Environmental Biology of Fishes **60:** 117–129, 2001.

Surfactants as chemical shark repellents: past, present, and future

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Received 26 January 1999 Accepted 21 February 2000

Key words: SDS, *Cephaloscyllium ventriosum*, shark chaser, pardaxin, dodecyl sulfate

Synopsis

The development of Shark Chaser by the U.S. Navy during World War II was the first serious effort to develop a chemical shark repellent. In the decade following the war reports of Shark Chaser ineffectiveness led the Office of Naval Research to search for a more efficacious shark repellent. After years without success, ONR eventually canceled the use of Shark Chaser and abandoned the search for a chemical shark repellent. In the early 1970s, interest in chemical shark repellents was renewed by the discovery of pardaxin, a natural shark repellent secreted by the Red Sea Moses sole, *Pardachirus marmoratus*. The surfactant-like nature of pardaxin led investigators to test the potential of various surfactants as repellents. Subsequent studies indicated that the shark repellent efficacy of the effective alkyl sulfate surfactants was due to their hydrophobic nature. Here we report tests conducted on juvenile swell sharks, *Cephaloscyllium ventriosum*, to determine if the noxious quality of alkyl sulfates is affected by surfactant hydrophobicity [carbon chain length and ethylene oxide (EO) groups] and counterions. Our results indicate that the aversive response of sharks to alkyl sulfate surfactants increases with carbon chain length from octyl to dodecyl, decreases with the addition of EO groups and is not affected by counterions. This study confirms that dodecyl sulfate is the most effective surfactant shark repellent, but it does not meet the Navy's potency requirement for a nondirectional surrounding-cloud type repellent of 100 parts per billion ($0.1\ \mu g\, ml^{-1}$). Thus, dodecyl sulfate is only practical as a directional repellent such as in a squirt application. Future research should test the action of alkyl sulfates on cell membranes, the potential of other biotoxic agents, and semiochemicals in the search for an effective chemical shark repellent.

Introduction

Over the last 50 years various antishark measures were employed to protect humans from shark attack (Nelson 1983). Such antishark measures include electrical repellent devices (Gilbert & Springer 1963, Gilbert & Gilbert 1973), acoustical playbacks (Myrberg et al. 1978, Klimley & Myrberg 1979), visual devices (Doak 1974) and chemical repellents (Tuve 1963, Clark 1974, Gruber & Zlotkin 1982). Although none of these procedures are totally effective in preventing shark attacks, previous work on surfactant chemical shark repellents indicates some promise (Gruber et al. 1984, Zlotkin & Gruber 1984, Smith 1991, Sisneros 1993, Nelson & Strong 1996). In this paper we present a brief history of chemical shark repellents, report current work on the potential use of surfactants as shark repellents, and discuss new directions for the search of a truly effective chemical shark repellent.

Past chemical repellency research

The first serious effort to develop a chemical shark repellent did not occur until the early years of World War II. The conflict at sea and in the air in the Pacific campaign put many wounded servicemen and airmen in shark infested waters. Many deaths were attributed to shark attack as the war developed (Server 1989). The most infamous incident occurred after the 30 July 1945 Japanese torpedo attack which sunk the heavy crusier U.S.S. Indianapolis. This incident was one of the worst tragedies in U.S. naval history. In addition to the hundreds of deaths that occurred in the incident, an estimated 60–80 deaths were attributed to shark attacks on the remaining survivors who were adrift at sea for 5 days (Brown 1980). Subsequent speculation by U.S. servicemen on the threat of shark attacks became widespread enough to create a significant morale problem. In order to alleviate anxiety and renew confidence in service personnel, the U.S. Navy hurriedly began serious efforts to develop a shark repellent. The ultimate result of their rushed efforts was the development of the chemical packet known as Shark Chaser (Figure 1), sometimes more formally labeled Life Jacket Shark Repellent Compound Packet (Baldridge 1990).

Figure 1. Shark Chaser, the original U.S. Navy chemical 'shark repellent', was primarily a package of copper acetate and nigrosine dye issued to all naval personnel but later proven to be ineffective against repelling sharks. Scale bar = 5 cm.

Shark Chaser was composed of approximately 80% nigrosine-type black dye and 20% copper acetate held together by a waxy binder that allowed it to dissolve in a controlled manner over a period of 3–4 h (Tuve 1963). The special nigrosine dye component produced a black cloud visible to the user and was said to mimic the natural defensive secretions of marine mollusks such as the squid and octopus. The copper acetate component served two additional functions. First, it was recognized that copper ions inhibited the feeding of teleost fishes, and secondly, ammonium acetate, a major constituent in decomposing shark flesh, was also shown to be a feeding deterrent in the dogfish, *Mustelus canis* (Gilbert & Springer 1963, Tester 1963). It was therefore concluded that the combination of copper and acetate ions would produce an increased deterrent effect (Tuve 1963).

Due to the urgency at the time for a repellent, the testing of Shark Chaser's effectiveness was limited. Nevertheless, Shark Chaser soon became an integral component in every naval serviceman's survival gear. Following World War II, reports[1,2] of Shark Chaser's ineffectiveness began to appear. Such reports led the Office of Naval Research to reconsider the matter of chemical shark repellents in the 1950s and renew the screening and testing of possible candidate repellents (Zahuranec & Baldridge 1983). Hundreds of chemical substances were tested on sharks in an effort to find a chemical that would produce a quick and effective repellent response (Springer 1954, Gilbert & Springer 1963, Tester 1963). These chemicals included powerful toxins that could kill a shark after brief exposure but none could elicit the desired repellent response. After many fruitless attempts, research support for a chemical shark repellent ended.

Pardaxin and surfactant research

The discovery of a natural shark repellent in the early 1970s renewed interest in chemical shark repellents. Clark & Chao (1973) discovered that the milky fluid secretion from pores at the base of the dorsal and

[1] Fogelberg, J.M. 1944. Final report on the use of chemical materials as shark repellents. NRL report No. P-2373, Naval Research Laboratory, Anacostia Station, Washington, D.C. 28 pp.

