



Contribution to the Chemoreception Capacity of Juvenile Loggerhead Sea Turtles (*Caretta caretta*, L.)

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Master Thesis

Funchal, 2010



REGIÃO AUTÓNOMA DA MADEIRA



REPÚBLICA PORTUGUESA



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FSE

I dedicate this thesis to my parents, Fernando and Ana Bela (Zitos), for the love, education and support in all the moments of my life!

ACKNOWLEDGMENTS

This study would not have been possible without the precious help of some persons who in many ways gave me support and strength to continue. To these people I really want to express my sincerest gratitude.

I would like to thank to my supervisor Prof. Thomas Dellinger for the ideas, the support, the long hours of *brainstorming* (sometimes it was like a real storm in my brain 😊) and for his sympathy.

To all of the people in the Marine Biology Station who helped me with this work and for their constant sympathy. I would like to emphasize the indispensable help of Mr. Loreto Andrade and Mr. Filipe França in the construction of the experimental tank.

I would also thank the people of Madeira University for their help and support, especially to Renato Barradas for the support, motivation and friendship. To Ramon Vilardell Corral that helped me with the DMS solutions and also for his friendship.

To the voluntary Nico that helped me with cleaning of the tanks and feeding of the turtles.

To the Center of Science and Technology of Madeira (CITMA) for the scholarship that allowed me to do this Master.

I am very grateful to all of my friends for the patience and support, especially to Sandra Ferreira and Ruben Agrela 😊

The last but not least, I would like to thank to my family for all of the support, especially to my brother Nuno, my sister-in-law Susana, to my grandfather Manuel and to my little nephew Joãozinho 😊

Thank you!

ABSTRACT

Loggerhead sea turtle juveniles (*Caretta caretta*), pelagic stage, are found in waters of Madeira archipelago. Pelagic turtles are in the main growth phase of their life cycle and consequently higher energy needs. However, knowledge about the ecology of pelagic loggerhead sea turtles is still quite rudimentary, mainly about the mechanisms that lead them to find food in the vast ocean.

Studies with other pelagic species, such as procellariiform birds, revealed that the olfactory system play an important role for the detection of feeding areas, through the detection of concentration peaks of DMS (dimethylsulfide), a scent compound that naturally exists in the marine environment and it is related to areas of high productivity. Based on the assumption that loggerhead sea turtles use a similar mechanism, behavioural experiments were conducted in order to analyze the chemoreception capacity to DMS (airborne chemoreception - theoretically responsible for the long distance detection of areas with food patches; and aquatic chemoreception - theoretically responsible for the short distance detection of preys). The first step was to observe if pelagic loggerheads demonstrate sensitivity to DMS and the second was to verify if they really use the DMS, in natural conditions, as an airborne cue to find areas where food patches might be available.

Four juveniles of loggerhead sea turtles were tested in captivity and three wild turtles in the open ocean. The results of airborne chemoreception experiments in captivity revealed that one turtle clearly demonstrated sensitivity to DMS and the sea experiments confirmed this result. However, the experiments were not conclusive on the question whether the pelagic turtles actually use the DMS as an airborne cue to detect long distance food patches. In aquatic chemoreception experiments was not observed sensitivity to DMS by the three sea turtles tested. In the classical conditioning experiment, where DMS and food were given nearly at the same time revealed that after a certain period of time, the sea turtle tested did not associated the DMS stimulus with a possible food reward.

The main cause of mortality of loggerhead sea turtles in Madeira waters is due to the accidental capture (*bycatch*) by deep pelagic longlines fishery which the target species is the black-scabbard (*Aphanopus carbo*) fish. Chub mackerel (*Scomber japonicus*) is one of the baits used in this fishery. Aquatic chemoreception experiments were conducted in order to evaluate the attractiveness of the chub mackerel for sea turtles. For the three sea

turtles tested, the results showed that in 90% of the cases the sea turtles were extremely attracted by the underwater smell of this fish.

Key-words: chemoreception, olfaction, dimethylsulfide, feeding ecology, sea turtles, *Caretta caretta* pelagic stage, *bycatch*, Madeira.

RESUMO

Os juvenis da tartaruga marinha comum (*Caretta caretta*), na fase pelágica, são encontrados nas águas do arquipélago da Madeira. As tartarugas pelágicas encontram-se na principal fase de crescimento do seu ciclo de vida e conseqüentemente de maiores necessidades energéticas. No entanto, os conhecimentos sobre a ecologia das tartarugas marinhas comuns pelágicas ainda são bastante rudimentares, nomeadamente o modo como elas encontram o alimento numa área tão vasta como o oceano.

Estudos efectuados com outras espécies pelágicas, nomeadamente com aves procelarifórmes, revelaram que o sistema olfactivo desempenha um importante papel na detecção de áreas alimentares, através da detecção de picos de DMS (dimetilsulfido), um composto aromático que existe naturalmente no ambiente marinho e que está relacionado com áreas de elevada produtividade. Partindo do pressuposto que as tartarugas marinhas comuns possuem e utilizam semelhante mecanismo, foram efectuadas experiências comportamentais de modo a avaliar a capacidade de quimiorrecepção do DMS (quimiorrecepção aérea - teoricamente responsável pela detecção a longa distância das áreas onde o alimento se encontra agregado; e quimiorrecepção aquática - teoricamente responsável pela detecção do alimento a curta distância). O objectivo era por um lado averiguar se as tartarugas marinhas comuns possuíam sensibilidade ao DMS e por outro tentar compreender se realmente utilizam esta substância em condições naturais para a detecção de áreas onde o alimento possa estar disponível.

Foram testadas 4 tartarugas em cativeiro e 3 tartarugas selvagens em mar aberto. Os resultados das experiências de quimiorrecepção aérea, em cativeiro, revelaram que uma das tartarugas possuía claramente sensibilidade ao DMS e as experiências de mar confirmaram este resultado. Contudo, as experiências não foram conclusivas relativamente à questão se as tartarugas pelágicas usam efectivamente o DMS como pista aérea olfactiva na detecção a longa distância das áreas onde o alimento se encontra. Nas experiências de quimiorrecepção aquática nenhuma tartaruga, de 3 testadas, revelou sensibilidade ao DMS dissolvido na água. A experiência de condicionamento clássico, em que o DMS e o alimento eram apresentados sensivelmente ao mesmo tempo revelaram que, após um certo período de tempo, a tartaruga não associava o estímulo de DMS com uma possível recompensa de alimento.

A principal causa de mortalidade das tartarugas marinhas comuns no arquipélago da Madeira é devida essencialmente à captura acidental (*bycatch*) pela pesca dirigida ao peixe-espada preto (*Aphanopus carbo*) em que é utilizado o palangre horizontal derivante. Um dos iscos utilizados nesta pesca é a cavala (*Scomber japonicus*). Foram efectuadas experiências de quimiorrecepção aquática de modo a avaliar a atractividade que a cavala poderia ter para as tartarugas marinhas. Os resultados de 3 tartarugas testadas revelaram que em 90% dos casos as tartarugas marinhas eram bastante atraídas pelo odor (dissolvido na água) deste peixe.

Palavras-chave: quimiorrecepção, olfacto, dimetilsulfido, ecologia alimentar, tartarugas marinhas, *Caretta caretta*, fase pelágica, captura acidental, Madeira.

INDEX

1. INTRODUCTION	1
1.1. Ecology of the Pelagic Stage of Loggerhead Sea Turtles	1
1.2. Orientation and Navigation in Sea Turtles	2
1.2.1. Chemoreception in Sea Turtles	2
1.2.1.1. Aquatic Chemoreception	2
1.2.1.2. Airborne Chemoreception	3
1.3. Dimethylsulfide – DMS (C ₂ H ₆ S) – the “smell of the sea”	4
1.3.1. DMS – an airborne olfactory cue used by pelagic species	5
1.3.1.1. Procellariiform Birds	5
1.3.1.2. Harbour seal (<i>Phoca vitulina vitulina</i>)	6
1.3.1.3. African Penguins (<i>Spheniscus demersus</i>)	7
1.4. Revision of the Sea Turtles Biology	8
1.4.1. Sea Turtles – Past and Present	8
1.4.2. Sea Turtles in the North Atlantic Ocean	11
1.4.3. Loggerhead Sea Turtle – <i>Caretta caretta</i>	11
1.4.3.1. Morphological Features	11
1.4.3.2. Distribution	12
1.4.3.3. Life Cycle	12
1.4.3.4. Diet	13
1.4.3.5. Current Status and Legal Protection	14
1.4.4. Main Threats	14
1.4.4.1. Actual Threats in Madeira waters	15
1.5. Objectives	16
2. MATERIAL AND METHODS	17
2.1. Sea Turtles used in the Captivity Experiments	17
2.2. Acclimation Period Before the Experiments	18
2.3. Diet in Captivity	18
2.4. Preliminary Experiments	19
2.4.1. Airborne Chemoreception	19

2.4.2. Aquatic Chemoreception	19
2.5. Experiments	20
2.5.1. Airborne Chemoreception of DMS	20
2.5.1.1. DMS solutions	21
2.5.1.2. Trial tank	21
2.5.1.3. Experimental Protocol	22
2.5.2. Aquatic Chemoreception of DMS and CMJ	23
2.5.2.1. Substances	23
2.5.2.2. Trial Tank	24
2.5.2.3. Experimental Protocol	24
2.5.3. Classical Conditioning Experiments	24
2.5.3.1. Substances	25
2.5.3.2. Trial Tank	25
2.5.3.3. Experimental Protocol	25
2.5.4. Sea Experiments with DMS	25
2.5.4.1. Modified Sea Buoy	25
2.5.4.2. Protocol	26
2.6. Statistical Analysis	26
3. RESULTS	28
3.1. Airborne Chemoreception of DMS	28
3.1.1. Testing Environmental Stimuli and Individual Preference	28
3.1.2. Testing if There was Reaction for the DMS stimuli	29
3.1.3. Behavioural responses vs. DMS concentration	30
3.2. Aquatic Chemoreception of DMS and CMJ	33
3.3. Classical Conditioning Experiments	34
3.4. Sea Experiments with DMS	34
3.4.1. Sea Experiment 1 (8 August 2009)	36
3.4.2. Sea Experiment 2 (11 September 2009)	36
3.4.3. Sea Experiment 3 (29 September 2009)	37
4. DISCUSSION	38
4.1. Airborne Chemoreception of DMS	38

4.2.	Aquatic Chemoreception	41
4.3.	Why do Juvenile Loggerhead Sea Turtles Occur Near Madeira?	45
5.	FUTURE DIRECTIONS	46
6.	BIBLIOGRAPHY	47
7.	ANNEX I – EXPERIMENTS DATA	54
8.	ANNEX II – EXAMPLE OF A DATA SHEET USED IN AIRBORNE CHEMORECEPTION EXPERIMENTS	55
9.	ANNEX III – BIRD SIGHTINGS IN SEA EXPERIMENTS	56

FIGURE INDEX

- Figure 1** – Juvenile Loggerhead Sea Turtle in waters of Madeira Archipelago. 1
- Figure 2** – Diagram of the experimental set-up (not to scale). The fan gently moved air through the PVC pipe, across the t-joint and opening of the cup, and into the arena where the turtle was tested. On the far side of the arena a small opening between the cover and the top of the arena permitted air to escape (Endres *et al.* 2009). 3
- Figure 3** – Dimethylsulfide (DMS) emissions increase when phytoplankton are grazed by zooplankton. Its precursor dimethylsulfoniopropionate (DMSP) is a metabolite of phytoplankton, and an excretion product of zooplankton and other predators (Dacey and Wakeham *in* Nevitt 2008). 4
- Figure 4** - A theoretical olfactory landscape in the atmosphere reflects biological activity over a seamount. This is an area of *upwelling* where primary productivity tends to be high. A seabird may be alerted to a potentially productive foraging area as it encounters a change in the olfactory landscape. Elements are not drawn to scale (Nevitt 2000). 5
- Figure 5** – Harbour seal (*Phoca vitulina vitulina*) - Tony Holm[©]/Azote[©]. 6
- Figure 6** – African penguin (*Spheniscus demersus*) - Etienne Marais[©]. 7
- Figure 7** – Holotype (IVPP V 15639) of *Odontochelys semitestacea* gen. et sp. nov. Skeleton in dorsal view (Li *et al.* 2008). 8
- Figure 8** – Holotype of *Santanachelys gaffneyi*, gen. et sp. nov. (THUg1386). The median length of the preserved carapace is 145 mm. Dorsal view (Hirayama 1998). 9
- Figure 9** – Sea Turtles Taxonomy. Sea Turtles Loggerhead sea turtle illustration (Márquez Millán 1990). 10
- Figure 10** – Loggerhead Sea Turtle (FAO 2009). 11
- Figure 11** - Loggerhead sea turtles (*Caretta caretta*) distribution (FAO 2009). 12
- Figure 12** – Map showing locations of sin nesting populations of loggerhead sea turtles: northeast Florida to North Carolina (NEFL-NC), south Florida, northwest Florida (NWFL), Mexico, Brazil and Greece. The two pelagic sampling sites (Azores and Madeira) are indicated with stars. Arrows show the North Atlantic gyre (Bolten *et al.* 1998). 13

Figure 13 – Loggerhead sea turtle entangled in plastic - Martijn de Jonge [©] .	15
Figure 14 – Loggerhead sea turtle as <i>bycatch</i> victim of longline fisheries (left); Black-Scabbard Fish at Mercado dos Lavradores (Funchal).	15
Figure 15 – Loggerhead sea turtle entangled in lost / abandoned fishing gear - TDellinger [©] .	16
Figure 16 – Madeira and Desertas islands – TDellinger [©] .	17
Figure 17 – Chub Mackerel (<i>Scomber japonicus</i>).	18
Figure 18 – Experimental tank.	19
Figure 19 – Localization of the trial tank (3 meters diameter) in the Marine Biology Station of Funchal (Google Earth [®] - 19/04/2010).	21
Figure 20 – Compartments numeration of the trial tank. Hypothetic positions of DMS evaporation bottle (blue spot) and Control evaporation bottle (orange). The colors are merely representative, the solutions were transparent.	22
Figure 21 – Plastic bottles used in the experiments.	23
Figure 22 – Modified Fishing Buoy.	26
Figure 23 – Cumulative time (observed and expected) spent by all of the sea turtles in each compartment of the tank.	28
Figure 24 – Cumulative time spent by each turtle in each compartment of the tank.	29
Figure 25 – Cumulative time spent by all of the sea turtles in each compartment of the tank.	30
Figure 26 - Cumulative time that each turtle spent in the different compartments of the tank.	30
Figure 27 – Cumulative time spent by all of the sea turtles in each compartment of the tank for the different DMS concentrations.	31
Figure 28 – Cumulative time spent by all of the sea turtles only in DMS compartment of the tank for the different DMS concentrations.	31
Figure 29 – Cumulative time spent by T#1735 only in DMS compartment of the tank for the different DMS concentrations.	32

Figure 30 – Cumulative time spent by T#1735 in DMS compartment of the tank in each experiment for the different DMS concentrations ($R^2 = 64,6\%$).	32
Figure 31 – Analysis of aquatic chemoreception by loggerhead sea turtle juveniles ($n = 3$). Two substances were used: DMS solution (67 mmol/L) and CMJ (“Chub Mackerel Juice”).	33
Figure 32 – Cory’s shearwater – TDellinger [©] .	34
Figure 33 - Bulwer’s petrel - Awei Lee [©]	34
Figure 34 – Common tern - Dave Provencher [©] .	35
Figure 35 - Yellow–legged gull – TDellinger [©] .	35
Figure 36 – Main types of hooks used by longliners (FAO 2009).	44

TABLE INDEX

Table I – Sea Turtles Data (SCL – Straight Carapace Length).	18
Table II – DMS Solutions.	21
Table III – Sea Turtles reaction to CMJ and DMS dissolved in water. “0” – Lack of reaction to the stimulus; “1” – Presence of reaction to the stimulus.	33
Table IV – Bird species sighted during the sea experiments.	35
Table V – Number of bird sightings in sea experiments of each species.	36

1. INTRODUCTION

1.1. Ecology of the Pelagic Stage of Loggerhead Sea Turtles

Loggerheads spend most of their lives at sea, and most investigations have focuses on the brief terrestrial portion of the life cycle – egg laying, incubation, and hatchling (Plotkin *et al.* 1993). In fact, a significant proportion of a loggerheads life is spent as a pelagic turtle (Figure 1). It is a critical life history period where most growth occurs and, therefore, with higher energy needs (Dellinger and Freitas 2000) and the areas around Azores and Madeira archipelagos might prove to be the most important developmental habitat for North Atlantic loggerheads (Dellinger and Encarnação 2000).



