# Predator Emitted Volatile Organic Compounds in New Zealand

Subjects: Others

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The volatile organic compounds (VOCs) emitted by the bodies and secretions of introduced mammalian predators in New Zealand forests are covered, with a specific focus on mice, rats, ferrets, stoats, and possums.

Keywords: invasive species ; VOCs ; biomarkers ; pest management

## 1. Introduction

The native ecosystems of New Zealand are unique in many ways, one being that it boasts a remarkable absence of native mammalian predators with the exception of two species of bat. Fostering a favourable environment for the thriving evolutionary diversity of endemic species, New Zealand boasts a wide range of both extinct and extant ground-dwelling birds (e.g., moa, kiwi, and kakapo), and an ancient lizard, the tuatara [1][2][3][4]. Unfortunately, the introduction of mammalian predators to New Zealand, including rodents, possums, and mustelids, has had a detrimental impact on the local ecosystems, posing an ongoing threat to native prey species. This ecological disturbance has disrupted the delicate balance that once allowed for the flourishing diversity of New Zealand's unique flora and fauna [5]. It was reported that the invasion of mammalian predators in New Zealand, such as stoats, possums, and rats, has been directly linked to the decline or even complete extinction of numerous native species within New Zealand forests [3][5][6]. This alarming trend highlights the devastating impact these animals have had on the delicate ecological balance, resulting in a loss of biodiversity and the gradual decline of native species' populations [5][6][7][8]. For instance, the invasion of brushtail possums, mustelids, and rodents is largely responsible for the extinction of many native bird species in New Zealand, including the brown kiwi, blue duck, weka, kaka, parakeet, and cuckoo [6][9]. O'Donnell and co-workers estimate that native birds in New Zealand lose nearly 27 million eggs and nestlings each year due to introduced mammalian predators [5]. In addition, rats and mice pose a significant threat to the ecological systems within forests and islands, as they actively hunt nestling birds, eggs, and lizards [10][11][12]. Another study indicates that brushtail possums not only inflict severe damage on bird reproduction and the overall ecological environment of the forest, but they also act as disease vectors, including the transmission of bovine tuberculosis [13].

The task of reducing or eradicating mammalian predators has emerged as a formidable challenge for New Zealanders, culminating more recently in the national 'Predator Free 2050′ conservation mission [14][15][16]. To date, New Zealand has employed various strategies to reduce predator populations, including traps, poison and lure baits, dog tracking, and hunting. All these methods necessitate human involvement, which carries potential risks and costs [17]. For example, automated trapping systems and camera monitoring have emerged as a popular method for predator eradication efforts. These traps use mechanical triggers, pressure pads, or infrared light to capture images or videos of animals. However, the subsequent identification of captured species usually requires manual assessment by experienced professionals [18][19]. Alternatively, employing an advanced AI-driven framework for image recognition could reduce labour requirements but it might come at the cost of increased power consumption [20][21][22]. There are also techniques developed for tracking and detecting predators, such as faecal sample collections followed by deoxyribonucleic acid (DNA) or messenger ribonucleic acid (mRNA) extractions and identifications [23]. Although the method demonstrates impressive accuracy based on gene alignment, the rapid degradation of DNA and RNA samples in the environment poses significant challenges to the sample collection process. Moreover, human intervention is often necessary during sample collection and the unpredictable environmental conditions in the wild could amplify potential risks for operators. Additionally, further identifications, which are based on specific genes of different species, demand not only a high-cost instrument and database but also professionally trained operators [24][25][26][27]. The challenge of exploiting these current methods includes the real-time monitoring and the sample collection processes within intricate forest environments, thereby resulting in potential risks and costs associated with human involvement, such as the risk of accidental injuries during tracking and sample collection procedures, along with the consideration of the associated time and cost.

Volatile organic compounds (VOCs) are organic molecules with low boiling points at room temperature, and they contribute to the characteristic scents of various objects [28]. It is suggested that general body odours or specific odours from glands or secretions (such as urine or faeces), can persist in the environment and continue to disseminate olfactory information after the predator has left the area [29][30]. Certain VOCs are emitted by animals and plants, serving as semiochemicals that facilitate information exchange between conspecific or different species [31]. These VOCs play crucial roles in processes such as sex attraction and species identification, and their potential applications have been discussed and studied in lure and bait formulations [17][32]. These odours emitted from predators and their secretions consist of a diverse array of unique VOCs that could theoretically serve as unique biomarkers for detecting the presence of mammalian predators in the forest.

