Skin ulcerations in *Holothuria scabra* can be induced by various types of food

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Abstract

Skin ulceration disease and skin ulceration syndrome (SKUD-SUS) affecting sea cucumbers have been described from various regions in the world. Both are attributed to bacteria and/or viruses. *Holothuria scabra* is, as many sea cucumbers are, a deposit feeder that feeds on organic matter from the sediment. Here, we demonstrate that sediments enriched with different types of organic matter can induce skin ulcerations in *H. scabra*. The various tested organic matter added to the sediment included: i) crushed integument ulcerations from ulcerated *H. scabra* adults; ii) crushed integument from healthy *H. scabra* juveniles; iii) crushed skin from healthy fishes; and iv) crushed healthy red seaweed. The three first types of organic matter induce skin ulcerations in less than three days of exposure, sometimes leading to the death of an individual. The last, non-animal, organic matter did not induce skin ulcerations. Our results indicate that: i) skin ulceration is not a single disease but a symptom of bad health, and it occurs in various diseases induced by either biotic agents or abiotic factors (e.g. the ingestion of non-adequate food); and ii) fish meal comprising animal organic matter, which when added to food, can be deleterious for sea cucumbers.

Introduction

Skin ulceration disease and syndrome (SKUD-SUS) affect the integument of sea cucumbers. The first sign of these diseases is the appearance of whitish spots on the skin. These spots are actually the result of the breakdown of the cuticle, and the epidermis putting the partially degraded underlying dermis in contact with the water. Microscopic observation reveals disorganised collagen fibers and scattered ossicles (Eeckhaut et al. 2004). The first spots are often on the periphery of the cloaca of the affected individual (Becker et al. 2004), but then colonise more and more areas until they become widespread after 24 hours in a large posterior lesion. New spots appear all over the body until the entire surface is covered with them. After three days, affected individuals die. During the evolution of the disease, the individual is characterised by a state of general weakness and an inability to retract its tentacles and to adhere to the substrate. It can also present anorexia, swollen mouth and uncontrolled tremors (Eeckhaut et al. 2004; Wang et al. 2004).

Two main causes of the disease mentioned in the literature are a viral agent (Deng et al. 2008; Hongzhan et al. 2010) or bacteria, with some research on *Apostichopus japonicus* showing that both agents can induce ulcerations in this species. Previously, members of our team showed that bacteria in skin ulcerations of *H. scabra* juveniles could not be transmitted through contact, and that most bacteria present in the lesions are probably opportunistic bacteria (Becker et al. 2004). With regard to bacteria, *Vibrio* (Proteobacteria, Gammaproteobacteria) are the most commonly observed species in lesions, and some can induce ulceration in *A. japonicus* (Becker et al. 2004; Eeckhaut et al. 2004; Deng et al. 2008; Hua et al. 2010; Liu et al. 2012; Ma et al. 2006; Morgan 2001; Yancui et al. 2011; Yancui et al. 2012). Other Gammaproteobacteria, represented by the genera *Pseudoalteromonas* and *Pseudomonas*, which are common and abundant in muddy sands, are also observed in lesions (Hongzhan et al. 2010; Hua et al. 2010). *Bacteroidetes* bacteria are usually present in the intestinal flora and can, in certain cases, be pathogenic (Becker et al. 2004, Eeckhaut et al. 2004). Alphaproteobacteria are also commonly found in marine sediments and have some representatives that are abundant in the digestive system of *H. scabra* (Eeckhaut et al. 2004; Plotieau et al. 2013).

The hypothesis in this work was that skin ulcerations could be induced by factors other than bacteria and viruses. For that purpose, we tested whether sediments that are ingested by *H. scabra* can induce skin ulcerations when they are enriched with different types of organic matter.

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Materials and methods

The experiments were performed in the R & D Department (Madagascar Holothurie) of the Indian Ocean Trepang company in Madagascar in 2016. Four types of organisms were crushed using a manual food grinder: 1) diseased *H. scabra* adults taken from the Indian Ocean Trepang sea pens; 2) healthy *H. scabra* juveniles taken from the Indian Ocean Trepang land ponds; 3) healthy, small perciform fishes (caught in the harbour); and 4) healthy *Kappaphycus alvarezii* (red seaweed) taken from the farms of the company Ocean Farmer. The fluid obtained was mixed with healthy sediment from marine enclosures where the disease has never been reported. Daily monitoring of the sea cucumbers (i.e. healthy sea cucumbers with no ulcerations) placed on the various sediments was carried out in order to observe the potential emergence of ulcerations and other symptoms.