Figure 1 – Juvenile Loggerhead Sea Turtle in waters of Madeira Archipelago.

Knowledge about the ecology of sea turtles in pelagic stage is still quite rudimentary, mainly due to logistical difficulties. It is unknown how these animals find their food in the vast ocean.

Many pelagic animals locate food patches in the wide ocean through the olfactory sense, especially procellariiform birds, sphenisciformes and also pinnipeds. This study is based on the hypothesis that loggerhead sea turtles use similar sensory mechanisms to detect their food.

1.2. Orientation and Navigation in Sea Turtles

For logistical reasons, most studies on orientation mechanisms in sea turtles have focused on hatchlings rather than adults, because it is much easier to manipulate hatchlings instead of adults that often exceed one hundred kilograms (Lohmann *et al.* 1997). In fact, there is a lack of knowledge about the mechanisms of orientation and navigation that enable juvenile and adult sea turtles to navigate to specific targets, such as feeding areas, or to complete long, seasonal migrations (Arens and Lohmann 2004).

The vast stretches of oceans are almost featureless and migrating animals, such as sea turtles, probably have not only one but a multiple cue-based orientation system (Rozhok 2008). This system could include magnetic, hydrodynamic (wave direction and ocean currents), celestial or chemical cues (reviewed by Lohmann *et al.* 2008).

1.2.1. Chemoreception in Sea Turtles

In tetrapods, chemoreception is accomplished by olfaction, vomerolfaction (chemical detection using the vomeronasal organ), or gustation (Southwood *et al.* 2008).

Based on anatomical studies, loggerhead sea turtles present well developed olfactory and vomeronasal organs. The vomeronasal epithelium seems to play an important role in aquatic chemoreception and the olfactory epithelium is related to the airborne chemoreception (Saito *et al.* 2000).

Several studies were conducted in order to verify if sea turtles are able to detect chemical cues carried by water, air or both (Manton *et al.* 1972a; Manton *et al.* 1972b; Owens *et al.* 1986; Southwood *et al.* 2008; Endres *et al.* 2009).

1.2.1.1. Aquatic Chemoreception

A rhythmic “throat-pumping” behaviour was observed in loggerhead sea turtles, while underwater. Probably the turtles were passing water through the nostrils for olfaction (Walker 1959).

Using operant conditioning techniques, the ability of four immature green turtles (*Chelonia mydas*) to detect various chemical substances dissolved in water was studied. The sea turtles were able to detect underwater chemoreception of β -phenethylalcohol, iso-pentyl acetate, triethylamine and cinnamaldehyde at approximate concentrations of 5×10^{-6} M or 5×10^{-5} M, but not of L-serine or glycine at an approximate concentration of 10^{-4} M. It was tested a method of reversibly interrupting olfaction for a period of days by treating the olfactory

epithelium with a 0,35 M solution of ZnSO₄ (zinc sulfate). After this treatment, the sea turtles were unable to distinguish the chemical from the control, revealing that they were using olfaction and not taste for chemoreception. These results present evidence to support the theory that soluble compounds in the ocean from the vicinity of nesting sites might be detectable by green turtles, and that this could assist in navigation (Manton *et al.* 1972a).

1.2.1.2. Airborne Chemoreception

Recently, was proven for the first time that loggerhead sea turtles can detect airborne odours, supporting the theory that airborne chemical cues might be involved in navigation and/or foraging. In this experiment were tested 8 loggerhead sea turtles, raised in captivity, with curved carapace length (CCL) between 25,3 cm and 31,6 cm (approximately 1,5 years). The behaviour of the turtles about the food odour was tested. For this purpose was used an experimental set-up (Figure 2). Gel food was placed in a cup and the odour carried by a fan through the PVC pipe to the tank where the turtle were being tested. As a control a cup with distilled water was placed, instead of gel food. Experiments in which the cup contained the gel food has been detected an increase in turtles activity (measured by the number of transversals by the turtles in the tank during the trials) after they surfaced to breathe. In control experiments no significant differences were detected for an increase in activity. Although, the way in which this ability is used under natural conditions is unknown. A fascinating possibility is the possible presence of certain substances (airborne odours) that can function as a signal of feeding areas. One of these substances that might play an important role in detection of oceanic food patches is dimethylsulfide (DMS) (Endres *et al.* 2009).

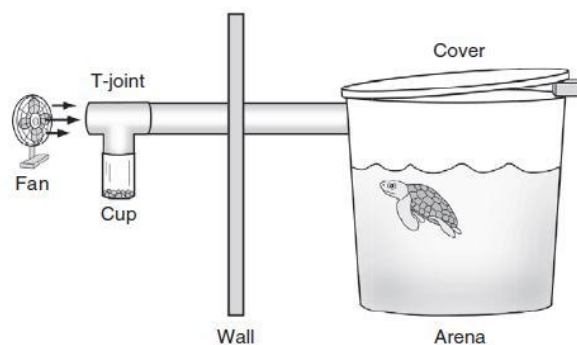


Figure 2 – Diagram of the experimental set-up (not to scale). The fan gently moved air through the PVC pipe, across the t-joint and opening of the cup, and into the arena where the turtle was tested. On the far side of the arena a small opening between the cover and the top of the arena permitted air to escape (Endres *et al.* 2009).

1.3. Dimethylsulfide – DMS (C_2H_6S) – the “smell of the sea”

Dimethylsulfide (DMS) and its precursor molecule, dimethylsulfoniopropionate (DMSP) dominate the natural sulfur cycle in the marine environment. (Scarratt *et al.* 2007) and has been studied in global climate change area (Beardall *et al.* 2009).

DMS is an odour produced by phytoplankton that is associated with areas of high primary productivity where prey are likely to be found (Nevitt *et al.* 1995) and because of its volatile nature it is lost from the surface waters by ventilation to the atmosphere (Scarratt *et al.* 2007).

Local emissions of marine DMS can be predictable features in the environment, reflecting bathymetric features such as shelf breaks and seamounts (Berresheim 1987). Furthermore, the rate of DMS emissions have been shown to greatly increase when the phytoplankton are subject to grazing by zooplankton (Dacey and Wakeham 1986) – Figure 3.

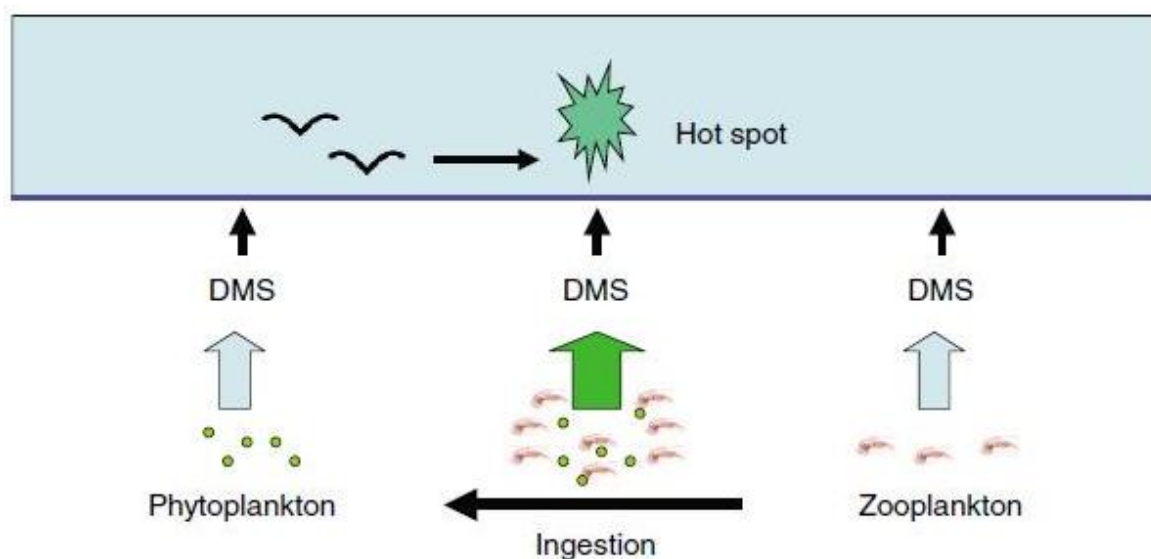


Figure 3 – Dimethylsulfide (DMS) emissions increase when phytoplankton are grazed by zooplankton. Its precursor dimethylsulfoniopropionate (DMSP) is a metabolite of phytoplankton, and an excretion product of zooplankton and other predators (Dacey and Wakeham *in* Nevitt 2008).

1.3.1. DMS - an airborne olfactory cue used by pelagic species

1.3.1.1. Procellariiform birds

The tube-nosed seabirds (order: Procellariiformes) are recognized for their wide ranging pelagic lifestyle. These birds spend most of their lives in flight over the ocean, and are tied to land for merely a few months each year or every other year to breed and rear a single offspring (Nevitt 2008).

Most procellariiforms forage over immense areas of the ocean for patchily distributed prey, including various species of fish, squid and krill. These birds use changes in the olfactory landscape to identify potentially productive foraging opportunities as they fly over them (Figure 4) (Nevitt *et al.* 1995). Such changes in the olfactory landscape reflect bathymetric features, which tend to accumulate phytoplankton and therefore prey. Thus, areas where the phytoplankton are abundant (for example *upwelling* zones, seamounts and shelf breaks) tend to aggregate seabirds and other marine predators for foraging (Nevitt *et al.* 1995; Nevitt and Bonadonna 2005).

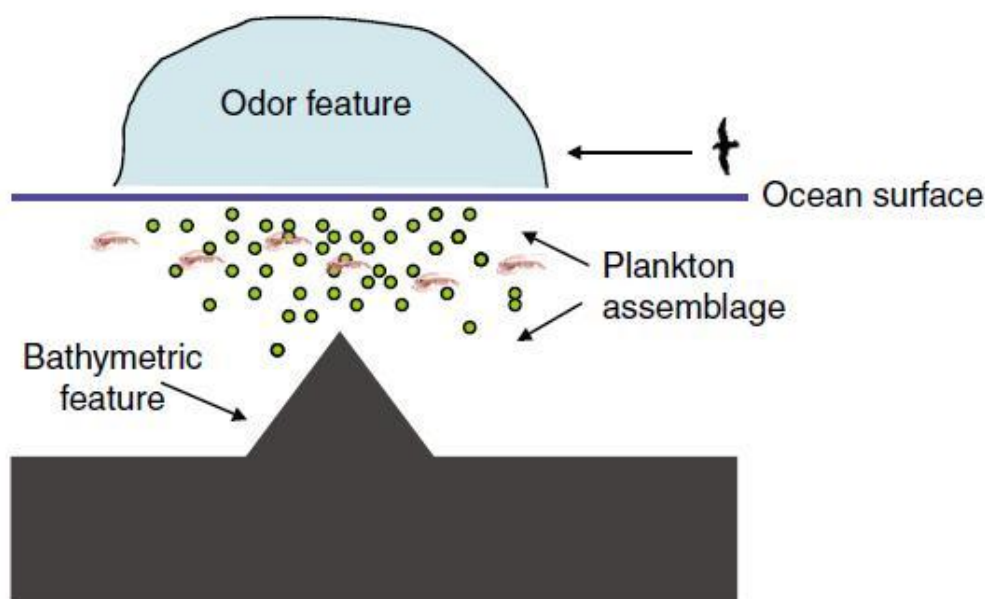


Figure 4 - A theoretical olfactory landscape in the atmosphere reflects biological activity over a seamount. This is an area of *upwelling* where primary productivity tends to be high. A seabird may be alerted to a potentially productive foraging area as it encounters a change in the olfactory landscape. Elements are not drawn to scale (Nevitt 2000).

Based in this assumptions was proposed that procellariiform birds build up a map of these features over time. The natural scent cues in the marine environment present guideposts

to assist seabirds in foraging and navigation (Nevitt 2000). DMS is one of these natural scents and had special attention. In order to test this hypothesis several studies were conducted with procellariiforms, adults and chicks, which confirmed that these birds tend to be attracted not to prey scents *per se*, but rather to odours such as DMS that are released during feeding interactions (Nevitt *et al.* 1995; Nevitt 1999a; Nevitt 1999b; Nevitt 2000; Cunningham *et al.* 2003; Nevitt and Haberman 2003; Bonadonna *et al.* 2006).

1.3.1.2. Harbour seal (*Phoca vitulina vitulina*)

Pinnipeds are known to travel on direct routes and return regularly to particular feeding areas. Nevertheless, the environmental information seals use to perform this navigation is still unknown. Two harbour seals (Figure 5) were tested in order to verify their ability to smell DMS at concentrations typical for the marine environment (Kowalewsky *et al.* 2006).



Figure 5 – Harbour seal (*Phoca vitulina vitulina*) - Tony Holm®/Azote®.

DMS stimuli from $8,05 \times 10^8$ to $8 \text{ pmol (DMS) m}^{-3}$ (air) were tested against a control stimulus using a custom-made olfactometer adapted to the experiments. The results of these experiments revealed that harbour seals have a high olfactory sensitivity for DMS, which may provide the sensory basis for the identification and orientation to more favorable feeding areas (Kowalewsky *et al.* 2006).

1.3.1.3. African Penguins (*Spheniscus demersus*)

The African penguins (Figure 6) are listed as a “vulnerable” species (Birdlife International, 2004), breeding only on the coast and coastal islands of Namibia and South Africa (Shannon and Crawford 1999).

Penguins are close relatives of procellariiform birds and similar to procellariiform adults, chicks have a tube-nose (Kinsky 1960). Therefore, it seems plausible that the penguins also have a functional olfactory sense (Cunningham *et al.* 2008).



Figure 6 – African penguin (*Spheniscus demersus*) - Etienne Marais[©].

Two separate experiments were carried out in order to investigate whether adult African penguins (*Spheniscus demersus*) could respond to artificial sources of DMS. For that wild penguins on Robben Island were tested, South Africa, by deploying μ molar DMS solutions in the colonies. The results indicated that the penguins slowed down their walking speeds. Captive penguins were tested in a Y-maze. In both cases, the data show credibly that the African Penguins have a functional olfactory sense and were attracted by the DMS. The inference of this study is that the detection of changes in the odour landscape (DMS) may aid penguins in the identification of productive areas for foraging in the ocean. This was the first study that clearly demonstrated, through experimentation, that penguins can detect an odour. However, this study did not test directly the DMS as a foraging cue. More investigation is needed related to sea experiments, in order to demonstrate definitively that penguins, like procellariiforms, use odours to forage (Cunningham *et al.* 2008).

1.4. Revision of the Sea Turtles Biology

1.4.1. Sea Turtles - Past and Present

The oldest fossil of a prehistoric turtle (*Odontochelys semitestacea* sp. nov) was found in China and lived about 220 million years ago (Figure 7). Phylogenetic analysis places the new species basal to all known turtles, fossil and extant. The marine deposits that yield the fossils indicate that this primitive turtle inhabited marginal areas of the sea or river deltas (Li *et al.* 2008).

The second oldest turtle fossil is *Proganochelys* found in Germany (Gaffney 1990) from the Late Triassic period.



Figure 7 – Holotype (IVPP V 15639) of *Odontochelys semitestacea* gen. et sp. nov. Skeleton in dorsal view (Li *et al.* 2008).

However, the oldest known sea turtle fossil (*Santanachelys gaffneyi*, gen. et sp. nov.) is much more recent, from the Early Cretaceous stage about 110 million years ago and it was found in eastern Brazil (Figure 8). This specimen represents a new taxon, extending the history of chelonoids by 10 million years, and it shed new light on the early evolution of the group. This fossil has some features that support the occupation of a marine habitat such as the paddle-like limbs and the existence of large interorbital foramina that indicate the presence of lachrymal salt glands surrounding the eyes (Hirayama 1998).



Figure 8 – Holotype of *Santanachelys gaffneyi*, gen. et sp. nov. (THUg1386). The median length of the preserved carapace is 145 mm. Dorsal view (Hirayama 1998).

Actually there are only two families of sea turtles (Cheloniidae and Dermochelyidae) and these are divided in a total of seven species (Figure 9). The family Cheloniidae is characterized by an extensively roofed skull with well-developed rhamphothecae; secondary palate present, incompletely retractile or nonretractile head. The extremities are in the form of nonretractile flippers covered with numerous small scales and the forelimbs having highly elongate digits firmly bound together by connective tissue. The claws being reduced to one or two on each limb, and the radius and ulna immobilized against independent movement by juxtaposed rugose surfaces. The shell is covered with horny scutes, variable in number, but commonly including five vertebrals and six pairs of plastral scutes, together with an unbroken series of three or four pairs of inframarginals, multiple axillary scutes, and usually both an interregular and an interanal scute. The plastron often with persistent fontanelles, one in the middle and others in the entoplastral and xiphiplastral regions, hyoplastra and hypoplastra not suturally connected mesially, but each with a series of coarse spikes that interdigitate mesially. The plastron not cruciform, and posterior plastral lobe relatively long and wide (Lutz and Musick 1997).

