the fields, but showed no preference for either, learning and habituation would be possible. However, if the catsharks were actually incapable of discriminating between the fields, they may not be able to identify which fields related to previously rewarding and unrewarding encounters without responding to both. Nevertheless, use of other senses such as vision, olfaction and the mechano-sense, in addition to electroreception, would most probably permit discrimination in the latter scenario and thus adaptive flexibility would still be possible.

The findings from Chapter 5 also demonstrate that *S. canicula* possess a reasonably short memory window with regard to foraging behaviour of between 12 hours and 3 weeks. Learned and habituated behaviours towards E fields were not continued after a three week experimental interval and behaviour levels were independent of whether rewards were offered before or after the experimental interval. These results suggest that elasmobranchs' adaptations in electroreceptive responses towards ORED E fields and prey bioelectric fields will likely be forgotten within a few days or weeks. Hence, whilst the catsharks may be able to detect and avoid anthropogenic E fields and focus foraging on prey over short time and small spatial scales (e.g. within hours or days; within patches and between nearby patches), over long time scales and large spatial scales (e.g. after weeks or months; between distant patches) they are likely to respond to both types of E field equally. A trade-off would ensue, in which the catsharks would effectively be reducing possible missed opportunities but would also be expending time and energy upon non-rewarding foraging. The balance between these two scenarios would ultimately dictate long term effects on individual success and Darwinian fitness.

Renewable energy, including wind, is crucial to global efforts to minimise the use of fossil fuels and release of carbon dioxide. Therefore widespread ORED development should, and will, continue. Indeed, the installation of wind turbines to the coastal, marine environment is likely to have positive, as well as negative, environmental impacts (Gill, 2005). For example, elasmobranchs are likely to benefit from improved prey availability and choice via increased biota abundance and diversity. These increases may occur due to a) enhanced habitat heterogeneity arising from turbine shafts, bases and scour protection and their subsequent colonisation, and b) locally reduced fishing effort around wind farms allowing communities to flourish (Gill &

Kimber, 2005; Figure 31). Furthermore, scour protection may offer ideal refuges for segregating, female *S. canicula*.

However, there is a requirement to consider all ecological consequences of a development; not least a human responsibility, but also legal requirements including Environmental Impact Assessments (a European Union directive) and the Food and Environmental Protection Act (U.K. Government licensing and monitoring requirements). There is also an opportunity for cooperative management to attempt to address issues relating to both the environment and the industries in question (Gill & Kimber, 2005). Precautionary measures, during early stages of development, are far more effective than reactionary measures, once full scale implementation is complete. Despite increasing concerns over possible interactions between electrically sensitive fish and ORED (Sutherland *et al.*, 2008), Environmental Impact Assessments, designed to identify possible effects of developments on the surrounding area based on available information, have largely overlooked possible interactions between elasmobranchs and ORED (Gill, 2005). Many have focused instead upon effects on birds, sea mammals and the value of ocean front property.