Ten kilos of wet sediment were deposited in experimental basins measuring 2 m^2. In the enriched sediments, 50 g kg^{-1}or 25 g kg^{-1} of crushed, diseased *H. scabra* adults was added against 50 g kg^{-1} for healthy *H. scabra* juveniles, healthy fishes and healthy *K. alvarezii*. Thirteen sea cucumbers were placed on normal sediment (i.e. i.e. free of crushed organisms) and were monitored for three days as a negative control. Sediment enriched with diseased *H. scabra* adults was tested on 26 sea cucumbers: 21 for the first condition (50 g kg^{-1}) and 5 for the second (25 g kg^{-1}). Sea cucumbers were monitored for two days. The experiment involving the highest concentration of 50 g kg^{-1} was carried out twice, the first time with 16 individuals and the second time with 5 individuals. Sediment enriched with healthy *H. scabra* juveniles was tested on four sea cucumbers and monitored for two days. Sediment enriched with crushed fish was tested on five sea cucumbers and monitored for two days. *K. alvarezii*-enriched sediment was tested on five sea cucumbers and monitored for two days.

Pictures of individuals were taken every day. Ulceration coverage within the bivium and trivium was estimated by measuring the total ulceration surface based on dorsal and ventral pictures using ImageJ (Schneider et al. 2012). The integument ulceration coverage was expressed as a percentage of the total integument surface.

Results

In the control and in the sediment enriched with *K. alvarezii*, none of the individuals showed any degradation of their integument. No signs of disease onset or evisceration could, therefore, be detected after six days of observation.

All healthy sea cucumbers placed on sediment enriched with diseased *H. scabra* adults, at a concentration of 50 g kg^{-1}, died during the night following contact with the sediment. All individuals were found eviscerated and with a strongly disintegrated integument in the majority of cases (Fig. 1). Thirteen out of sixteen individuals present in the first batch showed a particular behaviour a few minutes after

Figure 1. Eviscerated and dead sea cucumbers placed on sediment enriched with diseased *H. scabra* adults at a concentration of 50 g kg^{-1} after one day of experiment. Scale: 3 cm

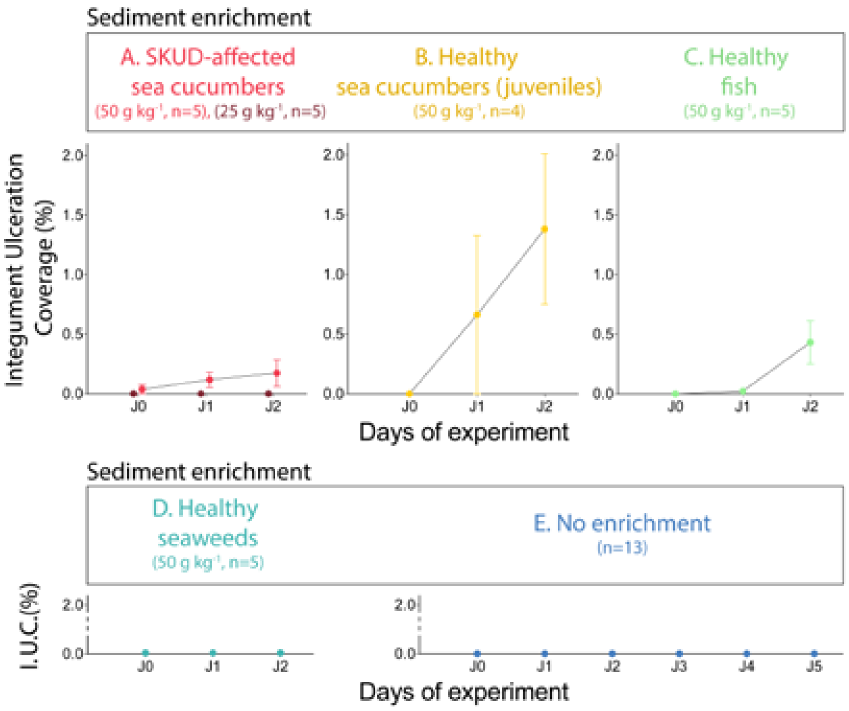

Figure 2. Integument ulceration coverage observed in *Holothuria scabra* sea cucumbers placed on an enriched sediment. The different enrichment treatments were: A) sediment enriched with crushed diseased adults of *H. Scabra*; B) sediment enriched with crushed healthy juveniles of *H. scabra*; C) sediment enriched with healthy fishes; D) sediment enriched with healthy red seaweed (*Kappaphycus alvarezii*); E) no enrichment.

