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# Chemical shark repellent: Myth or fact? The effect of a shark necromone on shark feeding behavior

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ABSTRACT

Since 1942, the search for an effective chemical shark repellent has been ongoing research concern in the United States. A long-standing anecdote that sharks avoid areas containing decomposing shark tissue has initiated new interest in identifying trace chemical alarm signals produced during decomposition (necromones). A commercially-sourced shark necromone produced from putrefied shark tissue was evaluated over a five-year period in South Bimini, Bahamas. Competitively-feeding populations of Caribbean reef sharks (*Carcharhinus perezi*) and blacknose sharks (*Carcharhinus acronotus*) were exposed to necromones using pressurized aerosol canisters at the surface. Shark density estimations were made at the initial, 1 min and 5 min intervals after preliminary exposure along with continuous exposure of feeding stimulus. In both species, an unambiguous halt in feeding behavior was observed within 1 min after exposure of the necromone. For aerosol delivery, a 150 mL dose of the necromone from a single aerosol canister is able to halt all feeding activity in a combined population of *C. perezi* and *C. acronotus*. Shark necromones induced a spectacular alarm response in interacting sharks resulting in a temporary evacuation of an area containing feeding stimuli. Additionally, sharks were not deterred by alternative treatment presentations of 10% weight percent (w/w) aqueous urea, 10% w/w oleic acid in ethanol, or water buffered to pH 8.5. Habituation to the necromone was not observed for repeated tests at the same location. In all experiments, the presence of a shark necromone did not produce a similar aversion response for teleosts as observed in *C. perezi* or *C. acronotus*; however, anecdotal observations demonstrate that teleosts increased their feeding rate in the presence of the necromone. Experimental controls using denatured ethanol or water confirmed that feeding sharks were not deterred by bubbles, sound, or the solvents used to extract the necromones. Comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry indicates that the necromone is a complex solution rich in amino acids and putrefaction products. Experiments demonstrate that the key chemical component responsible for the alarm response is within these amino acids and/or putrefaction products, but further experimentation is needed to more accurately identify the active ingredient. Shark necromones hold particular promise for use in shark bycatch reduction and conservation. The existence of a putative chemical shark repellent has been confirmed.



## 1. Introduction

The term semiochemical broadly describes molecules used for animal communication, resulting in specific behaviors such as orientation, survival, and reproduction (Law and Regnier, 1971). In terrestrial organisms, notably insects, semiochemicals are volatile and have a molecular weight between 80 and 300 (Wilson and Bossert, 1963). Semiochemicals that induce repellent behavior are particularly interesting for pest management and bycatch reduction practices. Certain semiochemicals, such as the odor emitted by dead conspecifics, trigger strong avoidance behaviors. For example,

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the unsaturated fatty acids, oleic acid (one double bond) and linoleic acid (two double bonds) induce necrophoric corpse removal in ants and bees (Wilson et al., 1958; Akino and Yamaoka, 1996). In cockroaches and termites, blood, intact corpses, and alcohol extracts of conspecifics are also repellent, and these samples contain the common oleic and linoleic acid moieties (Rollo et al., 1994, 1995). In the aquatic environment, isopods also share oleic and linoleic acid as necrophoric behavior-inducing semiochemicals (Yao et al., 2009). These phenomena also exist in vertebrates. For example, the sea lamprey, an ancient cartilaginous fish, is also chemically aware of its dead and will avoid odors emitted by dead conspecifics (Wagner et al., 2011).

Based largely on anecdotal information, the existence of a novel alarm cue has been speculated for sharks. Commercial fishermen have long purported that shark fishing dramatically decreased in areas where decomposing shark tissue was present (Baldridge, 1990). Development of the Shark Chaser, a time-release chemical shark repellent, focused on the acetate anion (from ammonium acetate). The acetate anion was the major constituent identified in decomposing shark tissue. The existence of a true shark repellent semiochemical was first considered by Rasmussen and Schmidt (1992). The findings from this study suggested that sharks may be chemically aware of the presence of potential danger through the sensing of bodily secretions from predators. Rasmussen and Schmidt (1992) hypothesized that lemon sharks (*Negaprion brevirostris*), especially juveniles, inherently recognize chemical exudates produced by the American crocodile (*Crocodylus acutus*), a known predator of these shallow water coastal sharks. The concentrations needed to produce aversive responses in lemon sharks ranged from $10^{-7}$ to $10^{-9}$ M, which was near the functional limit of shark chemoreceptors (Hodgson and Mathewson, 1978). Both Rasmussen and Schmidt (1992) and Sisneros (2001) proposed that semiochemicals exist in extremely low concentrations within decaying shark flesh and act as alarm substances for other sharks in the proximity. Since these signals are produced following death and decomposition, they are broadly described in this study as "necromones".