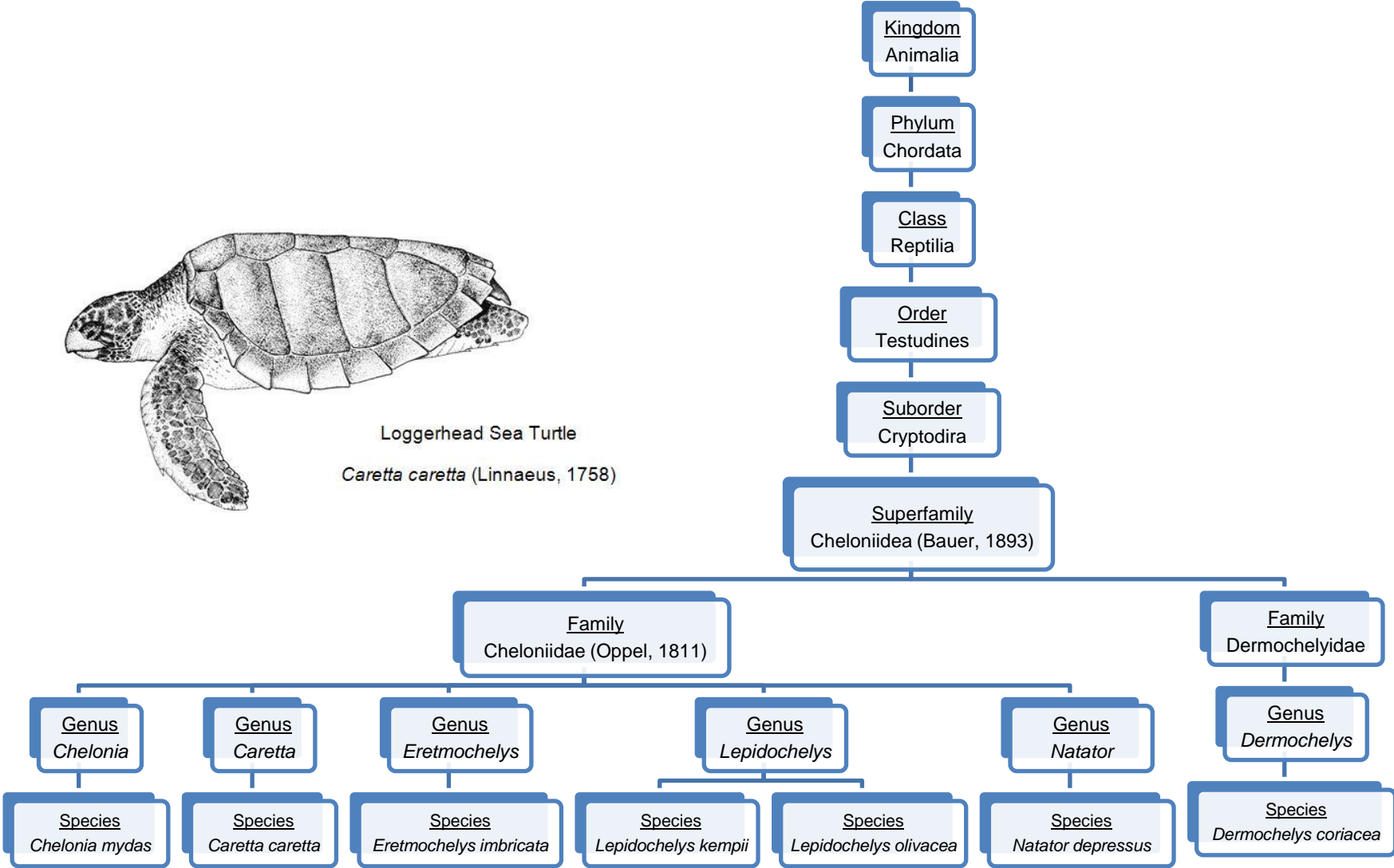


Figure 9 – Sea Turtles Taxonomy. Loggerhead sea turtle illustration (Márquez Millán 1990).

1.4.2. Sea Turtles in the North Atlantic Ocean

In North Atlantic Ocean occurred six of the seven species of sea turtles in the world (Brongersma 1982; Márquez Millán 1990; Dellinger 2008). In Portugal there are registers of five of them (Brongersma 1968; Oliveira *et al.* 2005; Dellinger 2008) but the most common species are loggerhead sea turtle (*Caretta caretta*) followed by leatherback sea turtle (*Dermochelys coriacea*) (Oliveira *et al.* 2005; Dellinger 2008).

1.4.3. Loggerhead Sea Turtle – *Caretta caretta*

1.4.3.1. Morphological Features

In loggerhead adults, the carapace in dorsal view is heart-shaped and its width corresponds to approximately 76 to 86% of its length (Márquez Millán 1990) (Figure 10).

The size of the sea turtles is commonly indicated as the straight carapace length (SCL - usually measured from the nuchal notch to the tip of the posterior marginal). In general, the loggerhead rookeries with the largest individuals are found in the Atlantic Ocean. The sizes of sea turtles are thought to be affected by genetics, energy consumption, food quality and abundance, and age (Kamezaki 2003). In general, the mean SCL of the mature females is between 81,5 and 105,3 cm (n = 3502), with a mean weight near to 75 Kg (65,7 to 101,4 Kg, n = 153). The carapace length (SCL) in nesting females varies within a limited size range, but always over 70 cm (Márquez Millán 1990).

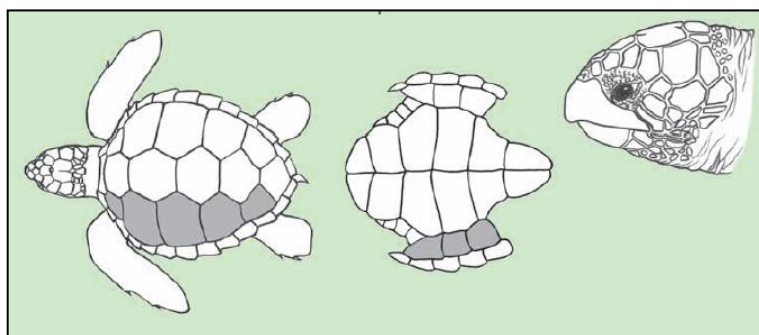


Figure 10 – Loggerhead Sea Turtle (FAO 2009).

The head is large, broad and subtriangular corresponding to 23 to 28% of carapace length and that is the reason for the name of this sea turtle, with 2 pairs of prefrontal scales, and commonly one inter-prefrontal. This species have a horny beak very strong, although is thicker comparative to the other sea turtles. The carapace include 5 pairs of lateral scutes with the anterior touching the precentral scute, 5 central scutes (also called neurals) and

commonly 12 or 13 pairs of marginals, including the postcentral or pygal scute. Underneath the bridge of the plastron, there are 3 pairs of inframarginal scutes which rarely have pores. The fore flippers are relatively short and thick, each one, with 2 visible claws on the anterior margin. The rear flippers have 2 or 3 claws. Hatchlings and juvenile loggerhead sea turtles have blunt spines on the carapace scutes, forming 3 longitudinal keels that disappear during the juvenile stage (Márquez Millán 1990).

Loggerhead adults generally have a constant dorsal pattern, easily recognizable by the reddishbrown coloration, sometimes with dark streaks (South African turtles), that become orange-creamy on the flanks and yellow-creamy underneath. The hatchlings are darkbrown dorsally, with the flippers pale brown marginally and underneath, and the plastron usually is much paler (FAO 2009).

1.4.3.2. Distribution

The loggerhead sea turtle is widely distributed in coastal tropical and subtropical waters (16 - 20°C) around the world (Figure 11). Commonly this species wanders into temperate waters and to the boundaries of warm currents (Márquez Millán 1990).

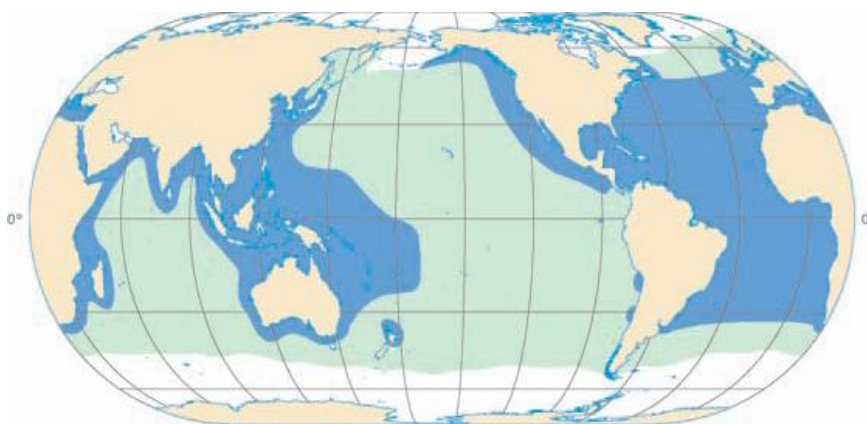


Figure 11 - Loggerhead sea turtles (*Caretta caretta*) distribution (FAO 2009).

1.4.3.3. Life Cycle

The live of the sea turtles consists of a series long-distance migrations and involve some of the most extraordinary mechanisms of orientation and navigation in the animal kingdom (Lohmann and Lohmann 1996). Prince Albert the First of Monaco (1898), Brongersma (1972) and Carr (1986) suggested that posthatchlings are transported by the North Atlantic system around the Atlantic ocean, and that the small loggerheads reported to occur in large numbers in the eastern Atlantic around the Azores and Madeira (Brongersma 1972) are derived from the nesting beaches in the western Atlantic.

An analysis of mitochondrial (mt) DNA sequence comparing mtDNA haplotypes of pelagic juvenile loggerhead sea turtles (*Caretta caretta*) from Azores and Madeira supported the hypothesis proposed and estimated that 100 % of these pelagic juveniles are from the nesting populations in the southeastern United States and adjacent Yucatan Peninsula, Mexico (Bolten *et al.* 1998) – Figure 12.

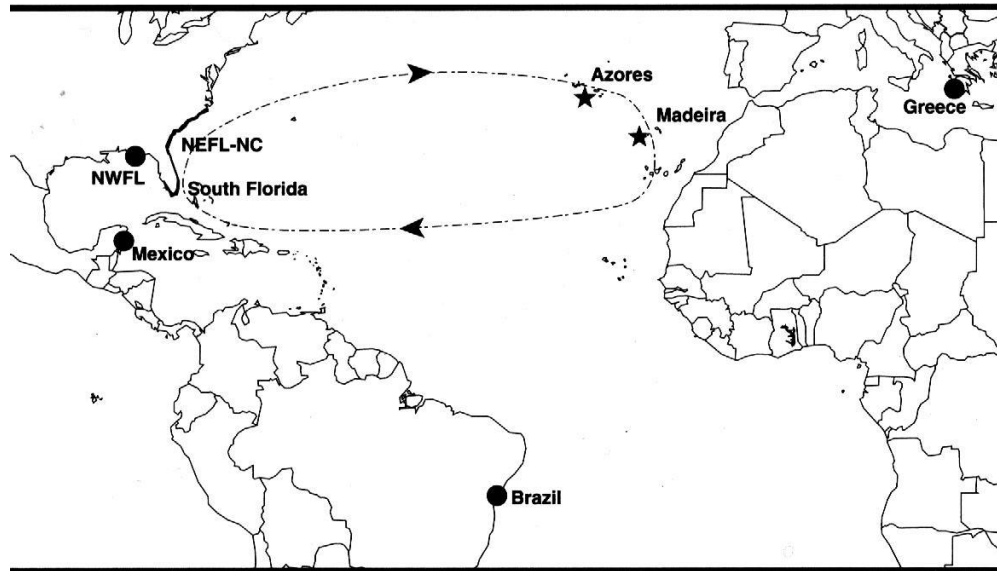


Figure 12 – Map showing locations of sin nesting populations of loggerhead sea turtles: northeast Florida to North Carolina (NEFL-NC), south Florida, northwest Florida (NWFL), Mexico, Brazil and Greece. The two pelagic sampling sites (Azores and Madeira) are indicated with stars. Arrows show the North Atlantic gyre (Bolten *et al.* 1998).

Larger juvenile loggerheads return to their natal areas in the western Atlantic with an average size of approximately 50 cm SCL (46 - 64 cm SCL, or 6,5 – 11,5 years, as reported by (Bjørndal *et al.* 2000), where they enter a neritic juvenile stage before reaching maturity (Bolten 2003).

1.4.3.4. Diet

Loggerhead sea turtles are the most generalist among sea turtles, feeding upon a great variety of animal *taxa* (Bjørndal 1997; Bjørndal 2003). The diet of loggerhead sea turtle were recorded in several studies in different areas of the world (Brongersma 1968; van Nierop and den Hartog 1984; Burke and Standora 1993; Plotkin 1996; Tomás *et al.* 2001; Parker *et al.* 2005; Revelles *et al.* 2007; Casale *et al.* 2008). Two studies about loggerhead sea turtle diet in Madeira were conducted and the main gut contents found were salpae, small fishes (fish parts or incidentally ingest fish) (Brongersma 1968) but the favoured items

by loggerheads in the pelagic stage seems to be coelenterates and cephalopod mollusks (van Nierop and den Hartog 1984).

1.4.3.5. Current Status and Legal Protection

Loggerhead sea turtle (*Caretta caretta*) is classified as Endangered (EN) in the Red List of Threatened Species of International Union for Conservation of Nature (IUCN 2010). Listed on Appendix I of Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) and Appendix I of the Convention of Migratory Species (CMS or Bonn Convention) that prohibits international trade from or to signatory countries.

Since 1979, loggerhead sea turtle is considered Strictly Protected by the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention) in Appendix II.

Furthermore is listed in the Habitats Directive (more formally known as Council Directive 92 / 43 / EEC on the Conservation of Natural Habitats and of Wild Fauna and Flora) that aims to protect the wild plants, animals and habitats that make up our diverse natural environment (in Portugal lead to Decree-Law n.º 140 / 99 modified by the Decree-Law n.º 49 / 2005, with *Caretta caretta* in Appendix B-II and B-IV).

The Law n.º 1 / 87, modified by the Law n.º 13 / 2002, refers specifically migratory species, a several regulatory laws for the fishing activity, especially for those that execute fishing in Exclusive Economic Zones (EEZ) such as the Council Regulation (EC) n.º 1954 / 2003. Loggerhead sea turtle is also listed in the Red List of Vertebrates of Portugal of Nature Conservation Institute (ICN) (Oliveira *et al.* 2005).

The Regional Legislative Decree n.º 18 / 85 / M (7 of September) prohibits capture or intentional killing of turtles in EEZ of Madeira and any form of sea turtles trade.

1.4.4. Main Threats

Sea turtles have a long life span, a life cycle that requires several habitat types, and an extensive distribution in terms of the distance they cover. They are affected by a range of different factors, some natural and others caused by human activities, at all stages of their life cycle. These factors have an impact both in the terrestrial part of their habitat as well as in the marine environment. Impacts in the nesting environment (on sandy beaches) include the direct take of adults for meat, oil, shells etc.; the collection of eggs by humans; the predation of eggs by animals (for example: dogs, pigs); climate change which may affect embryo development; sea - level rise, a consequence of global warming that in some

circumstances results in a reduction of nesting beach habitat; loss of nests due to hurricanes; and heavy utilization of nesting beaches by humans (FAO 2009).

Loggerhead turtles face a number of threats in the pelagic waters of the eastern Atlantic. The same physical forces that tend to concentrate young turtles in convergence zones (the biologically active boundaries between currents and / or water masses) also concentrate floating debris (Figure 13), thus increasing the chance of ingestion or entanglement (Carr 1987). The major threat to the survival of these pelagic juveniles is the risk of incidental capture in commercial fisheries (Bolten 2003).



Figure 13 – Loggerhead sea turtle entangled in plastic - Martijn de Jonge[®].

1.4.4.1. Actual Threats in Madeira waters

In Madeira archipelago, the deep pelagic longlines (set between 700 – 1300 m depth, around 15 Km long) targeting black-scabbard fish (*Aphanopus carbo*) had the greatest impact on sea turtles (Figure 14) compared to the other fishing methods. Based in Catch per Unit Effort (CPUE) values there is an estimation of slightly over 500 turtles are caught, each year, by this activity as *bycatch* (Dellinger and Encarnação 2000).

