Vol. 38: 71–74, 1999 | DISEASES OF AQUATIC ORGANISMS Dis Aquat Org | Published October 11

NOTE

Sea urchin *Meoma ventricosa* die-off in Curaçao (Netherlands Antilles) associated with a pathogenic bacterium

I. Nagelkerken[1,2,*], G. W. Smith[3], E. Snelders[1], M. Karel[1], S. James[3]

[1]Carmabi Foundation, PO Box 2090, Piscaderabaai z/n, Curaçao, Netherlands Antilles
[2]Laboratory of Aquatic Ecology, University of Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands
[3]Department of Biology, University of South Carolina - Aiken, 171 University Parkway, Aiken, South Carolina 29801, USA

ABSTRACT: During January 1997 a die-off occurred in the sea urchin *Meoma ventricosa* in Curaçao, Netherlands Antilles. The mortalities were characterized by a progressive loss of spines and death. Microscopy of affected tissue revealed amorphous catch connective tissue of the spines, containing Gram-negative bacterial cells. Deliberate infections of the urchin *Lytechinus variegatus* with 1 of the 2 bacterial isolates resulted in signs similar to those observed in affected *M. ventricosa* in the field. The *M. ventricosa* die-off was restricted to an area of 3.5 km along the coast just down-current of the main harbor of Curaçao, and caused a significant decrease in population size of *M. ventricosa* in this area. In the impact area the percentage of affected urchins was similar, but the percentage of dead urchins dropped from 83 to 0% with distance down-current from the harbor. It is likely that polluted water from the harbor increased the density of pathogenic bacterial strains causing the infection.

KEY WORDS: Mortality · Pathogenic bacterium · Sea urchin · *Meoma ventricosa* · Infection · *Lytechinus variegatus*

Mass mortalities of echinoids have been recorded worldwide. They can be caused by unfavorable environmental conditions, diseases, or a combination of both (e.g. Scheibling & Hennigar 1997). Mortalities resulting from unfavorable environmental conditions are often caused by sharp or sudden changes in any of a wide variety of abiotic factors. In the Caribbean region, mortalities have been associated with factors such as earthquakes, strong water motion from storms and hurricanes, exposure during low tides, high sea water temperature, decreased seawater salinity after heavy rain fall, phytoplankton toxins and pollutants (Cortés et al. 1992, Lawrence 1996).

Mass mortalities resulting from diseases are often caused by microorganisms (Jangoux 1987a, b). However, the causative agent has been identified for only a few of the reported mass mortalities. In the bald-sea-urchin disease (Maes & Jangoux 1984) the causative agent was of bacterial nature (Gilles & Pearse 1986), while a marine amoeba was suspected as the causative agent of mass mortalities of *Strongylocentrotus droebachiensis* (Jones & Scheibling 1985).

In the Caribbean, (mass) mortalities have been reported for a variety of echinoid species (see review by Lawrence 1996), such as *Diadema antillarum* (Lessios et al. 1984), *Astropyga magnifica* and *Eucidaris tribuloides* (Williams et al. 1986), *Tripneustes ventricosus* and *Lytechinus variegatus* (Williams et al. 1996), *Brissus unicolor, Echinometra lucunter, E. viridis, Echinoneus cyclostomus* and *Paraster floridiensis* (Hendler 1977), and *Plagiobrissus grandis* (B. Boekhoudt, Department of Agriculture, Husbandry and Fishery, Aruba, pers. comm.). In the present study we report a die-off in the sea biscuit *Meoma ventricosa* (Echinoidea, Spatangidae) which occurred in Curaçao (Netherlands Antilles) during 1997, and appeared to be associated with a pathogenic bacterium.

Materials and methods. Quantitative data on urchin mortality were collected in Curaçao during February 1997, at 5 different sites located down-current of the harbor (Table 1). At each site (except at Holiday Beach), 3 or 4 belt transects of 5 × 15 m were placed at variable water depth in sand patches on the coral reef. Urchins were localized by searching the sandy bottom by hand. The condition of the urchins was categorized into: dead (only skeleton left), affected (showing loss of spines) and normal (no signs). The densities of normal urchins in the impact and reference area were compared using a nested ANOVA on log transformed data. Homogeneity of variances was tested with a Bartlett test, while normality was tested with a 1-sample Kolmogorov-Smirnoff test (Sokal & Rohlf 1995). In the impact area, the percentages of dead urchins per site were compared using a Chi-square test, while the estimated pre-mortality urchin densities were compared

*E-mail: carmabi@ibm.net

with the post-mortality densities using a Student's *t*-test (Sokal & Rohlf 1995).

