CHAPTER ONE

Physiological traits of the Greenland shark *Somniosus microcephalus* obtained during the TUNU-Expeditions to Northeast Greenland

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1.1 Introduction

Arctic regions are inhabited by cold-adapted stenothermal or eurythermal species. Unlike in the Antarctic, eurythermal species predominate, because of opportunities for migrations to temperate latitudes. In the Antarctic sea, the modern chondrichthyan genera are scarcely represented. In contrast, in the Arctic, sharks and skates are present with about 8% of the species (Mecklenburg et al., 2011; Lynghammar et al., 2013). The distribution of the Greenland shark *Somniosus microcephalus* is quite wide; in fact, this species typically thrives in deep and extremely cold waters, seasonally covered by sea ice (MacNeil et al., 2012), but is also known to enter more temperate waters in the North Atlantic (Bigelow & Schroeder, 1948; Skomal & Benz, 2004; Campana et al. 2015). Widespread climate changes in the arctic ecosystem have led to increased attention on trophic dynamics and on the role of this apex predator in the structure of arctic marine food webs (MacNeil et al., 2012).

The Greenland shark (Bloch & Schneider, 1801; Campana et al. 2015) (Squaliformes, Somniosidae) is distributed across the North Atlantic and the adjacent Arctic from surface waters to >2900-m depth (Porteiro et al., 2017; Mecklenburg et al., 2018). It is among the largest fish species known with a verified total length (TL) of 375 cm for males and 550 cm for females (Campana et al., 2015). The Greenland shark is considered an scavenger and predator that eats practically anything, including marine mammals (Nielsen et al., 2014). But recent studies also suggest that Greenland shark may hunt actively (Leclerc et al., 2012) with an ontogenetic shift in prey from cephalopods for juveniles to fishes and seals for adults (Nielsen et al., 2019).

The Greenland shark has received increasing scientific attention because its life history, abundance and demography are largely unknown (MacNeil et al., 2012). Climate change likely affects the fishes of the North Atlantic and the Arctic Seas, and among them, the Greenland shark. Moreover, the species often turns up as unaccounted bycatch and is viewed as a nuisance in commercial bottom longline fisheries. The Greenland shark is categorised as 'near threatened' (NT) in the Red List of the International Union for Conservation of Nature (IUCN, www.iucnredlist.org/). In the Norwegian Red List (2015, www.artsdatabanken.no/) the given status is 'data deficient' (DD), flagging the precautionary principle and raising pertinent conservation issues and questions about the species' ecological resilience and physiological adaptation.

The ongoing TUNU-Programme (since 2002) at UiT The Arctic University of Norway primarily investigates the diversity and adaptation of arctic marine fishes and so conducts regular expeditions to the fjords and shelves in Northeast Greenland (Christiansen, 2012). In 2010, TUNU became affiliated with the independent Old & Cold Project on the biology

of the Greenland shark (headed by John F. Steffensen at the University of Copenhagen, http://bioold.science.ku.dk/jfsteffensen/OldAndCold/). Within TUNU, the Greenland shark has become the target of joint investigations, including migration and genetic structuring, life history, physiology, organotropism and age/gender difference of trace elements and organic pollutants, and sensory capability. In 2010, the first three animals from Northeast Greenland were sampled. The following TUNU-Expeditions in 2013 and 2015 landed an additional eight specimens – altogether 11 animals (Table 1.1).

The specimen from Peters Bugt (#5) is of particular interest (Table 1.1). The core of the eye lens disclosed an independent radiocarbon marker depicting the onset of the bomb pulse from the atmospheric nuclear tests that took place around year 1960 (Campana et al., 2002; Nielsen et al. 2016). In other words, this particular specimen could be aged to about 50 years at the time of capture in 2013. This finding ignited the Greenland shark chronology from which the age of larger and presumably older animals could be estimated. The Greenland shark attracted worldwide attention in the media and gained iconic status when a Science-paper gave age estimates for the largest animals (TL: c. 500 cm) of at least 272 years – i.e. the oldest among vertebrates (Nielsen et al., 2016).

Recent studies provide new information about the diet and trophic relationships of the Greenland shark (Nielsen et al., 2019), and its whereabouts in time

Table 1.1 *Greenland sharks* Somniosus microcephalus *obtained from scientific longlines during the TUNU-Expeditions and sampled for organs and tissues. N/A indicates that data are not available due to partly cannibalised animals. During TUNU-V, two additional animals were equipped with archival pop-up tags (PSAT) and released*

Animal	Expedition	Date	Location	Latitude N	Longitude W	Depth (m)	Sex	Total length (cm)
1	TUNU-IV	11 Aug 10	Ella Ø	72.47	24.40	442	M	290
2	(2010)	12 Aug 10	Rødsten	73.26	23.48	473	N/A	N/A
3		12 Aug 10	Rødsten	73.26	23.48	473	F	330
4	TUNU-V	13 Aug 13	Kap Herschell	74.05	19.32	320	F	320
	(2013)							
5		14 Aug 13	Peters Bugt	75.16	20.21	325	F	220
6	TUNU-VI	9 Aug 15	Shelf	74.39	13.55	395	M	291
7	(2015)	9 Aug 15	Shelf	74.39	13.55	395	M	266
8		11 Aug 15	Shelf	75.04	12.42	377	M	271
9		11 Aug 15	Shelf	75.04	12.42	377	F	322
10		11 Aug 15	Shelf	75.04	12.42	377	F	320
11		11 Aug 15	Shelf	75.04	12.42	377	N/A	N/A

and space from satellite tracking (Campana et al., 2015; Hussey et al., 2018; Fisk et al. 2012). A recent review addresses main knowledge gaps, especially reproduction biology such as generation span (age-at-maturity estimates >134 years, Nielsen et al., 2016), Nielsen et al. in press

Physiological studies on the metabolic capacity of the Greenland shark have just started to emerge (Costantini et al., 2016; Herbert et al., 2017; Shadwick et al., 2018), but further physiological insights are imperative both from *in vitro* assays on tissues and from tests on live animals. In light of the extreme life span of the Greenland shark, we sampled a range of organs and tissues from the TUNU animals (Table 1.1), and here address little known physiological traits such as structure and function of haemoglobins (Hbs), sensory capability from anatomical proxies and tissue-specific loads of pollutants. Thus, this contribution mostly deals with oxygen transport: Hbs and ligand-binding properties, ecotoxicology of trace elements and organic contaminants, and sensory capability: the sense of olfaction.

1.2 Oxygen transport: haemoglobins and ligand-binding properties

The evolutionary success of elasmobranchs has raised considerable interest in their respiratory control mechanisms (Butler & Metcalfe, 1988). Structural information on Hbs, unlike in polar teleosts, is scarce. Erythrocytes are larger than those of most vertebrates, which may limit the efficiency of the oxygen-transport system.

Herewith we summarise the structural and functional properties of the Hb system of *S. microcephalus*, with regard to Hb oxygen-binding properties and their modulation by physiological effectors. Hbs have also been structurally characterised by a combination of spectroscopic techniques (UV-Vis and resonance Raman spectroscopy, autoxidation kinetics and CO-rebinding kinetics) to gain information on the heme cavity, heme oxidation and coordination states. This part is described in detail in Russo et al. (2017), in which all experimental details are outlined.

Shark Hb systems see the occurrence of Hb isoforms (Manwell & Baker, 1970; Fyhn & Sullivan, 1975). The erythrocytes of the Greenland shark (Russo et al., 2017) contain three major Hbs, made of two copies of the same α chain combined with two copies of three β chains (β^1, β^2 and β^3), with very similar primary structures (Figure 1.1).

The chain compositions of Hb 1, Hb 2 and Hb 3 are $(\alpha\beta^1)_2$, $(\alpha\beta^2)_2$ and $(\alpha\beta^3)_2$, respectively. The α chain has 141 residues (Figure 1.1a). The β chains have 142 residues (Figure 1.1b). The sequence identity between the β chains approaches 100%. Despite separation by ion-exchange chromatography, β^1 and β^2 appear identical, but the sequences have portions in the CD corner and helix F that could not be sequenced, and some differences may be placed in these portions.