Furthermore, debris and lost / abandoned fishing gear (Figure 15) which has negatively impacts on sea turtles are commonly found in Madeira waters.



Figure 14 – Loggerhead sea turtle as *bycatch* victim of longline fisheries (left); Black-Scabbard Fish at Mercado dos Lavradores (Funchal).



Figure 15 – Loggerhead sea turtle entangled in lost / abandoned fishing gear – TDellinger[®].

1.5. Objectives

This study is expected to contribute about:

- (1) Chemoreception capacities (airborne and aquatic) of loggerhead sea turtle juveniles (*Caretta caretta*);
- (2) Better understanding in the way how pelagic loggerheads detect food patches in the vast ocean;
- (3) The extent that bait used and discarded in Madeira fishery activities (mainly longline fishery) can be the stimulus that leads to sea turtles *bycatch*.

2. MATERIAL AND METHODS

2.1. Sea Turtles used in Captivity Experiments

Four juvenile loggerhead sea turtles were collected between 2 and 4 nautical miles (NM) off Funchal on the southern coast of Madeira island ($\approx 32^{\circ} 40' N 17^{\circ} W$) – Figure 16. Capture of turtles was most efficient on calm seas with bright sunlight, when turtles bask. They were approached by the boat at slow speed from behind, and caught by hand following the methodology described in Dellinger *et al.* (1997).

Turtles were kept in captivity for the experiments at the Marine Biology Station of Funchal during the period between July and December of 2009.

It was used the Body Condition Index (CI), that measures fitness and health, described in the equation below. Body Condition Index (Table I) relates the weight (Kg) of the sea turtles with their SCL (cm) as described in (Bjorndal and Bolten 2010).

$$\text{Condition Index (CI)} = \frac{\text{Weight (Kg)}}{\text{SCL}^3 \text{ (cm)}} \times 10^4$$



Figure 16 – Madeira and Desertas islands – TDellinger[©].

Table I – Sea Turtles Data (SCL – Straight Carapace Length).

ID	Name	SCL (cm)	Weight (Kg)	Condition Index (CI)	Rank	Capture Date	Release Date
1735	Maggie Kutchi	21,3	1,536	1,59	2	11/07/2009	12/11/2009
1740	Selvagem	32,6	5,622	1,62	1	08/08/2009	12/11/2009
1742	Cracas	26,5	2,098	1,13	4	08/08/2009	13/12/2009
1744	Mancha	35,1	6,362	1,47	3	08/08/2009	12/11/2009

2.2. Acclimation Period Before the Experiments

In order to minimize stress to the sea turtles, the process of capture and transportation was conducted with maximal care. Once in the tanks, turtles had an acclimation period of 2 weeks before the experiments, serving the purpose to establish a captivity routine.

2.3. Diet in Captivity

Sea turtles were fed with chub mackerel (*Scomber japonicus*) – Figure 17, every other day. Prior to the experiments sea turtles were fasted for two days.



Figure 17 – Chub Mackerel (*Scomber japonicus*).

2.4. Preliminary Experiments

2.4.1. Airborne Chemoreception

Five different concentrations of DMS (35 mmol/L; 67 mmol/L; 135 mmol/L; 270 mmol/L and 539 mmol/L) were tested with two different experimental methodologies.

The tank used was a circular tank (3 meters diameter) divided equally in 4 compartments (Figure 18). The tank was filled with seawater until 45 cm height.

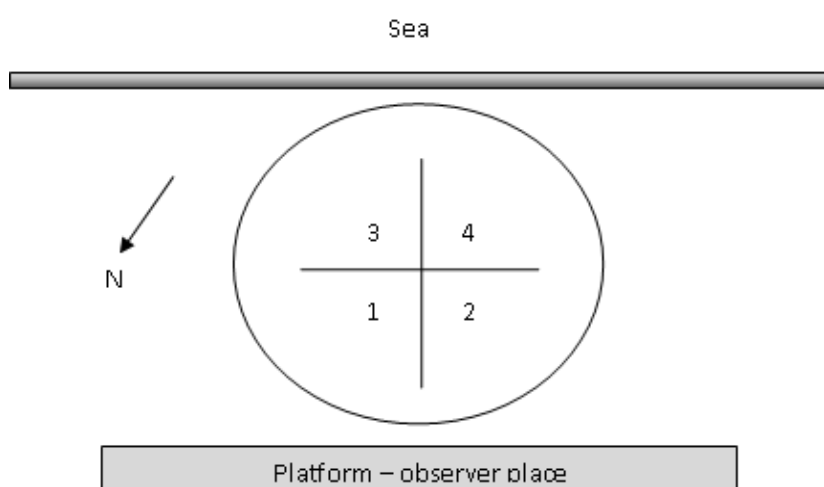


Figure 18 – Experimental tank.

The methodologies used are explained below:

- 1) On the top of a floating container was placed a moist ball of cotton with DMS solution and the container was positioned in one of the compartments of the tank. In the opposite compartment was placed a similar floating container, but the cotton was moist only with distilled water. All of the concentrations above were tested;
- 2) A tea ball infuser was suspended by a string in one of the compartments with a moist ball of cotton with DMS solution inside, about 5 cm from the water level of the tank. All of the concentrations above were tested.

2.4.2. Aquatic Chemoreception

Three different protocols were conducted related to aquatic chemoreception experiments and the procedures are explained below:

The turtle was placed inside the PVC experimental tank (95 cm wide; 115 cm long; 55 cm high, filled with seawater to height of 45 cm). During the experiments, the water volume in the tank was approximately 492 L. The DMS concentration used in these experiments was always 67 mmol/L.

- 1) Ice prepared with DMS solution and ice made of only distilled water were made. These two types of ice were inserted, alternately, in a tea ball infuser and placed inside the tank. This procedure was conducted in order to promote a slowly diffusion of the DMS in the sea water;
- 2) CMJ (Chub Mackerel Juice) that was obtained by pressing chub mackerels including their viscera was used. This preparation was sieved through a spoon colander in order to obtain a homogeneous liquid mixture. A similar procedure in the previous experiment with DMS ice was carried out, but the DMS ice was prepared with a red food coloring (without flavour) in order to investigate if the visual attraction was responsible for any reaction by the sea turtles and also to observe the speed of DMS dispersion in the sea water of the tank;
- 3) In this procedure, it was used CMJ and instead of DMS ice (with red food coloring) was used the red liquid DMS solution.

2.5. Experiments

2.5.1. Airborne Chemoreception of DMS

In order to analyze if loggerhead sea turtle juveniles could detect DMS as an airborne odour, three different concentrations of DMS (67 mmol/L; 135 mmol/L and 270 mmol/L) and distilled water as control were used.

Since the experiments were performed outdoors with potentially large dilution factors of the attractant and there were no means to measure air concentrations of the DMS and also due to the fact that no previous study had tested sea turtles for DMS, it was used highly elevated liquid DMS concentrations as compared to natural airborne concentrations. The choice for these concentrations was based on preliminary experiments. The use of three different DMS concentrations instead a single one was also due to the lack of knowledge about the capacity of the sea turtles to detect DMS through the air.

The experiments were conducted during the second fast day and at daylight hours. Each experimental trial consisted of up to three 20 minute experiments with short recesses in between to change the positions of the attractants. Subsequent experiments with the same animal started after an interval of at least 3 days, usually much longer.

2.5.1.1. DMS Solutions

DMS is insoluble in water. Due to this fact it was first pre-diluted in ethanol 70 % and then this solution was diluted in distilled water. The protocol to the preparation of the different DMS solutions is in the following table – Table II. It was used 200 mL of DMS solution and 200 mL of distilled water in each experiment.

Table II – DMS Solutions.

DMS Concentration (mmol/L)	DMS 99 %	Ethanol 70 %	Distilled Water	Total Volume
67	1 mL	4 mL	195 mL	200 mL
135	2 mL	8 mL	190 mL	200 mL
270	4 mL	16 mL	180 mL	200 mL

2.5.1.2. Trial Tank

The trial tank was a circular pool with 3 meter in diameter and 90 cm of depth. It was filled with seawater until 45 cm height and it was equally divided in four compartments as shown in Figure 19 and Figure 20. During the experiments, the numeration of the compartments was always the same.



Figure 19 – Localization of the trial tank (3 meters diameter) in the Marine Biology Station of Funchal (Google Earth® - 19/04/2010).

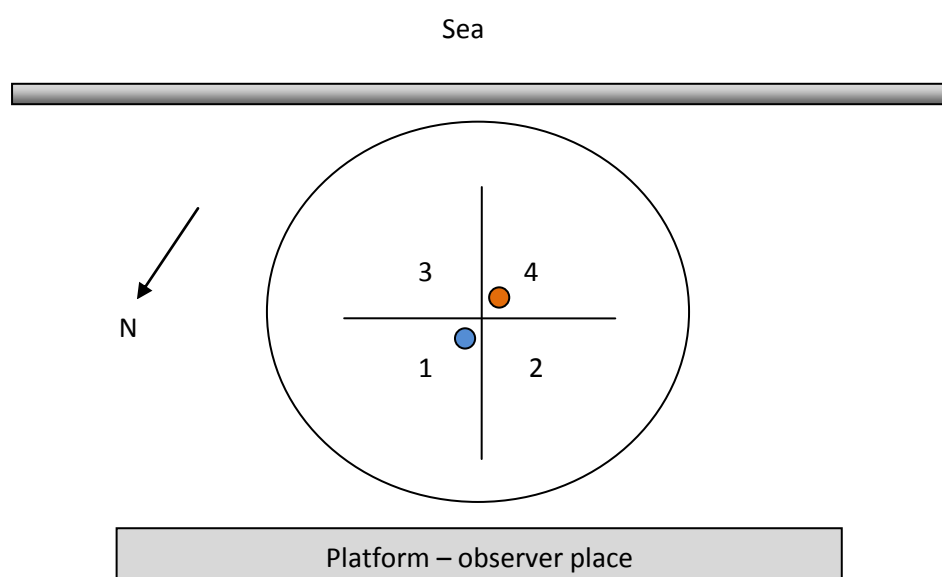


Figure 20 – Compartments numeration of the trial tank. Hypothetic positions of DMS evaporation bottle (blue spot) and Control evaporation bottle (orange). The colors are merely representative, the solution were transparent.

2.5.1.3. Experimental Protocol

The four sea turtles were tested 3 times each for the 3 different concentrations of DMS, in a total of 36 experiments, using always simultaneously the distilled water as control. The turtle in test was placed inside the trial tank and an acclimation period was performed during 30 minutes before the experiment, in an attempt to avoid stress.

All the experiments were done in the second fast day in order to allow them to become more sensitive to the odours. Attractants as well as the distilled water were placed in plastic bottles (500 mL) which in turn were fixed at the centre of the compartment separator and were suspended by a strand about 2 cm from the water level of the tank.

Control evaporation bottles and attractant evaporation bottles were always placed in opposite compartments (Figure 20). Both evaporation bottles were filled with 200 mL of solution. The bottles also had several 3 mm wide holes to make them permeable to the dispersion of the scent through the air (Figure 21). The time that turtles remained in each of the 4 compartments of the tank was recorded during 20 minutes (example of a data sheet used in these experiments – see annex II).



Figure 21 – Plastic bottles used in the experiments.

2.5.2. Aquatic Chemoreception of DMS and CMJ

These experiments were conducted in order to analyze if pelagic loggerheads could detect DMS in aquatic environment and also to analyze if they demonstrate any behaviour to chub mackerel odour underwater.

2.5.2.1. Substances

Two substances were used in this experiment: DMS solution at a concentration of 67 mmol/L and chub mackerel “juice” (CMJ). There is no toxicological information available about the effect of high concentrations of DMS in reptiles, but for rats it can lead to toxic reactions [LD50 (Oral) – 535 mg/Kg]. As a precaution this value served as a reference.

As explained above, DMS is insoluble in water, so it was first pre-diluted in ethanol and this solution diluted in distilled water. One hundred milliliters of DMS solution (67 mmol/L) were used in each experiment.

The CMJ was obtained based in the same methodology explained in the preliminary experiments. In each experiment 100mL of CMJ were used.

2.5.2.2. Trial Tank

Turtles were tested in two experimental PVC tanks 95 cm wide and 115 cm long and 55 cm high, filled with seawater to height of 45 cm. During the experiments, the sea water volume in the tank was approximately 492 L.

2.5.2.3. Experimental Protocol

Three sea turtles were tested each one 3 times for each substance, in a total of 18 experiments. During experiment days, turtles were tested separately for the two substances: DMS (67 mmol/L) and CMJ. The animals were carefully transported to the trial PVC tank 30 minutes before the beginning of the experiment. This period, as mentioned before, had the main purpose to give them the required acclimation. The experiments were conducted during the second fast day and at daylight hours.

The presentation of both attractants was alternated to avoid habituation. The attractant solution was carefully introduced into the tank, avoiding observation by the turtle. During the following 5 minutes the animal reaction to the tested substance was recorded by behavioural observation. A positive reaction was scored when the turtles turned to the substance and opened and closed several times their mouth (trying to bite – behaviour observed when they were eating). Otherwise, if the turtle did not demonstrate any reaction at all, remained lying over the bottom of the tank and barely showed movements, the test was considered as “lack of reaction to the stimulus”.

When each experiment was completed, the turtle was moved to another tank containing clean fresh seawater. After another 30 minutes of acclimation, the turtle was tested again in the same way, using a different attractant.

2.5.3. Classical Conditioning Experiments

The aim of these experiments was to observe if juveniles of loggerhead sea turtles could associate the stimulus of DMS odour, dissolved in the sea water of the tank, with a possible food reward.

In a first period of the experiments, the DMS stimulus was always followed by a food reward (chub mackerel). In the second period of the experiments the DMS stimulus, dissolved in the sea water of the tank, was presented without the food reward. In the “experimental protocol” these procedures are explained in detail. Only one sea turtle was tested in these experiments (T#1742).

2.5.3.1. Substances

The substance tested in these experiments was DMS (35 mmol/L).

2.5.3.2. Trial Tank

The sea turtle was tested in a PVC tank 95 cm wide and 115 cm long and 55 cm high, filled with seawater to height of 30 cm.

2.5.3.3. Experimental Protocol

First Period of Experiments

Every day, for a period of 15 days, a DMS solution (35 mmol/L; 100 mL) was placed in the trial tank and approximately 1 minute later was given a small chub mackerel to the sea turtle. After this 15 days of DMS-Chub Mackerel stimulus the sea turtle was fasted for three days in order to make it more sensitive to aquatic odours.

Second Period of Experiments

In experiment days, a DMS solution (35 mmol/L; 100 mL) was placed in the trial tank and the behaviour of the sea turtle (if the turtle opened and closed several times the mouth - trying to bite – behaviour observed when sea turtle were eating) was recorded during the next 2 minutes. Only after 1 - 2 hours the experiment was concluded the turtle were fed with chub mackerel. In each experiment the sea turtle was fasted for 2 days. Eight experiments were conducted.

2.5.4. Sea Experiments with DMS

Three experiments were done at sea, in the south coast of Madeira Island, with wild free-ranging juvenile pelagic basking sea turtles. Sea turtles were spotted as described in Dellinger *et al.* (1997) and under the same weather and sea conditions, but, instead of capturing them, turtles were observed from a distance and all interactions of the turtle with the observed or boat was avoided. Experiments took place between August and September of 2009, with mostly calm seas and clear skies.

2.5.4.1. Modified Sea Buoy

A modified fishing buoy (Figure 22) was used, which had a plastic net compartment fitted to its top. A cotton ball moistened with DMS solution (135 mmol/L; 200 mL) was placed within this compartment at the beginning of each experiment. The buoy also had a short string attached to its top as an indicator of wind direction.



Figure 22 – Modified Fishing Buoy.

2.5.4.2. Protocol

Once a basking sea turtle was sighted, the speed of the boat was reduced to its minimum in order not to disturb or awaken the animal. The buoy fitted with DMS was released at an estimated distance of 100 meters windward to the animal. Then the boat moved away from buoy and animal and assumed a position at the third tip of an equilateral triangle with the other tips formed by the turtle and the buoy.

The motor of the boat was turned out, except for short position readjustments. Behaviour and mainly orientation of the turtle were recorded using “*ad libitum*”- sampling (Martin and Bateson 1994).

Additionally, bird sightings in the area surrounding the buoy were recorded during the experimental period.

2.6. Statistical Analysis

The data collected during experiments was analyzed with Excel 2007 and SPSS Statistics 17.0 software. Kruskal-Wallis test, chi-square test and linear regression were applied in statistical analysis. The manual (Zar 1998) was used as support material.

The expected time, presented in the graphs on the Results section, is the hypothetically time that turtles would spend in each compartment if it was equally distributed. It was calculated through the division of the total number of hours spent in the experiments by the number of the compartments.