Through preliminary field and laboratory experimentation, evidence exists for the efficacy of a shark necromone since a private corporation first publicized the efficacy of a putative shark necromone (National Geographic News, 2004). The present study aims to replicate the preliminary work done in 2004 to determine the efficacy of the semiochemicals on two species of shark, *Cacharhinus perezi* and *Carcharhinus acronotus*. We hypothesize that the putative shark necromone will induce a "schreckreaction-like" response in sharks similar to that evoked by Schreckstoff in ostariophysian fish (von Frisch, 1938), whereby chemically stimulated sharks will rapidly cease feeding and disperse from the treated site.

## 2. Materials and methods

### 2.1. Aerosol canister preparation

All shark repellent aerosol canisters were obtained from Repel Sharks, LLC (Charlestown, Nevis) and were supplied in nominal 177 mL steel aerosol canisters. According to the manufacturer, the model RS-IM-S canister is charged with 150 mL of necromone and pressurized to 150psig with argon gas. The necromone mixture, labeled "CP/BCOMP/BGCOMP", is a composite mixture of extractions from putrefied blue shark (*Prionace glauca*), *C. perezi*, and Galapagos shark (*Carcharhinus galapagensis*) tissue. The canisters are positively buoyant and therefore have a lead metal band near the canister top (i.e. content ejection point) to ensure the can is slightly negatively buoyant and inverted in the water after deployment. This arrangement allows a rapid evacuation of canister contents and the minimization of gas bubble release. The canister is designed to fully evacuate within 60 s, producing a plume in the water column as the can gradually rises to the surface. All aerosols were stored at ambient temperature and out of direct sunlight until testing, per the manufacturer's instructions.

The concentration of the stock necromone was consistent for all field experiments because a single batch (lot CP/BCOMP/BGCOMP) was purchased from the supplier and all canisters had a uniform fill volume. The dried solids basis of the necromone extract was approximately 3%w/w. At the time of the field tests, the necromone active or its physical state were not elucidated, therefore the precise concentration of the necromone active at the time of delivery was unknown.

Control canisters contained 150 mL of either water or denatured ethanol (EtOH). Denatured ethanol was chosen to establish that the necromone solvent system alone was not repellent. Deionized water was chosen to establish that the ejection of fluid and argon gas from the canister and the accompanying sound and motion was not repellent.

### 2.2. Preliminary treatments

Additional canisters were obtained for a preliminary evaluation of the efficacy of necromone components. The necromone solution, as obtained directly from the aerosol canister, has an average pH of 8.5 at room temperature. A corresponding buffer solution (pH 8.5) was selected to establish that the slightly more basic pH of the necromone than seawater (pH 8.1) was not repellent. A 10% w/w aqueous urea solution and a 10%w/w oleic acid solution in ethanol samples were chosen as both urea and oleic acid were identified in the necromone using instrumental analysis. Oleic acid is essentially insoluble in water and its high viscosity at room temperature presents a challenge for aerosol delivery. A 10%w/w solution in ethanol was selected to reduce viscosity and aid in the dispersion of oleic acid droplets in water.

### 2.3. Study location

This study was conducted at South Bimini, Bahamas, in association with the Bimini Biological Field Station. More specifically, all experimental trials were conducted at a shallow reef location, known as Triangle Rocks (25°37′58.29″N, 79°18′52.48″W). The site affords large populations of adult *C. perezi* and adult *C. acronotus* throughout much of the year. Visual observations of sharks are easily made at this site due to its 6 m depth, sandy substrate, and excellent water visibility. Tests were conducted under Bahamas Government research permits, held by the Bimini Biological Field Station BBFS (2005–2009) and SharkDefense (2010). The BBFS supplied boats, bait, photography, and supervision for the experiments.

### 2.4. Field trials

Following anchorage at the Triangle Rocks site, sharks were stimulated into competitive feeding behavior at the surface with the use of chum and small pieces of locally captured fish. An actively feeding population was typically established within 10 min at the surface using this method. Feeding stimuli were continuously added to the test site during an experimental trial. Once sharks were actively feeding at the surface, researchers estimated shark density by determining the number of sharks feeding on the chum. If the feeding population remained intact after 5 min, the experimental trial commenced. Researchers randomly selected a can to deploy, either a control or treatment canister. If treatment canisters were selected first and sharks were repelled, the researchers returned to the test site later in the day following a tide change to

conduct control tests. Density estimates were made before and after canister deployment.

The number of cans deployed was varied as a means to obtain information on the necessary quantity of repellent needed to repel sharks. One to five canisters of control solution or the necromone were actuated and thrown into a 5 m-wide area where the population density was greatest. The cans were thrown with enough spacing to permit dispersion of a wide plume of contents (i.e. control or necromone). Care was taken to ensure that cans did not physically contact the sharks. Density estimates for feeding sharks were made before and after the presentation of controls and treatment compounds. All tests were conducted within 20 min of establishing a feeding population to avoid satiation. Feeding stimuli were continuously added to the test site during the experimental trial and for at least 5 min following a canister deployment. No more than two tests were conducted in a single day, and at least one month of rest period was provided between excursions to South Bimini for necromone testing.