## 2. Mice

Mice exhibit a strong sense of territory and mark their individual territories using scent, typically through urine and faeces [33]. These scent marks contain specific volatile compounds that play a crucial role in information transfer among conspecifics. For example, several studies indicated that mice can assess the age of other individuals based on the volatile compounds emitted in their urine [34][35][36]. Mice also possess the ability to discern individuals that have been afflicted by parasites or diseases [37]. Female mouse urine has been found to contain volatile compounds that serve as sex-attractive pheromones to male mice [38][39]. In an investigation reported by Varner and co-workers, gas chromatograph-mass spectrometry (GC-MS) was employed to analyze bedding materials contaminated with mouse secretions. Their study revealed the presence of 28 VOCs in these samples. Among them, four specific VOCs, namely butyric acid, 2-methyl butyric acid, 3-methyl-butyric acid, and 4-heptanone, were exclusively detected in samples collected from female mice [40].

In recent decades, there has been a gradual increase in research focused on exploring volatile pheromones and unravelling the intricate functions of these compounds [38][39][40]. In a more recent investigation reported by Tang and co-workers, the volatile profile of female mouse urine was examined, resulting in the identification of 77 unique VOCs. The identified compounds encompass a wide range of chemical classes, including hydrocarbons, alcohols, aldehydes, ketones, and some aromatic compounds, which can also be found in the urine of other rodent species [41].

Among these compounds, six specific volatiles in relatively high concentrations may function as prospective biomarkers for mice [42]. 2-sec-butyl-4,5-dihydrothiazole, previously believed to function as an alarm pheromone in mice, shares a structural similarity with heterocyclic sulfur-containing compounds present in stoat and ferret anal sac secretions [43]. This volatile compound, along with 2,3-dehydro-exo-brevicomin, can be found in the urine of all adult male mice, which can elicit aggression in other males and possess properties that are attractive to females [44][45][46][47]. 2,5-dimethylpyrazine is a female-specific compound known for its role in suppressing estrus. It is utilized by females during mate selection processes [48].

Additionally, distinct volatile profiles could also be distinguished among various mouse species. Soini and co-workers identified 47 VOCs and eight unknown volatile compounds in the urine of a species of mouse, *Mus Spicilegus*, and suggested five kinds of main VOCs emitted from *Mus Spicilegus* that are distinct to that of *Mus Domesticus* [49].

It has been reported that the quantity of volatiles emitted from mouse urine is influenced by the physiological and psychological conditions of mice [50]. Studies indicate variations in the volatiles emitted by mice, distinguishing between healthy and sick individuals [34][37][51]. Notably, male mice have been observed releasing EE-α- and E-β-farnesenes to attract female mice and announce aggressive signals to other male mice; these are not found in female individuals [52]. Another gender-dependent volatile, Trimethylamine, serves as an attractive scent signal to mice of the opposite sex. It can be identified in the urine of a range of mammals and be found in high concentrations in mouse urine. Importantly, male mouse urine is reported to contain a concentration approximately 20-fold higher than that of female mouse urine [53].

Investigations also suggest the presence of volatile compounds in sex-attractant pheromones deposited by female mice. In a field experiment reported by Musso and co-workers, it was observed that corn cob bedding contaminated with secretions from female laboratory mice had a significant impact on their attraction to wild mice of the opposite sex [54]. During the estrus period of female mice, a high concentration of 2,5-dimethylpyrazine can be found in volatiles released by mouse urine to spread the estrus signals and attract male individuals [55]. Furthermore, 1-ido-2-methylundecane has been specifically identified in the urine of female mice during the proestrus and estrus stages [56]. Dehydro-exo-brevicomin, in either its 2,3- or 3,4-isomer form, is a well-known semiochemical found in both male and female mouse urine, and a rise in its concentration in the urine of female mice was identified during the estrus phase [57]. Tang and co-workers reported that several compounds, including 3,4-dehydro-exo-brevicomin, butanoic acid, pent-1-ene/cyclopentane, 1,2,3-/1,2,4-trimethylbenzene, heptadecane, dioctyl ether, dodecane-1-ol, and 2-ethylhexyl salicylate, were identified to be more abundant or exclusively present in samples collected during the fertile phase [41].

The preputial gland, also known as the clitoral glands in females, releases secretions that can be mixed into the urine. It is another source of odour in mice. Zhang and co-workers conducted a GC-MS analysis to investigate the differences in volatile compounds emitted from the preputial gland secretion and urine of house mice [50]. They identified a total of 42 volatile compounds in the preputial gland secretions, including 32 esters, eight alcohols, and two sesquiterpenes. The result exhibited differences from the identified VOCs in the mouse urine while the urine itself serves as a rich source of additional compounds. Moreover, research by Röck and co-workers demonstrated that aliphatic aldehydes, such as pentanal and decanal, play significant roles in the mouse body scent. Additionally, other compounds, including nitromethane, propanoic acid, dimethyldisulphide, 1-octene, 1-hexanol, hexanoic acid, indole, and α- and β-farnesene, were found in the air surrounding mice while 1-methoxy-2-propane, 6-hydroxy-6-methyl-3-heptanone, phenol and 4-methyl phenol compounds could be found in both the body scent and the urine of mice [52].