[2] Fogelberg, J.M. & F.E. Brinnick. 1944. First partial report on the use of chemical materials as shark repellents. NRL report No. P-2230, Naval Research Laboratory, Anacostia Station, Washington, D.C. 35 pp.

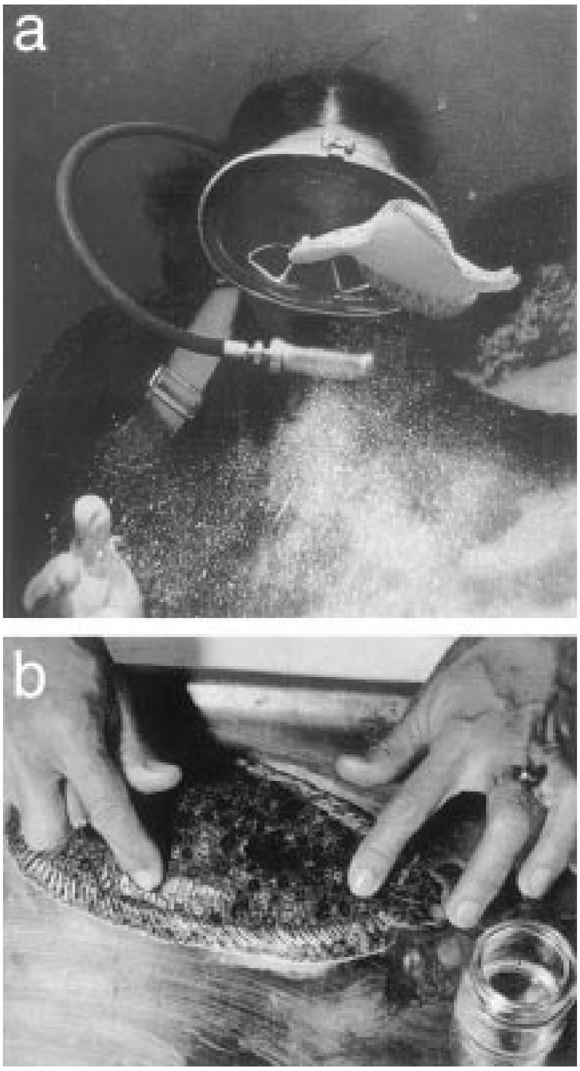

Figure 2. a – The Moses sole, *Pardachirus marmoratus*, was first demonstrated by Eugenie Clark (shown here) to produce a natural shark repellent that is both toxic and repellent to sharks. b – The Moses sole secretes a milky fluid known as pardaxin from poison glands located along the sole's anal and dorsal fins (photographs courtesy of David Doubilet).

anal fins of the Red Sea Moses sole, *Pardachirus marmoratus*, was both toxic and repellent to teleost fishes (Figure 2). Their initial observations led to a series of experiments in which Clark (1974, 1983) demonstrated the repellent effects of the secretion on captive reef whitetip sharks, *Triaenodon obesus*, and on Red Sea reef sharks, *Carcharhinus amblyrhinchus* (=*C.wheeleri*), in open water. Subsequent studies by Clark & George (1979) also revealed similar defense secretions produced by the Japanese peacock sole, *Pardachirus pavoninus*. The active ingredient of the secretion produced by *P. marmoratus* was later isolated and identified as pardaxin, an acidic protein composed of 162 amino acids with an approximate molecular weight of 17 kDa (Primor et al. 1978). Biochemical studies revealed that pardaxin affected a number of cellular targets and pathways including the neuromuscular systems of frogs (Spira et al. 1976), the gills of fishes (Primor et al. 1980) and the ion transport systems of dogfish (Primor et al. 1984).

Although pardaxin was an effective shark repellent, there were three major drawbacks to its practical use: (1) sufficient quantities were unavailable from natural sources, (2) at the time it was very difficult to synthesize, and (3) it had an extremely short shelf-life unless it was freeze-dried which reduced its potency. However, structure–activity analyses of pardaxin revealed promising alternatives to its use. Sequence analyses of the first 10 amino acids of the N-terminal primary structure of pardaxin revealed that it is extremely hydrophobic, positively charged and of amphiphatic character (Zlotkin & Barenholz 1983). Observations performed during the process of purification indicated that pardaxin possessed surface active (surfactant-like) properties by its ability to cause strong foaming and reduce water surface tension (Parness 1975). It was therefore suggested that relatively inexpensive synthetic surfactants that possess characteristics of pardaxin should be screened and tested as chemical shark repellents (Zlotkin & Barenholz 1983).

Subsequent studies revealed that certain synthetic surfactants do possess significant shark repellent properties (Gruber et al. 1984, Zlotkin & Gruber 1984, Smith 1991). In these studies, investigators tested a wide variety of different anionic, cationic and nonionic synthetic surfactants (Gruber et al. 1984, Zlotkin & Gruber 1984). Of these various surfactants, the anionic alkyl sulfate sodium dodecyl sulfate (SDS) was the most effective in repelling sharks. SDS was found to be five times more ichthyotoxic and four times more repellent to lemon sharks, *Negaprion brevirostris*, than a typical freeze-dried secretion of pardaxin (Gruber et al. 1984). The shark repellent efficacy of SDS was hypothesized to be due to the formation of large amphiphilic monolayers of surfactant alkyl chains that interact at the hydrophobic surface of lipid bilayers on buccal and gill epithelia (Tachibana & Gruber 1988). This chemical mode of action of SDS is presumably similar to pardaxin and other closely related defensive skin secretions such mosesins and pavoninins, which are also postulated to have a hydrophobic interaction with lipid membranes of buccal and gill epithelia, and ultimately elicit the repellent response (Tachibana & Gruber 1988). Other studies also suggested that the hydrophobicity of surfactants affect both the toxic and irritation effects in teleost fish (Mann 1955, Herbert et al. 1957, Pickering 1970, Parker 1971). Parker (1971) reported an increased rate of respiratory movements and air gulping in goldfish as the number of ethylene oxide (EO) groups on nonionic surfactants decreased. This increased respiratory distress was hypothesized to be due to an increase in hydrophobicity of surfactants with fewer EO groups. Thus, if shark repellency is related to the hydrophobic nature of anionic surfactants then we predict that the irritant/repellent efficacy of alkyl sulfate surfactants should increase with surfactant hydrophobicity.