Necropsies were performed on normal, affected, and dead *Meoma ventricosa*. Intestinal tract tissue was removed from 2 normal and 5 affected urchins; dry weights were determined and results were normalized by dividing tissue weights with tests weights. Spine counts were made on the aboral surface of each animal from 3 areas of 18 to 80 cm^2 (mean ± SD = 41 ± 18 cm^2) depending on the size of the urchin. Intestinal tract weights and spine counts were compared between normal and affected urchins using a 1-way ANOVA and a nested ANOVA, respectively, after testing for homogeneity of variances and normality (Sokal & Rohlf 1995). Comparative microscopy was performed on various tissue samples from affected and normal animals. Affected tissue was Gram-stained to look for the presence of microbial cells

Two types of bacteria isolated from the base of the spines of affected *Meoma ventricosa* (from catch connective tissue), which were not present from normal specimens, were used to test their potential for initiating the infection signs as observed in the field. Healthy sea urchins (5 each per aquarium) of the species *Lytechinus variegatus* were deliberately infected with subcultures of both bacterial types in separate aquariums (along with 5 controls). The inoculum (each bacterial treatment) was introduced by spiking lettuce with each strain (lettuce was immersed in a 10^6 concentration of each strain) and by adding suspended bacteria (1.0 ml of a 10^8 suspension) directly to separate aquaria (containing the urchins in sterile artificial seawater).

Results. At the end of January 1997, numerous skeletons of *Meoma ventricosa* were observed at the Holiday Beach reef in Curaçao, Netherlands Antilles. Over 125 skeletons were found scattered on the reef in a large sand patch approximately 300 × 20 m in size and situated at a depth between 15 and 25 m. Underwater surveys at this and other sites revealed normal, affected, as well as dead urchins. Affected animals were distinguished by loss of spines in patches of variable size on the aboral side of the skeleton. The patches always had a darker coloration than the remaining part of the skeleton and were purplish/brownish in color. Affected urchins were found buried about 1 to 5 cm under the substrate together with normal animals. Affected animals were still able to direct their spines upon manipulation. Progressive loss of spines resulted in a completely spineless aboral side of the skeleton. These animals were often found lying on top of the substratum or partly buried in the sediment. Such animals appeared to be moribund and responded very slowly to tactile stimulation using only few of their spines. Ultimately, the spines on the underside of the skeleton were also lost, but at this time the animals were already dead. Dead specimens and skeletons were always found above the substrate and were never buried under the sediment.

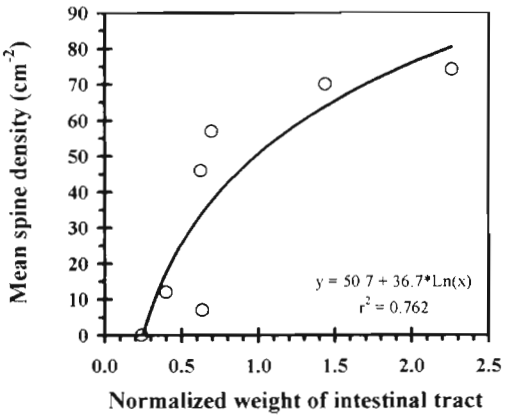

Fig. 1. *Meoma ventricosa*. Mean spine density as a function of the intestinal tract weight (normalized for the test weight)

Necropsies on affected *Meoma ventricosa* showed significant reductions in spine densities (nested ANOVA, $F_{1,5}$ = 120.200, $p < 0.001$) and intestinal tract weights (1-way ANOVA, $F_{1,5}$ = 25.850, $p = 0.004$), which were positively correlated (Fig. 1). Comparative microscopy of various tissue samples from affected and normal urchins revealed differences only in the spine catch connective apparatus. In affected urchins, tissue was amorphous and contained bacterial cells that stained Gram-negative, whereas in unaffected tissue bacterial cells were absent and intact collagen fibers were present.

Two types of bacterial isolates (VL-1 and PL-1) were obtained from affected tissue of *Meoma ventricosa*, which were not found with unaffected tissue. Deliberate infections of the urchin *Lytechinus variegatus* with the VL-1 strain caused signs similar to those in *M. ventricosa* (progressive loss of spines and death) within 3 d (feeding experiment) or within 2 wk (direct seawater inoculations). All controls and PL-1 inoculated *L. variegatus* remained unaffected. A more complete description of isolation techniques, identity of the putative pathogen, possible pathogenic mechanisms and host range studies are reported in Ritchie et al. (unpubl.).

Inquiries addresed to dive-operators and several surveys along the coast revealed that the die-off was restricted to an area of approximately 3.5 km along the coast and was situated just down-current of the Willemstad harbor. The CARICOMP network, a research and monitoring network of marine laboratories, parks and reserves (CARICOMP 1997), and the Caribbean Aquatic Animal Health Project (E. H. Williams pers. comm.) were contacted, but other reports of mortalities in *Meoma ventricosa* in other Caribbean islands or countries were not encountered.