3. RESULTS

3.1. Airborne Chemoreception of DMS

The main goal of these experiments was to determine if sea turtles could detect DMS solutions as an airborne scent, through the registration of the time that each turtle remain in the different compartments of the tank during the experiment period.

3.1.1. Testing Environmental Stimuli and Individual Preference

To analyze if DMS was the only stimulus that influences the behaviour of the sea turtles in study, it was necessary to verify if there were other stimuli that might influence the turtle's position. To test for possible geostationary stimuli, such as the direction of the sound of the sea or magnetic stimuli, the preference of turtles for specific compartments was tested. It is important to mention again, that the position of the compartments of the tank was always the same during all experiments, only the DMS evaporation bottle and the Control evaporation bottle were rotated through each compartment.

The compartments that were closer to the sea were 3 and 4. Consequently, if turtles were attracted to the sound or smell of the sea, they should spend more time in these compartments instead of compartments 1 and 2. The analysis of the cumulative observed time spent by all of the sea turtles in each compartment, during each experiment, revealed no statistically significant differences (Kruskal-Wallis $H = 2,959$; $df = 3$; $p = 0,398$), although turtles spent slightly more time in compartment 3 (Figure 23).

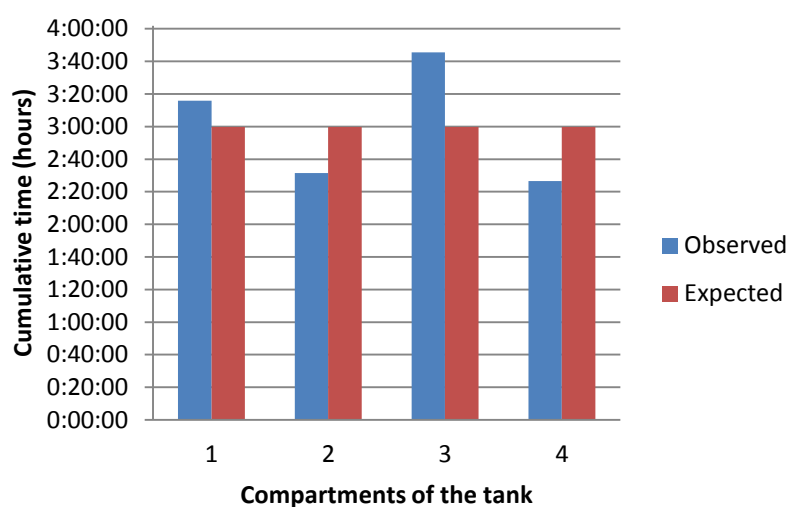


Figure 23 – Cumulative time (observed and expected) spent by all of the sea turtles in each compartment of the tank.

However, when testing each turtle individually (Figure 24), only T#1735 did not divide equally its time between the 4 compartments (Kruskal-Wallis $H = 11,276$; $df = 3$; $p = 0,010$), showing a preference for compartment 3. The other 3 turtles used in the experiments did not revealed any statistically significant preference for a specific compartment.

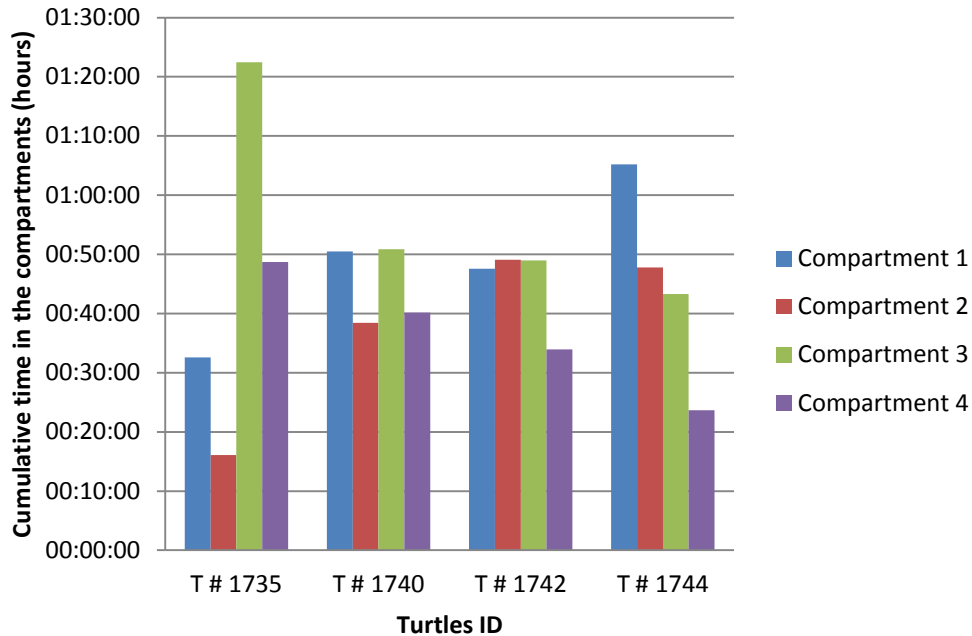


Figure 24 – Cumulative time spent by each turtle in each compartment of the tank.

3.1.2. Testing if there was reaction for the DMS stimulus

Airborne scents are carried by the wind. Thus the empty compartments may not be equivalent for the turtles since the leeward compartment might have more attractant than the windward one. Since the prevalent wind direction comes from the sea, compartments 1 and 2 are Leeward and 3 and 4 windward.

An analysis of the cumulative time spent in each of the 4 compartments (DMS, Control and Empty Compartments) by all of the sea turtles in study indicate no statistically significant results (Kruskal-Wallis $H = 2,444$; $df = 3$; $p = 0,485$), but besides that it is possible to notice in Figure 25 that they spent more time in the Leeward – Empty Compartment, compared to the other three compartments.

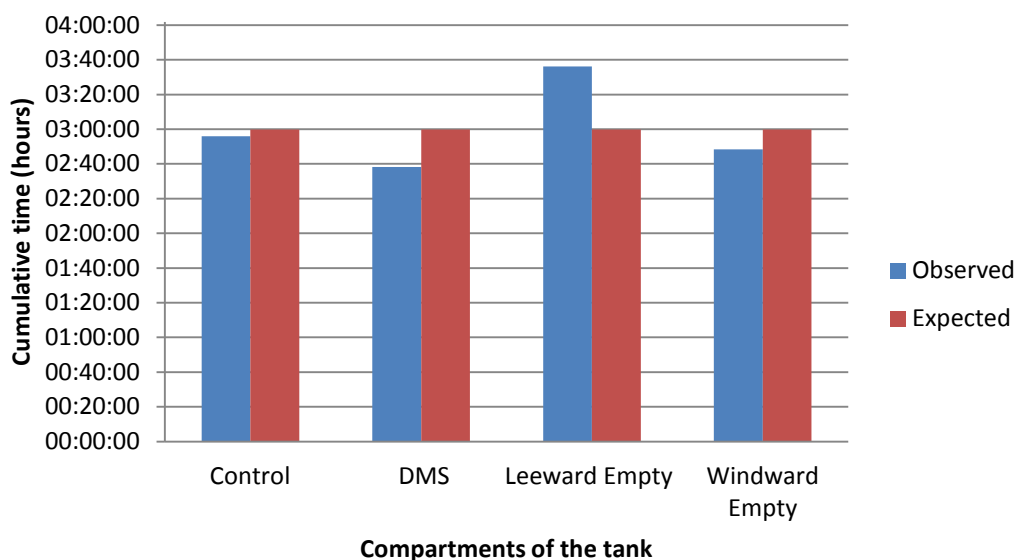


Figure 25 – Cumulative time spent by all of the sea turtles in each compartment of the tank.

The analysis of the cumulative time spent by each turtle in each of the four compartments revealed no statistically significant differences by Kruskal-Wallis tests. Only turtle T#1744 showed a tendency for spending more time in the “Leeward - Empty Compartment” (Kruskal-Wallis $H = 5,605$; $df = 3$; $p = 0,132$) – Figure 26.

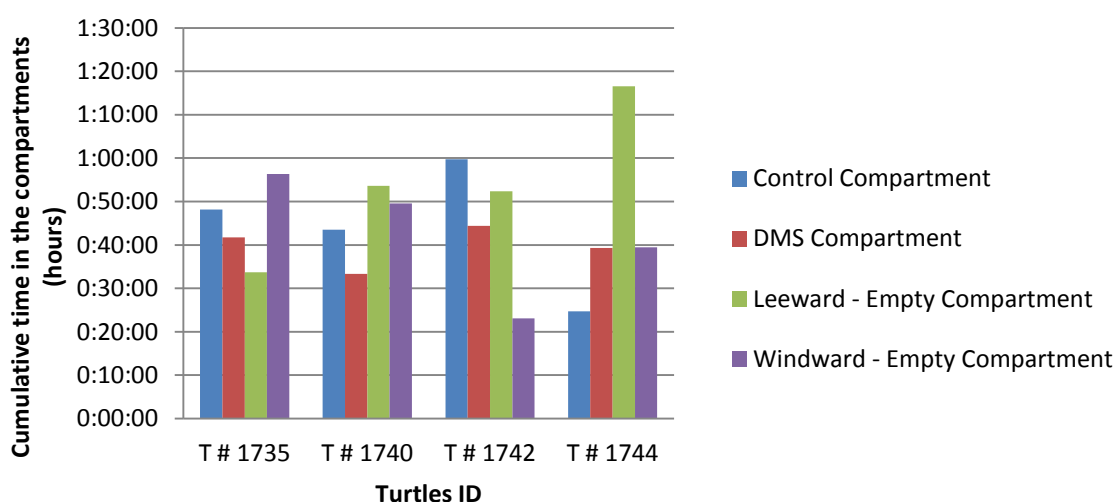


Figure 26 - Cumulative time that each turtle spent in the different compartments of the tank.

3.1.3. Behavioural responses vs. DMS concentration

For the different concentrations of DMS used (67 mmol/L; 135 mmol/L and 270 mmol/L) there was no significant differences in the cumulative time spent in each of the 4 compartments (DMS, Control and the two Empty compartments), by all of the sea turtles –

Figure 27, though, for the 67 mmol/L DMS concentration the result of Kruskal-Wallis test was almost statistically significant (Kruskal-Wallis $H = 7,652$; $df = 3$; $p = 0,054$) for the Leeward – Empty Compartment.

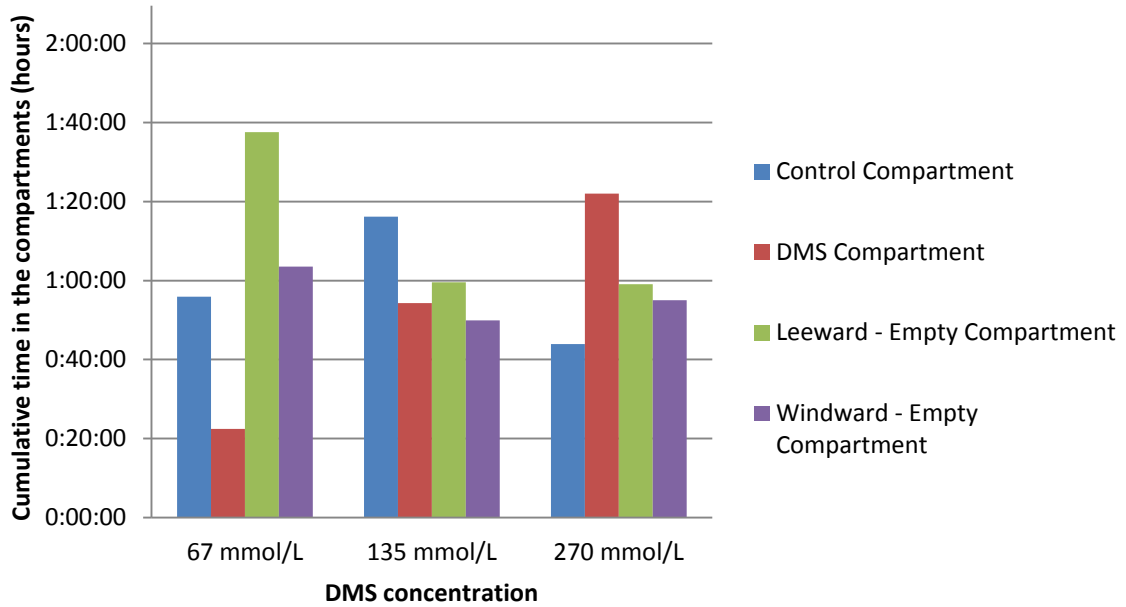


Figure 27 – Cumulative time spent by all of the sea turtles in each compartment of the tank for the different DMS concentrations.

However, if only the cumulative time spent in the DMS compartment by all of the sea turtles are analyzed for the different concentrations (67 mmol/L; 135 mmol/L and 270 mmol/L) – Figure 28 – an obvious increase with the DMS concentration is observable, but not significant using a test for averages (Kruskal-Wallis $H = 3,677$; $df = 2$; $p = 0,159$).

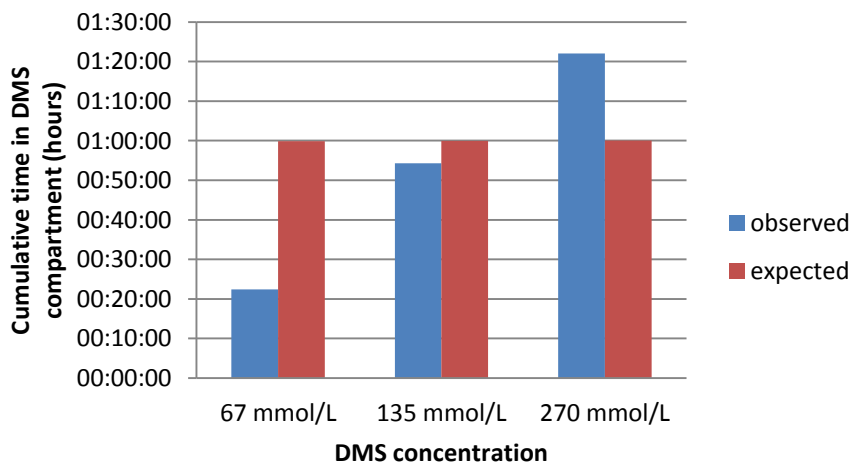


Figure 28 – Cumulative time spent by all of the sea turtles only in DMS compartment of the tank for the different DMS concentrations.

In contrast, a regression confirmed this increase of the cumulative time per experiment spent in the DMS compartment and the DMS concentration ($R^2=15,7\%$, $F = 6,330$; $df = 1$; $p = 0,017$). Because only 15,7% of the variation in DMS residency - time is explained by the DMS concentration, each turtle was tested separately. Only T#1735 actually spent more cumulative time in DMS compartment with the increase of DMS concentration (Figures 29 and 30). This result is statistically almost significant for a test using averages (Kruskal-Wallis $H = 5,422$; $df = 2$; $p = 0,066$), but highly significant in a regression ($R^2 = 64.6\%$, $F = 12,794$; $df = 1$; $p = 0,009$). The other 3 turtles in study did not present any significant results in this analysis.

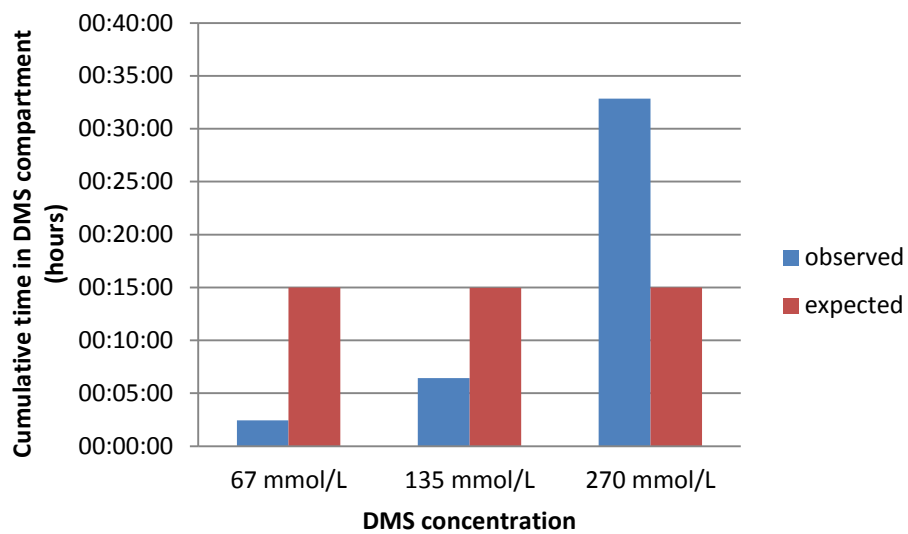


Figure 29 – Cumulative time spent by T#1735 only in DMS compartment of the tank for the different DMS concentrations.