Here we present the test results of seven homologous alkyl sulfate surfactants assayed as irritants/repellents on the swell shark, *Cephaloscyllium ventriosum*. The major goal of this study was to determine the effect of surfactant hydrophobicity on the shark irritant efficacy of alkyl sulfate surfactants. We investigate how carbon chain length and EO groups affect hydrophobicity and irritant efficacy. In addition, we also test whether counterions which influence surfactant solubility affect the irritant efficacy of alkyl sulfates.

Methods

Thirty-seven juvenile swell sharks, *Cephaloscyllium ventriosum*, 20.4–36.1 cm total length (TL) ($\bar{x}$ = 26.2 $\pm$ 2.9 SD cm TL) were used in tests conducted between August 1992 and April 1993. Juvenile sharks were raised from egg cases oviposted by captive adults or obtained from local collectors and public aquaria. Sharks were maintained in temperature controlled aquaria (15.0–20.0°C) and fed 2–3 times a week an *ad libitum* diet of squid, anchovies, mackerel and shrimp.

All tests were performed in a custom built roundabout test tank similar to that used by Smith (1991). The experimental tank (Figure 3) consisted of an oval channel and a removable inner holding pool built on a rectangular 1.2 $\times$ 2.4 m plywood base. The tank was covered with an inner coat of polyester laminating resin and an outer coat of light gray, gel-coat resin

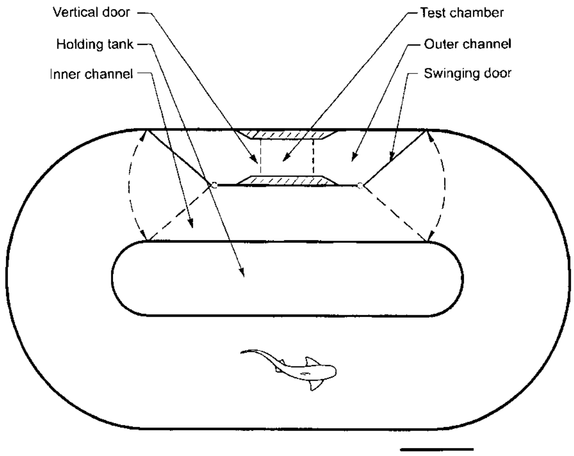

Figure 3. Experimental tank used to test surfactants as chemical shark repellents on swell sharks, *Cephaloscyllium ventriosum*. The outer channel and test chamber were used during tests while the holding tank was used prior to tests to acclimate sharks to water temperature. Swinging doors were used to direct sharks to either the inner or outer channels while the vertical doors were used to isolate chemical solutions prior to tests. Scale bar = 30 cm.

to form a smooth surface. A 5 l test chamber (20.0 cm long $\times$ 12.5 cm wide $\times$ 20.0 cm deep) made of 6.4 mm clear Plexiglass was built into the outer channel raceway (Figure 4). Sharks were directed into either the inner channel or outer channel, which contained the test chamber, by two swinging doors. The test chamber had two vertical, watertight doors which could be raised for presentations. This arrangement permitted the chamber to be isolated from the main raceway while chemicals were prepared prior to tests.

The primary test chemicals consisted of anionic surfactants sodium octyl sulfate (SOS), sodium decyl sulfate (SDecS), sodium dodecyl sulfate (SDS), sodium tetradecyl sulfate (STDS) and sodium octadecyl sulfate (SODS) (Figure 5a). SDS in 99% pure powder form (Sigma, St. Louis, MO); aqueous solutions of SDS (30% by volume), SOS (33%) and SDecS (31%) (Henkel Co., Hoboken, NJ); powdered STDS (95% pure) and SODS (93% pure) (Aldrich Chemical Co., Milwaukee, WI); and aqueous solutions of sodium dodecyl ether sulfate (SDES) (29% by volume) and magnesium dodecyl sulfate (MDS) (30%) (Figure 5b) (Lonza Inc., Long Beach, CA) were tested. Surfactants were either diluted or added to filtered seawater to produce a 1% (by volume) stock solution that was then added to the test chamber to achieve the desired concentration.

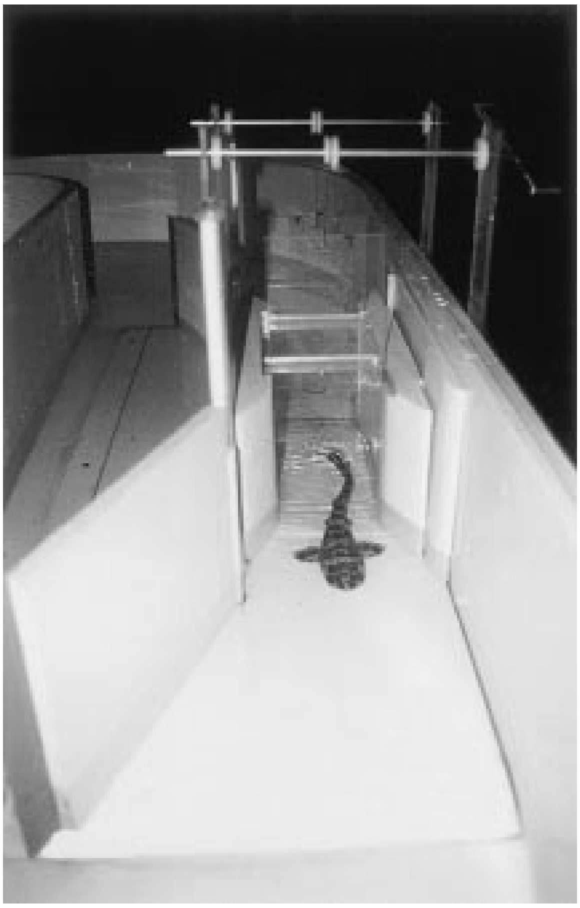

Figure 4. Test chamber used to test surfactants as chemical shark repellents on swell sharks, *Cephaloscyllium ventriosum*.