In the impact area, located 1.0 to 3.5 km down-current of the harbor, the percentage of aberrant urchins (i.e. sum of dead and affected urchins) was similar at the 3 sites and ranged between 70.0 and 84.8% (Table 1). The percentage of dead urchins showed a strong decrease down-current along the coast dropping from 83.1 to 0.0% (Chi-square test, $\chi_2^2 = 88.8$, $p < 0.01$), while the percentage of affected urchins showed an increase from 1.7 to 72.7%. Reference sites located further down-current at 4.5 and 8.0 km from the harbor revealed no affected urchins (Table 1).

The post-mortality densities of normal urchins at the 3 affected sites were much lower compared to those of normal urchins at the 2 reference sites (Table 1). Variability in density among sites within areas was negligible (nested ANOVA, $F_{3,11} = 1.8$, $p = 0.197$), but the mean post-mortality density of normal urchins in the impact area was 90% lower than in the reference area (mean ± SD): 2.7 ± 3.4 versus 27.1 ± 7.5 per 100 m^2 (nested ANOVA, $F_{1,3} = 40.0$, $p < 0.001$). This suggests that the die-off had a significant negative effect on the population size of *Meoma ventricosa* in the impact area, although this conclusion may be disputable as the pre-mortality densities in the impact area were not known and may also have been low before the die-off.

To overcome this problem the pre-mortality densities in the impact area were estimated by adding up all urchins of each transect (i.e. dead, affected and normal). These densities are probably minimum densities, as moribund and dead urchins may have been lost from the transects as a result of fish predation or disintegration of the skeleton. Although the mean pre-mortality densities are an estimated minimum (10.5 ± 10.2 per 100 m^2), they were significantly higher (*t*-test, $p = 0.038$) than the post-mortality densities of normal urchins (2.7 ± 3.4 per 100 m^2) in the impact area (Table 1). This result confirms the negative effect of the die-off on the population size of *Meoma ventricosa* in the impact area.

Discussion. The present study is apparently the first report of a die-off in *Meoma ventricosa* associated with a pathogen. Mortalities of a variety of echinoid species have been recorded in the Caribbean (Lawrence 1996) but, so far, the causative agent(s) of these mortalities remain(s) unknown.

The mortality of *Meoma ventricosa* was positively correlated with distance to the Willemstad harbor, and affected urchins were only found in a relatively small area down-current of the harbor. Further down-current and up-current of the entrance to the harbor no affected urchins were found. Furthermore, the degree of infection in the impact area was similar for all sites, but the percentage of dead urchins was highest near the entrance of the harbor and dropped relatively fast to zero further down-current of the harbor. These data suggest that the mortalities were related to the outflow of harbor water. A similar observation was made by Bak et al. (1984) during the Caribbean-wide mass mortality of *Diadema antillarum* in 1983. They observed that the *D. antillarum* mortality in Curaçao originated at the harbor and spread much faster down-current than up-current.

The putative pathogen, a tetrodotoxin-producing strain of the genus *Pseudoalteromonas* (Ritchie et al. unpubl.), is common in marine waters but normally associated with various macro-organisms (algae, fish, etc., Gauthier et al. 1995). Based on the observations that ingestion of these bacteria produced infections more efficiently than direct inoculation into the water mass, it is possible that outflow of polluted water from the harbor increased the density of these strains residing in sediment (or associated with algae in sediment) It is interesting that although ingestion seemed to be required for rapid infection, the bacteria concentrated

Table 1 *Meoma ventricosa*. Percentage of normal, affected, dead and aberrant (damaged + dead) sea urchins at various reef sites. The pre-mortality density was estimated by summing all urchins for each transect. na = not applicable

Location	Impact area			Reference area	
	Holiday Beach	Corredor	Sonesta Beach	Carmabi Buoy 1	St. Michiel Bay
No. of km down-current from harbour	1.0	2.0	3.5	4.5	8.0
Water depth (m)	15–23	6	8–19	6–12	5
No. of transects (mean area in m^2)	4 (280)	3 (75)	3 (75)	3 (75)	3 (75)
Sample size of urchins	59	50	11	73	49
% of normal urchins	15.2	30.0	18.2	100.0	100.0
% of affected urchins	1.7	52.0	72.7	0.0	0.0
% of dead urchins	83.1	18.0	9.1	0.0	0.0
% of aberrant urchins	84.8	70.0	81.8	0.0	0.0
Mean post-mortality density (±SD) of normal urchins ($100 m^{-2}$)	0.6 ± 0.5	6.7 ± 5.8	0.9 ± 1.5	32.4 ± 14.7	21.8 ± 9.1
Estimated mean pre-mortality density (±SD) of normal urchins ($100 m^{-2}$)	4.3 ± 4.8	22.2 ± 4.1	4.9 ± 6.2	na	na

in the catch connective tissue. Studies are continuing to determine the specific route of infection. Nevertheless, it appears that increased densities of these strains may have caused the epizootic rather than a lowered resistance of the urchins.

