iScience study: Most frequently asked Q&As on dolphins and skin care

I. Behavioral study (Ziltener *et al.* part)

How can your team be certain that the dolphins are self-medicating using the coral, vs. playing in the coral/rubbing their bodies on a certain species because it feels good?

Dolphins appear to be selectively matching certain body parts to specific corals. While the whole body is rubbed into gorgonians (*Rumphella aggregata*), dolphins select leather corals (*Sarcophyton sp.*) and sponges (*Ircinia sp)* to rub mostly their head region, ventral side and fluke. Particular hard corals (*Favia sp*.) are only used for rubbing the edges of pectoral fins but this behaviour is not describe in this publication. Leather corals and sponges are more compact and harder in their texture than the soft gorgonian coral branches, so the dolphins push one particular body part strongly into the selected substrate. The dolphins are not rubbing on one of these mentioned organisms when they are surrounded by other corals, which could harm their skin, such as the fire coral. They seem to be very aware of what they are choosing. The more sensitive calves aged under one year have not been observed engaging in the group rubbing on these particular organisms, instead they watch the adults doing the rubbing.

How long have scientists known about this rubbing behavior practiced by *T. aduncus*? Was it widely thought to be a form of self-medication, or were there competing theories as to its function?

In 2009, when Angela Ziltener, a wildlife biologist at the University of Zurich, and her team started to study the Indo-Pacific bottlenose dolphins, they observed the rubbing behaviour on corals and sponges. They wanted to find out more about these selected marine organisms in particular their bioactive compounds. The gorgoning behaviour was observed by other divers, before the scientists started the research project on site. Please check following links:

https://www.dolphinwatchalliance.org/index.php/en/projects/self-rubbing-behaviour

https://www.dolphinwatchalliance.org/index.php/en/projects/selective-self-rubbing-behaviour

Based on your observations, do you suspect the dolphins do this regularly (more as a preventative against infection) or reactively, only after picking up an infection?

We observed it more regularly, juveniles and adults are also doing the rubbing behaviour without having any infection and the younger calves are watching the adults doing the rubbing on gorgonian corals or the sponge. Our hypothesis that dolphins utilize these secondary metabolites against dermal disease-causing pathogens in the sense of preventative or curative self-medication appears plausible. The linking of the observation with the effect detection is new. Although similar gorgonian coral species are known to produce antimicrobial as well as cytotoxic secondary metabolites and leather coral species are reported to contain bioactive metabolites, the findings were not connected with the corals and sponges that the dolphins selectively or targeted accessed. Self-medication is also known in primates and other animals but not in cetaceans.

Please explain what kind of skin conditions makes the dolphins rub their skins against corals? (Kindly, tell us in detail about the particular skin conditions)

Dolphins queue up behind each other and wait for their turn to approach the invertebrate. This group event has been observed for the gorgonian coral and sponge, but not for the leather coral. The leather coral rubbing appears more randomly mostly alone. The more sensitive calves aged under one year have not been observed engaging in the group rubbing on these particular organisms, instead they watch the adults doing the rubbing. The rubbing behavior is observed for juvenile and adult dolphins, they don't need to have a particular skin condition.

Could you please tell us something about the behavioral and biological characteristics of the polyps that form the coral community (which is also very relevant to your research)? Do the polyps also get benefitted in some way when dolphins rub their skin against them?

On the gorgonian coral, dolphins slide into the branches of the coral and rub several body parts against it. Upon rubbing, the gorgonian coral polyps start to secrete mucus and to close and the mucus secreted by the corals can then be transferred to the skin of the dolphin. Through the closed polyps and resultant harder and rougher surface of the coral, skin contact via abrasion and subsequent absorption might be even more efficient. For example, the dolphin rubs its ventral, lateral or dorsal body part on the leather coral and its head and fluke often touches the coral, too. As a further example, the dolphin rubs its ventral or dorsal body part and fluke on the sponge and pushes its head strongly against it and twists it around. We do not know so far, if the polyps also are benefitted in some way, but it could be possible.

You said in your research that "the dolphins knew exactly which coral they wanted to use". Does that mean only particular coral species are used by the dolphins for self-medication? If so, please mention some of those coral species.

Dolphins appear to be selectively matching certain body parts to specific corals. While the whole body is rubbed into gorgonians (*Rumphella aggregata*), dolphins select leather corals (*Sarcophyton sp.*) and sponges (*Ircinia sp)* to rub mostly their head region, ventral side and fluke. Particular hard corals (*Favia sp*.) are only used for rubbing the edges of pectoral fins but this behaviour is not described in this publication. Leather corals and sponges are more compact and harder in their texture than the soft gorgonian coral branches, so the dolphins push one particular body part strongly into the selected substrate.