Sharks were exposed to a geometric series of chemical concentrations (Table 1) to determine the threshold concentration for each surfactant as an effective irritant/repellent. Initial concentrations for each geometric series were determined in preliminary trials. In this study sharks were exposed to only one chemical concentration per night (i.e. a single swim-through test per night) to reduce desensitization or habituation. After each test, sharks were returned to aquaria and not tested for a minimum period of at least 7 days. Due to the nocturnal nature of swell sharks, all trials were conducted at night under the illumination of dim red light.

Sharks in each night experimental session were placed in the inner pool of the tank and acclimated to the tank temperature (17.0–20.5°C) for 30 min. For each trial, a shark was randomly selected from the inner holding tank, placed in the roundabout, and allowed to swim for a minimum of 20 min before the experimental procedure was initiated. This pretest acclimation allowed the shark to adjust to a normal swimming motion.

The experimental procedure began with first isolating the test chamber by closing the vertical doors and moving the swinging doors to direct the shark through the inner channel. The appropriate amount of treatment chemical was then added to the water in the test chamber and stirred to obtain the desired concentration. After the test chemical was mixed, the test chamber solution was allowed to settle for 2–5 min before a trial was run. An experimental trial began when the swinging doors were moved to direct the shark to the outer channel. The vertical doors of the test chamber were raised when the animal approached to within approximately one body length of the chamber. Immediately after the shark exited the test chamber, the vertical doors were closed to isolate the chamber and minimize outward spread of the test chemical. Immediately following each trial, the shark was returned to its aquarium, the vertical doors of the test chamber were lifted and the entire tank emptied through a drain in the tank floor. The tank was then rinsed and refilled with fresh seawater and prepared for the next trial.

All trials were recorded on videotape using a Sony EV-C8 recorder and a CV110 Precision black-and-white video camera (0.2 lux) mounted approximately 2 m above the test chamber. Behavioral responses were classified into two levels of unconditioned aversive responses (see Results) based upon Smith (1991). The effective concentration for 50% of the test population (EC_{50}) for each chemical was determined using the Spearman–Karber method (Hubert 1980) and represents the concentration at which 50% of the population shows a categorical response. A one-way ANOVA with a Neuman–Keuls test and two-tailed t-tests (Sokal & Rohlf 1981) were used to test for differences between the surfactant EC_{50}s for each category.

Results

Response levels

Two distinct levels of unconditioned averse responses were identified in sharks exposed to the alkyl sulfate surfactants, and are similar to the classification used by Smith (1991). Level 1 was defined as a minimum aversion response (MAR) to the surfactant. The MAR consisted of a 'cough' or an expulsion

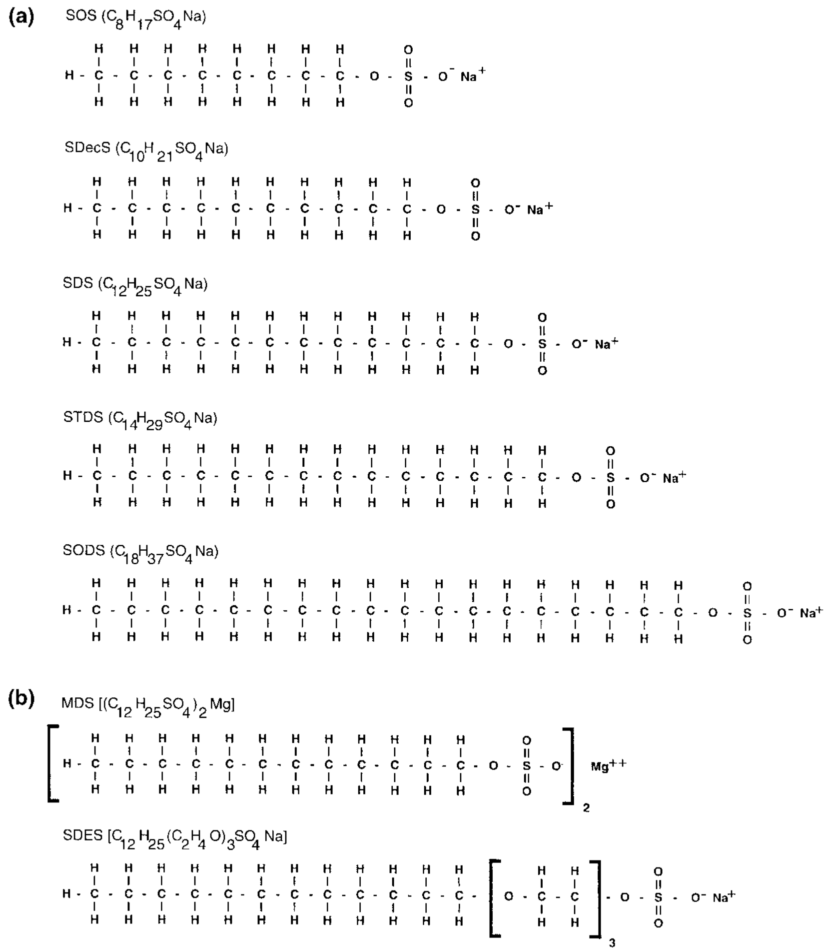

Figure 5. a – Chemical structures of the primary test chemicals: SOS, SDecS, SDS, STDS, SODS. Note that the abbreviation SDecS is used for sodium decyl sulfate to avoid confusion with the standard abbreviation of SDS for sodium dodecyl sulfate. b – Chemical structures of MDS and SDES. Note that these surfactants share the same alkyl carbon chain length of 12 carbons with SDS. MDS possess the counterion magnesium while SDES has a sodium counterion. Note that SDES has 3 ethylene oxide groups attached to the alkyl carbon chain and sulfate group.

reflex (Satchell & Maddalena 1972), and/or headshaking accompanied by a slight forward acceleration. The 'cough' response was considered to be a modified exhalation that forcefully expelled water from the mouth, gill slits and spiracles. Level 2, the vigorous aversion response (VAR), consisted of rapid and exaggerated headshaking, mouth gaping, and either a rapid acceleration forward or a 180° turnaround. The 'mouth gaping' response was characterized by a repeated opening and closing of the mouth together with large buccal and opercular expansions, presumably to flush out the buccal cavity.