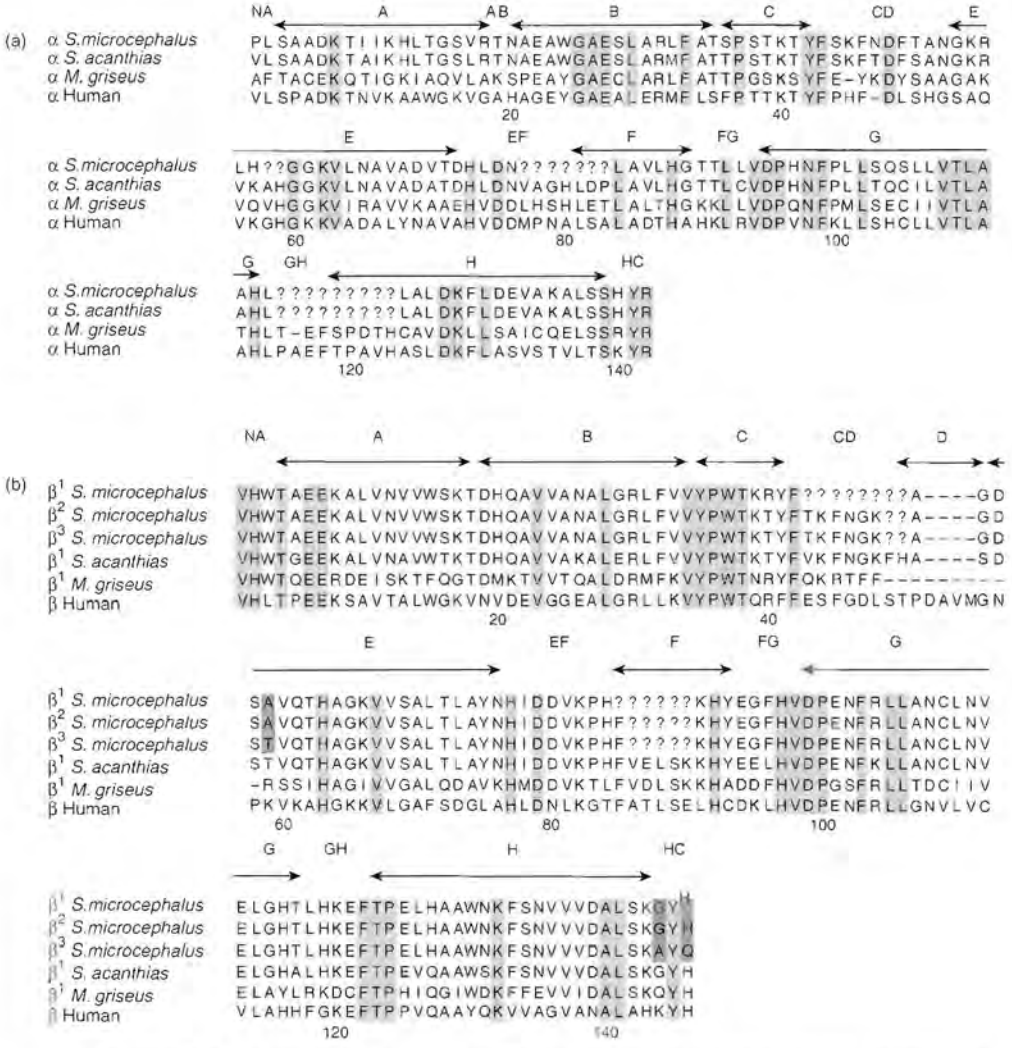

Figure 1.1 Amino acid sequence alignment of the α (a) and β (b) chains of *Somniosus microcephalus* (Greenland shark) Hbs with *Squalus acanthias*, *Mustelus griseus* and *Homo sapiens* α and β chains. Identical residues are in grey; different residues in the β globins of Greenland shark are in dark grey. The chains were aligned using Clustal OMEGA; in the β chains of *S. microcephalus* the position of residues in CD and D have been manually aligned with the sequences of *M. griseus* (Naoi et al., 2001), *S. acanthias* (Aschauer et al., 1985) and HbA (accession numbers: P69905 for α chain and P68871 for β chain). The question mark indicates unsequenced regions; dashes indicate deletions.

The sequence identity of Greenland shark Hbs and human HbA is 50% for the α chain and between 44 and 47% for the β chains, with many replacements of functionally important residues. Compared to other sharks, the globin

chains of the *Somniosus microcephalus* (Greenland shark) have much higher identity with those of *S. acanthias* than those of *M. griseus*. The highly conserved C-terminal sequence Tyr-Arg is found in α chains. In all β chains, the four residues of helix D are missing. The lack of β helix D is a feature that distinguishes cartilaginous from teleost Hbs.

The Hbs of several polar skates and sharks (Nash et al., 1976; Fisher et al., 1977; Aschauer et al., 1985; Naoi et al., 2001) and of a temperate skate (Chong et al., 1999) do not display β helix D. Site-directed mutagenesis suggested that this deletion is a neutral modification, neither exerting large functional effect(s) on oxygen binding nor affecting the assembly of cooperative tetramers (Komiyama et al., 1991). The high number of His in Greenland shark Hbs confirms the hypothesis that a high Hb buffer capacity was ancestral in all jawed vertebrates, similar to contemporary elasmobranchs (Berenbrink et al., 2005).

The isoforms display similar Bohr effects (Table 1.2). The Bohr coefficient φ ($\Delta \log P_{50}/\Delta pH$), i.e. the mean number of protons released upon heme oxygenation, is higher in the presence of ATP (around −0.20 without ATP, −0.60 with ATP), with oxygen-linked dissociation of ~0.8 and ~2.0 protons in the absence and presence of ATP, respectively, *per* Hb tetramer. The enhancement by ATP is high in all Greenland shark Hbs, indicating that binding of negatively charged ATP to the low-affinity conformation enhances H^+ uptake.

Greenland shark Hbs show P_{50} values similar to *M. griseus* Hb at pH 7.4 and 6.7 without ATP, under the same conditions (Naoi et al., 2001). P_{50} values of Greenland shark Hbs at 0°C, calculated by extrapolation from vant'Hoff plots (Fago et al., 1997), show the same behaviour as those at 15°C and 25°C, in the absence and presence of ATP, indicating that the oxygen affinity increases with decreasing temperatures up to the physiological conditions of cold waters.

The higher Bohr effect in the presence of ATP found in Greenland shark Hbs in comparison to temperate sharks suggests evolution of molecular adaptations. In Greenland shark Hb 1 and Hb 2, HisβHC3 and LysαC5 are conserved; Hb 3 has Gln at position βHC3, whereas AspβFG1 is replaced by Glu, as in *M. griseus* Hb (Naoi et al., 2001). Valβ1 and Hisβ2, which contribute to the Bohr effect in adult human Hb (HbA) together with HisβHC3, forming salt bridges with Asp94βFG1 and LysαC5 (Perutz, 1998), are also present in the Greenland shark (Figure 1.1b). These residues are present as well in *S. acanthias* and *M. griseus* Hbs, which however show a smaller Bohr effect and weaker cooperativity (Aschauer et al., 1985; Naoi et al., 2001).