What concrete steps has the Dolphin Watch Alliance taken to protect the animals? What else can be done?

Angela and her colleagues founded Dolphin Watch Alliance in 2011 to support the research, educational and conservation projects on site and spread more awareness about the dolphins and their environment. The team gave over 100 workshops and seminars to the public, tourists, boat captains and tour guides. With their projects, they are bringing sustainable change to the area of marine wildlife tourism such as creating a Code of Conduct (guidelines for responsible dolphin encounters) with other organizations together and proposing protected reef areas which the dolphins visit during the daytime to rest, socialize

and clean their skin. The aim of Dolphin Watch Alliance is respectful and regulated encounters between humans and animals in the wild, taking into account the needs of all parties. Due to their studies over the years, the Northern Red Sea Islands was declared as Important Marine Mammal Areas in 2019, see link: https://www.marinemammalhabitat.org/portfolio-item/northern-red-sea-islands/

Were there any particular challenges that arose during the fieldwork for this study (either to do with observing the dolphins or collecting coral samples)?

It needs time to understand a dolphin population, their activity states, behaviour patterns and social structure. If you collect data (dolphin behaviour, social structure, distribution and abundances) over years, the data is more significant. It also needs time to get the trust of the dolphins to observe them very close and follow them over a longer time during Scuba diving. Because the dolphins accepted the scientists in the group, they could see which particular corals and sponges they are using. Also the process of paperwork to get the permission to sample the invertebrates takes time, and of course, the analysis of the bioactive compounds as well. In the last decade, Gertrud Morlock and her team have worked hard on the development of a straightforward procedure, combining chemistry (separation of mixtures) with biology (biological effect detection) on the same surface.

How might the dolphins be affected if the coral populations decline, due to climate change, for instance?

The reefs are essential for many marine species, everything depends on each other. It is crucial to get more understanding on invertebrate and vertebrate interaction to protect them.

You mention the importance of protecting these animals in light of tourists/other human disturbances. Is there any way to know how not having the corals would affect the dolphins' health?

So far, we don’t know.

II. Analytical investigation (Morlock *et al.* part)

On a chemical level, what is happening that is "medicating" the dolphins?

Our hypothesis that dolphins utilize these secondary metabolites against dermal irritations or pathogens in the sense of preventative or curative self-medication appears plausible. Repeated rubbing allows the bioactive metabolites to come into direct contact with the skin of the dolphins, which in turn could help them support their skin homeostasis and be useful for prophylaxis or auxiliary treatment against microbial infections, says Gertrud Morlock, analytical chemist and Professor of Food Science at Justus Liebig University Giessen in Germany. The innovative analytical technique combines the separation of the mixture with an on-surface bioassay, which links to the bioactive compounds in the marine organism. First this provided understanding on a potential purpose why they are doing so.

The linking of the observation with the effect detection is new. Although similar gorgonian coral species are known to produce antimicrobial as well as cytotoxic secondary metabolites and leather coral species are reported to contain bioactive metabolites, the findings were not connected with the corals and sponges that the dolphins selectively/targetedly accessed.

In the last decade, Gertrud Morlock and her team have worked hard on the development of a straightforward platform, combining chemistry (separation of mixtures) with biology (biological effect detection) on the same surface. We found 17 biologically active substances with antimicrobial, antioxidant, hormonal and toxic properties. We subjected only few microliters of the extract (e.g., 1 cm sponge in 1 mL solvent) to the analysis and already saw biological effects. We found 17 bioactive compounds overall across all three species. There were effect mechanism in common, meaning out of the 17, 10 were antibacterial/antimicrobial. In contrast, there were also differences observed, e.g., preferably the leather coral produced estrogen-like (hormonal-active) compounds.

Regarding the bacterial assays, were the bacterial species selected because they're known to be relevant to dolphin health? Did any of the results of these assays stand out as particularly surprising/notable to you?

The potpourri of different effect that we observed was surprising. We used one Gram-negative strain and one Gram-positive bacterial strain, to cover both bacterial cell wall types for the antibacterial bioassays. In both, antibacterial compounds were observed, thus acting against both types of bacteria. Moreover, we used well-known mammalian hormonal receptors for the bioassay detection of hormonal effects and the very sensitive planar SOS-Umu-C bioassay for detection of genotoxic compounds. The acetylcholinesterase/butyryl-cholinesterase inhibition assays were used to detect neurotoxic compounds.