Effect of alkyl carbon chain length on aversion

The irritant efficacy of SOS, SDecS, SDS, STDS and SODS was tested on 30 sharks at various chemical concentrations (Table 1) to determine the effect of surfactant hydrophobicity (which is proportional to carbon chain length) on irritant efficacy. Results show that the shark irritant efficacy of the alkyl sulfate surfactants increased as carbon chain length increased from octyl to dodecyl (Figure 6). The EC_{50}s for the MAR were 2028.1 $\mu g\,ml^{-1}$ for SOS, 175.9 $\mu g\,ml^{-1}$ for SDecS, and 36.4 $\mu g\,ml^{-1}$ for SDS. The EC_{50} of

Table 1. Concentrations of alkyl sulfate surfactants tested on juvenile swell sharks, *Cephaloscyllium ventriosum*. Note that a geometric series of concentrations was used to determine the effective dose for each surfactant.

Surfactant	Concentrations ($\mu g\,ml^{-1}$)
SOS	320, 640, 1280, 2560, 5120
SDecS	80, 160, 320, 640
SDS	20, 40, 80, 160
STDS	20, 40, 80, 160, 320, 640
SODS	20, 40, 80, 160, 320, 640
MDS	20, 40, 80, 160, 320
SDES	20, 40, 80, 160, 320

SDS was approximately 1/50th that of SOS and 1/5 that of SDecS (one-way ANOVA, Neuman–Keuls test, $p < 0.001$). The EC_{50}s for the VAR were 374.6 $\mu g\,ml^{-1}$ for SDecS, 82.6 $\mu g\,ml^{-1}$ for SDS, and inconclusive for SOS due to the lack responses at concentrations as high as 5120 $\mu g\,ml^{-1}$. The estimated 95% confidence limits (95% CL) and the number of sharks used to estimate MAR and VAR EC_{50}s are shown in Table 2. For the VAR, the EC50 of SDS was approximately 1/5 that of SDecS (two-tailed t-test, df $= 20$, $p < 0.01$). We were unsuccessful in a number of attempts to test the irritant efficacy of STDS and SODS due to the lack of solubility of these chemicals in seawater. In the 14 trials conducted using STDS and SODS no aversive responses were observed at concentrations as high as 640 $\mu g\,ml^{-1}$. In addition, no responses were observed in any of the 110 control trials performed. These results show that the intensity of the aversive response to alkyl sulfate surfactants is associated with increased carbon chain length and hydrophobicity (from octyl to dodecyl) until the surfactants become insoluble in seawater.

Effect of EO groups on aversion

The irritant efficacy of SDES was also tested on 5 sharks at 4 different chemical concentrations (Table 1) to determine the effect of hydrophobicity on the shark irritant efficacy of alkyl sulfates. SDES has the same number of carbon atoms (12) as SDS but has 3 additional EO groups that link the carbon chain to a sulfate group (Figure 5b). This arrangement of the EO group attached to the carbon chain effectively reduces the hydrophobicity of the surfactant (Satkowski et al. 1967, Kastner 1980), and the results show that SDES is less effective in producing aversion responses in sharks than SDS. The MAR EC_{50} of SDES was 67.3 $\mu g\,ml^{-1}$

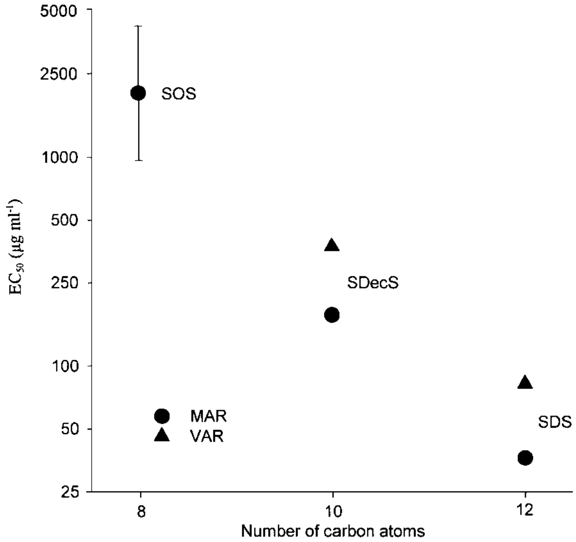

Figure 6. Relationship between surfactant EC_{50} of the MAR and VAR and alkyl carbon chain length in juvenile swell sharks, *Cephaloscyllium ventriosum*. EC_{50} represents the effective concentration for 50% of the test population for each chemical determined using the Spearman–Karber method (Hubert 1980). Note EC_{50} decreases as number of carbon atoms in the alkyl molecule increases. Standard errors for EC_{50}s are plotted but for most points are obscured by symbols.

($n = 5$, 95% CL = 63.8–70.9 $\mu g\,ml^{-1}$) and for the VAR 113.1 $\mu g\,ml^{-1}$ ($n = 5$, 95% CL = 106.5–120.2 $\mu g\,ml^{-1}$). For the MAR, the EC_{50} of SDES was approximately twice that of SDS (two-tailed t-test, df $= 13$, $p < 0.01$). For the VAR, the EC_{50} of SDES was approximately 1.4 times that of SDS (two-tailed t-test, df $= 13$, $p < 0.05$). No responses were observed in any control trials ($n = 25$). Thus, these results confirm that an increase in surfactant hydrophobicity does increase the shark irritant efficacy of alkyl sulfates.

Effect of counterions on aversion

The irritant efficacy of MDS was tested on 5 sharks at 4 different chemical concentrations (Table 1) to determine if the surfactant counterion affects the irritant efficacy of alkyl sulfates. MDS has the same carbon chain length (12 carbon atoms) as SDS but has the counterion magnesium instead of sodium (Figure 5b). Results show that the irritant efficacy did not differ between MDS and SDS. The EC_{50} of MDS for the MAR was 37.3 $\mu g\,ml^{-1}$ (n = 5, 95% CL = 35.5–39.3 $\mu g\,ml^{-1}$) and 85.7 $\mu g\,ml^{-1}$ (n = 5, 95% CL =

Table 2. MAR and VAR for juvenile swell sharks, *Cephaloscyllium ventriosum*, exposed to alkyl sulfate surfactants.