The effect of ATP on the oxygenation of Greenland shark Hbs differs from that of the two polar rays *B. eatonii* and *R. hyperborea* (Verde et al., 2005), which show no Bohr effect and ATP sensitivity. The model of the

Table 1.2 *Values of* P_{50}, n_H, ΔH, φ *of* S. microcephalus *Hbs (1 kcal = 4.184 kJ)*

			P_{50} (Torr)		n_H		ΔH^a (kcal·mol^{-1})		φ
S. microcephalus Hbs	100 mM KCl	2 mM ATP	pH		pH		pH		pH
			7.4	6.7[b]	7.4	6.7[b]	7.4	6.7[b]	7.4–6.7[b]
25°C									
Hb 1	+	–	5.5	8.9	1.2	1.4	–7.9	–10.1	–0.2
	+	+	14.4	46.1	1.8	1.6	–0.7	–5.4	–0.5
Hb 2	+	–	5.7	8.6	1.6	1.9	–8.9	–8.5	–0.2
	+	+	12.5	40.8	1.8	1.6	–2.1	–6.6	–0.6
Hb 3	+	–	7.7	11.6	1.4	1.7	–8.4	–8.3	–0.2
	+	+	13.3	36.2	1.9	1.7	–0.9	–4.8	–0.6
	100 mM KCl	2 mM ATP	pH		pH			pH	
			7.4	6.8[c]	7.4	6.8[c]			7.4–6.8[b]
15°C									
Hb 1	+	–	2.9	4.1	1.5	1.7			–0.2
	+	+	11.6	28.2	2.1	1.3			–0.4
Hb 2	+	–	2.8	4.4	1.6	2.0			–0.2
	+	+	9.2	23.2	1.9	1.3			–0.4
Hb 3	+	-	3.9	6.0	1.8	1.7			–0.2
	+	+	11.0	22.9	2.0	1.7			–0.4
0°C[†]									
Hb 1	+	–	1.0	1.2					
	+	+	8.1	12.6					
Hb 2	+	–	0.9	1.5					
	+	+	5.6	9.3					
Hb 3	+	–	1.3	2.0					
	+	+	7.3	10.8					

[a] Extrapolated from LogP_{50} (measured at 15–25°C) versus 1/T.

[b] pH 6.08 for Hb 3 in the presence of ATP.

[c] pH 6.01 for Hb 3 in the presence of ATP.

P_{50}: O_2 partial pressure at 50% saturation; n_H, Hill coefficient; ΔH, enthalpy change; Φ, Bohr coefficient

Experimental errors are within 10%

ATP binding site in teleost Hbs (Perutz & Brunori, 1982; Perutz, 1983) indicates that Valβ1, Hisβ2, LysβEF6 and HisβH21, which bind 2,3-diphosphoglycerate (DPG) in human HbA, participate in ATP binding. These residues are conserved in Greenland shark Hbs, with the exception of HisβH21, replaced by Lys, consistent with the allosteric effect of ATP on oxygen affinity and Bohr effect. In *M. griseus* Hb, although the canonical binding site is not preserved, ATP works as allosteric effector by lowering the oxygen affinity and stabilising the T-state structure (Naoi et al., 2001).

Greenland shark Hbs show cooperativity at all pH values investigated and at both 15°C and 25°C, with n_H ranging from 1.2 to 2.0. Cooperativity increases in the presence of ATP.

Looking at the oxygen affinity between 15 and 25°C (Table 1.2), ΔH (which includes the heat of oxygen solubilisation and the heat of proton and anion dissociation, i.e. processes linked to oxygen binding) shows that the change of oxygenation is constant in the absence of ATP over the whole pH range explored (6.7–7.4), with the exception of Hb 1 for which it is more negative (i.e. oxygenation is more exothermic) at low pH. In the presence of ATP, the heat of oxygenation progressively decreases with decreasing pH, reflecting the endothermic contribution of the heterotropic effectors released upon oxygen binding. The data show that the oxygenation-enthalpy change in Greenland shark Hbs is lower than that of temperate fish Hbs and very similar to that of polar fish Hbs (di Prisco et al., 1991; Verde et al., 2006). The expression of Hbs with reduced ΔH seems a frequent evolutionary strategy of cold-adapted fish, resulting in improved oxygen release to tissues at low temperatures. The low temperature effect on oxygen affinity thus suggests that oxygen delivery may be facilitated by lower heat of oxygenation of Hb, as also reported in polar mammals (Weber & Campbell, 2011).

No important differences were observed in the oxygen-binding parameters in the absence and presence of urea (a very important compound in the physiology of marine elasmobranchs) either in terms of P_{50} or cooperativity. A cooperating effect of urea and ATP on the oxygen affinity has also been found in other elasmobranchs, e.g. the dogfish *S. acanthias* and the carpet shark *Cephaloscyllium isabella* (Weber et al., 1983a,1983b; Tetens & Wells, 1984).

The three isoforms of the Greenland shark display identical electronic absorption and resonance Raman spectra (Russo et al., 2017), indicating identical or highly similar heme-pocket structures.

Thus, the Hbs are functionally quite similar, in keeping with the high sequence identity, as seen in other elasmobranchs (Weber et al., 1983a),

suggesting microheterogeneity without major functional specialisation of isoforms associated with migrations within wide latitudinal ranges. Teleosts often exhibit multiplicity of Hbs that display structural and functional differences (Riggs, 1970; Gillen & Riggs, 1973). Multiplicity is usually interpreted as a sign of phylogenetic diversification and molecular adaptation, and generally is the result of gene-related heterogeneity and gene-duplication events (Dettaï et al., 2008; Giordano et al., 2010). However, in cartilaginous fishes, there is no evidence of the functional differentiation often found in teleosts, and no information on the functional consequences of Hb multiplicity is available for sharks. The absence of functional heterogeneity in Greenland shark Hbs is in keeping with the low tolerance of sharks to oxygen-pressure variation, and their low capability to maintain constant uptake rates as oxygen tension falls (Speers-Roesch et al., 2012b). The fact that they have very similar features also suggests that the shark may play regulatory control of oxygen transport at other levels. Alternatively, the isoforms may protect against deleterious mutational gene changes, thus providing higher total Hb concentration in the erythrocyte, and increasing the gene expression rate (Speers-Roesch et al., 2012a).

The very similar ligand-binding properties suggest that regulatory control of oxygen transport may be at the cellular level and may involve changes in the cellular concentrations of allosteric effectors and/or variations of other systemic factors. Compared to temperate sharks, the Hbs of the Greenland shark have evolved adaptive decreases in oxygen affinity, similar to that of some antarctic and sub-antarctic teleosts of the suborder Notothenioidei (di Prisco et al., 1991) and of the arctic fish *Arctogadus glacialis* (Verde et al., 2006), thus suggesting that the P_{50} values might be linked to the high oxygen concentration in cold waters, implying some adaptation to cold temperatures (di Prisco et al., 2007). In fact, these observations highlight an important difference from temperate cartilaginous Hbs, and, in contrast, some similarity between a polar shark and cold-adapted teleosts thriving in both polar environments.

Although amino acid sequences and ligand binding do not reveal special features associated with cold environmental conditions, the decrease in oxygen affinity evolved by the Hb isoforms of the Greenland shark may have adaptive implications. Physiological differences in oxygen transport between polar and temperate sharks, both dispersed across wide latitude and temperature gradients, may be governed at the physiological plasticity level.

1.3 Ecotoxicology of inorganic and organic contaminants

Despite the limited ecotoxicological data available, this species can be considered at high risk of bioaccumulating toxic and persistent organic

pollutants (POPs), endocrine-disrupting compounds (EDCs) and trace elements. Most of the organs were investigated for the presence of these contaminants. Studies were focused on assessing the contaminant organotropism and the differences between age classes, gender and sampling areas in Greenland seawaters (NE, E, S and W Greenland) (Corsolini et al., 2014, 2016; Ademollo et al., 2018; Cotronei et al.. 2018a, 2018b). POP levels were determined also in its prey to evaluate the biomagnification and related biomagnification factor (BMF), taking into account that shark prey such as cods and Greenland halibuts, seals and polar bears are all important food resources for the Greenland shark and they play a key ecological role in the POP transfer through the shark food web.

The dioxin-like compound (DLC) and Toxic Equivalent (TEQ) concentrations were also evaluated using the Toxic Equivalency Factors (TEF) method (Van den Berg et al., 1998, 2006) in order to assess the possible risk for the analysed specimens.

The study of this interesting and peculiar species may suggest speculations in the light of global change, being this shark is strictly dependent on deep and cold seawaters. For instance, the stomach content of specimens caught in 2015 in NE Greenland seawaters (74°–78°N, 5–14°W) during the TUNU VI Expedition (Nielsen et al., 2019) included Atlantic cod *Gadus morhua*, a boreal species distributed from temperate to sub-arctic seas (Righton et al., 2010); this cod was reported to move northward to more arctic seawaters following warming (Fossheim et al., 2015; Ingvaldsen et al., 2017). Therefore, the shark might be affected by global change by shifting its diet and migrations.