In summary, mice establish individual territories through the scent of their secretions, including urine and faeces. Among over 80 VOCs identifiable in mouse urine, six specific VOCs have been found in higher concentrations. Significantly, diverse VOC profiles are identifiable among different mouse species. Moreover, certain VOCs display sex-specific characteristics or manifest variations in concentrations between different genders. These differences can be attributed to the various sexual stages of mice and the secretions from specific preputial glands, which play an essential role in sexual attraction and information exchange between mice of different genders.

## 3. Rats

Rats, like mice, exhibit similar behavioural traits such as territoriality and communication through scent signals. Rat urine contains specific compounds, including squalene, 2-heptanone, and 4-ethylphenol, which play a crucial role in transmitting information among individuals [17]. A study by Zhang and co-workers reported that male rats in the mature stage release a mixture of VOCs in their urine, including squalene, 2-heptanone, 4-ethylphenol, 4-heptanone, and phenol. These compounds are suggested to have sex-attractive functions in female rats [58]. In a similar study, Takács and co-workers collected bedding materials contaminated with rat secretions (urine and faeces) and identified nine male-specific VOCs in these samples [59]. Furthermore, they exploited six of these VOCs, namely 2-heptanone, 4-heptanone, 3-ethyl-2-heptanone, 2-octanone, 2-nonanone, and 4-nonanone, to attract female rats. The results showed a significant increase, approximately ten-fold, in the attraction of female rats compared to the control group.

It is noteworthy that while 2-heptanone and 4-ethylphenol are primarily found in the urine of male rats, they can also be detected in the urine of female rats, though in significantly lower concentrations [58]. Osada and co-workers also reported the attractiveness of 2-heptanone and 4-ethylphenol to female rats and they further identified 4-methyl phenol, which exhibits similar functions in female rats, emitted by adult male rats [60]. Squalene, a compound synthesized by the preputial glands of rats, is naturally present in the nest of female rats and areas where they conduct their activities. However, its concentration significantly increases during the pre-estrus and estrus stages to transmit mating information to the opposite sex [61]. Furthermore, male rat urine has been found to contain compounds such as 2-(octylthio) ethanol, and 1-chlorodecane, known to attract female conspecifics. Female rats in the estrus stage produce compounds like hydroperoxide, 1-nitropentane, and 4-azidoheptane, which are particularly attractive to male rats. Interestingly, 1-nitropentane also elicits attraction in female rats [62].

A scientific study has revealed the presence of distinct volatile compounds in rat urine, including 1-chlorodecane, 2-methyl-N-phenyl-2-propenamide, hexadecane, and 2,6,11-trimethyl decane [63]. Notably, these compounds form complexes with major urinary proteins within rat urine. This binding mechanism serves to prolong the lifespan of the volatiles in the air, allowing for their sustained presence while controlling their overall concentration [3][63]. Another study revealed the presence of specific compounds in the preputial glands of ship rats, including cyclohexene, beta-bisabolene, 1-pentene, hexadecatetraene, 3-cyclohexene, farnesol 1, and farnesol 2. Among these compounds, only the farnesol compounds were found to be bound with major urinary proteins within rat urine [64].

Byrom and co-workers report that over 20 kinds of volatile compounds can be emitted from the body of the rat. Further simulation experiments investigated four VOCs that make the greatest contribution towards creating rat body odour (pyrazine and thiazole-related compounds) [17]. Another study conducted by Schneeberger and co-workers identified a total of 27 biologically relevant VOCs in rat odour, comprising 11 carboxylic acids, 10 aldehydes and ketones, four alkanes, one ester, one alcohol, one sulfone, and one terpene [65]. The investigation also involved a comparison of the mean relative abundance of these compounds, as determined by the ratio of a particular compound’s peak area to the total peak area in the chromatographic profile. It is noteworthy that the study revealed substantial variations in the relative concentrations of seven specific volatiles present in rat odours between individuals in a hungry state and those in a satiated state. Notably, butyl acetate and 3-methyl butanoic acid were exclusively released by hungry rats, while pentanoic acid was identified solely in the odour of satiated rats.