Acknowledgements. In Curaçao the work was funded by the Island Government of Curaçao and the Central Government of the Netherlands Antilles, by their annual subsidy to the Carmabi Foundation. M. Engelsma, B. van den Heuvel, F. Isabela, and P. Visser provided logistic support in the field. We thank P. Seupel (Eden Roc Dive Center) and E. Motjee (Atlantis Diving) for providing information on the die-off in Curaçao, Dr J. Woodley for his inquiry at the CARICOMP network, and Drs A. Debrot and E. Williams for their comments on the manuscript. The manuscript furthermore benefited from the comments of 3 anonymous reviewers.

LITERATURE CITED

Bak RPM, Carpay MJE, de Ruyter van Steveninck ED (1984) Densities of the sea urchin *Diadema antillarum* before and after mass mortalities on the coral reefs of Curaçao. Mar Ecol Prog Ser 17:105–108

CARICOMP (1997) Caribbean coastal marine productivity (CARICOMP): a research and monitoring network of marine laboratories, parks, and reserves. Proc 8th Int Coral Reef Symp 1:641–646

Cortés J, Soto R, Jiménez C (1992) Earthquake associated mortality of intertidal and coral reef organisms (Caribbean of Costa Rica). Proc 7th Int Coral Reef Symp 1:235–240

Gauthier G, Gauthier M, Christen R (1995) Phylogenetic analysis of the genus *Alteromonas*, *Shewanella*, and *Moritella* using genes coding for small-subunit rRNA sequences and division of the genus *Alteromonas* into two genera, *Alteromonas* (emended) and *Pseudoalteromonas* gen. nov., and proposal of twelve new species combinations. Int J System Bacteriol 45:755–761

Gilles KW, Pearse JS (1986) Disease in sea urchins *Strongylocentrotus purpuratus*: experimental infection and bacterial virulence. Dis Aquat Org 1:105–114

Hendler G (1977) The differential effects of seasonal stress and predation on the stability of reef-flat echinoid populations. Proc 3rd Int Coral Reef Symp 1:217–223

Jangoux M (1987a) Diseases of Echinodermata. I. Agents microorganisms and protistans. Dis Aquat Org 2:147–162

Jangoux M (1987b) Diseases of Echinodermata. IV. Structural abnormalities and general considerations on biotic diseases. Dis Aquat Org 3:221–229

Jones GM, Scheibling RE (1985) *Paramoeba* sp. (Amoebida, Paramoebidae) as the possible causative agent of sea urchin mass mortality off Nova Scotia. J Parasitol 71: 559–565

Lawrence JM (1996) Mass mortality of echinoderms from abiotic factors. Echinoderm Studies 5:103–137

Lessios HA, Robertson DR, Cubit JD (1984) Spread of *Diadema* mass mortality through the Caribbean. Science 226:335–337

Maes P, Jangoux M (1984) The bald-sea-urchin disease: a biopathological approach. Helgol Meeresunters 37:217–224

Scheibling RE, Hennigar AW (1997) Recurrent outbreaks of disease in sea urchins *Strongylocentrotus droebachiensis* in Nova Scotia: evidence for a link with large-scale meteorologic and oceanographic events. Mar Ecol Prog Ser 152:155–165

Sokal RR, Rohlf FJ (1995) Biometry, 3rd edn. WH Freeman and Company, New York

Williams EH Jr, Bunkley-Williams L, Bruckner RJ, Bruckner AW, Ortiz-Corps EAR, Bowden-Kerby WA, Colón-Jones DE (1996) Recurring mass mortalities of the white-spined sea urchin, *Tripneustes ventricosus*, (Echinodermata: Echinoidea) in Puerto Rico. Caribb J Sci 32:111–112

Williams LB, Williams EH Jr, Bunkley AG Jr (1986) Isolated mortalities of the sea urchins *Astropyga magnifica* and *Eucidaris tribuloides* in Puerto Rico. Bull Mar Sci 38: 391–393

Editorial responsibility: Albert Sparks, Seattle, Washington, USA

Submitted: November 4, 1998; Accepted: July 27, 1999
Proofs received from author(s): September 28, 1999