The longevity, predatory habits and general biological and ecological features make important the ecotoxicological study of the Greenland shark. In the peculiar arctic environment, due to low temperatures and winter darkness, contaminants degrade very slowly; moreover, they can be trapped in snow and ice and eventually be released during summer melting; once in the seawater, they can enter trophic webs and bioaccumulate in the tissues of organisms (Corsolini, 2009).

POPs include a list of a diverse class of synthetic and ubiquitous contaminants. Due to their persistence in the environment, they show long-range transport potency (LRTP) and can bioaccumulate in organisms where they can elicit toxic effects (SC-POPs, 2013). POPs have been used extensively worldwide as pesticides in agriculture and home applications, and in industry. Exposure to POPs can lead to serious health effects among humans and wildlife, and they occur in ecosystems worldwide including the arctic region (Muir & de Wit, 2010; Rigét et al., 2010). For

these reasons, the TEQs of most toxic chemicals were calculated by applying WHO1998 TEFs for fish (Van den Berg et al., 1998) and the most recent WHO2005 TEFs for mammals and humans (Van den Berg et al., 2006).

Some POPs are also EDCs, which include a wide range of both natural and synthetic (not only listed as POPs) chemicals that can adversely deregulate the hormone systems with multiple effects, mostly through steroid receptors on peptide/protein hormones. Most EDCs interfere with reproduction acting as either agonists or antagonists of the steroidal sex hormones, estrogens or androgens. In the literature, there are few papers on the distribution and transport of these EDCs in the arctic marine food webs and no such studies have been carried out on the Greenland shark.

Nonylphenols (NPs) are metabolites of nonylphenol ethoxylates (NPEOs), belonging to the group of non-ionic surfactants used as detergents, solubilisers, emulsifiers and dispersants. NPEOs are biodegraded during sewage treatment processes in wastewater treatment plants (WWTPs) and partially in the environment by the loss of the ethoxy-groups, resulting in NPs and other mono- and di-ethoxylate precursors (NP1EO, NP2EO), which are more toxic and oestrogenic than NPEOs (Soares et al., 2008). Once NPs and NPEOs enter aquatic systems, they can elicit an oestrogenic action against the reproductive system of aquatic organisms binding to oestrogen receptors and can block or alter endogenous endocrine functions in various reproductive and developmental stages, also affecting aromatase activity and the function of the aryl hydrocarbon receptor (AhR) (Hong et al., 2004). Bisphenol A (BPA) is an intermediate in the production of epoxy resins and polycarbonate plastics. NPs and BPA have multiple detrimental effects, such as endocrine disorder, developmental abnormalities and reproduction dysfunctions (Zhou et al., 2009). Over time, aquatic organisms are chronically exposed to synergistic effects between these compounds and other xenobiotics in a global change scenario that includes harvesting, habitat degeneration and competitive exclusion. The potential of endocrine disruption, even at low NP concentrations, is likely to be enhanced by additive or synergistic effects due to co-occurrence with other xenoestrogens (Soares et al., 2008) like some POPs are.

Metals, including trace elements, occur naturally and are released into the environment from anthropogenic sources as well. Climate-mediated shift in food web structure may also influence body burdens of primarily dietary-driven contaminants (e.g. mercury, Hg) in arctic food webs.

Despite concern over trace-element toxicity and the potential for biomagnification in arctic wildlife, very limited data are available on the Greenland shark (Dietz et al., 2000; McMeans et al., 2007). A number of essential and non-essential elements were analysed only in liver of Greenland sharks from Cumberland Sound (McMeans et al., 2007). A contribution to fill this data gap was published on the presence of non-essential and essential metals in several organs and tissues of specimens from NE Greenland (Corsolini et al., 2014).

Among POPs, EDCs and trace elements, the following contaminants were studied in the Greenland shark samples: aldrin, chlordanes (CHLs), dichloro diphenyl trichloroethane (DDT), dieldrin, endrin, hexachlorobenzene (HCB), mirex, heptachlor, toxaphenes, hexachlorocyclohexanes (HCHs), pentachlorobenzene (PeCB), polychlorobyphenyls (PCBs) including mono- and non-*ortho* substituted congeners, polychlorinated dibenzo-dioxins (PCDDs) and -furans (PCDFs), the flame retardants polybromodiphenylethers (PBDEs) and hexabromocyclododecane (HBCDs), perfluorooctane sulfonate (PFOS), and perfluorooctanoic acid (PFOA), 4-NP, its mono- (NP1EO) and di-ethoxylate (NP2EO) precursors and bisphenol A (BPA), cadmium (Cd), lead (Pb), mercury (Hg) and selenium (Se).

These chemicals were also investigated in sample tissues of Greenland sharks from E, S and W Greenland seawaters, collected during other expeditions, thanks to projects led by Prof. J.F. Steffensen (University of Copenhagen) and to the collaboration with Dr J. Nielsen (University of Copenhagen).

The main results of investigations on inorganic and organic contaminants in specimens of Greenland shark, with regard to organotropism and age and gender differences, are summarised here. All these results are discussed in detail in Corsolini et al., 2014, 2016; Cotronei et al., 2017, 2018a, 2018b; Ademollo et al., 2018; in which all experimental details are also described. Very briefly, the analyses of POPs were performed by gas-chromatography coupled to mass spectrometry and high-performance liquid chromatography with electrospray ionisation tandem mass spectrometry; the analyses of EDCs implied liquid chromatography coupled to mass spectrometry equipped with a fluorescence detector; graphite furnace atomic absorption spectrometry was used for Cd and Pb determination, cold vapor flow injection for total Hg, and graphite furnace atomic absorption coupled with hydride generation spectrometry for Se. Several tissues (red and white muscle, brain, gonads, adipose tissue, liver, pancreas, spleen, gonad and epidermis) of the shark specimens were analysed, and, prior to residue analyses, their moisture and lipid contents were measured (Corsolini et al., 2014), in order to normalise the inorganic and organic

contaminants to dry and lipid weight, respectively; these calculations are useful for comparisons. In Corsolini et al. (2014), the tissue lipid content showed high values (47±6%–24±2%, with brain > liver > gonad > spleen > red muscle > abdominal fat > white muscle), confirming it as an adaptation to cold seawaters in polar fish; moreover, the fatty liver helps buoyancy in the sharks (Malins & Barone, 1970). Interestingly, in the three specimens caught during the TUNU-IV Expedition (August 2010), the lipid content in the fat was lower than in liver, an unusual pattern already reported in the blue shark *Prionace glauca* (Kannan et al., 1996).

POPs used now or in the past were detected in the sharks from NE Greenland with variable concentrations depending on the tissue and contaminant. Results are reported in Corsolini et al. (2014, 2016): pesticides were detected in most tissues and specimens, except for Dieldrin and Aldrin (<0.001 ng/g wet weight (wet wt)); HCB was <0.001 ng/g in spleen and pancreas and HCHs were <0.002 ng/g wet wt in all tissues except brain and white muscle; DDTs (sum of *p,p'*- and *o,p'*-DDD, DDE, DDT isomers) were the most abundant chlorinated pesticides and concentrations ranged from 0.02 to 0.59 ng/g wet wt in pancreas and white muscle, respectively. Shark specimens collected in E, S and W Greenland seawaters showed 1095 ± 818 ng/g lipid wt in muscle and 761 ± 416 ng/g lipid wt in liver samples (Cotronei et al., 2018a,b). These findings confirm that Greenland sharks bioaccumulate DDTs available in the environment because of their long-range global transport from countries where DDT-based insecticides for controlling the malaria disease vector, the *Anopheles* sp. mosquitoes, have been or are used (SC-POPs, 2013). Alternatively, being this shark alive before POP global production and use started in 1940s, the detection of these chemicals may be due to low biotransformation activity of the parent compound *p,p'*-DDT to *p,p'*-DDE.