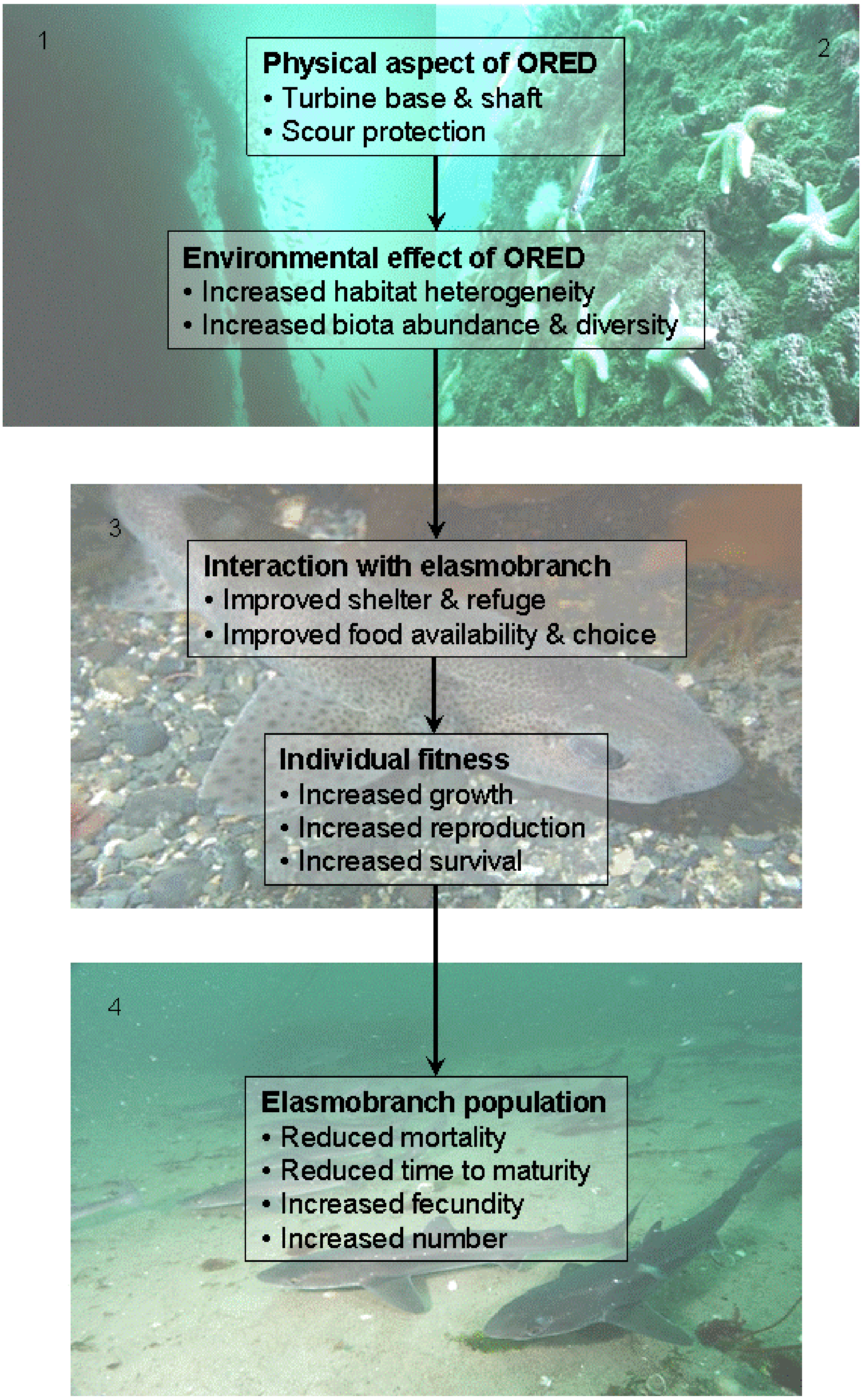


Figure 31. Flow diagram illustrating how ORED could potentially affect elasmobranchs through increased prey abundance and diversity (adapted from Fox *et al.*, 2006). (1 & 2 = colonised turbine base photographs, © MarineSeen & CMACS, courtesy of NPower Renewables; 3 & 4 = individual *S. canicula* and school of *Squalus acanthias* photographs, © Andy Murch, from www.elasmodiver.com)

The previously discussed findings from this study offer valuable, novel information with regards to how one group of electrically sensitive fish, the elasmobranchs, may interact with ORED (Figure 32). The findings from Chapters 4 and 5 demonstrate how elasmobranchs interact with and chose between artificial and prey E fields (Electroreceptive behaviour box; Figure 32). Considering these behavioural data alongside information concerning ORED cabling from the industry and modelling and measurements of induced E fields from technical reports (Specification box; Figure 32), provides better understanding of how elasmobranchs may interact with ORED over short and long time scales (Interaction oval; Figure 32). The information should contribute to crucial baseline knowledge required by scientists, fisheries managers, policy makers, conservationists and coastal developers to proceed with cooperative coastal zone management (Cooperative initiatives oval; Figure 32). A good example of this concept is illustrated by an incident in the mid eighties during which shark bites were found to be causing costly power shortages along an AT&T, transatlantic, fibre-optic cable (Marra, 1989; Chapter 1). Following an in depth investigation of the shark species involved, it was concluded that, rather than raising the entire length of the cable and improving protective shielding at great cost, only certain parts of the cable needed to be protected owing to the habitat depths occupied by the sharks causing the problem.