Repellency was defined as having at least half (50%) of the initial density halt feeding and remain beyond visual counting range for 5 min following a release of necromone while feeding stimuli is present. Observations on teleost behavior were also recorded.

During experimentation, additional observations were made as a means to determine the behavioral responses of interacting sharks and teleosts. Although each individual behavior was not quantified, researchers carefully noted behavioral responses such as: increase in feeding rate, decrease in feeding rate, position in the water column (e.g. swimming at surface or substrate), accelerations away from chemical or control plume, and biting of cans. The recording of these behaviors was made to assist in the overall description of both teleost and elasmobranch behavior in response to the canisters.

### 2.5. Chemical analysis

The shark necromone, as retrieved from the production aerosol canisters, is a complex volatile mixture in an ethanol-water matrix. Gas chromatography was selected as the primary analytical method because of the mixture's inherently high volatility. In single-dimensional gas chromatography, however, the complexity of the necromone would likely cause a number of co-elutions. As a result of this limitation, the authors selected comprehensive two-dimensional gas chromatography (GC × GC), wherein the complex volatile mixture is separated along a long non-polar column followed by a shorter polar column connected in series through a liquid nitrogen cryogenic modulator (Dallüge et al., 2003). The high selectivity provided by two columns of differing polarity and the high peak capacity of this configuration ensure that co-elutions are minimized upon presentation to the mass detector. A time of flight mass spectrometer (ToFMS) was used as the detector. In a TofMS, ions are formed and accelerated and their flight times measured to determine their mass. The ToFMS employs electron impact ionization, often resulting in high fragmentation, permitting rapid comparison of the full mass spectra to spectral databases.

A representative sample of the necromone was obtained for comprehensive chemical analysis using an equal weight composite from four aerosol cans of the "CP/BCOMP/BGCOMP" production lot. The composite sample was subsequently passed through an Acrodisc™ (Pall, Port Washington, NY) polytetrafluoroethylene (PTFE) 0.45 μm syringe filter into 1.5 mL clean glass autosampler vials (Waters, 186000307C 12 × 32 mm, Columbia, MD). Vials were sealed with PTFE/silicone septum caps and staged in a MPS2™ (Gerstel, St. Joseph, MI) autosampler. All samples were analyzed using a LECO® comprehensive two dimensional gas chromatograph (GC × GC) and a ToFMS. The total run time was 50 min. The injection volume was 1 μL ± 0.1 μL. Injection needle wash containers were replenished daily with fresh methanol. Two neat methanol purge samples were injected between each shark extract sample to eliminate analyte carryover. The instrumental method and operational parameters are defined in Table 1, Table 2, and Table 3. All library assignments were processed using a custom structured query language (SQL) algorithm to remove column artifacts and septae, liner, fitting, and needle contaminants. Further refinement was performed manually to remove implausible assignments.

### 2.6. Statistical analysis

Working under the null hypothesis ($H_o$) of no change in the shark feeding population, we compared the shark population at $t = 0$, defined as the instant immediately prior to treatment deployment, to shark population at $t = 1$ min, $t = 5$ min and $t = 10$ min. We used a Student's $t$-test to compare population means at $t = 0$ and $t = 1$ min. Comparisons between shark populations at $t = 0$ to $t = 5$ min and $t = 10$ min were reported as a percent of the initial population density. For graphical results, uncertainty was indicated using the standard error (SE) of the mean.

## 3. Results

### 3.1. Necromone canister experiment

For the thirteen field tests conducted between 2005 and 2010, all necromone canister deployments produced significant changes ($p = 0.00001$) by reducing *C. perezi* and *C. acronotus* feeding populations to zero within 1 min (Fig. 1). No significant differences were observed for any control trial (water, EtOH, Oleic acid in 10% EtOH, pH 8.5, and urea) and results for individual field trials are summarized in Table 4. No variation in repellent behavior was observed when data was grouped by year or by month (Fig. 2 and Fig. 3, respectively).

Complete repellency was observed for all three trials in 2005 using an average treatment dosage of 250 ± 87 mL. For the corresponding water control tests, a 250 ± 87 mL dose did not reduce the feeding population. Population density was reduced 100% at the 1 min and 5 min intervals following necromone release. Up to 40% of the initial shark density returned after the 10 min interval, with an average rate of return of 20%, but then would not feed at the surface and remained close to the substrate. Teleosts remained present and consumed bait following the release for the 10 min study duration.