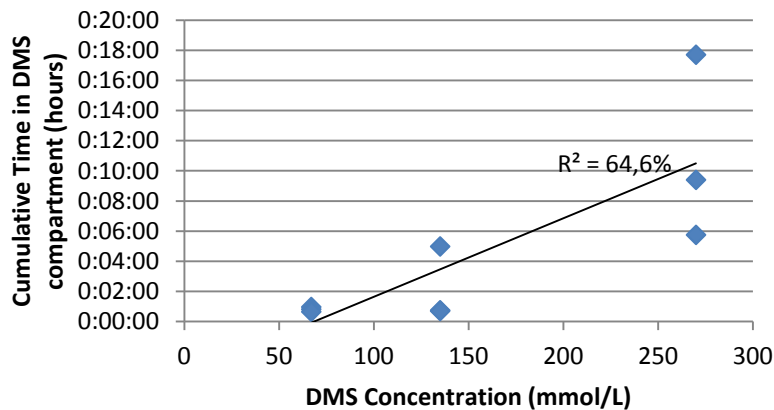


Figure 30 – Cumulative time spent by T#1735 in DMS compartment of the tank in each experiment for the different DMS concentrations ($R^2 = 64,6\%$).

3.2. Aquatic Chemoreception of DMS and CMJ

Nine experiments with DMS solution (67 mmol/L) diluted in the sea water of the tank were conducted and none of the 3 turtles in study demonstrated a positive reaction to this substance. However, in the nine experiments using CMJ (“Chub Mackerel Juice”), as attractant, the sea turtles had a positive reaction ($\chi^2 = 5,444$; $df = 1$; $p = 0,020$), as shown in the Figure 31 and in Table III. The maximum DMS dilution in the sea water of the tank reached approximately 137 $\mu\text{mol/L}$.

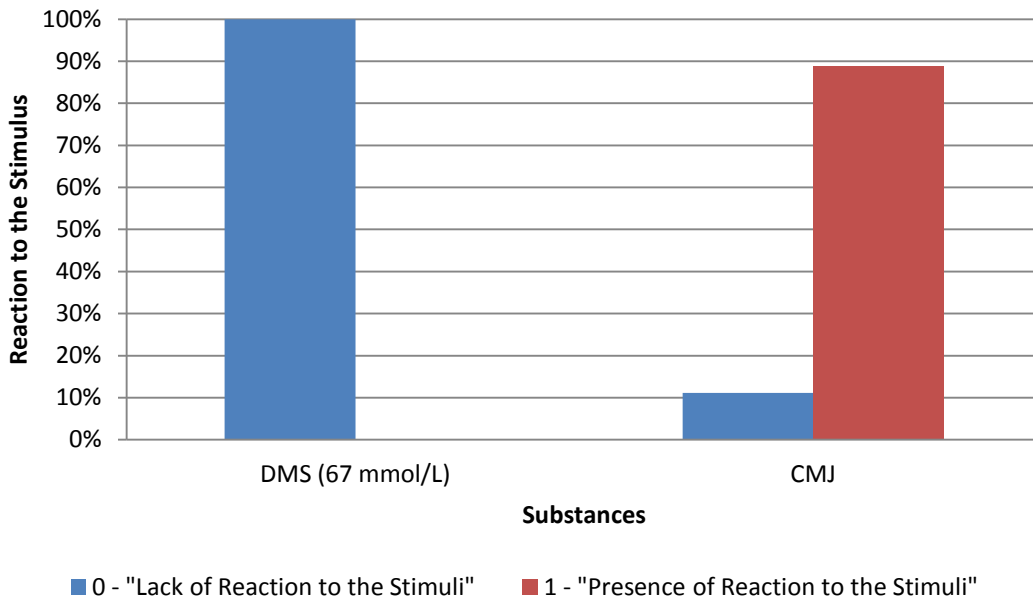


Figure 31 – Analysis of aquatic chemoreception by loggerhead sea turtle juveniles (n = 3). Two substances were used: DMS solution (67 mmol/L) and CMJ (“Chub Mackerel Juice”).

Table III – Sea Turtles reaction to CMJ and DMS dissolved in water. “0” – Lack of reaction to the stimulus; “1” – Presence of reaction to the stimulus.

Turtle ID	Substance	Reaction	Substance	Reaction
T#1735	CMJ	1	DMS	0
	CMJ	1	DMS	0
	CMJ	1	DMS	0
T#1740	CMJ	1	DMS	0
	CMJ	1	DMS	0
	CMJ	1	DMS	0
T#1744	CMJ	1	DMS	0
	CMJ	0	DMS	0
	CMJ	1	DMS	0

3.3. Classical Conditioning Experiments

Eight experiments with DMS solution (35 mmol/L) diluted in the sea water of the tank were conducted and the sea turtle tested did not demonstrate any positive reaction to this substance, suggesting that this animal did not associated the DMS stimulus with a possible food reward.

3.4. Sea Experiments with DMS

At sea experiments with wild basking turtles showed that orientation towards the buoy fitted with DMS occurrence in all cases. Furthermore the buoy was overflown more frequently by seabirds (Figures 32 - 35 and Table IV), mainly foraging Procellariiforms (Cory's Shearwater and Bulwer's Petrels).



Figure 32 – Cory's shearwater – Tdellinger[®].



Figure 33 - Bulwer's petrel - Awei Lee[®].



Figure 34 – Common tern - Dave Provencher[®].



Figure 35 - Yellow-legged gull – Tdellinger[®].

Table IV – Bird species sighted during the sea experiments.

Common Name		Species Name	Procellariform
English	Portuguese		
Cory's shearwater	Cagarra	<i>Calonectris diomedea borealis</i> (Cory, 1881)	Yes
Bulwer's Petrel	Alma-Negra	<i>Bulweria bulwerii</i> (Jardine and Selby, 1828)	Yes
Common tern	Garajau	<i>Sterna hirundo</i> (L., 1758)	No
Yellow-legged Gull	Gaivota de Patas Amarelas	<i>Larus cachinnans atlantis</i> (Clements, 1991)	No

3.4.1. Sea Experiment 1 (8 August 2009)

The turtle was observed for over 1 hour, starting at 13h12 and finishing at 14h15. It woke up from its basking about 40 minutes after the onset of the observation, slowly turned to the buoy approaching it at a very slow speed using sinuous path regularly breathing with its head out of the water. It reached the buoy 10 minutes later and then increased the distance again to 5 meters.

After the first hour wind direction changed, the sea turtle began to move further away, and the observation was terminated.

During the observation time 27 sea birds overflew the experiment (Table V and Annex III) which appeared to be more than usually when sea turtles are captured and measured.

Table V – Number of bird sightings in sea experiments of each species.

Birds	Quantity
Cory's shearwater	15
Bulwer's Petrel	10
Common tern	1
Yellow-legged Gull	1

3.4.2. Sea Experiment 2 (11 September 2009)

This experiment had 1 hour duration, starting at 12h35 and finish at 13h35. The turtle was basking and became active about 25 minutes later the experiment began. At this time, the turtle presented a similar behaviour observed in the first experiment. Thus, the turtle began to swim slowly to the buoy (not directly but with a sinuous path) and breathing with its head out of the water. Fifteen minutes later, approximately, the turtle was closer to the buoy, around 8 meters, where it remained for more or less 5 minutes. After this, the sea turtle began to move further away from the buoy and for the last 15 minutes of the experiment there was not another approaching.

During the experiment period 3 seabirds were sighted: 2 Cory's shearwater and 1 Bulwer's petrel (Annex III).

3.4.3. Sea Experiment 3 (29 September 2009)

This experiment lasted approximately 25 minutes, starting at 12h48 and finish at 13h12. The sea conditions were not the best to perform this experiment, due to this the DMS buoy was placed closer to the turtle than in the other two experiments, around 30 meters, instead of 100 meters.

The turtle turned to the buoy with DMS and began to approach, about 3 minutes later the experiment began. At that time the sea turtle was about 15 meters to the buoy and continued to swim in DMS buoy direction, with a sinuous path, and breathing with its head out of the water, a behavior observed in the two previous experiments. Approximately 3 minutes after, the sea turtle was around 8 meters from the buoy. However, the wind direction had changed and the sea turtle moved away. Due to the sea conditions, at 13h12 we lose sea turtle sight.

During the experiment period, two Cory's shearwaters were observed (Annex III).

4. DISCUSSION

Olfactory-guided search strategies over distances of hundreds or thousands of kilometers has not been rigorously studied in most organisms. Green sea turtles (*Chelonia mydas*) nesting on Ascension Island, in the middle of the Atlantic Ocean, are guided there from feeding grounds off the coast of South America, presumably by a redundant set of mechanisms that possibly includes an ability to smell their island birth place (Lohmann and Lohmann 1996).

In order to explain such behaviours, it is generally assumed that animals are able to recognize and follow odours emanating from a distant source. This logic predicts that a recognizable odour signature emanates from a site, forming a gradient that can be detected thousands of kilometers away. By some adaptative behavioural mechanisms such as turning or swimming upstream in response to the odour cue, the animal focuses its directional movement to locate the source of the odour plume (Nevitt 2000).

Juvenile pelagic sea turtles inhabit the oceanic domain of the subtropical ocean basins of the world. In such areas the food is patchily distributed both in space and time. Thus turtles have to rely largely on sensory input to locate exploitable food resources. It is this thesis hypothesis, that chemosensory orientation is one of the main mechanisms for food location by sea turtles. Specifically it is hypothesized that turtles may detect DMS, a byproduct from zooplankton grazing on phytoplankton. This is the first time that sea turtles were tested for DMS chemoreception.

4.1. Airborne Chemoreception of DMS

The most significant result of these experiments was the fact that one – T#1735 -, of the four sea turtles in study, increased the residence time in the DMS compartment with the increasing of DMS concentration. This result shows that this juvenile loggerhead sea turtle exhibited sensitivity to this compound. In support of the turtles ability to detect DMS is the fact that all turtles spent more time in the Leeward - empty compartment than in the windward one. This result can only be explained if the volatile nature of DMS made it accumulate more in downwind compartments as compared to the windward ones. However, though in tendency this was true, only with a DMS concentration of 67 mmol/L was the result significant. Based on the anatomy of loggerhead sea turtles Saito *et al.* (2000) suggested airborne chemoreception through the olfactory epithelium, which was later confirmed through the experiments by Endres *et al.* (2009) that showed that loggerhead sea turtles can indeed detect airborne odours, in his case using food gel as an

odour attractant. Moreover, the sea experiments conducted in the present study, corroborate the sensitivity for DMS by pelagic loggerheads, because (1) in all of the three experiments with wild basking sea turtles they showed curiosity for the DMS buoy and (2) they approached the buoy not in a direct line but used a *zig-zag* upwind swimming with regular breathing episodes with their head and nostrils out of the water. This *zig-zag* upwind search behaviour was also verified in procellariiform birds, which also have a wide ranging pelagic lifestyle, (Nevitt *et al.* 1995) and use DMS as an airborne foraging cue to detect patchily food resources in the wide ocean (Nevitt *et al.* 1995; Nevitt 1999a; Nevitt 1999b; Nevitt 2000; Cunningham *et al.* 2003; Nevitt and Haberman 2003; Bonadonna *et al.* 2006). Similarly to procellariiform birds there are other pelagic animals that also use this scent compound as an airborne foraging cue, such as African penguins (Cunningham *et al.* 2008) and Harbour seals (Kowalewsky *et al.* 2006). The main studies are summarized below:

- Blue petrel chicks (*Halobaena caerulea*), thin-billed prion chicks (*Pachyptila belcheri*) and common diving petrel chicks (*Pelecanoides urinatrix*) were exposed to DMS concentrations of 1 μ mol/L through the vaporization of the nostrils (Cunningham *et al.* 2003);
- Antarctic prions (*Pachyptila desolata*) were tested for DMS. Physiological experiments were conducted where the bird's beak was positioned in a handblown glass ventilation tube. This tube provided the bird with a constant air stream (1500 ml s⁻¹). A second continuous (10 ml s⁻¹) air flux delivered DMS (10 μ m, but this concentration was diluted to a level of 3 - 4nm at the bird's bill) or control stimulus via a system of bypasses. Behavioural experiments in order to determine whether individual prions would orient to DMS in a non-foraging context were carried out. For this purpose a Y-maze set up was used which had in one of the arm a Petri dish containing either DMS (1 μ M, 4 mL) or control solution (Nevitt and Bonadonna 2005);
- It was used a go/no-go response paradigm to determined the DMS detection threshold in two harbour seals (*Phoca vitulina vitulina*). DMS stimuli from 8.05x10⁸ to 8 pmol (DMS) m⁻³ (air) were tested against a control stimulus using a custom-made olfactometer (Kowalewsky *et al.* 2006).

- Adult African penguins (*Spheniscus demersus*) were tested in wild and in captivity in order to investigate if they could detect DMS. The DMS concentrations used were 1 μ molar dissolved in 25 mL distilled water (Kowalewsky *et al.* 2006).

Though these experiments showed that one and thus probably all sea turtles can detect DMS, the fact remains that 3 turtles did not show any sign that DMS was attractive to them. The sheer sensorial ability of sea turtles must not necessarily mean that this capacity is actually used during foraging, though knowledge of their ecology suggest that this is one of the most probable ways to find food. Conditions of captivity, with food being brought to them regularly, may demotivate turtles to use DMS as a cue. The number of pelagic loggerheads in study may also have been insufficient, though other publications in this area generally used low numbers of experimental animals (Kowalewsky *et al.* 2006; Endres *et al.* 2009). Why did only T#1735 reveal sensitivity for DMS? Turtle # 1735 differed from the other 3 experimental animals in that it was the smallest turtle and it had the longest time in captivity before the onset of the experiments (~1 month). Compensatory growth has been described for this turtle population (Bjorndal *et al.* 2003), and being the smallest, might mean that it was more sensitive to environmental food cues. Its Body Condition Index (CI =1,59) was within the range of the other 3 turtles, meaning that it was not particularly meagre. Turtle # 1735 DMS sensitivity could of course be explained simply as a result of a high variation among individuals, which was observed in behavioural experiments by Grassman and Owens (1987).

In these experiments, evaporation bottles were used as DMS point sources. The concentrations of the evaporation solutions were chosen to be rather high, in order to counterbalance its volatile nature. Since no gas chromatographic (Scarratt *et al.* 2007) equipment was available, it was impossible to determine air concentrations of DMS over the tank and within each of the 4 compartments. In the natural environment DMS concentrations peaks of 8×10^3 pmol DMS m^{-3} (air) were measured in productive areas in the North Atlantic (north of 40° N) and English Channel (north of 48° N) (Bürgermeister *et al.* 1990). Furthermore, naturally occurring DMS may have added sensorial noise to experiments in the present study. No data exist on the DMS concentration in the atmosphere around Madeira archipelago.

Another aspect is that in the sea experiments a Control buoy (without DMS) was not used. Juvenile pelagic sea turtles are attracted to any floating object and avidly inspect it, probably to test it for food (Dellinger, pers. com., Dellinger *et al.* 1997). As a result, it might not have been just the olfactory sensory system that guided the sea turtles to the DMS buoy but other senses might be involved, such as vision. However, the *zig-zag*

upwind search path the turtles used speaks against this hypothesis, and makes an olfactory approach highly probable.

No turtles showed any preference for a specific compartment, which indicates that other geostatic stimuli (since the compartment were always at the same location) did not have any measurable effect. The only exception was T#1735, which spent more time in compartment 3. This compartment was located to the East, considering the center of the tank as the intersection of the cardinal points. Possible stimuli include wind and smell coming from the sea, some magnetic stimulation and / or by the solar position (Lohmann and Lohmann 1996) that may have been more intense in the compartment 3 during the experiments, though it is highly improbable that compartments meters away would experience differences in solar intensity. However, turtles around Madeira show a long distance tendency to migrate along a NW-SE axis and, during the late summer, most turtles migrated in the direction of the Mauritanian coast which is SE (Dellinger 2000; Dellinger and Freitas 2000). If this migratory tendency is maintained in captivity it could explain T#1735's tendency, but not the unexisting tendency of the others.

In summary, these experiments were not conclusive in answering the question if the pelagic loggerhead sea turtles actually use DMS as an airborne cue for the detection of the food patches, in the natural environment. Only one turtle showed clear signs behavioural reaction to DMS. In future works it will be very important to measure the DMS concentration present in the atmosphere around Madeira archipelago, and close to the animals nostrils. Furthermore experiments in a more controlled setting may prove better able to identify the actual sensitivity of turtles towards DMS.