Based on the behavioral observations and the profound analytical data obtained, we dared to hypothesize that the bioactive molecules can have an effect upon skin contact. Morlock says, “We thought it is like making a chamomile extract for human skin care, to mention one example of many that we use as humans or in traditional medicine. Dolphins are intelligent, but they have no arms and hands. So how can they make skin care otherwise?”

What could the antioxidative and hormonal properties of the dolphins provide - could it be something like a 'skincare regime' but for dolphins?

Exactly. We found it plausible because: Estrogen keeps our human skin hydrated and helps with its elasticity and oil production. Antioxidants are acting against cell stress. Antibacterial compounds and toxic compounds (the latter not so pronouncedly present than antibacterial or antioxidative compounds!) could help against parasites and pathogens on the skin surface.

Do any of the compounds identified suggest they could be useful on specifically dolphin skin, or to help with skin conditions that dolphins have?

We assume that the detected bioactive compounds suggest they could be useful on specifically dolphin skin, considering the many different polarities (from polar to apolar) of the bioactive compounds found in the three species using 10 different planar on-surface

bioassays. There were several effect mechanisms detected that are good for the skin, e.g., working against dermal irritations or pathogens in the sense of preventative or curative self-medication.

I understand you identified general antibacterial properties of some of the compounds released by the coral, but would you be able to point to any in particular that might have a specific affect on dolphins?

Based on the behavioral observations and the profound analytical data obtained, we dared to hypothesize that the bioactive molecules can have an effect upon skin contact. We thought it is like making a chamomile extract for human skin care, to mention one example of many that we use as humans. Dolphins are intelligent, but they have no arms and hands. So how can they make skin care otherwise?

What would you flag as limitations of this research?

We did not test the bioactive compounds discovered in vivo on a dolphin skin having a disease. Having only about 1 mL (0.2-1.5 mL) sample volume, there was not enough sample for performing structure elucidation.

Take home

Let it (the wealth of bioactive compounds in these invertebrates) be for the dolphins! As the dolphins cannot speak and talk to you, we should not forget that Analytical Chemistry is the basis of all knowledge, as it provides the insights, Morlock says. Although it is not seen and often overlooked, it is the essential part.

What inspired this research?

Natural food, my main study object, and nature in general is a fascinating source of inspiration for me. When you observe animals, you realize that they behave very much like humans, even better, because even the cruelties you see are mostly driven by survival and not by malicious intrigues. Even sessile nature is not sessile if you look closely. As nature is so amazingly sophisticated (too much complexity for artificial intelligence and human understanding), why do we destroy it?

In 2007, Franz Brümmer, associate professor of biodiversity and scientific diving at the University of Stuttgart, approached me to study the bioprofiles of sponges [1] and later worm snails [2]. In 2019, he asked me again to bioprofile invertebrates which are selectively accessed for rubbing by Indo-Pacific bottlenose dolphins in the Northern Red Sea. This behavior was observed by Angela Ziltener, a wildlife biologist at the University of Zurich, and her team already in 2009. Over a decade they wondered why and our current aim was to have a closer look. As the dolphins cannot speak and talk to you, we should not forget that Analytical Chemistry is the basis of all knowledge, as it provides the insights. Although it is not seen and often overlooked, it is the essential part to proceed.

Could you briefly describe your analysis method?

Over the last decade, we developed a straightforward image-based hyphenated technique [3] that can deal with unknown unknowns, combining chemistry (separation of mixtures)

with biology (biological effect detection) on the same planar surface. This combination of two disciplines is extremely helpful in prioritizing the important compounds among the thousands of compounds present in such natural samples. It is therefore a very powerful unique technique for detecting active substances found not only in invertebrate but also natural plant-based food and traditional medicines. We easily detect traces of substances that are important. Their characterization is performed highly targeted using the high-resolution mass spectrometry recording of interesting active zones only.

Why choose this particular analysis method?

An image is worth a thousand words, an effect-image even more! To understand an image, you need no language! The technique is very matrix-tolerant, so the raw sample extract can be used. Remind that every sample preparation step can alter the original sample and discriminate ingredients which are lost then. If you search for active unknown unknowns, you treasure especially this feature. Moreover, it is such a fast and cost-efficient technique which can easily be used in routine. The parallel analysis of 20 samples, calculates the costs per sample 0.5-0.8 Euro and the analysis time 5-15 min per sample, depending on the planar assay. Especially for costly samples, you can trim the system not to lose a microliter of your sample. This allowed us to perform chemical separation combined with 10 different assays, followed by high-resolution mass spectrometry recording of interesting active zones.

Can you summarize the main findings?