PCB concentrations in the shark tissues (2.01–65.6 ± 32.8 ng/g wet wt, Corsolini et al., 2014) were of the same order of magnitude as those reported by Molde et al. (2013) in the plasma of the Greenland shark from Svalbard (Norway), and lower than those detected in sharks from the NE Atlantic (Strid et al., 2007). Tetra-, penta- and hexa-CBs made up 65–75% of the total PCB residue and the class of isomer profile in the tissues was similar to that of Aroclor 1248, a technical mixture used in hydraulic fluids, lubricants, plasticisers and adhesives (IARC, 1979).

Brominated flame retardants were also studied in the Greenland sharks: HBCDs and PBDEs were detected in all tissues (Corsolini et al., 2016; Cotronei et al., 2017, 2018b). The γ-HBCD is the major component in the commercial mixtures, but α-HBCD made up 95% of the residue, likely due to its slow metabolism or to a possible bioisomerisation of all isomers mainly to α-HBCD (Janák et al., 2005). PBDEs were 426 pg/g wet wt in NE

Greenland specimens (Corsolini et al., 2016) and 20 ± 25 ng/g lipid wt in specimens from W, S and E Greenland (Cotronei et al., 2018b). BDE47 was the most abundant congener in sharks from all sampling areas and BDE99 was below 5%, although it is a major constituent of the technical Bromkal mixtures (Corsolini et al., 2016). These findings are in agreement with other authors (Strid et al., 2013) and suggest a debromination process may occur in this shark species, following the pattern BDE154→BDE99→BDE47 (Ikonomou et al., 2002). Levels differed significantly between muscle samples of sharks from NW and SE Greenland ($p < 0.05$); notwithstanding Greenland sharks migrate (Nielsen, 2018), the difference may be related to environmental levels as W and E Greenland are influenced by different water currents (Pedersen et al., 2004).

The biomagnification factors were calculated for most contaminants using predator-stomach content and predator–prey relationships and values ranged from 33 to 3892 for DDTs and PCBs and from 0.01 to 239 for flame retardants, confirming this shark is able to highly biomagnify POPs, and, for this reason, at risk. The risk assessment was performed using the TEF approach for the dioxin-like compounds (PCBs, PCDDs/DFs) (van den Berg et al., 1998, 2006) and TEQ values ranged from 28.23 to 45.15 pg/g lipid wt in sharks from E, S and W Greenland and 1.91–131 pg/g wet wt.

Although it is a top predator, pesticides, PCBs and PBDEs were lower than in the polar bear, the arctic apex predator (e.g. Gebbink, et al., 2008; Verreault et al., 2015).

Interestingly, younger specimens showed higher concentrations than older sharks and lipid content, sex and size were not significantly correlated to the POP concentrations ($p > 0.05$). Longevity may affect bioaccumulation more than diet, in a species where the detoxifying activity may be low: contaminants are transferred from mother to the next generation (Corsolini et al., 2014; Olin et al., 2014). Thus, at birth, offspring tissues already contain contaminants, which can be gradually diluted with body growth (Corsolini & Sarà, 2017), as the elimination rate may be more important than accumulation in a pristine environment, e.g. NE Greenland and deep-sea ecosystems. The presence of POPs in Greenland seawaters may be due to LRT and to release from the dump site located near villages: waste materials have been disposed of in dump sites located near each settlement and close to the sea for decades, and PCBs and other contaminants can be released from old equipment containing them. Contaminants can leach into the soil, beach and the sea, then entering the trophic webs. Concentrations in the Greenland shark from Greenland seawaters were generally lower in specimens collected in NE Greenland with respect to E, S and W (presence of settlements) Greenland seawaters,

and all levels were lower than in sharks from other areas (Figure 1.2) (Fisk et al., 2002; Strid et al., 2007, 2010, 2013; Molde et al., 2013); the presence of low levels in top predators from NE Greenland likely confirms this area to be among the most pristine regions of the northern hemisphere.

The EDC concentrations were higher in muscle than in liver samples of the sharks from SE and NE Greenland, and higher in liver than in muscles in those from W and SW Greenland. The 4-NP, NP1-2EO and BPA mean concentrations in liver of SW Greenland specimens were 43.5 ng/g, 288.5 ng/g and 8.2 ng/g wet wt, respectively; in muscle, the mean concentrations were 20.3 ng/g of 4-NP, 171.1 ng/g wet wt of NP1-2EO and 7.9 ng/g of BPA. Further details on concentrations are reported in Ademollo et al. (2018). Our results showed that, despite the highest lipophilicity of 4-NP, its ethoxylates were highly bioconcentrated in these sharks. These findings are in agreement with the evidence that NPEs are the main alkylphenols used in industry, accounting for about 90% of the global production, and that NP1-2EO are inefficiently removed in sewage treatment plants and very slowly degraded in the marine polar environments.

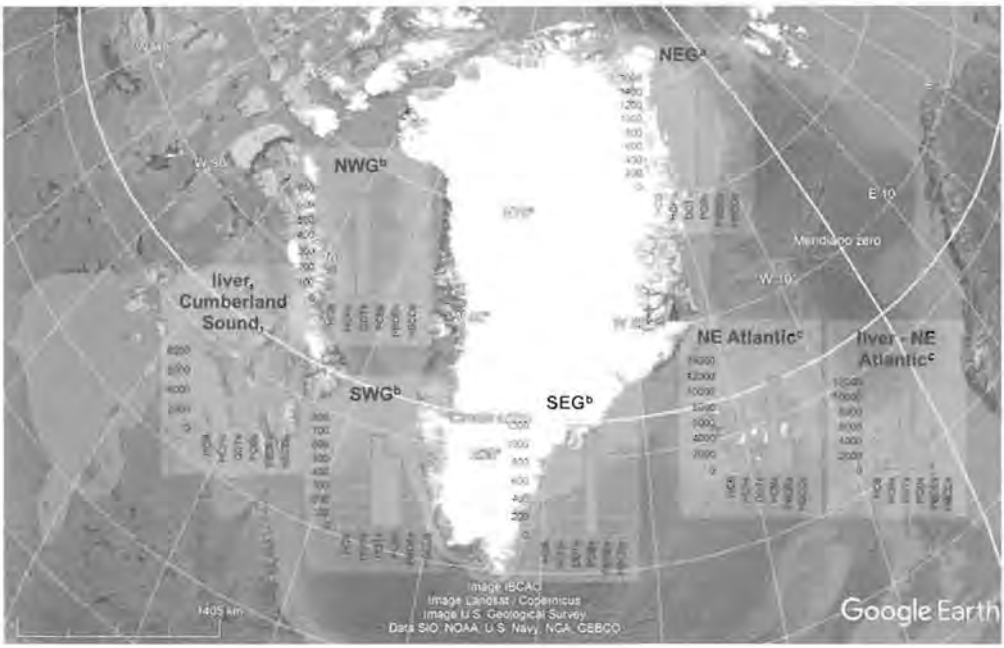

Figure 1.2 Average concentrations of HCB, HCHs, DDTs, PCBs, PBDEs and HBCDs (ng/g lipids) in Greenland shark white muscle (or liver where specified) samples from NE Greenland (NEG), NW Greenland (NWG), SW Greenland (SWG), SE Greenland (SEG), NE Atlantic and Cumberland Sound (data are from: a = Corsolini et al., 2014, 2016; b = Cotronei et al., 2017, 2018a, 2018b; c = Strid et al., 2007; d = Fisk et al., 2002). (A black and white version of this figure will appear in some formats. For the colour version, please refer to the plate section.)

The outcomes of this study suggest an ongoing input of NP1-2EO from human activities, especially from the North American arctic coasts.

The contamination pattern of organic contaminants in different tissues depends on several factors, such as age, gender, metabolism and diet (Corsolini et al., 2014), factors that may be more important than the sampling area of sharks. The liver is vital in detoxifying, storing and redistributing contaminants (Staniszewska et al., 2014), since it is well known that exposure to any contaminant will first result in metabolism by the liver, after which the compounds are stored in muscle (Mita et al., 2011). The usually high concentrations of these EDCs in the shark muscle could be explained by the fact that removal of these compounds from the liver occurs more rapidly with respect to other POPs. These compounds are metabolised and eliminated mostly via bile and faeces, but the high levels detected in muscle can be a result of continuous and chronic exposure. The high standard deviation values are owed to the intraspecific variability of EDC bioaccumulation depending on its opportunistic diet (MacNeil et al., 2012; Nielsen et al., 2014). The growth rate of this long-lived species and its reproductive characteristics are further factors affecting the bioaccumulation of contaminants.