Figure 3. Integument ulceration coverage observed in three *Holothuria scabra* individuals placed on sediment enriched with crushed diseased adults of *H. scabra* (A–C); crushed healthy juveniles of *H. scabra* (D–F); and healthy fishes (G–I). Two days of exposure are presented (J0 and J2). Scales A, B, D, E, G, H: 2 cm; Scales C, F, I: 1 cm.

their interaction with the enriched sediment: they were very agitated, stirred quickly and exposed their trivium upwards. They also tried to avoid the black patches that line the sediment where the density of crushed organisms was greater. The repetition of this experiment has yielded somewhat different results. At the end of the two days of observation, only 60% of individuals had moderate integumentary damage (Figs. 2A and 3), which resulted in the evisceration and death of two individuals.

None of the individuals placed on sediment enriched with diseased *H. scabra* adults at a concentration of 25 g kg^{-1} showed any sign of ulceration or evisceration (Fig. 2A).

The health of the five individuals placed on sediment enriched with crushed healthy *H. scabra* juveniles (Fig. 2B) and four individuals placed on sediment enriched with crushed healthy fishes (Fig. C) were significantly altered, even leading to the evisceration and death of one individual. All of the individuals placed on sediment enriched with crushed fishes also had ulcerated integuments, with two individuals that finally died. The other three died more slowly but still had whitish lesions on different parts of their body.

Discussion

Several studies conducted, mainly on *A. japonicus*, have suggested that certain bacteria and viruses are the agents of SKUD (Morgan 2001; Ma et al. 2006, Deng et al. 2008; Deng et al. 2009, Hongzhan et al. 2010, Hua et al. 2010; Li et al. 2010; Yancui et al. 2011; Liu et al. 2012; Yancui et al. 2012). SKUD symptoms are found in several species of sea cucumbers that have varying geographic distributions (Morgan 2001; Deng et al. 2008; Deng et al. 2009; Hongzhan et al. 2010; Yancui et al. 2011; Liu et al. 2012). Here, we have demonstrated that enriched sediments with various types of organic matter can induce skin ulcerations in *H. scabra*. The concept of SKUD does not refer to one single disease but to a group of diseases characterised by similar macrosymptoms, including skin ulcerations. In this context, this work refers to a specific SKUD that is present in the sea pens in Toliara, Madagascar.

Another point suggested by these results is that a high proportion of animal organic matter in ingested sediments does not seem to be good for the survival of *H. scabra*. Indeed, not only does sediment enriched with crushed sea cucumbers with skin ulcerations induce ulcerations in healthy individuals following ingestion, but also sediment enriched with healthy integument from sea cucumbers or with crushed fishes. On the other hand, seaweed-enriched sediment does not induce skin ulcerations. This result goes in the same direction as the results of Plotieau and colleagues (2014) who compared sea cucumber growth in two villages on the coast of Madagascar: Tampolove and Fiherenaramasay. Sea cucumber growth was better in Tampolove, where sediments contain a high proportion of primary producers, than in Fiherenaramasay, where organic matter contains fragments of organisms from a higher level of the food chain. They explained this situation by the presence of a very important population of ophiurids (*Ophiocoma scolopendrina*) at a density of > 3 ind. m^{-2} at Fiherenamasay, but not in the other three villages. Once they die, ophiuroids may greatly contribute to the formation of organic matter, a hypothesis supported by the high proportion of magnesian calcite found in the sediment, since the skeleton of echinoderms consists mainly of magnesian calcite.

Animal organic matter does not appear to be good for the survival and growth of *H. scabra*. In previous research (Rakotonjanahary et al. 2016), different foods were tested in ponds on juveniles that grew under the same conditions (i.e. temperature, salinity and density). The worst results were obtained with sediments with added provender. The provender was made with fish flour as a source of animal protein (30%), soya powder as a source of vegetable protein (26%), peanut pieces as a source of lipids (17%), and rice or cassava as a source of carbohydrates, and was added two times per week. After seven weeks, the survival rate was of 42% and the biomass was less than 50 g m^{-2}, both values being the worst results of the tested food.

These researches indicate that the presence of vegetal organic matter seems preferable than animal organic matter in the food of *H. scabra*. With regard to farming, we suggest checking the trophic level of the sediment through δN isotopic analysis, when possible, or at least checking the presence of well-developed seagrass beds in the vicinity of the farming sites that would bring vegetal organic matter during the natural recycling.

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