Complete repellency was observed for both trials in 2006 using an average treatment dosage of 675 ± 106 mL. For water controls, a 600 mL dose did not reduce the feeding population. An additional necromone trial (750 mL) that did not have a corresponding control trial was performed in May 2006. For 2006 trials, population density was reduced 100% at the 1 min and 5 min intervals following necromone release, and 27% of the initial density returned after the 10 min interval, with an average rate of return of 21%, but would not feed at the surface and remained close to the substrate. Teleosts remained present and consumed bait following

**Table 1**
Capillary configuration for orthogonal separation of shark necromones by GC × GC.

| Column | Length (m) | Diameter (μm) | Film thickness | Stationary phase | Bleed masses |
|---|---|---|---|---|---|
| 1 | 30 | 250 | 1.00 | RTX-5MS | 73, 207, 149 |
| 2 | 1.284 | 100 | 0.10 | Stabilwax | None |

**Table 2**
Oven temperature ramps for orthogonal separation of shark necromones by gas GC × GC.

| Oven | Step | Rate (°C/min) | Target temp (°C) | Duration (min) |
|---|---|---|---|---|
| 1 | 1 | Initial | 30 | 5 |
| 1 | 2 | 5 | 230 | 5 |
| 2 | 1 | Initial | 35 | 5 |
| 2 | 2 | 5 | 235 | 5 |

necromone release. The May 2006 trial represented the largest population of sharks tested in one instance, with twelve sharks competitively feeding.

Only one trial was conducted in 2007, and complete repellency was observed for a 300 mL dose. The corresponding water control dose of 300 mL did not produce repellency. For 2007 trial, population density was reduced 100% at the 1 min and 5 min intervals following necromone release, and 25% of the initial density returned after the 10 min interval but would not feed at the surface and remained close to the substrate. Teleosts remained present and consumed bait following necromone release.

Repellency was observed for all three trials in 2008, wherein a 250 ± 86.6 mL necromone dose produced repellency. A 300 mL water control dose did not produce repellency. Two additional necromone trials (150 ml and 300 ml doses) that did not have corresponding control trials were performed in February and April 2008. For 2008 trials, population density was reduced 100% at the 1 min and 5 min intervals following necromone release, and up to 34% of the initial density returned after the 10 min interval, with an average rate of return of 18%, but would not feed at the surface and remained close to the substrate. Teleosts remained present and consumed bait following necromone release.

Repellency was observed for both trials in 2009, wherein a 375 ± 106 mL necromone dose produced repellency. A 300 mL water control and a 300 mL denatured ethanol control did not produce repellency. An additional necromone trial (450 mL) that did not have a corresponding control test was performed in April 2009 for a film crew. Population density was reduced 100% at the 1 min and 5 min intervals following necromone release, and up to 27% of the initial density returned after the 10 min interval, with an average rate of return of 11%, but would not feed at the surface and remained close to the substrate. Teleosts remained present and consumed bait following necromone release.

Repellency was observed for both trials in 2010, wherein a 150 mL necromone dose produced repellency. A 150 mL dose of 10% aqueous urea, pH 8.5 buffer solution, and 10%w/w oleic acid in ethanol did not produce repellency. Population density was reduced 100% at the 1 min and 5 min intervals following necromone release, and 26% of the initial density returned after the 10 min interval, with an average rate of return of 24%, but would not feed at the surface and remained close to the substrate. Teleosts remained present and consumed bait following necromone, urea, buffer, and oleic acid releases.

**Table 3**
LECO® instrument configuration and parameters for GC × GC-TofMS analysis of shark necromones.

| | |
|---|---|
| *GC × GC parameters* | |
| Carrier gas | Helium |
| Injection | Split (front injector) |
| Column flow rate | 1.00 mL/min |
| Split Ratio | 25.00 |
| Inlet temperature | 250 °C |
| Over equilibration time | 1 min |
| Transfer line temperature | 200 °C |
| Acquisition delay | 0 s |
| *Modulator parameters* | |
| Modulator temperature offset | 20 °C |
| Second dimension separation time | 5 s |
| Hot pulse time | 0.90 s |
| Cool time between stages | 1.60 s |
| *Mass spectroscopy parameters* | |
| Start mass | 45u |
| End mass | 700u |
| Acquisition rate | 20 spectra/second |

### 3.2. Chemical analysis

Comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry successfully provided an orthogonal separation of the complex necromone mixture and a unique chemical profile of polar and non-polar compounds. Highly volatile putrefaction products responsible for odor, such as primary alkyl amines, were detected. Lipid components and amino acids that did not undergo putrefaction were also detected. For method repeatability, the following marker compounds were used to confirm a successful analysis for three separate sample injections:

- Butanoic acid, 2-methylbutanoic acid, and 3-methylbutanoic acid, with retention times between 568 and 1608 s on the nonpolar column and 1.8–1.9 s on the polar column.
- 2-Piperidinone, with a retention time of approximately 1024 s on the nonpolar column and 2.7 s on the polar column.
- Butanamines, with retention times between 600 and 1500 s on the nonpolar column and 1.6–1.8 s on the polar column.
- Oleic acid, with a retention times between 1692 and 1808 s on the nonpolar column and 1.9–2.1 s on the polar column.

A summary of the major functional groups identified by area percent is provided in Table 5. Fig. 4 provides a three-dimensional total ion chromatogram of the necromone.

## 4. Discussion

This study is a clear illustration of the repellency achieved using a shark necromone mixture on the behavior of both *C. acronotus* and *C. perezi*. Additionally, through comprehensive instrumental analysis, the active chemicals responsible for the repellent reactions are hypothesized to lie within the amino acid or putrefaction products.