The concentration and organotropism of the non-essential (Hg, Cd, Pb) and essential (Se) elements studied in the Greenland shark from NE Greenland are reported in Corsolini et al. (2014). Cd was found to be mainly accumulated in the liver (10.4±4.87 mg/kg dry wt), although the highest concentration was detected in the only sample of the pancreas (19.6 mg/kg dry wt). The order of Cd-organotropism was: pancreas > liver > spleen > brain > skin > red muscle > gonad > white muscle. The liver shows a particularly high capacity to biotransform pollutants (Klaassen, 1986). Kidney samples, usually rich in specific Cd-binding proteins (metallothioneins) like liver (Roesijadi, 1992), were not available for the analysis. The presence of Cd in marine food webs is related to a diet based on cephalopods, particularly at high latitudes (Bustamante et al., 1998). The Cd levels in the liver of the Greenland shark from NE Greenland were in agreement with data from the North Atlantic (McMeans et al., 2007) and showed values one order of magnitude higher than those reported for other shark species from lower latitudes (e.g. Storelli & Marcotrigiano, 2002). However, the diet of the Greenland shark is mainly based on fishes and mammals, and to a lesser extent on cephalopods and crustaceans (Fisk et al., 2002; Yano et al., 2007; McMeans et al., 2010; Nielsen et al., 2014).

The skin showed the highest Pb concentrations: 0.358±0.172 mg/kg. The order of Pb-organotropism was: skin > red muscle > pancreas > gonad > liver > spleen > white muscle > brain. The high Pb level in the skin may be a consequence of external contamination, as it may be adsorbed onto the skin surface rather than accumulate in the tissue itself: the dermal denticles

of the skin provide a rough surface to which particulate matter or sediment can attach, in agreement with studies on other shark species (Vas, 1991).

The highest Hg concentrations were found in red (6.91±3.65 mg/kg dry wt) and white (4.10±1.73 mg/kg dry wt) muscle; concentration in liver was 2.11 ±0.98 mg/kg dry wt. The Hg-organotropism was: red muscle > white muscle > spleen > pancreas > liver > gonad > brain > skin. High Hg concentrations are usually ascribed to a diet rich in fishes (Domi et al., 2005). Data on Hg levels in sharks are limited, and studies indicate a unique distribution pattern in the liver and muscle (Branco et al., 2007). Hg concentrations were higher in muscle than in the liver of immature tiger shark, *Galeocerdo cuvier*, whereas the opposite pattern was observed in mature specimens (Endo et al., 2008). Direct deposition from the atmosphere is the dominant pathway by which Hg reaches the oceans; exceptions are semi-enclosed basins such as the Mediterranean Sea and the Arctic Ocean, where river runoff and coastal erosion account for about half of Hg inputs (UNEP, 2013).

The highest Se concentration was detected in the pancreas (3.57 mg/kg dry wt) and ranged from 0.105 to 1.95 mg/kg dry wt in the other tissues. The order of Se-organotropism was: pancreas > spleen > gonad > brain > red muscle > liver > skin > white muscle. Selenium is known to detoxify Hg in marine organisms (Koeman et al., 1975). The mechanisms involved in this process are species- and tissue-specific (Cuvin-Aralar & Furness, 1991) and include the formation of inert Hg–Se complexes, the binding of Hg with selenoproteins, the indirect action of Se in preventing oxidative damage by Hg by increasing glutathione peroxidase activity, and the induction of metallothioneins. A positive correlation between Hg and Se is usually found in marine organisms and a molar ratio Hg:Se close to 1 is common in marine mammals and seabirds with high Hg concentrations in the liver (Koeman et al., 1975; Cuvin-Aralar & Furness, 1991; Dietz et al., 2000; Storelli & Marcotrigiano, 2002). It has been suggested that a Hg:Se ratio close to 1 occurs when Hg concentrations increase to >1000 mg/kg (Koeman et al., 1975). A ratio close to 1 has also been reported at Hg > 2 mg/kg wet wt (Dietz et al., 2000). Although the Hg concentration in the Greenland shark liver was 2.11±0.98 mg/kg dry wt, the mean Hg:Se ratio was 1.57±0.35, suggesting that the protective mechanism between Se and Hg is valid also in this species.

Hg and POPs showed different biomagnification patterns. McMeans et al. (2007) suggested that these differences may be due to a lower trophic position than expected or, more likely, to the different half-lives of Hg and POPs.

The Greenland shark is confirmed to bioaccumulate persistent inorganic and organic contaminants in all the studied tissues. In contrast to POPs and EDCs, trace elements occur naturally, although they are also released into the environment from anthropogenic sources. The Greenland shark may

eliminate Hg more efficiently than POPs by means of continuous exchange between the blood and seawater through the gills (Bacci, 1994): the different physicochemical features of Hg and POPs may allow more efficient Hg elimination with respect to POPs. Ecotoxicological baseline data on marine wildlife in Greenland including the Greenland shark are particularly important due to possible (or already running) prospective oil, gas and mining activities in the fjords and on the shelf (Christiansen et al., 2014), both of which are visited by this shark. Moreover, global warming and the concomitant declines in ice cover will continue to alter emission, transport and bioavailability of contaminants in the Arctic (AMAP, 2011).

1.4. Sensory capability: the sense of olfaction in the Greenland shark

Animals rely on their sensory system to obtain, process and act upon information from the environment where they live, including food availability as well as presence of competitors, predators and mates. Indeed, an animal's behavioural choices are based on its knowledge of resources availability, competition and predation risk, thus largely depending on the organism's capability to perceive and interpret the spatial structure of the environment (Kramer, 2001; Koy & Plotnick, 2007; Ferrari et al., 2010).

Although vision is generally known to play a central role among senses, in the marine realm the sensory system is stimulated by an extremely wide range of environmental cues, including light, sound, odour, water movement, temperature, pressure, and electric and magnetic field strength. In such a multifaceted environment, olfaction is at least as relevant as vision, sometimes being the principal sense through which a marine organism detects environmental cues (Tierney, 2015). The detection and discrimination of chemicals, and the behavioural reactions they evoke, are among the most primordial activities of living organisms, and chemoreception, which evolved 500 million years ago, can be considered the most ancient of sensory systems (Hara, 1992).

Olfaction is especially important as a distant or far-field chemoreception sense, since chemicals can be entrained in currents and transported far away through the water to the sensory cells. The spatial pattern of odours (odour landscape) on the one hand, and the olfactory organ morphology and functionality on the other, determine the fish behaviour in a particular environment and situation, thus indirectly influencing several aspects of its ecology. The extent of encountering olfactory signals depends upon the nature and concentration of the original source, the way the signal is conserved or modified during propagation in the aquatic environment, the swimming mode of the given species, and the morphology and arrangement of its nasal canals. The behavioural response is then determined primarily by the capability of the sensory system to detect a signal, transduce it into a nervous impulse and integrate it at the peripheral and central level. Therefore morphological

information on the olfactory system represents the necessary basis for attempting ecological inferences (Collin, 2012).

In cartilaginous fishes, olfaction is relevant for various survival tasks including prey localisation, predator avoidance and communication with conspecifics. Evidence of the importance of chemical sensing to elasmobranchs is abundant (reviewed by Hodgson & Mathewson, 1978; Smeets, 1998), and sharks in particular are often referred to as 'swimming noses' based on their olfactory abilities. In Chondrichthyes, the olfactory cues are received from the environment through a peripheral organ, the olfactory rosette, organised as an array of primary lamellae connected to a central support, named raphe (Meng & Yin, 1981; Meredith & Kajiura, 2010; Cox, 2013). The lamellae are covered by the olfactory epithelium, where the olfactory receptor neurons (ORNs) are located; axons from the ORNs project to the olfactory bulb in the telencephalon, where the signal undergoes a first step of integration (Dryer & Graziadei, 1996).