Carbon disulfide is a typical volatile compound identified in the exhaled breath of rodents. The specialized olfactory sensory neurons of rats can detect the presence of carbon disulfide emitted from their conspecifics and employ this chemical signal to acquire information regarding the safety of food sources [66]. In addition, it is reported that hexanal and 4-methyl pentanal can be identified in the odour released from anxious rats [67]. The identification of these VOCs provides insights into the relationship between VOC components and the emotional state and well-being of rats, which can also serve as potential biomarkers for anxiety in rats and offer a possible non-invasive approach for evaluating the emotional and physiological states of laboratory animals.

In addition to other sources, the glands of rats contribute significantly to overall rat odours. The scent glands of rats contain a mixture of alcohols, aldehydes, and acids derived from both saturated and unsaturated aliphatic or aromatic compounds. These compounds found in the scent glands function as pheromones in rats [68]. The cheek glands of rats play a significant role as a source of odour-producing secretions. A diverse range of compounds, including alkanes, aliphatic acids, esters, and alcohols, were discovered in the cheek gland secretions of laboratory rats [69]. Male rats' cheek glands were found to contain di-n-octyl phthalate to elicit attraction solely from females. Furthermore, the study identified two key components in the cheek gland secretions of female rats: 1,2-benzene dicarboxylic acid (2-methylpropyl) ester and 2,6,10 dedecatrien-1-ol, 3,7,11-trimethyl-(Z, E). Notably, these compounds demonstrated attractive properties for both male and female rats [69]. The study reported by Kannan and co-workers has indicated that the clitoral gland of female rats secretes specific compounds, including 6,11-dihydro-dibenzo-b,e-oxepin-11-one, 2,6,10-dodecatrien-1-ol-3,7,11-trimethyl(Z), and 1,2-benzene dicarboxylic acid butyl(2-methylpropyl) ester. These compounds are believed to serve as signals of attraction, playing a role in communication between conspecifics [68]. Early research also investigated that the preputial glands of male rats release volatile compounds, including 2,6,10-dodecatrien-1-ol-3,7,11-trimethyl, and di-n-octyl phthalate, to attract female rats [70]. Similarly, a higher concentration of E-E-α-farnesene and E-β-farnesene can be emitted from male rat glands compared to female rat glands, which plays a role in attracting opposite-sex conspecifics [58][68]. In another study investigating testosterone-dependent volatile compounds, researchers identified a total of 34 different volatiles in the preputial gland of rats, including 15 alkanes, six sterols and steroids, four terpenes and terpenoids, four fatty acid esters, one chlorinated compound, and four other compounds [71].

In comparison to mice, rats exhibit similar territorial instincts and behavioural characteristics. Three specific VOCs, namely squalene, 2-heptanone, and 4-ethylphenol, play a crucial role in delineating individual territories and facilitating inter-individual communications. As observed in mice, rats also exhibit sex-specific variations in the concentrations of certain VOCs. These differences are primarily attributed to specific preputial glands responsible for signalling sexual attraction. Moreover, the scent glands in rats contribute to their overall body odour, resulting in a more complex blend of VOCs. Notably, the concentrations of these specific VOCs can be different based on various physiological states, including different estrus states and satiety levels.

## 4. Mustelids

Before the 1990s, studies on mustelids, including stoats and ferrets, primarily focused on analyzing the odorous components of their anal secretions, which serve as strong sources of odours [72][73][74][75][76][77][78][79]. For example, Crump's study identified 2-propylthietane as the major component emitted from the anal gland secretions of stoats [74]. Subsequent studies in mustelid odours have revealed the presence of new thietanes in stoat anal glands [76]; and identified 11 volatile compounds, including 2,2-dimethylthietane, 2-propylthietane, 2-pentylthietane, quinoline, and indole, in ferret anal glands [75]. Indeed, a large number of various sulfur-related VOCs could be found in anal secretions of stoats and ferrets; and the abundance of these VOCs shows an association with the gender of mustelids [75][76]. For instance, higher concentrations of 2,3-dimethylthietane and 3,4-dimethyl-1,2-dithiolane can be found in anal secretions of female mustelids while 2-propylthietane is in higher abundance in that of male mustelids [58][74]. In addition, another study revealed the presence of two aldehydes, five ketones, benzothiazole, 2-methylquinoline, and 4-methyl quinazoline in secretions from both male and female mustelids, with a male-specific compound, o-aminoacetophenone, discovered in the secretions. Female secretions, on the other hand, contained 3-ethyl-1,2-dimethyl-1,2-dithiolane [80].