### 4.1. Field trials

The presentation of necromone to competitively feeding populations of *C. perezi* and *C. acronotus* produced unambiguous aversion behaviors and a 100% reduction in feeding behavior within the first minute of release. The aversive behaviors were often violent, with sharks rapidly accelerating away from the chemical plume. Ethanol, water, pH 8.5 buffer, urea, and oleic acid controls failed to produce any aversive behaviors and were suitable control materials. Urea is abundant in shark tissue for osmoregulation and is likely not to be a specific signal from death and putrefaction. Comprehensive GC × GC-ToFMS confirmed total urea content at approximately 2% by peak area. Oleic acid was also detected at approximately 0.8% by peak area, and is likely to originate from liver lipids which aid in buoyancy. Oleic acid was selected because it is a known necromone in insects and isopoda, although this functionality did not extend to elasmobranchs. Poor solubility and lack of specificity are proposed reasons for inefficacy of this acid.

The sharks at the test site were not tagged or uniquely identified. The authors were not able to determine if the identical population of sharks returned to the site for each test, yet this is

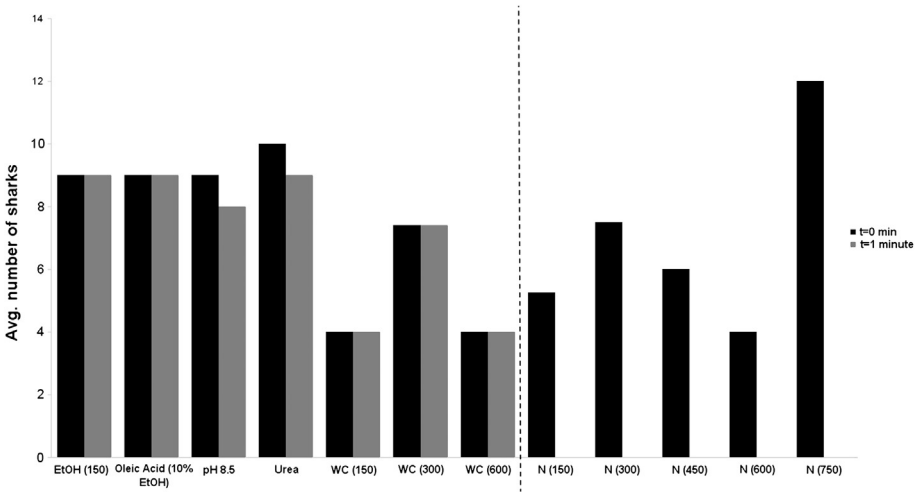


**Fig. 1.** Change in size of feeding populations at 1 min following presentation of various canisterized materials. Feeding populations were *Carcharhinus perezi* and *C. acronotus* at Triangle Rocks, South Bimini, Bahamas from 2005 to 2010. EtOH = ethanol; WC = water control; *N* = necromone; numbers in parenthesis indicate volumes in mL.

unlikely given the duration of the study. No habituation to the necromone was observed over the five year period. Given the same mode of delivery and same necromone material, the aversive responses were consistent. The 10-Jul-2010 trial was the most impressive result to date, with a single 150 mL dose producing strong aversive behaviors in a population of nine sharks. The sharks at the test site are subjected to organized shark feedings at least once per month and therefore, conducting repellent studies at these locations subjects these repellents to the highest form of experimental rigor and repellent effectiveness.

With continuous bait and chum present, a limited number of sharks began to return after 10 min. Returning sharks were always *C. perezi*; *C. acronotus* did not return after necromone exposure within this period. The returning *C. perezi* population was always less than half of the initial population and these sharks were noticeably disinterested in feeding. It appeared that sharks were investigating food that had fallen to the seafloor, a dramatic contrast to competitive surface feeding. Although a positive identification of returning sharks as those exposed to the necromone could not be determined, their behavior (i.e. lack of feeding and proximity to the seafloor) is consistent with the behavior of sharks exposed to necromone during preliminary trials and therefore we reported the return rate as a percent of the initial density.

Aerosol canisters produced an unexpected benefit for delivery. The motion and noise produced by the evacuating control canisters and empty control canisters floating at the surface appeared to heighten the sharks' interest in approaching the cans. The authors propose that the evacuating can either visually resembles a struggling prey item or the evacuation noise is attractive. Often, control canisters were bitten, nosed, or temporarily pulled underwater. The canisters therefore served as lures to ensure that sharks contacted the necromone plume.

Teleosts were the first organisms present during all trials, with the black durgon (*Melichthys niger*) being the predominant species and the first arrival. Other teleosts present in abundance included the oceanic triggerfish (*Canthidermis maculata*), queen triggerfish (*Balistes vetula*), Bermuda chub (*Kyphosus sectatrix*), and yellowtail snapper (*Ocyurus chrysurus*). These teleosts moved toward the surface in large numbers once bait was dispensed. Upon the arrival of sharks, the teleosts positioned themselves deeper but remain at the test site. The fish would prey upon any small particles of bait not consumed by the sharks. Many teleosts were observed swimming through the chemical plume after sharks had left the immediate area and before the canister completed its ejection. These fish displayed no aversion behaviors and would continue feeding long after the sharks had left the test site, thus, the necromone was observed to act selectively on sharks.