Test surfactant	MAR		VAR		n
	EC_{50} ($\mu g\,ml^{-1}$)	95% CL ($\mu g\,ml^{-1}$)	EC_{50} ($\mu g\,ml^{-1}$)	95% CL ($\mu g\,ml^{-1}$)	
SOS	2028.1	970.0–4256.8	—	—	6
SDecS	175.9	168.4–183.6	374.6	363.8–385.8	11
SDS	36.4	35.2–37.6	82.6	79.5–85.7	11

EC_{50} represents the effective concentration for 50% of the test population for each chemical (Hubert 1980) and is the concentration at which 50% of the population shows a categorical response. The 95% confidence limit (95% CL) is reported for each EC_{50}. MAR is the minimal noticeable response to the surfactant while VAR is more vigorous.

81.5–90.2 $\mu g\,ml^{-1}$) for the VAR. There was no difference between the EC_{50}s of MDS and SDS for either the MAR (two-tailed t-test, df $= 14$, p < 0.90) or VAR (two-tailed t-test, df $= 14$, $p < 0.85$). No responses were observed in any control trials ($n = 33$). Thus, these results are consistent with the notion that counterions do not affect the irritant efficacy of alkyl sulfates.

Discussion

This study is the first to investigate the effects of surfactant hydrophobicity on the shark irritant efficacy of alkyl sulfate surfactants. The goal of this study was to determine the optimum carbon chain length in alkyl sulfate surfactant molecules that produces the greatest aversive response in sharks. Our results show that shark irritant efficacy increases as the carbon chain of alkyl sulfates increases from octyl to dodecyl. Alkyl sulfates with carbon chain lengths greater than 12 (dodecyl) were insoluble under the test conditions of this study and thus did not produce any aversive responses. Additionally, we show that other factors that can influence surfactant solubility such as counterions did not affect the shark irritant efficacy of alkyl sulfates. In this discussion we interpret our results as they relate to shark irritant efficacy and chemical structure of alkyl sulfates and discuss future directions for the search of a more effective chemical shark repellent.

The important finding of this study is that the shark irritant efficacy of alkyl sulfate surfactants increases as carbon chain length increases from 8 (octyl) to 12 (dodecyl) carbons. Alkyl sulfates that have carbon chains greater than 12 are not soluble in seawater at testable temperatures between 17°C and 27°C. Thus, the 12-carbon hydrophobic chain of SDS is the optimum chain length that produces the greatest aversion response in sharks. Similar studies that have investigated the irritating effects of alkyl sulfates (ranging from octyl to octadecyl) on skin tissue have shown that dodecyl sulfates produce the greatest irritation and potential damage (Choman 1963, Imokawa et al. 1975, Bartnik & Kunstler 1987). A similar structure–effect relationship of alkyl carbon chain length is observed with mucous membranes where maximum irritation occurs at carbon chain lengths between decyl sulfate and tetradecyl sulfate (Kastner 1980). Schott (1973) hypothesized that the maximum irritation of skin and mucous membranes by octyl to octadecyl alkyl sulfates occurs at dodecyl due to two opposing factors inherit in these surfactants: specifically the oil/water partition coefficient and the limiting monomer concentration. Together these two factors are thought to control the amount of surfactant molecules available to interact and affect the lipid components of sensitive tissues and membranes. Skin and mucous membrane irritation increases with increases in the oil/water partition coefficient (i.e. carbon chain length). However, maximum monomer (single unclustered surfactant molecule) concentration in alkyl sulfates decreases with increases in the oil/water partition coefficient. Thus alkyl sulfates with longer carbon chains should have fewer monomers in solution available to interact with hydrophobic lipid membranes. As a result of these two opposing factors, peak skin and membrane irritation is hypothesized to occur at or near the optimized carbon chain length of 12 in alkyl sulfates. Thus our results support Schott's (1973) hypothesis since the greatest shark aversion response occurs with dodecyl sulfate in the alkyl sulfates tested in this study.

Our results also show that ethylene oxide (EO) groups effectively reduce shark irritant efficacy. SDES was approximately 1.8 and 1.4 times less effective than SDS in evoking both MAR and VAR, respectively. The difference in irritant efficacy between SDES and SDS is most likely due to their differences in surfactant hydrophobicity. SDES has three EO groups attached to the 12-carbon alkyl chain while SDS lacks EO groups. The attachment of the EO groups is known to effectively reduce the hydrophobicity of the surfactant (Satkowski et al. 1967, Kastner 1980). Similar studies have also shown that increasing levels of ethoxylation decrease the skin-irritating properties of alkyl ether sulfates (Opdyke & Burnet 1965). Parker (1971) reported that goldfish showed increased respiratory distress and accelerated rates of respiratory movements as the number of EO groups decreased. Parker attributed these effects to the increase in hydrophobicity as EO groups decreased and argued that the increase in hydrophobicity increased the affinity of the alkyl carbon chain to the lipid components of gill membranes. Thus our results that increased hydrophobicity increases the shark irritant efficacy of alkyl sulfate surfactants agrees with previous studies.

One chemical characteristic that does not affect the shark irritant efficacy of alkyl sulfates is the counterion. There was no difference between MDS (counterion = magnesium) and SDS (counterion = sodium) at either the MAR or VAR. Zlotkin & Gruber (1984) reported similar results with lithium dodecyl sulfate and SDS assayed on lemon sharks, *Negaprion brevirostris*. We also observed similar results in limited tests with ammonium dodecyl sulfate and SDS which were both equally effective in evoking strong responses in adult swell sharks (unpublished data). Although the results demonstrate that counterions do not affect irritant efficacy at the temperatures (17.0–20.5°C) tested in this study, we did notice that a 1% solution of SDS would begin to form surfactant crystals and become insoluble in seawater below 17°C. In contrast, we never did observe surfactant crystal formation in a 1% solution of MDS in seawater at temperatures as low as 12°C. This difference in surfactant solubility at lower temperatures becomes significant when testing these chemicals under various field conditions, especially in the cool temperate waters of Pacific Ocean. Thus based on our results and observations, we suggest MDS may be a more practical shark irritant/repellent than SDS due the higher solubility of MDS in seawater at lower temperatures.