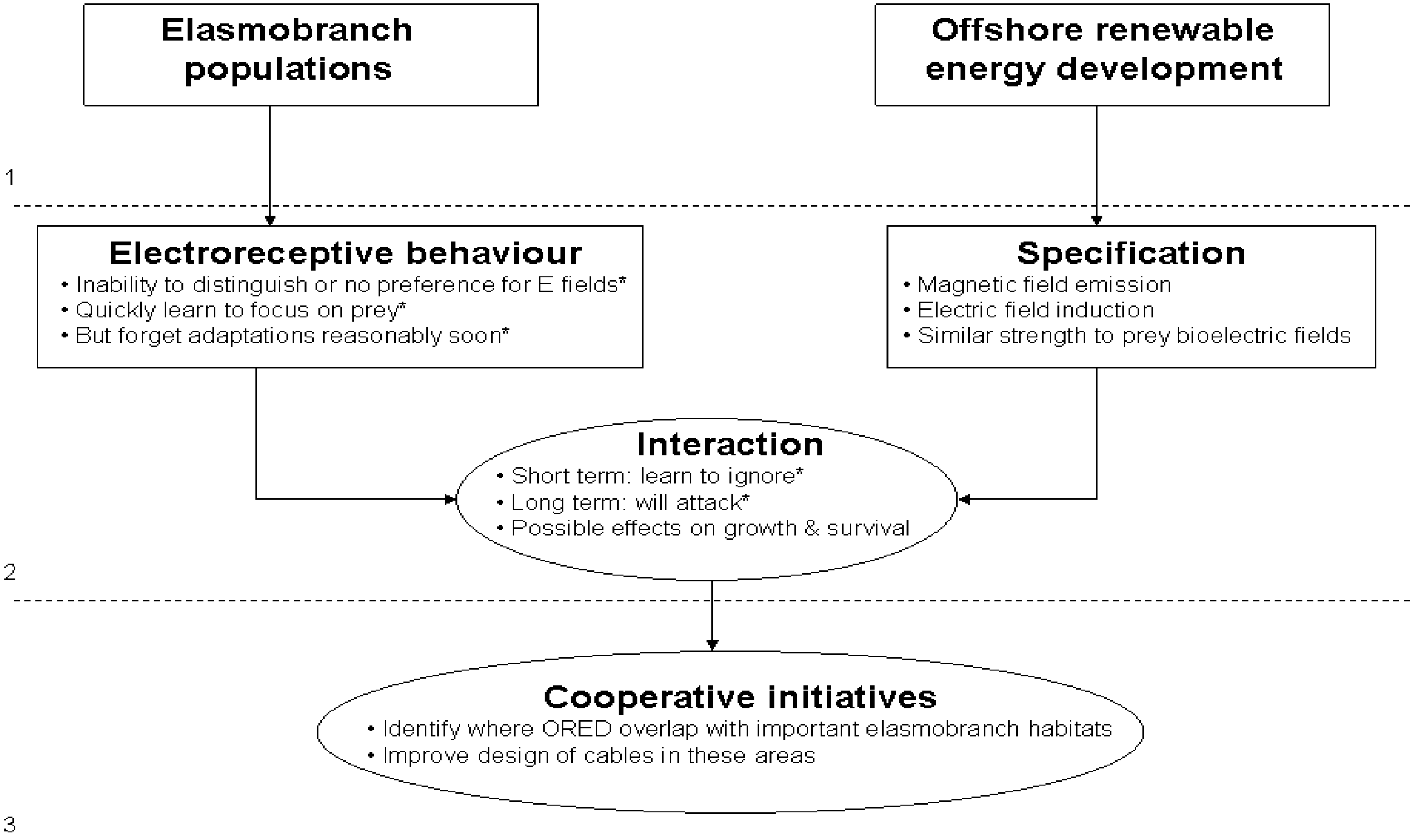


Figure 32. Diagram illustrating the potential to combine knowledge concerning elasmobranch populations and ORED (1) gained from elasmobranch behaviour research (including findings from this study) and ORED specification (level 2) to enable cooperative management of mutual benefit (level 3) (adapted from Gill & Kimber, 2005; asterisks mark where information was gained from present study).