While initial results suggest that the necromone repels two elasmobranch species, it may also have broad-spectrum efficacy within the Elasmobranchii subclass. The necromone used in aerosol canister tests was a composite mixture from the blue shark (*Prionace glauca*), the Caribbean reef shark (*C. perezi*), and the Galapagos shark (*C. galapagensis*). We observed aversive behaviors from *C. acronotus* despite the lack of a conspecific extract in the mixture. It is proposed that since the mixture was derived from Carcharhiniforms, the necromone may exhibit repellency across many other species of ground sharks.

### 4.2. Chemical analysis

Instrumental analysis shows that there are hundreds of volatile components constituting the commercial shark repellent mixture. The necromone has a dominant ammoniacal odor due to short chain volatile amines, predominantly 2-methyl-1-butanamine and 2-butanamine. Amines are the result of decarboxylation of amino acids during putrefaction. For example, 2-pentanamine was detected and is a plausible decarboxylation product of leucine. These short chain amines possess high pKa's, typically greater than 9.0, and are likely the source of the basicity of the necromone mixture. Trimethylamine was present as expected, since the osmoregulatory compound trimethylamine-n-oxide (TMAO) is abundant in elasmobranchs. The basic trimethylamine (pKa 9.8) is produced by the bacterial reduction of TMAO during putrefaction. Short chain amines resulting from decarboxylation of amino acids are unlikely to be the specific necromone actives, as bony fish also contain the common amino acids and would undergo putrefaction via similar pathways.

Over 30% of the necromone's detected peak area during chemical analysis was comprised of amino acids, with leucine and alanine comprising more than half (58%) of this fraction. Leucine (2-amino-4-methylpentanoic acid) and alanine (2-aminopropanoic acid) were present in the necromone because shark muscle tissue is used in the extractive process. These amino acids are abundant in nature and would be unlikely to produce a highly specific chemical signal.

**Table 4**
Results of field trials using canisterized necromone, water, ethanol and urea on on *Carcharhiuns perezi* and *C. acronotus* at Triangle Rocks, South Bimini, Bahamas from 2005 to 2010. Media references are provided for tests where film crews recorded the experiment. Reference 1: Miami Univision; 2: "Dirty Jobs, Jobs that bite", Discovery Channel; 3: Tigress productions; 4: BBC Oceans; 5: Tigress productions; 6: DiveBum Studios; 7: Discovery LLC.

| Test date | Test material | *C. perezi* population at $t = 0$ min | *C. acronotus* population at $t = 0$ min | Total population at $t = 0$ min | Total population at $t = 1$ min | Total population at $t = 5$ min | Observation at $t = 10$ min | Dose (mL) | Number of canisters deployed | Greater than 50% population reduction at $t = 5$ min? | Teleosts remain feeding after dose? | Media ref |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15-Sep-05 | Necromone | 3 | 1 | 4 | 0 | 0 | 0 | 150 | 1 | Yes | Yes | 1 |
| 15-Sep-05 | Water control | 3 | 1 | 4 | 4 | | | 150 | 1 | No | Yes | |
| 17-Nov-05 | Necromone | 5 | 1 | 6 | 0 | 0 | 2 | 300 | 2 | Yes | Yes | |
| 17-Nov-05 | Water control | 5 | 1 | 6 | 6 | | | 300 | 2 | No | Yes | |
| 10-Dec-05 | Necromone | 5 | 2 | 7 | 0 | 0 | 2 | 300 | 2 | Yes | Yes | |
| 10-Dec-05 | Water control | 5 | 2 | 7 | 7 | | | 300 | 2 | No | Yes | |
| 16-Feb-06 | Necromone | 3 | 1 | 4 | 0 | 0 | 1 | 600 | 4 | Yes | Yes | |
| 16-Feb-06 | Water control | 3 | 1 | 4 | 4 | | | 600 | 4 | No | Yes | |
| 02-May-06 | Necromone | 9 | 3 | 12 | 0 | 0 | 2 | 750 | 5 | Yes | Yes | 2 |
| 27-Mar-07 | Necromone | 6 | 2 | 8 | 0 | 0 | 2 | 300 | 2 | Yes | Yes | |
| 27-Mar-07 | Water control | 6 | 2 | 8 | 8 | | | 300 | 2 | No | Yes | |
| 16-Feb-08 | Necromone | 3 | 1 | 4 | 0 | 0 | 0 | 150 | 1 | Yes | Yes | 4 |
| 02-Apr-08 | Necromone | 6 | 2 | 8 | 0 | 0 | 2 | 300 | 2 | Yes | Yes | 3 |
| 10-Jul-08 | Necromone | 6 | 1 | 7 | 0 | 0 | 2 | 300 | 2 | Yes | Yes | |
| 10-Jul-08 | Water control | 6 | 1 | 7 | 7 | | | 300 | 2 | No | Yes | |
| 14-Jan-09 | Ethanol control | 7 | 2 | 9 | 9 | | | 300 | 2 | No | Yes | |
| 14-Jan-09 | Necromone | 7 | 2 | 9 | 0 | 0 | 2 | 300 | 2 | Yes | Yes | |
| 14-Jan-09 | Water control | 7 | 2 | 9 | 9 | | | 300 | 2 | No | Yes | |
| 15-Apr-09 | Necromone | 5 | 1 | 6 | 0 | 0 | 0 | 450 | 3 | Yes | Yes | 5 |
| 06-Apr-10 | Necromone | 4 | 0 | 4 | 0 | 0 | 1 | 150 | 1 | Yes | Yes | 6 |
| 06-Apr-10 | Oleic acid 10% EtOH | 8 | 1 | 9 | 9 | | | 150 | 1 | No | Yes | |
| 06-Apr-10 | pH 8.5 | 7 | 2 | 9 | 8 | | | 150 | 1 | No | Yes | |
| 06-Apr-10 | Urea 10% aq | 7 | 3 | 10 | 9 | | | 150 | 1 | No | Yes | |
| 15-Jul-10 | Necromone | 7 | 2 | 9 | 0 | 0 | 2 | 150 | 1 | Yes | Yes | 7 |