In summary, we have shown that the shark irritant efficacy of alkyl sulfate surfactants increases as the alkyl carbon chain increases from octyl to dodecyl, decreases with the addition of EO groups and is not affected by counterions. However, we suggest that MDS has the potential to be a more practical chemical shark repellent than SDS due to its higher solubility in seawater at lower temperatures. This study also confirms past studies that dodecyl sulfate is the most effective surfactant irritant/repellent tested to date. The MAR EC_{50} we report for SDS (36.4 $\mu g\,ml^{-1}$) is very similar to the effective concentration of SDS (35.6 ± 3.4 SD $\mu g\,ml^{-1}$) needed to terminate tonic immobility in lemon sharks, *Negaprion brevirostris* (Gruber et al. 1984, Zlotkin & Gruber 1984). In addition, the VAR response reported for SDS and the other alkyl sulfates tested in this study was very similar to the responses observed by us in blue sharks, *Prionace glauca*, which were effectively repelled in field tests (unpublished data). However, SDS still does not meet the potency requirement for a nondirectional surrounding-cloud type repellent of 100 parts per billion (0.1 $\mu g\,ml^{-1}$) set forth by Johnson & Baldridge[3] for the U.S. Navy. The potency range of SDS reported in our study and by Smith (1991) indicates that it could be used as a directional repellent such as in a squirt application. One device that has been suggested for divers is a 'chemically enhanced' shark billy club (Figure 7) that could be used initially as a billy club, but then employ a chemical squirt of dodecyl sulfate if necessary (Nelson 1991, Nelson & Strong 1996). Thus this study supports early findings that alkyl sulfates are impractical as a nondirectional surrounding-cloud type repellent.

Future direction of chemical shark repellent research

Future candidate chemical shark repellents will need to meet the criteria set forth by Johnson & Baldridge[3] and be effective at a concentration no greater than 0.1 $\mu g\,ml^{-1}$ to be considered practical as a classical, nondirectional, surrounding-cloud type repellent. The 0.1 $\mu g\,ml^{-1}$ effective concentration represents the concentration at the boundary of a protected water volume of about 6 m^3 maintained by release from a central point source at approximately 100 $g\,h^{-1}$ over a 3.5 h period

[3] Johnson, C. S. & H. D. Baldridge. 1985. Analytic indication of the impracticability of waterborne chemicals for repelling an attacking shark. A second, confirming look. Technical document 843, Naval Oceans Systems Center, San Diego. 12 pp.

Figure 7. 'Chemical enhanced' shark billy club developed by D.R. Nelson that can deliver a rapid squirt of sodium dodecyl sulfate (SDS) to repel sharks. a – The device contains 250 ml of the SDS test solution to the right of the piston (P) and 60 psi of compressed air to the left of the piston. The device (122 cm in length) is discharged by a thumb operated valve that allows the compressed air to drive the cylinder piston to the right, thereby pushing surfactant solution out of the barrel in approximately 1 s (photograph courtesy of W.R. Strong). b – Shark researcher W.R. Strong tests the 'chemical enhanced' shark billy club on a bait-attracted white shark from the stern platform of the R/V *Alcyone* (photograph courtesy of the Cousteau Society).

under steady state conditions (Johnson & Baldridge[3]). Since surfactants are an order of magnitude less effective than the target concentration needed for a practical repellent, we suggest that other areas of research should be examined and investigated in future research. There are three potential areas of research that need to be addressed in chemical shark repellency research. These areas are the determination of the morphological target sites of chemical and natural shark repellents, further investigation of natural bioactive toxins as shark repellents, and the identification and purification of possible semiochemicals to be used as possible shark repellents. We identify and discuss three hypotheses that should be tested in future work.

The membrane lipid layer hypothesis

The site of action and biochemical mode of chemical shark repellents in elasmobranchs is one area of research that has been neglected in previous work. Various hypotheses have been proposed for the site of action and biochemical mode of pardaxin and alkyl sulfates (Primor et al. 1983 a,b, Smith 1986, Tachibana & Gruber 1988). Primor et al. (1983 a,b) hypothesized that the target sites of pardaxin were the membranal lipid components of the gills. Similarly, Tachibana & Gruber (1988) also proposed that the lipid membranes of buccal and gill epithelia and/or some unknown shark sense organ(s) were the targets affected by pardaxin and alkyl sulfates. In contrast, Smith (1986) speculated that the numerous 'pits', presumably chemoreceptors, located over the entire internal structure of the buccal cavity in swell and horn sharks were the targets of alkyl sulfates. Clearly, experimental studies that test these hypotheses will need to be performed before a truly effective chemical shark repellent can be developed. Future physiological studies that directly test the function of elasmobranch buccal 'pits' will need to be performed to determine how chemical and natural substances influence repellent behavior in sharks. The lipid membranes found in the gill and buccal cavity of elasmobranchs should also be examined to determine if the mode of action of pardaxin and alkyl sulfates is due to either a general lytic effect or due to the interaction with specific receptors that mediate sensory input for pain, olfaction, or gustation. Thus, future experiments that test this hypothesis will provide a better understanding of how shark repellent behavior is biochemically mediated and possibly lead to the better design of future chemical shark repellents.