Despite similar overall morphological organisation, the olfactory sense organ in various species shows a degree of variability in size, shape, position of the nares, number and surface area of lamellae, and extent of the sensory epithelium (Bell, 1993; Schluessel et al., 2008, 2010; Meredith & Kajiura, 2010). The presence and variation in size and shape of secondary folds on the surface of the primary lamellae (Ferrando et al., 2019) is another feature to be taken into account when using the olfaction organ structure to infer information on the sensory capabilities of a species.

Until recently, little was known about the sensory biology of the Greenland shark, besides the inference of a poor relevance of vision among the senses based on this shark's propensity for infection of the cornea by the ectoparasitic copepod *Ommatokoita elongata* (Berland, 1961). The important role of non-visual sensory systems is also consistent with the evidence that the Greenland shark prefers to live in low-light environments such as deep waters or ice-covered areas. This is further confirmed by studies based on baited remote underwater videos (BRUVs), which have reported that the Greenland shark is the primary consumer of the bait (Devine et al., 2018), presumably enabled by a sensitive olfactory system.

Anatomical studies on the peripheral olfactory organ and olfactory bulb of the Greenland shark have recently been performed (Ferrando et al., 2016, 2017a). The olfactory rosette is an oval-shaped structure anatomically organised as an array of primary lamellae, arranged in parallel, attached to a central raphe, and decreasing in size towards both ends (Figure 1.3); such a linear lamellar organisation is widespread among sharks. In Greenland shark the lamellae at the two sides of the raphe do not touch each other, showing a 'type II parallel lamellae array' (*sensu* Cox, 2013) typically found in rather sedentary species. The lamellar

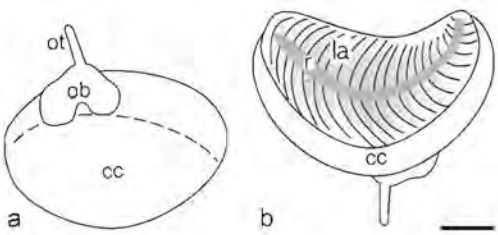

Figure 1.3 Scheme of the olfactory organ and bulb of (Greenland shark) (specimen of 230 cm total length). (a) dorsal view; (b) ventral view. cc=connective capsule; la=primary lamellae; ob=olfactory bulb; ot=olfactory tract; r=raphe. Scale bar 1 cm.

structure is enveloped by a connective capsule, opened ventrally to expose the olfactory lamellae to the water entering the nasal cavity, and in continuity with the olfactory bulb and peduncle in its dorsal part. The olfactory bulbs are subdivided into two sub-bulbs that converge, and are kept together in their central parts, from where a ribbon-like olfactory tract emerges. In a specimen of 230-cm total length, the number of primary lamellae, according to a general definition of primary lamella established in 2017 (Ferrando et al., 2017b), was 44 (Ferrando et al., 2016, 2017b). The total surface area of the primary lamellae for the same specimen was estimated to be 117 cm^2, a remarkably high value compared to other elasmobranch species (Schluessel et al., 2008). Since most of that area is covered by sensory epithelium, the estimate of surface area is considered a good proxy for olfactory potential. The relatively high value found in the Greenland shark suggested a rather good olfactory capability for the species. Such a sensory potential might sustain the shark scavenging behaviour (Beck & Mansfield, 1969; Leclerc et al., 2011), but also relevant ecological features of the species such as in shore/off shore movements across life stages (Campana et al., 2015), long migrations, as recently documented by using mark-report pop-up archival tags (Hussey et al., 2018), and vertical movements from deep waters to the undersea-ice cover (Skomal & Benz, 2004).

To further support the functional inferences on the Greenland shark olfactory potential made on the morphological bases, and in order to evaluate and discern features related to specific ecological traits, comparative analysis with taxonomically related species is needed. The morphology of the olfactory organ of the congeneric little sleeper shark *Somniosus rostratus* has been investigated and compared to that of Greenland shark in a wider comparative context (Ferrando et al., 2019). The gross morphology and relative size of the olfactory organs of the two *Somniosus* species were similar to each other and also to that of several other elasmobranchs. Nevertheless, they share a noteworthy and unique shape of the secondary folds, which are epithelial folds on the surface of the primary lamellae, increasing the sensory surface

area in the olfactory organ. In *S. rostratus* and *S. microcephalus* the secondary folds are very branched, with a very complicated and maze-like three-dimensional shape (Figure 1.4a,b). Secondary folds in the olfactory organs of other elasmobranch species investigated to date have little branching (Figure 1.4c) or none at all (Figure 1.4d,e). Such a peculiar organisation found in *S. rostratus* and *S. microcephalus* results in an increased surface area of lamellae in their olfactory peripheral organs, with potential effects on the water dynamics in the olfactory chamber, but this remains to be investigated.

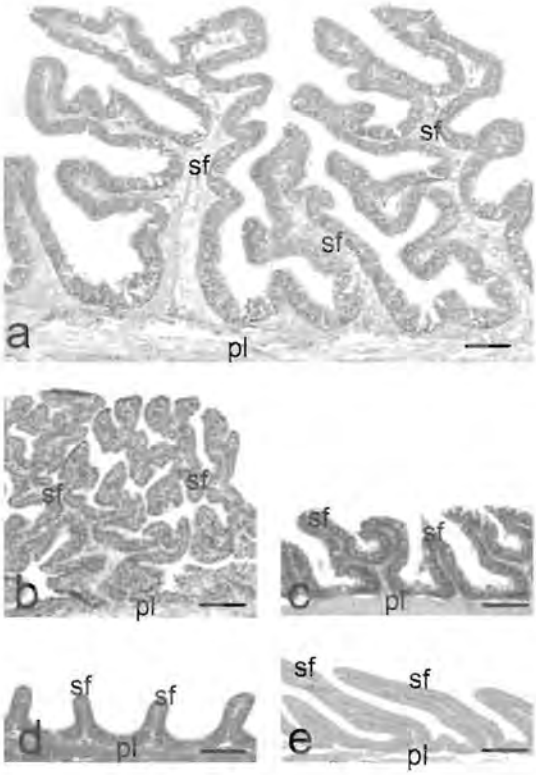

Figure 1.4 Histological sections of olfactory organs of elasmobranchs. Haematoxylin-eosin stain. (a) *S. microcephalus* (230-cm total length). Some secondary folds (sf) on the side of a primary lamella (pl). Almost all of the secondary folds present several branches. (b) *S. rostratus* (96-cm total length). Also in this species the secondary folds are very branched. (c) *Raja miraletus* (41-cm disc width). The secondary folds branch once or not at all. (d) *Pteroplatytrygon violacea* (107-cm disc width). The secondary folds are small, not branched and quite distant from one another. (e) *Scyliorhinus canicula* (27.5-cm total length). The secondary folds are not branched and elongated. Scale bars 200 μm. (A black and white version of this figure will appear in some formats. For the colour version, please refer to the plate section.)

Given the scarcity of information available to date on olfactory system organisation in the family Somniosidae, any conclusion based on the features of the two *Somniosus* species is premature. According to present data, the overall similarity of the peripheral olfactory organ in the Greenland shark and *S. rostratus* might be ascribed to their taxonomic relationship, although a possible ecological explanation for the peculiar organisation of the olfactory secondary folds in both species cannot be excluded. Further comparative studies in the family Somniosidae are needed to fully understand the possible ecological implications of an olfactory organ with the highly complicated structure summarised here in the two congeneric species of *Somniosus*. It would be interesting to explore a possible correlation between olfactory organ structure and relative olfactory bulb sizes. Indeed, according to the present available information on 58 species of elasmobranchs, three benthopelagic bathyal species of Somniosidae, the longnose velvet dogfish *Centroselachus crepidater*, the plunked shark *Proscymnodon plunketi* and the roughskin dogfish *Centroscymnus owstonii*, have enlarged olfactory bulbs compared to other squalomorph species of similar ecological niche (Yopak et al., 2015). Other important behavioural aspects should be taken into account in the ecology of the *Somniosus* species when attempting to infer ecological implications from their olfactory organ structure, intraspecific interactions being the most relevant. Unfortunately, as in other elasmobranchs, the social behaviour of these species remains largely unknown.