We found 17 biologically active substances with antimicrobial, antioxidant, hormonal and toxic properties across all three invertebrate species. The potpourri of different effects that we observed was surprising. We used one Gram-negative and one Gram-positive bacterial strain, to cover both bacterial cell wall types for the planar antibacterial bioassays. Antibacterial compounds acting against both types of bacteria were observed in the three invertebrate species. Moreover, we used well-known mammalian hormonal receptors in the bioassay to detect hormonal effects and the latest, very sensitive planar SOS-Umu-C bioassay for detection of genotoxic compounds. The acetylcholinesterase/butyrylcholinesterase inhibition assays were used to detect neurotoxic compounds. We subjected only few microliters of the extract (e.g., 1 cm sponge in 1 mL solvent) to the analysis and already saw biologically effective compounds. There were effect mechanisms in common across the three species, such as antibacterial/antimicrobial compounds of different polarities. In contrast, there were also clear differences between the species observed, e.g., preferably the leather coral produced hormonal-active compounds. Based on the long-term behavioral observations and the profound analytical data obtained, we dared to hypothesize that the bioactive molecules can have an effect upon skin contact. The contact with the dolphin skin (coloring it green-yellowish and staying there, as they have no hands to wipe it away) was clearly proven by the videos taken. I thought it is like making a chamomile extract for human skin care, to mention one example of many that we use as humans or in traditional medicine. Dolphins are intelligent - how else can they do skin care if they have no arms and hands?

Did you encounter any difficulties with sample collection?

Not at all. We used minimally invasive sampling (e.g., 1 cm sponge in 1 mL solvent) to minimize disturbance to nature. Having only about 1 mL sample extract volume, we successfully performed 10 different assays and detected differently active compounds. Some we could assign due to literature data, which we consider as proof of principle of the technique used, but there was not enough sample for structure elucidation of the discovered active unknowns, which we could not assign due to lack of literature data.

Do you have any plans for future research?

Too much than hands (like all devoted colleagues). I would like to give analytical chemistry even more efficiency through innovative thinking. For example, latest planar multiplex assay developments first provide the important information, which is necessary for understanding complex mixtures [4], or as another example, the transfer of the developed straightforward image-based hyphenated technique to a miniaturized open source 2Labs2Go system that can be used for Citizen Science [5]. Participating in the discovery of the secrets of nature is great because it makes you so incredibly humble! Let it (the wealth of bioactive compounds in these invertebrates) be for the dolphins!

[1] Klöppel, A., Gasse, W., Brümmer, F., Morlock, G. HPTLC Coupled with Bioluminescence and Mass Spectrometry for Bioactivity-Based Analysis of Secondary Metabolites in Marine Sponges, J. Planar Chromatogr. 21 (2008) 431-436

[2] Klöppel, A., Brümmer, F., Schwabe, D., Morlock, G. Detection of bioactive compounds in the mucus nets of Dendropoma maxima, Sowerby 1825 (prosobranch gastropod Vermetidae, Mollusca), J. Marine Biology (2013) 283506, doi.org 10.1155 2013 283506

[3] Morlock, G.E. High-performance thin-layer chromatography combined with effect-directed assays and high-resolution mass spectrometry as an emerging hyphenated technology: A tutorial review, Anal. Chim. Acta 1180 (2021) 338644, doi.org/10.1016/j.aca.2021.338644.

[4] Ronzheimer, A., Schreiner, T., Morlock, G.E. Multiplex planar bioassay detecting phytoestrogens and verified antiestrogens as sharp zones on normal phase, Phytomedicine, in print.

[5] Sing, L., Schwack, W., Göttsche, R., Morlock, G.E. 2LabsToGo – Recipe for building your own chromatography equipment including biological effect-detection, in submission

Contacts of first authors

Prof. Dr. Gertrud Morlock (analytical investigation)
Justus Liebig University Giessen
Full Professor of Food Science, and Analytical Chemist
Director of TransMIT Center for Effect-Directed Analysis
Board Member of the Interdisciplinary Research Center (iFZ)
Heinrich-Buff-Ring 26-32
35392 Giessen, Germany
Tel. +49-(0)641-99-39141
Fax +49-(0)641-99-39149
www.uni-giessen.de/food

Angela Ziltener
Research Guest University Zurich (behavioral study)
https://www.aim.uzh.ch
Research Guest Technical University Berlin, Campus El Gouna
https://www.campus-elgouna.tu-berlin.de
CH +41 76 411 27 90
EG +20 109 633 71 41
Founder and president of Dolphin Watch Alliance
https://www.dolphinwatchalliance.org