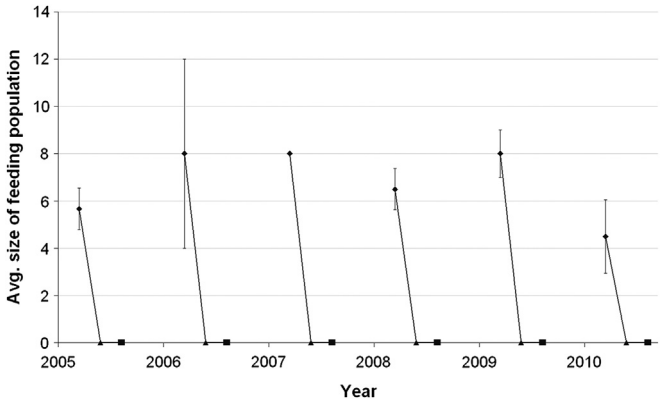


**Fig. 2.** Annual summary of canisterized necromone tests on *C. perezi* and *C. acronotus* at Triangle Rocks, South Bimini, Bahamas from 2005 to 2010. Diamond (◆) points indicate the initial population density prior to canister release. Triangular (▲) points indicate the population density within visual range at 1 min after necromone deployment. Square (■) points indicate the population density within visual range at 5 min after necromone deployment. Lines illustrate the density decrease following necromone exposure. Uncertainty is indicated using the standard error (SE) of the mean.

**Table 5**
Major functional groups in shark necromone identified by orthogonal separation and comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry.

| Major functional group | Area percent |
|---|---|
| Amino acids | 30.41 |
| Acids | 12.61 |
| Esters | 8.94 |
| Amines | 7.34 |
| Sulfurs | 7.04 |
| Amides | 5.05 |
| Ketones | 4.70 |
| Alcohols | 3.51 |
| Alkyls | 2.98 |
| Ureas | 2.36 |
| Nitro compounds | 0.85 |
| Aldehydes | 0.52 |
| Enals | 0.47 |
| Alkenyls | 0.22 |
| All others | 13.00 |
| Sum | 100 |

Acids constitute more than 12% of the detected peak area of the shark necromone. Most are short-chain carboxylic acids, which are expected putrefaction end products due to the β-scission of the alkoxy radicals formed from unsaturated fatty acids. These short-chain acids also contribute to the characteristic odor of the necromone. Acetic acid ($C_3$), and the isovaleric acids ($C_5$, 3-methyl-butanoic acid and 2-methyl-butanoic acid) were the most abundant. Fatty acids were also successfully detected, and these are likely extracted from shark liver. As discussed earlier, the known insect necromone oleic acid was found. The ethyl ester of stearic acid ($C_{18}$) was detected, resulting from the esterification of the acid with the extraction solvent. Capric acid ($C_{10}$), myristic acid ($C_{14}$) and palmitic acid ($C_{16}$) were also found, along with their ethyl esters. These fatty acids are unlikely to produce a highly specific signal because they are ubiquitous and have low solubilities in seawater. A multitude of aldehydes, enals, ketones, and organosulfur products were also identified. These are the result of the degradation of squalene and other unsaturated lipids present in the liver, likely by oxidative pathways. As hundreds of plausible compounds were identified, fractioning the necromone mixture by functional group is the recommended approach for the further elucidation of the shark necromone active.