1.5. Conclusions

Sharks comprise about half of contemporary chondrichthyans, a monophyletic group of predatory fishes that originated about 423 million years ago. Their evolution and life history suggest that sharks have been a relatively stable force in ocean ecosystems over evolutionary time and possess a particular combination of ecological traits. Although sharks are morphologically and phylogenetically related to bony fishes, their life histories resemble those of marine mammals, specifically with respect to their large size, low rate of reproduction and late maturity. These features render the survival of sharks highly sensitive to a number of factors, e.g. predation and fishing. To date, shark populations in the Arctic are still thought to be relatively abundant, although comprehensive survey data to confirm this hypothesis are lacking. The chondrichthyan evolution appears steady, with low origination and extinction rates. Their high resilience might be related to high evolutionary adaptability.

Due to their slow growth rate and late maturity, Greenland sharks are especially vulnerable to human encroachment. There is little knowledge about their physiology and biochemistry. This contribution is an effort to try and fill this gap, by shedding light on parts of the physiology of the Greenland shark and reach better understanding of important biological

tools, functions and capacity to withstand increasing loads of environmental contaminants.

Concerning respiration, our results on the mechanisms of oxygen transport indicate the presence of three Hb isoforms in the Greenland shark, which lack significant structural and functional differentiation. However, *in vivo* regulation of oxygen transport can be achieved *via* local blood pH shifts (Bohr effect) and changes in the concentration of physiological allosteric modulators, such as ATP, within erythrocytes. Additionally, differences in gene expression and overall protein synthesis may also contribute to regulate oxygen transport in the short term.

In terms of sensory capability, the overall similarity of the peripheral olfactory organ in the Greenland shark and other sharks, e.g. *S. rostratus*, might be due to a taxonomic relationship, although an ecological meaning of the specific organisation of the olfactory secondary folds cannot be excluded. Further comparative studies in the family Somniosidae are needed, for instance to verify correlations between organ structure and olfactory bulb sizes. Other important behavioural aspects in the ecology of the *Somniosus* species, e.g. largely unknown intraspecific interactions, need to be taken into account.

The Greenland shark accumulates persistent inorganic and organic contaminants in all its tissues. Trace elements occur naturally, although they are also released through anthropogenic sources, similar to POPs and EDCs. Hg can be efficiently eliminated by exchange through the gills (Bacci, 1994). Ecotoxicological baseline data on marine wildlife in Greenland are particularly important due to mining activities in the fjords and on the shelf (Christiansen et al., 2014), both of which are visited by the Greenland shark. Moreover, global warming and declines in ice cover will continue to alter emission, transport and bioavailability of contaminants in the Arctic (AMAP, 2011).

In conclusion, in order to evaluate resilience and vulnerability of this species, we badly need additional information on biochemistry and physiology, including information on contaminant bioaccumulation and organotropism processes in the context of the shark's metabolism. In fact, a detailed understanding of the underlying mechanisms of biochemical and physiological adaptations are key for management and conservation.

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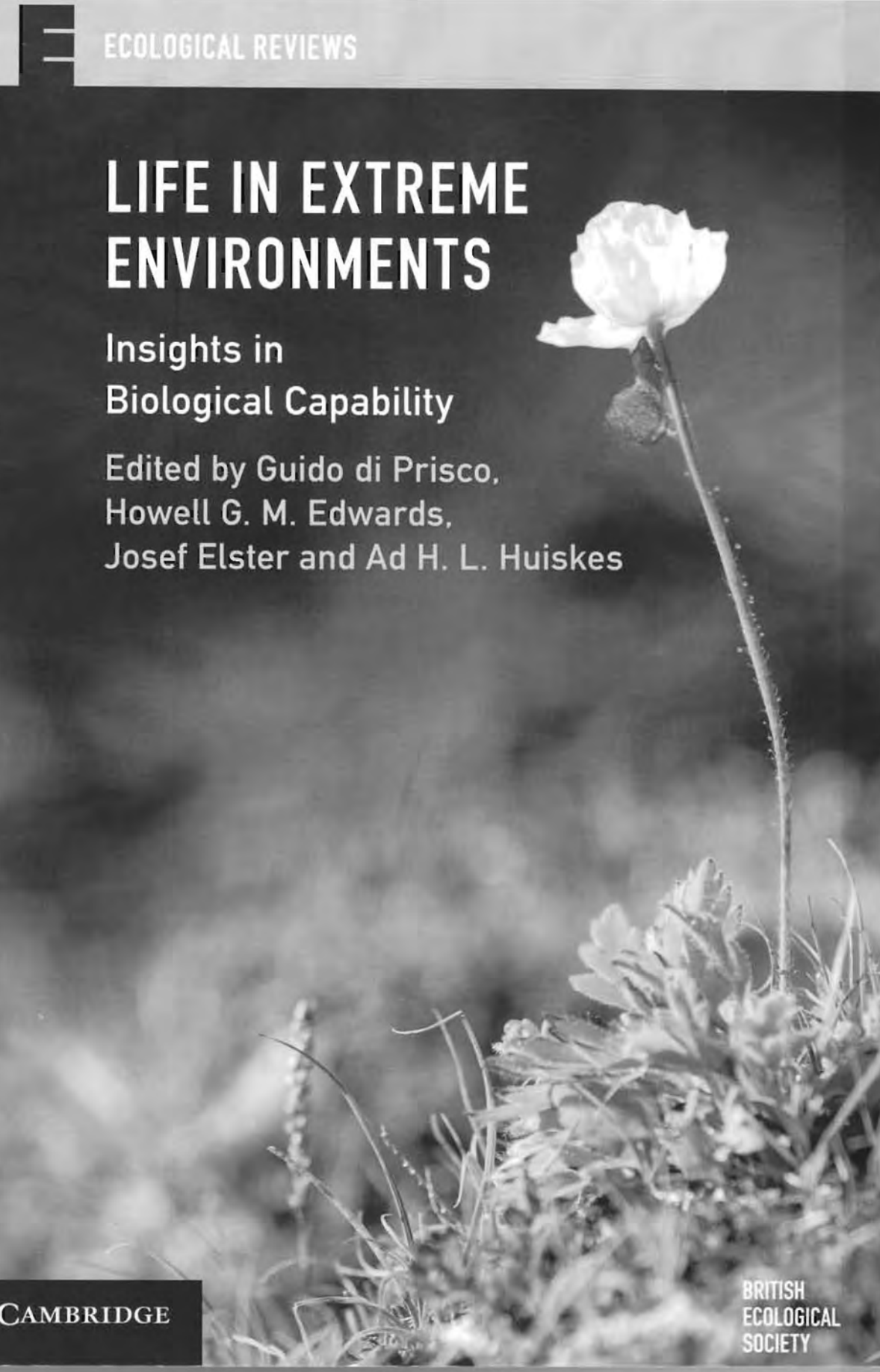
ECOLOGICAL REVIEWS

LIFE IN EXTREME ENVIRONMENTS

Insights in Biological Capability

Edited by Guido di Prisco, Howell G. M. Edwards, Josef Elster and Ad H. L. Huiskes



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From deep ocean trenches and the geographical poles to outer space, organisms can be found living in remarkably extreme conditions. This book provides a captivating account of these systems and their extraordinary inhabitants, 'extremophiles'. A diverse, multidisciplinary group of experts discuss responses and adaptations to change; biodiversity, bioenergetic processes, and biotic and abiotic interactions; polar environments; and life and habitability, including searching for biosignatures in the extraterrestrial environment. The editors emphasise that understanding these systems is important for increasing our knowledge and utilising their potential, but this remains an understudied area. Given the threat to these environments and their biota caused by climate change and human impact, this timely book also addresses the urgency to document these systems. It will help graduate students and researchers in conservation, marine biology, evolutionary biology, environmental change and astrobiology better understand how life exists in these environments and its susceptibility or resilience to change.

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