In subsequent studies, the focus on mustelids has shifted towards investigating the pheromones emitted from their fur and urine, given the abundance of preputial and sebaceous glands beneath the skin. The secretions from these scent glands can contaminate the mustelid urine, contributing to its overall odour [81][82][83]. Similar to rodents, male mustelids have larger preputial glands compared to females [84]. A detailed urinary profile for ferrets has been established by Zhang and co-workers, which includes 41 identified compounds and seven unidentified compounds [80]. It is important to highlight the major compounds found in anal secretions, which consist of sulfur-related compounds, differ from the constituents identified in urine. The urine contains higher concentrations of eight identified nonsulfur components. Among these volatile compounds, 2-methylquinoline is exclusively found in male ferret urine, potentially contributing to sex attraction and scent marking of male territories [80][85].

In contrast to rodents, mustelids heavily rely on their anal glands and secretions as prominent sources of odours. These emissions encompass a wide array of VOCs, including numerous sulfur-related compounds, along with certain VOCs based on different genders such as o-aminoacetophenone and 3-ethyl-1,2-dimethyl-1,2-dithiolane. These specific compounds play a crucial role in conveying scent signals related to sexual information exchange between different genders. Furthermore, anal glands and other plentiful subcutaneous glands contribute to the overall body odour and can contaminate mustelid urine, resulting in the identification of nearly 50 different VOCs in mustelid urine. Some of these specific VOCs exhibit distinctions between different species and genders.

## 5. Possums

*Trichosurus vulpecula*, commonly known as the brushtail possum, is a browsing marsupial species that was introduced into New Zealand in the 19th century for the fur industry [3][86]. However, the introduction of brushtail possums had significant ecological consequences, as they became a major pest in New Zealand, posing a threat to forest ecosystems [87].

Compared to rodents, scent communication among marsupials, such as the brushtail possum, is believed to be more complex. It involves the release of sophisticated pheromones from various secretions and numerous glands, suggesting a potential for intricate pheromonal communication at the neurological level [88][89].

The brushtail possum is equipped with various specialised glands dedicated to producing scent marks and olfactory signals [90][91]. For example, paracloacal glands of brushtail possums secrete an oily white liquid with onion or garlic odours, which consists of tetradecanyl hexadecanoate, octadecenoate, $C_5$–$C_{30}$ fatty acids, and alcohols [92][93][94]. A study by McLean and co-workers identified nearly 150 different VOCs in the cloaca secretion of brushtail possums, comprising 81 acids and alcohols, 27 esters (2,6- and 2,7-dimethyloctanol related), and 39 species of sulfur-related compounds; especially, a relatively high concentration of 2-Methyl-3-pentanol (over 20% relative abundance in the chromatographic profile) and hexadecanoate (over 16%) can be found within the cloacal secretions of possums [86]. Woolhouse and co-workers reported that esters of $C_{16}$ and $C_{18}$ fatty acids could be found in the sternal glands of brushtail possums, and Salamon refined the finding that more complex components (23 compounds) can be identified in male possums than in female possums (4 compounds) [94][95]. Zabaras and co-workers reported that acetic acid, 1,1-bis-(p-tolyl)-ethane, $C_6$–$C_{10}$ aldehydes, and long alkyl-chain compounds could be considered as general components of sternal gland secretions [96]. The other glands of the brushtail possum, including labial glands, apocrine glands, and sebaceous glands, also contribute to the production of odours [91][97][98].

Urine is considered to be another significant source of marsupial odours, containing various pheromones from glands. In the case of the brushtail possum, urine is believed to facilitate the dispersion of cloacal secretions, enabling the incorporation of pheromones from the paracloacal glands into the urine [99][100]. Analysis of possum urine reveals interesting differences between male and female possums. In male possum urine, pyrazine and methyl ketone derivatives are present, while aldehydes are exclusively found in female possum urine. Notably, both male and female possum urine contain methyl ketones [100]. Additionally, olfactory communication among marsupials may exhibit similarities, prompting researchers to actively pursue the identification of key semiochemicals for the optimal formulations of lures [17][101][102]. A prior investigation conducted by Toftegaard and colleagues on another marsupial (Brown Antechinus) may provide valuable insights. The study identified 16 VOCs in marsupial urine, including two pyrazine derivatives, six ketones, three aldehydes, and five miscellaneous compounds. These compounds play a crucial role in olfactory communication among marsupials and may serve as the most effective formulations for influencing marsupial behaviour [103].

In conclusion, the presence of a diverse array of glands leads to a more complex volatile profile found in possums' odours. The secretions from these glands contain a multitude of compounds and potentially contaminate urine and faeces. Furthermore, specific preputial glands are implicated in the production of sex-specific VOCs, such as aldehyde derivatives detected in female urine.

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