The necromone active would be immediately relevant in the commercial fishing industry, where high rates of accidental shark catch (bycatch) occur. The authors envision that the necromone active would be incorporated into a time-release matrix and inserted into longline baits, providing a protection window for each baited hook. Since the necromone is selective to sharks, the target fish catch rates should remain unaffected. Ideally, the target fish catch rates would increase, because more hooks would become available for marketable fish given reduced shark capture.

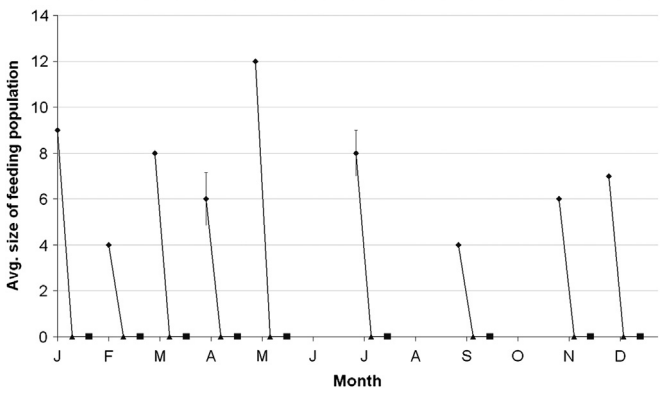


**Fig. 3.** Monthly summary of canisterized necromone tests on *C. perezi* and *C. acronotus* at Triangle Rocks, South Bimini, Bahamas from 2005 to 2010. Diamond (◆) points indicate the initial population density prior to canister release. Triangular (▲) points indicate the population size within visual range at 1 min after necromone deployment. Square (■) points indicate the population size within visual range at 5 min after necromone deployment. Lines illustrate the density decrease following necromone exposure. No tests were conducted in the months of June due to the unavailable of sharks during their mating period. No tests were conducted in August and October due to unfavorable weather conditions. Uncertainty is indicated using the standard error (SE) of the mean.

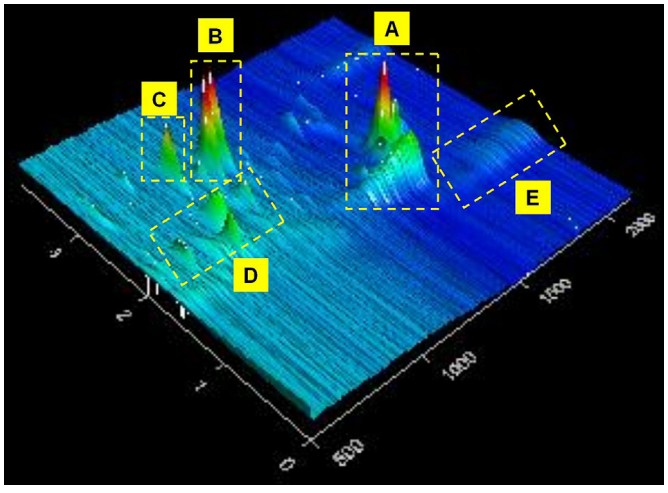


**Fig. 4.** Three-dimensional chromatogram of commercial shark repellent necromone, *X*-axis is retention time (seconds) on column #1, *Y*-axis is retention time (seconds) on column #2, area intensity is on the *z*-axis. Compounds of interest are indicated by large peak area. A: Methionine, leucine, and $C_5$ carboxylic acids. B: Nitrogen heterocycles and alkyl ureas. C: Amides. D. $C_3$–$C_5$ acids, alkyl amines, ketones, diols and piperidinone, E: Oleic acid and long chain fatty acids.

## 5. Conclusions

A necromone produced from putrefied shark tissue has shown to be 100% repellent to competitively feeding *C. perezi* and *C. acronotus* at South Bimini, Bahamas. Dosages as small as 150 mL, delivered by a pressurized aerosol canister, were able to induce aversive responses in feeding populations of up to nine sharks. For all tests conducted at Triangle Rocks, South Bimini over a five year period, aversion behaviors were unambiguous and sudden, with sharks losing interest in feeding and accelerating beyond visual range within 1 min of local dispersion of the necromone. Control aerosol canisters containing water, ethanol, aqueous urea, pH 8.5 buffer or 10%w/w oleic acid in ethanol did not illicit aversion. Teleosts present at the test site showed no aversion with controls and with necromone presentations, indicating that the necromone is specific to *C. perezi* and *C. acronotus*. The necromone may not require a conspecific to be effective, because *C. acronotus* responded to a necromone mixture that lacked its conspecific tissue. Further research is needed to identify species-specific variations, and this will require necromone extracts produced from other species of shark.

Comprehensive GC × GC-ToFMS is repeatable method for separating and identifying compounds in the complex shark necromone mixture. Both polar and non-polar molecules were identified successfully. The shark necromone was found to be rich in amino acids, short chain and fatty carboxylic acids, amines, and short-chain lipid oxidation products. Because hundreds of compounds were tentatively assigned, fractioning and grouping by functionality is required for the elucidation of the necromone active(s).

A highly selective shark necromone will find purpose in commercial fisheries, where accidental shark catch rates can be reduced without affecting target fish catch rates.

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