The biotoxin hypothesis

The identification and testing of natural bioactive toxins from marine organisms as potential shark repellents is an area of research that should be further investigated. This area of research led to the discovery of the natural shark repellent pardaxin and a series of closely related ichthyotoxins known as pavoninins from the peacock sole, *Pardachirus pavoninus* (Primor et al. 1978, Clark & George 1979, Tachibana et al. 1984, Thompson et al. 1986). One ichthyotoxin that has received little attention as a potential shark repellent is saponin. Saponins also known as holothurins are ichthyotoxins that were first identified in sea cucumbers which use these toxins to protect themselves from being eaten by predators. Only crude tests regarding the toxicity of saponins have been conducted using sharks. Sobotka (1965) reported that a 1 $\mu g\, ml^{-1}$ solution of saponin (i.e. holothurin) collected from the sea cucumber *Actinopyga agassizi* killed a 22 kg lemon shark within 50 min. More recently, a naturally occurring saponin has been synthesized and identified in a class of mosesins isolated from the Red Sea Moses sole, *Pardachirus marmoratus* (Gargiulo et al. 1989). Hence, saponins are potential candidate repellents that should be screened and tested but to date have not been rigorously assayed for shark repellency. In addition to sea cucumbers, there is a plethora of other toxic marine organisms that have been tested for toxicity but have yet to be screened as shark repellents (Bakus 1983). Thus, the further investigation of the chemical defensive toxins of marine organisms may lead to the discovery of more efficacious shark repellents.

The semiochemical hypothesis

Perhaps the most encouraging area of shark repellency research is in the study of semiochemistry. This area of research was first proposed by Rasmussen & Schmidt (1992) who suggested that sharks may be chemically aware of the presence of potential danger by sensing the bodily secretions from potential predators. Semiochemicals found in the bodily secretions of predators may convey survival information to a shark and elicit rapid flight from an area that is potentially dangerous. Rasmussen & Schmidt (op. cit.) hypothesized that lemon sharks, especially juveniles, inherently recognize chemical exudates produced by the American crocodile, *Crocodylus acutus*, a known predator of sharks. Further, they demonstrated that the lemon shark, *Negaprion brevirostris*, shows aversive responses to 3 identified crocodile exudates (2-ethyl-3-methylsuccinimide, 2-ethyl-3-methylmaleimide, and 3-ethylidene-4-methylpyrrolidine-2,5-dione) produced most likely from the crocodile's chin gland, feces and blood. The concentrations needed to produce aversive responses in lemon sharks ranged from 10^{-7} to 10^{-9} M, which is near the functional limit of shark chemoreceptors (Hodgson & Mathewson 1978). Another proposed potential source for shark repellent semiochemicals may perhaps be found in decomposing shark flesh (Baldridge 1990, Rasmussen & Schmidt 1992). Anecdotal information exists from fishermen who claim that sharks will avoid areas containing decomposing carcasses of previously caught sharks. This was the reason why the U.S. Navy originally included acetate in Shark Chaser primarily due to the fact that ammonium acetate was found to be the major constituent in decomposing shark flesh. Perhaps there are semiochemicals found in extremely low concentrations in decaying shark flesh that act as alarm pheromones and provide warning signals to nearby sharks. Thus, in the search for shark repellent semiochemicals the re-examination of old ideas and the pursuit of new ones may offer the best hope for the discovery of a practical chemical shark repellent.

Acknowledgements

We thank John Reider for artwork, Michael Martinez and Jim McKibben for laboratory assistance, Shane Anderson, Jeff Landesman, John Rupp, and Mike Shaw for providing some of the animals used in this study, A.Z. Mason and R.N. Bray for comments on an earlier draft of this manuscript, and T.C. Tricas for technical comments on the final version of the manuscript. The CSULB Richard B. Loomis Memorial Fund provided partial support for this study and a student travel award from the American Elasmobranch Society (AES) enabled the author (J.A.S.) to present this paper at the AES Nelson Symposium in July 1998. This work was in partial fulfillment of a Master degree in Science for J.A.S. in the department of Biological Sciences at California State University, Long Beach.

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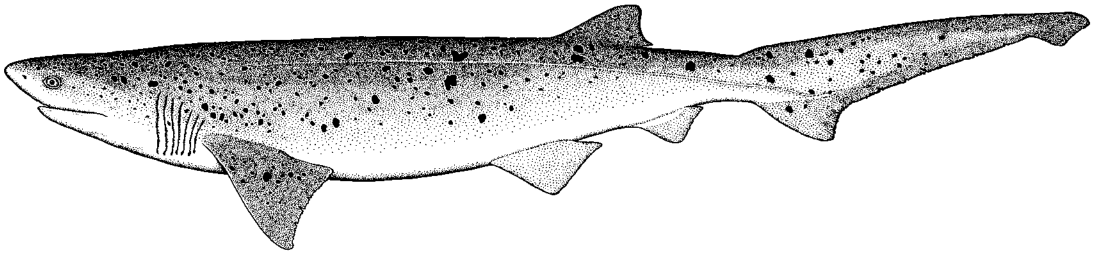
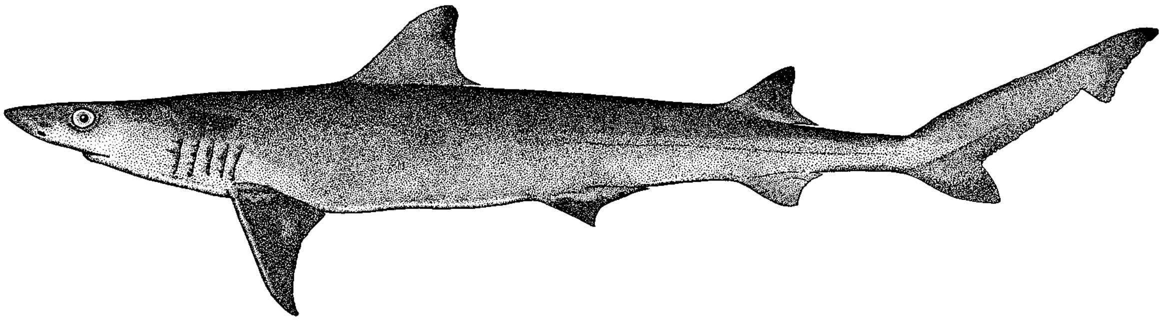


Female of the broadnose sevengill shark, *Notorychus cepedianus*, 180 cm long, captured off the coast of Japan (PV).



A female of the pencil shark, *Hypogaleus hyugaensis*, 62 cm long, from the Arabian Gulf (SH).