4.2. Aquatic Chemoreception

Sea turtles have demonstrated no reaction / sensitivity for DMS solution (67 mmol/L) dissolved in the sea water of the tank. According to the literature, the average value of dissolved DMS in surface waters of the northwest Atlantic is 1,85 nM (Scarratt *et al.* 2002) which is lower than the concentration used by a factor of approximately 36433633. Even if the sample had been diluted within the tank (sea water volume approximately 492L) the maximum diluted concentration reached would be 137 μ mol/L, a value that is still well above the average ambient concentrations, thus a lower concentration should be used in future works. Although, the lack of reaction by the sea turtles, might be explained by the fact that DMS had quick and large dispersion in the sea water of the tank (tested in preliminary experiments with red food coloring) and the turtle could not detect a certain

peak concentration as might happen in the natural environment. In fact, certain areas of the ocean have more DMS dissolved than others (Scarratt *et al.* 2007). Another suggestion is that the DMS could not be a signal substance involved in short-distance detection of food by the sea turtles, but other aromatic compounds (such as prey odours). Besides that other senses could also be involved, such as vision (Nevitt 2008). The classical conditioning experiments of the present study sustain this theory that probably the DMS dissolved in the ocean waters is not used at short-distance food detection, otherwise the sea turtle should have associated the DMS odour dissolved in the water of the tank to the probable found of food resources (chub mackerel reward, in the case of the experiments conducted).

According to Nevitt (1995) procellariiform seabirds, at large spatial scales, use changes in the olfactory landscape to recognize potentially productive foraging opportunities as they fly over them (mainly DMS peaks in the environment). Once more this corroborates the long distance orientation by pelagic loggerheads to food patches, through the DMS airborne chemoreception. At small scales, procellariiforms must pinpoint and capture prey using whatever proximate cues are available to them. The bird knows that has arrived to a productive area by a predictable variation in the way the ocean smells. This change in the background scent triggers the bird to begin ARS (Area Restricted Search) might using olfactory, visual or a combination of signals (Nevitt 2008). Pelagic loggerheads might use the same short-distance mechanism for food detection. Summarizing, at long-distance they might use DMS as an airborne chemical cue to detect productive areas as was explained before, and at short-distances they might use other odours in the environment to detect effectively the preys.

Sea turtles are aquatic animals, that allow other chemoreception capacities that procellariiform seabirds might not have or do not use so often, the aquatic chemoreception. Thus, the pelagic loggerheads might use both airborne and aquatic chemoreception for the detection of preys at short-distance, probably the DMS is not the signal cue involved in this detection, but otherwise the airborne and aquatic odour of the preys, besides the visual signals. These assumptions are supported by the aquatic chemoreception experiments with CMJ (Chub Mackerel Juice) conducted in the present study. In fact, the experiments using CMJ dissolved in the sea water revealed that in 90% of the cases sea turtles present aquatic chemoreception for the chub mackerel. The aquatic chemoreception by sea turtles was already demonstrated in previous studies (Manton *et al.* 1972a; Grassman and Owens 1981) and it was suggested that this type of chemoreception are provided by the vomeronasal epithelium (Saito *et al.* 2000). The experiments of the present study had confirmed this once again. The sensitivity and the

feeding behaviour (trying to bite the CMJ solution) observed in the experiments of the present study revealed that CMJ was recognized as potential food.

Another important issue is related to the *bycatch* of loggerhead sea turtle juveniles in Madeiran waters. One of the baits used in fishing activities is the chub mackerel (*Scomber japonicus*). The great attractiveness confirmed by the aquatic chemoreception experiments using CMJ could explain the mortality of these animals in the fishery gears. However, these results may have been due to the fact that the chub mackerel was the diet used during the captivity period. In fact, sea turtles that are fed with a particular diet have the capacity to readily adapt to a new diet under laboratory conditions (Grassman and Owens 1982). Then the results obtained in this study may have been due to habituation to chub mackerel odour during the feeding periods.

Besides the diet habituation could influenced the results, it is a fact that the main cause of mortality of loggerhead sea turtles in the waters of Madeira archipelago is due to *bycatch* by the deep pelagic longlines fishery targeting black-scabbard fish (*Aphanopus carbo*) and the chub mackerel is actually one of the baits used in this fishery. It was estimated that approximately 500 loggerhead sea turtles are caught, each year, in black-scabbard fishery (Dellinger and Encarnação 2000). The most important aspect in sea turtles chemoreception for interactions with longline fisheries is the role of chemical signals involved in detection, recognition and locating food. This is particularly important for species of sea turtles that ingest the bait used in longline fisheries, such as loggerhead sea turtles. Hatchlings of loggerhead sea turtles revealed a high response to the odour of fish, an ability to distinguish between different types of food, based on chemical signals (Grassman and Owens 1982). Similarly to pelagic fishes, the chemical signals play an important role on the decision between "biting and not biting" one food item that was located visually by sea turtles. It was demonstrated that loggerhead sea turtles have the ability to distinguish between the bait used in fisheries through the odour. When it was given artificial plastic baits, squid-shaped, without smell and other similar bait but with a piece of fish (*Scomber* spp.), it was observed that those containing the fish were chosen more often (Piovano *et al.* 2004). Therefore it is extremely important the creation of effective measures to reduce the *bycatch* of these sea turtles that are classified as endangered on the IUCN Red List. It is essential to emphasize that the pelagic stage of this animals that are found in Madeira waters represents the main growth phase of this species. Some of these reducing *bycatch* measures could be the changing of the bait to another one that could reduce the sea turtles *bycatch*, but not decreasing the target species capture; the use of wide circle hooks, instead of J hook actual used in black-

scabbard fishery in Madeira waters (Figure 36) and setting hooks deeper than turtle abundant depths.

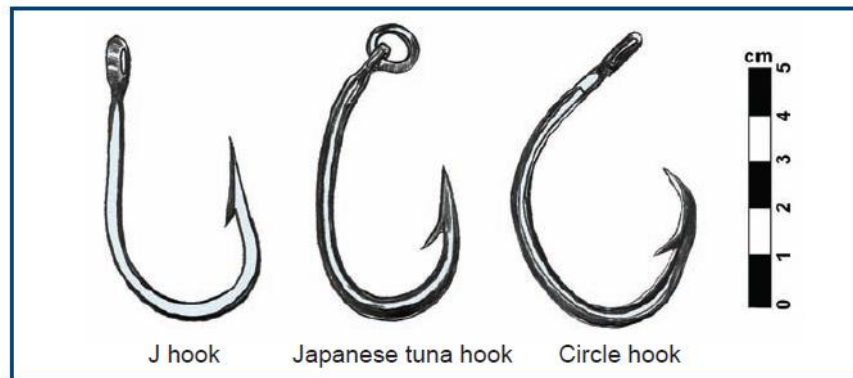


Figure 36 – Main types of hooks used by longliners (FAO 2009).

Experiments suggest that circle hooks are effective at reducing captures of harselled sea turtles, that is the case of loggerheads, because they are wider at their narrowest point than J hooks. Moreover, in order to reduce sea turtle capture rates, the use of circle hooks has been shown to reduce the number of turtles that are deeply-hooked (the hook is swallowed into the esophagus or deeper, rather than being hooked in the mouth or foul hooked on the body). Mouth-hooked turtles probably have a greater chance of surviving a hooking than deeply hooked turtles. The disadvantages of this kind of hook are related to the possible lower catch rates of certain target and commercially important incidental species and also the possible increase in accidental shark catch rates (FAO 2009).

A study conducted in Madeira waters revealed that juveniles of loggerhead sea turtles remain just below the surface (10 - 25m) (Dellinger and Freitas 2000). Setting the gear deeper than 100 m in longline fisheries could be a *bycatch* avoidance method. The main advantage of this method is the substantially fewer sea turtle interactions, since sea turtle *bycatch* rates are higher by an order of magnitude in shallow set pelagic longline fisheries. Although this method may not be economically viable and turtles caught in deep-set gear may drown before gear is hauled (FAO 2009).

Accurate information on the feeding habits of loggerhead sea turtles during their pelagic stage is crucial in order to reduce mortality.

4.3. Why do Juvenile Loggerhead Sea Turtles Occur Near Madeira?

Juvenile loggerhead sea turtles, in the pelagic stage, are commonly found in Madeiran waters. Madeira is an oceanic archipelago that lies within an oligotrophic marine area. Why do pelagic loggerheads “choose” these waters poor in nutrients to spend the most important growth phase of their life cycle?

In fact Madeiran waters might not be so unproductive after all. Today’s islands are actually to be found at the end of a chain of seamounts that start at Cape S. Vicente in mainland Portugal (Geldmacher *et. al.* 2000). Both the island as well as the surrounding seamounts function as marine productivity centres within these oligotrophic waters. In fact, Madeira waters are considered as an “Oasis in the Atlantic” due to the “island mass effect” - defined as the disturbance caused by an island in the ocean currents and their effects on marine ecosystem (Mann and Lazier, 2006). Coastal Zone Colour Scanner (CZCS) images and historical data *in situ* revealed high levels of chlorophyll-a in coastal waters of Madeira, Desert and Porto Santo islands that leads to a consequent increase in the zooplankton biomass, which most of the times are followed with an increase of fish schools in the area. The presence of *eddies* and frontal systems, between April and September, in the west part of Madeira island were often observed and fish discard data revealed higher captures during this period (Caldeira and Lekou 2000). This data provide important information about the high productivity of Madeira waters compared to the rest of the open ocean.

The more productive waters around Madeira are probably also associated to an increased DMS concentration in the atmosphere. There is no data available about the DMS concentrations for this area of the Atlantic Ocean. According to Kettle *et al.* (1999), the concentrations of DMS greatly fluctuate and the peak concentrations of this aromatic compound is often associated with areas of coastal *upwelling* or other oceanic regions of high productivity. Given this assumption, if sea turtles can and do detect DMS peaks and are attracted to them, this might be one reason for their abundance around Madeira and choose this area to forage and grow. The spring and summer months are the period when more juvenile loggerhead sea turtles sightings occur (Dellinger, pers. com.). Moreover, a study conducted in the Pacific Ocean about the feeding habits of pelagic stage of loggerhead sea turtles revealed that these animals are commonly encountered in main frontal areas where a sharp gradient in surface chlorophyll is observed. This frontal area present concentrated phytoplankton that also collects and attracts a diverse of neustonic and oceanic organisms, many of which may be potential preys (Parker *et al.* 2005).

5. FUTURE DIRECTIONS

This was a pilot study with the aim of providing new perspectives for new methodologies that could lead to future research in this important area that is the detection of food by the pelagic juvenile loggerhead sea turtles. Thus, more studies should be carried out in order to investigate whether chemoreception, the short, long distance or both, does have a fundamental role in the location of food patches by the loggerhead juveniles.

The role of DMS in airborne chemoreception should be further explored, since this scent compound is used by other pelagic species as chemical signal for olfactory detection of feeding areas.

More experiments should be conducted in order to investigate the role of aquatic chemoreception in the detection of prey at short distance. It is also very important to study if DMS serve as a chemical sign in short distance detection of the preys or if sea turtles are guide for other olfactory cues, such as the prey odour, or even for other sensory mechanisms, for example visual cues.

In order to create effective measures to reduce the *bycatch* of sea turtles should be conducted studies concerning the existence of other baits or other efficient methods that do not decrease the capture of the fish target species, but which reduce the *bycatch* of sea turtles.

Increasing the knowledge about chemoreception capacities in sea turtles is especially desirable in order to create satisfactory conservation efforts for these endangered animals.

6. BIBLIOGRAPHY

- Albert 1^{er} de Monaco (1898). "Sur le développement des tortues (*T. caretta*)."
Comptes Rendus de la Société de Biologie **50**: 10-11.
- Avens, L. and K. J. Lohmann (2004). "Navigation and seasonal migratory orientation in juvenile sea turtles." Journal of Experimental Biology **207**(11): 1771-1778.
- Beardall, J., S. Stojkovic and S. Larsen (2009). "Living in a high CO₂ world: impacts of global climate change on marine phytoplankton." Plant Ecology & Diversity **2**(2): 191-205.
- Berresheim, H. (1987). "Biogenic Sulfur Emissions from the Sub-Antarctic and Antarctic Oceans." Journal of Geophysical Research-Atmospheres **92**(D11): 13245-13262.
- Birdlife International. (2004). Threatened Birds of the World CD ROM. Cambridge: Birdlife International.
- Bjorndal, K. (1997). Foraging Ecology and Nutrition (Chpt.8). The Biology of Sea Turtles I. P. L. Lutz and J. A. Musick. Boca Raton, USA, CRC Press. **I**: 199-231.
- Bjorndal, K. A. (2003). Roles of loggerhead sea turtles in marine ecosystems. Chapter 15. Loggerhead sea turtles. A. B. Bolten and B. E. Witherington. Washington, D.C., USA, Smithsonian Institution Press: 235-254.
- Bjorndal, K. A. and A. B. Bolten (2010). "Hawksbill sea turtles in seagrass pastures: success in a peripheral habitat." Marine Biology **157**(1): 135-145.
- Bjorndal, K. A., A. B. Bolten and H. R. Martins (2000). "Somatic growth model of juvenile loggerhead sea turtles *Caretta caretta*: duration of pelagic stage." Marine Ecology Progress Series **202**: 265-272.
- Bjorndal, K. A., A. B. Bolten, T. Dellinger, C. Delgado and H. R. Martins (2003). "Compensatory growth in oceanic loggerhead sea turtles: response to a stochastic environment." Ecology **84** (5): 1237-1249.
- Bolten, A. B. (2003). Active swimmers - passive drifters: the oceanic juvenile stage of loggerheads in the Atlantic system. Chapter 4. Loggerhead sea turtles. A. B. Bolten and B. E. Witherington. Washington, D.C., USA, Smithsonian Institution Press: 63-78.
- Bolten, A. B., K. A. Bjorndal, H. R. Martins, T. Dellinger, M. J. Biscoito, S. E. Encalada and B. W. Bowen (1998). "Transatlantic developmental migrations of loggerhead sea

- turtles demonstrated by mtDNA sequence analysis." Ecological Applications **8**(1): 1-7.
- Bonadonna, F., S. Caro, P. Jouventin and G. A. Nevitt (2006). "Evidence that blue petrel, *Halobaena caerulea*, fledglings can detect and orient to dimethyl sulfide." Journal of Experimental Biology **209**(11): 2165-2169.
- Brongersma, L. D. (1968). "Notes upon some turtles from the Canary Islands and from Madeira." Koninkl. Nederl. Akademie van Wetenschappen Amsterdam, Proceedings **C 71**(2): 128-136.
- Brongersma, L. D. (1972). "European Atlantic turtles." Zoologische Verhandelingen **121**: 1-318.
- Brongersma, L. D. (1982). Marine turtles of the eastern Atlantic Ocean. Biology and Conservation of Sea Turtles. K. A. Bjorndal. Washington, D.C., Smithsonian Institution Press: 407-416.
- Bürgermeister, S., Zimmermann, R. L., Georgii, H.W., Bingemer, H. G., Kirst, G. O., Janssen, M. and Ernst, W. (1990). "On the biogenic origin of dimethylsulfide: relation between chlorophyll, ATP, organismic DMSP, phytoplankton species, and DMS distribution in Atlantic surface and atmosphere." J. Geophys. Res. **95**: 20 607 – 20 615.
- Burke, V. J. and E. A. Standora (1993). "Diet of juvenile Kemp's Ridley and loggerhead sea turtles from Long Island, New York." Copeia **1993**(4): 1176-1180.
- Caldeira, R.M.A. & Lekou, S. (2000). Madeira, um Oásis no Atlântico - Um introdução aos estudos oceanográficos no Arquipélago da Madeira. Direcção Regional de Formação Profissional, Funchal, Madeira, Portugal.
- Carr, A. (1986). New perspectives on the pelagic stage of sea turtle development. NOAA Technical Memorandum. NMFS-SEFC-190: 35.
- Carr, A. (1987). "New Perspectives on the Pelagic Stage of sea turtle development." Conservation Biology **1**: 103-121.
- Casale, P., G. Abbate, D. Freggi, N. Conte, M. Oliverio and R. Argano (2008). "Foraging ecology of loggerhead sea turtles *Caretta caretta* in the central Mediterranean Sea: evidence for a relaxed life history model." Marine Ecology-Progress Series **372**: 265-276.