6.5. Future work

The work undertaken during this study significantly increases knowledge of elasmobranch electroreceptive behaviours and their ecological implications. As clear from the previous section (6.4.), it also contains valuable information that may be of use to fisheries and ORED managers. However, as previously highlighted in the thesis, the behaviour and ecology of sharks, skates and rays (especially those associated with the electric sense) are areas of marine biology that have received surprisingly little attention considering the importance and appeal of these top predators. Whilst this study (and similar recent investigations, e.g. Filer *et al.*, 2008) is a step in the right direction, considerable questions still remain concerning electroreceptive behaviour, some of which are raised when considering the research in this thesis:

6.5.1. General

- There is an urgent requirement to more accurately measure electric fields associated with different species (prey, predators, symbionts and conspecifics) with consistent methods (see Chapters 1 & 4). The same applies to those E fields associated with anthropogenic devices and developments (see Chapters 1, 4 & 5). Whilst difficult to achieve, this would be extremely worthwhile as it would permit better understanding of E fields in the marine environment and how electrically sensitive organisms may interact with their electrical environment.
- It is important to determine whether findings from this study are species specific or characteristic of a wider range of species. Thus, electroreceptive foraging behaviour should be examined across a wider range of elasmobranch species to enable comparison between alternative taxa and their respective ecologies. For example, contrary to the findings relating to *S. canicula* in Chapter 3, foraging behaviour may

be unaffected by the presence of the opposite sex among species in which sexual conflict and segregation do not occur. Similarly, whilst male and female *S. canicula* showed similar sensitivities to different E fields (Chapters 3 & 4), in species that locate conspecifics by detecting their bioelectric fields or that have different diets the sexes may exhibit differing sensitivities to different E fields. Furthermore, investigation of a broader range of taxa would enable the questions of whether benthic species are likely to possess more developed and acute electro-senses than pelagic species (Chapter 4), and whether learning rates and memory windows vary according to environmental heterogeneity (Chapter 5) to be addressed.

6.5.2. Male-female interactions

- It would be interesting to perform experiments similar to those documented in Chapter 3, but with some habitat heterogeneity offering refuges added to arenas. This would determine whether female activity levels and responses to E fields decrease even further when in the presence of males as a result of being able to avoid them more effectively. If so, this would strengthen the evidence of sexual conflict affecting foraging behaviour and, in turn, being one of the causes of sexual segregation in *S. canicula*.
- Another fascinating question raised by this study arises from the link between Chapters 3 & 5; given that male harassment and subsequent female avoidance seem to cause increased female foraging intensity, possibly via competitive release (Chapter 3), one may also expect more rapid learning and habituation among females. Theoretically, this would permit even more successful foraging during their relatively brief forays from refuges (Sims *et al.*, 2001; Sims *et al.*, 2005).

6.5.3. Choice

The novel methodology used during this study to examine preferences for different E fields presented simultaneously (Chapter 4) could be utilised to:

- Determine whether the catsharks are, in actual fact, unable to distinguish between similar artificial and natural E fields or whether they simply show no preference. In doing so, the answer as to whether these elasmobranchs would be able to learn to reduce wasted effort by avoiding “hunting” artificial E fields would become more clear. This could be achieved by conditioning the catsharks; positively reinforcing responses to one E field (e.g. by food reward) and negatively reinforcing responses to the other E field (e.g. by shaking the electrodes).
- Determine whether the catsharks are able to differentiate between different natural prey and therefore more precisely identify the level of control the electro-sense permits on elasmobranch diet selection and therefore maximisation of energy intake. This could be achieved by placing different prey animals and sizes under each agar chamber (for example a crab and a small, teleost fish or a large and a small crab).

6.5.4. Cognitive ability

The novel method of rewarding the catsharks for responding to E fields (via the hydraulically operated trap door, Chapter 5) could be utilised further in order to:

- Discover whether learning rates are faster when more than one elasmobranch co-habits an experimental tank. Observation of conspecifics has been demonstrated to improve foraging improvement via learning in some species of teleosts (Pitcher & Magurran, 1983; Pitcher & House, 1987; Jain & Sahai, 1989; Ozbilgin & Glass, 2004).

- Precisely identify the length of memory window in *S. canicula* by repeating learning experiments after differing lengths of intervals (between 12 hours and 3 weeks) to determine how long altered foraging behaviour is retained. This would allow the memory of this species to be more accurately compared with other elasmobranch species, and even with teleost fishes, birds and mammals.

6.6. Conclusion

The experimental work, analyses and discussions detailed within this study significantly improve current understanding of elasmobranch electroreceptive foraging behaviour with regards to sexual conflict, choice and cognitive ability. Such information, when considered in conjunction with research covering other aspects of elasmobranch behaviour, physiology and ecology, will better prepare scientists, conservationists, fisheries managers and policy makers to confront elasmobranch management concerns by improving previously poor baseline knowledge.

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