- Cunningham, G. B., V. Strauss and P. G. Ryan (2008). "African penguins (*Spheniscus demersus*) can detect dimethyl sulphide, a prey-related odour." Journal of Experimental Biology **211**(19): 3123-3127.
- Cunningham, G. B., R. W. Van Buskirk, F. Bonadonna, H. Weimerskirch and G. A. Nevitt (2003). "A comparison of the olfactory abilities of three species of procellariiform chicks." Journal of Experimental Biology **206**(10): 1615-1620.
- Dacey, J. W. H. and S. G. Wakeham (1986). "Oceanic Dimethylsulfide - Production During Zooplankton Grazing on Phytoplankton." Science **233**(4770): 1314-1316.
- Dellinger, T., J. Davenport and P. Wirtz (1997). "Comparisons of social structure of Columbus crabs living on loggerhead sea turtles and inanimate flotsam." Journal of the Marine Biological Association of the United Kingdom **77**: 185-194.
- Dellinger, T. 2000. Conservation support project for North Atlantic *Caretta caretta** sea turtles - Life Nature Project contract no. B4-3200/96/541 (Life96Nat/P/3019). Final Technical Activity Report. CITMA, Funchal, pp. 56.
- Dellinger, T. and H. Encarnação (2000). Accidental capture of sea turtles by the fishing fleet based at Madeira Island, Portugal. Proceedings of the Nineteenth Annual Symposium on Sea Turtle Biology and Conservation, South Padre Island, Texas, USA, NOAA Technical Memorandum NMFS-SEFSC-443.
- Dellinger, T. and C. Freitas (2000). Movements and diving behaviour of pelagic stage loggerhead sea turtles in the North Atlantic: preliminary results obtained through satellite telemetry. Proceedings of the Nineteenth Annual Symposium on Sea Turtle Biology and Conservation, South Padre Island, Texas, USA, NOAA Technical Memorandum NMFS-SEFSC-443.
- Dellinger, T. (2008). Tartarugas marinhas. Atlas dos Anfíbios e Répteis de Portugal. A. Loureiro, N. Ferrand de Almeida, M. A. Carretero and O. S. Paulo. Lisboa, Instituto da Conservação da Natureza e Biodiversidade: 193-209.
- Endres, C. S., N. F. Putman and K. J. Lohmann (2009). "Perception of airborne odors by loggerhead sea turtles." Journal of Experimental Biology **212**(23): 3823-3827.
- FAO, F. D., Ed. (2009). Guidelines to reduce sea turtle mortality in fishing operations. Rome.
- Gaffney, E. S. (1990). "The Comparative Osteology of the Triassic Turtle *Proganochelys*." Bulletin of the American Museum of Natural History(194): 5-263.

- Geldmacher, J., Bogaard, P., Hoernle, K. & Schmincke, H. 2000. "The $^{40}\text{Ar}/^{39}\text{Ar}$ age dating of the Madeira Archipelago and hotspot track (eastern North Atlantic)." Geochem. Geophys. Geosyst. **1**, 109-122.
- Grassman, M. and D. Owens (1981). "Evidence of Olfactory Imprinting in Loggerhead Turtles." Marine Turtle Newsletter **19**: 7-10.
- Grassman, M. A. and D. W. Owens (1982). "Development and Extinction of Food Preferences in the Loggerhead Sea Turtle, *Caretta-Caretta*." Copeia(4): 965-969.
- Grassman, M. and D. Owens (1987). "Chemosensory Imprinting in Juvenile Green Sea-Turtles, *Chelonia-Mydas*." Animal Behaviour **35**: 929-931.
- Hirayama, R. (1998). "Oldest known sea turtle." Nature **392**(6677): 705-708.
- IUCN (2010). Red List of Threatened Species. Version 2010.1.
- Kamezaki, N. (2003). What is a loggerhead turtle? The morphological perspective. Chapter 2. Loggerhead sea turtles. A. B. Bolten and B. E. Witherington. Washington, D.C., USA, Smithsonian Institution Press: 28-43.
- Kettle, A. J., Andreae, M. O., Amouroux, D., Andreae, T. W., Bates, T. S., Berresheim, H., Bingemer, H., Boniforti, R., Curran, M. A. J., DiTullio, G. R. et al. (1999). "A global database of sea surface dimethylsulfide (DMS) measurements and a procedure to predict sea surface DMS as a function of latitude, longitude, and month." Global Biogeochem. Cycles **13**: 399-444.
- Kinsky, F. C. (1960). "The yearly cycle of the Northern Blue Penguin (*Eudyptula minor novaehollandiae*) in the Wellington harbour area." Rec. Dom. Mus. Wellington **3**: 145-218.
- Kowalewsky, S., M. Dambach, B. Mauck and G. Dehnhardt (2006). "High olfactory sensitivity for dimethyl sulphide in harbour seals." Biology Letters **2**(1): 106-109.
- Li, C., X.-C. Wu, O. Rieppel, L.-T. Wang and L.-J. Zhao (2008). "An ancestral turtle from the Late Triassic of southwestern China." Nature **456**: 497-501.
- Lohmann, K. J. and C. M. F. Lohmann (1996). "Orientation and open-sea navigation in sea turtles." Journal of Experimental Biology **199**(1): 73-81.
- Lohmann, K. J., C. M. F. Lohmann and C. S. Endres (2008). "The sensory ecology of ocean navigation." Journal of Experimental Biology **211**(11): 1719-1728.

- Lohmann, K. J., B. E. Witherington, C. M. F. Lohmann and M. Salmon (1997). Orientation, navigation, and natal beach homing in sea turtles (Chpt. 5). The Biology of Sea Turtles I. P. L. Lutz and J. A. Musick. Boca Raton, USA, CRC Press. I: 107-136.
- Lutz, P. L. and J. A. Musick, Eds. (1997). The Biology of Sea Turtles I. Boca Raton, USA, CRC Press.
- Mann, K. H. and L. J. R. N. (2006). Dynamics of marine ecosystems: biological-physical interactions in the oceans. Oxford, Blackwell Publishing.
- Manton, M., A. Karr and Ehrenfeld, D. W. (1972a). "Chemoreception in Migratory Sea Turtle, *Chelonia-Mydas*." Biological Bulletin **143**(1): 184-195.
- Manton, M. L., Ehrenfeld, D. W. and A. Karr (1972b). "Operant Method for Study of Chemoreception in Green Turtle, *Chelonia-Mydas*." Brain Behavior and Evolution **5**(2-3): 188-&.
- Márquez Millán, R. (1990). "Sea turtles of the World: an annotated and illustrated catalogue of sea turtle species known to date. FAO species catalogue Vol. 11." FAO Fisheries Synopsis **125**(11): 1-81.
- Martin, P. and P. Bateson (1994). Measuring Behaviour - An introduction guide. Cambridge, Press Syndicate of the University of Cambridge.
- Nevitt, G. (1999a). "Foraging by seabirds on an olfactory landscape." American Scientist **87**(1): 46-53.
- Nevitt, G. (1999b). "Olfactory foraging in Antarctic seabirds: a species-specific attraction to krill odors." Marine Ecology-Progress Series **177**: 235-241.
- Nevitt, G. A. (2000). "Olfactory foraging by Antarctic procellariiform seabirds: Life at high Reynolds numbers." Biological Bulletin **198**(2): 245-253.
- Nevitt, G. A. (2008). "Sensory ecology on the high seas: the odor world of the procellariiform seabirds." Journal of Experimental Biology **211**(11): 1706-1713.
- Nevitt, G. A. and F. Bonadonna (2005). "Sensitivity to dimethyl sulphide suggests a mechanism for olfactory navigation by seabirds." Biology Letters **1**(3): 303-305.
- Nevitt, G. A. and K. Haberman (2003). "Behavioral attraction of Leach's storm-petrels (*Oceanodroma leucorhoa*) to dimethyl sulfide." Journal of Experimental Biology **206**(9): 1497-1501.
- Nevitt, G. A., R. R. Veit and P. Kareiva (1995). "Dimethyl Sulfide as a Foraging Cue for Antarctic Procellariiform Seabirds." Nature **376**(6542): 680-682.

- Oliveira, M. E., (coord.), J. C. Brito, T. Dellinger, N. Ferrand de Almeida, A. Loureiro, H. R. Martins, J. Pargana, O. S. Paulo, P. Rito and J. Teixeira (2005). Tartaruga-comum *Caretta caretta* (Linnaeus, 1758). Livro Vermelho dos Vertebrados de Portugal. M. J. Cabral, (coord.), J. Almeida, P. R. Almeida et al. Lisboa, Instituto da Conservação da Natureza: 123-124.
- Owens, D., D. C. Commuzie, M. Grassman and M.-S. D. e. S. R. M. e. Duvall D. (1986). "Chemoreception in the homing and orientation behavior of amphibians and reptiles, with special reference to sea turtles." Chemical Signals in Vertebrates IV: 341-355.
- Parker, D. M., W. J. Cooke and G. H. Balazs (2005). "Diet of oceanic loggerhead sea turtles (*Caretta caretta*) in the central North Pacific." Fishery Bulletin **103**(1): 142-152.
- Piovano, S., E. Balletto, S. DiMarco, A. Dominici, C. Giacomina and A. Zannetti (2004). "Loggerhead turtle (*Caretta caretta*) bycatches on longlines: the importance of olfactory stimuli." Ital J Zool **2**: 213-216.
- Plotkin, P. T. (1996). "Occurrence and diet of juvenile loggerhead sea turtles, *Caretta caretta*, in the northwestern Gulf of Mexico." Chelonian Conservation and Biology: 78-80.
- Plotkin, P. T., M. K. Wicksten and A. F. Amos (1993). "Feeding ecology of the loggerhead sea turtle *Caretta caretta* in the northwestern Gulf of Mexico." Marine Biology **115**(1): 1-5.
- Revelles, M., L. Cardona, A. Aguilar and G. Fernandez (2007). "The diet of pelagic loggerhead sea turtles (*Caretta caretta*) off the Balearic archipelago (western Mediterranean): relevance of long-line baits." Journal of the Marine Biological Association of the United Kingdom **87**(3): 805-813.
- Rozhok, A. (2008). Orientation and Navigation in Vertebrates, Springer Berlin Heidelberg
- Saito, K., T. Shoji, I. Uchida and H. Ueda (2000). "Structure of the olfactory and vomeronasal epithelia in the loggerhead turtle *Caretta caretta*." Fisheries Science **66**(2): 409-411.
- Scarratt, M. G., M. Levasseur, S. Michaud, G. Cantin, G. M. and S. J. de Mora (2002). "Influence of phytoplankton taxonomic profile on the distribution of dimethylsulfide and dimethylsulfoniopropionate in the northwest Atlantic." Mar. Ecol. Prog. **244**: 49-61.

- Scarratt, M. G., M. Levasseur, S. Michaud and S. Roy (2007). "DMSP and DMS in the Northwest Atlantic: Late-summer distributions, production rates and sea-air fluxes." Aquatic Sciences **69**(3): 292-304.
- Shannon, L. J. and Crawford, R. J. M. (1999). "Management of the African Penguin (*Spheniscus demersus*) – insights from modeling. *Mar. Ornithol.* **27**: 119-128.
- Southwood, A., K. Fritches, R. Brill and Y. Swimmer (2008). "Review: Sound, chemical, and light detection in sea turtles and pelagic fishes: sensory-based approaches to bycatch reduction in longline fisheries." Endang Species Res **5**(2-3): 225-238.
- Tomás, J., F. J. Aznar and J. A. Raga (2001). "Feeding ecology of the loggerhead turtle *Caretta caretta* in the western Mediterranean." Journal of Zoology, London **255**: 525-532.
- van Nierop, M. M. and J. C. den Hartog (1984). "A study on the gut contents of five juvenile loggerhead turtles, *Caretta caretta* (Linnaeus)(Reptilia, Cheloniidae), from the south-eastern part of the North Atlantic Ocean, with emphasis on Coelenterate identification." Zoologische Mededelingen **59**(4): 35-54.
- Walker, W. F. (1959). "Closure of the nostrils in the Atlantic loggerhead and other sea turtles." Copeia **3**: 257-259.
- Zar, J. H. (1998). Biostatistical Analysis New Jersey, Prentice Hall.

7. ANNEX I - Experiments Data

Experiments	Turtle ID	Date	DMS concentration (mmol / L)	Control Compartment	DMS Compartment
1	1735	14-09-2009	67	1	4
2	1735	14-09-2009	135	4	1
3	1735	14-09-2009	270	2	3
4	1735	18-09-2009	67	2	3
5	1735	18-09-2009	135	3	2
6	1735	18-09-2009	270	4	1
7	1735	12-10-2009	67	4	1
8	1735	12-10-2009	135	1	4
9	1735	12-10-2009	270	2	3
10	1740	14-09-2009	67	3	2
11	1740	14-09-2009	135	2	3
12	1740	14-09-2009	270	1	4
13	1740	18-09-2009	67	4	1
14	1740	18-09-2009	135	1	4
15	1740	18-09-2009	270	2	3
16	1740	09-10-2009	67	2	3
17	1740	09-10-2009	135	3	2
18	1740	09-10-2009	270	4	1
19	1742	12-09-2009	67	3	2
20	1742	12-09-2009	135	2	3
21	1742	12-09-2009	270	4	1
22	1742	15-09-2009	67	2	3
23	1742	15-09-2009	135	3	2
24	1742	15-09-2009	270	1	4
25	1742	19-09-2009	67	4	1
26	1742	19-09-2009	135	1	4
27	1742	19-09-2009	270	2	3
28	1744	12-09-2009	67	4	1
29	1744	12-09-2009	135	1	4
30	1744	12-09-2009	270	3	2
31	1744	15-09-2009	67	1	4
32	1744	15-09-2009	135	4	1
33	1744	15-09-2009	270	2	3
34	1744	19-09-2009	67	2	3
35	1744	19-09-2009	135	3	2
36	1744	19-09-2009	270	4	1

8. ANNEX II – Example of a Data Sheet used in Airborne Chemoreception Experiments

Date ___ / ___ / _____

Start ___h ___min

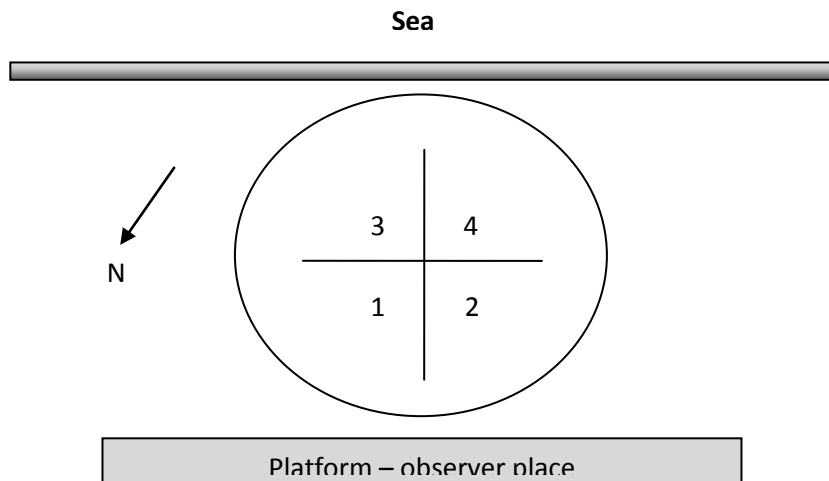
End ___h ___min

Sea Turtle Name / ID Number _____ / _____

Concentration of the DMS solution _____

DMS Compartment (1 - 4) ____

Control Compartment (1 - 4) ____



Experiment Period: 20 minutes

Compartment 1		Compartment 2		Compartment 3		Compartment 4	

9. ANNEX III – Bird Sightings in Sea Experiments

Bird sightings in sea experiments (8 August 2009)

Hour	Quantity	Common Name
13h22	1	Cory's shearwater
13h23	1	Cory's shearwater
13h25	2	Cory's shearwater
	1	Bulwer's Petrel
13h30	1	Bulwer's Petrel
13h31	1	Cory's shearwater
13h33	1	Cory's shearwater
	1	Bulwer's Petrel
13h34	1	Bulwer's Petrel
13h35	1	Cory's shearwater
13h36	2	Cory's shearwater
13h37	2	Bulwer's Petrel
13h41	1	Cory's shearwater
13h42	3	Cory's shearwater
13h55	2	Bulwer's Petrel
13h57	1	Bulwer's Petrel
14h01	1	Cory's shearwater
14h02	1	Cory's shearwater
14h03	1	Bulwer's Petrel
14h04	1	Bulwer's Petrel
14h05	1	Common tern
14h13	1	Yellow-legged Gull

Bird sightings in sea experiments (11 September 2009)

Hour	Quantity	Common Name
12h47	1	Cory's shearwater
12h51	1	Bulwer's Petrel
13h21	1	Cory's shearwater

Bird sightings in sea experiments (29 September 2009)

Hour	Quantity	Common Name
12h49	1	Cory's shearwater
13h01	2	Cory's shearwater



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