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MASS MORTALITY AND RECOLONIZATION OF *PYURA STOLONIFERA* (HELLER) ON THE SOUTH COAST OF SOUTH AFRICA

N. HANEKOM, J. M. HARRIS†, G. M. BRANCH‡ and J. C. ALLEN§*

A mass mortality of the ascidian *Pyura stolonifera* (red-bait) was recorded along the Tsitsikamma coast of South Africa in May 1991, following the infection of a large proportion of the population with a white microbial growth. At nine subtidal red-bait beds, reductions in percentage cover of red-bait were greatest on the crests of reefs (42 ± 17%:1*SD*) and lowest on the landward edges (17 ± 8%). The test of *P. stolonifera* appeared to be the main site of the microbial infection. Scanning electron and epifluorescent microscopy revealed much higher numbers of bacteria on the tests of infected individuals ($64.3 \pm 5.5 \times 10^6 \cdot mm^{-2}$) compared to healthy ones ($5.1 \pm 0.1 \times 10^6 \cdot mm^{-2}$). Fat, curved rod bacteria and thick chains, which accounted for more than 45% of the bacteria on infected red-bait tests, were absent from the tests of healthy red-bait. Standard characterization techniques, coupled with API tests, showed that the isolates from infected red-bait tests belonged exclusively to the genus *Vibrio*, whereas isolates from healthy tests included the genera *Vibrio*, *Pseudomonas*, *Aeromonas* and *Flavobacterium/Cytophaga*. The mass mortality therefore appeared to be associated with the proliferation of large curved rod bacteria of the genus *Vibrio*. Recolonization by *P. stolonifera* into areas naturally denuded of red-bait was significantly faster than for experimental plots cleared of all organisms during the mid 1980s. A mean cover of 33 ± 4% was recorded for *P. stolonifera* after 38 months following natural mortality, whereas it took 71 months to achieve a comparable recovery of 35 ± 20% in experimental plots. Possible reasons for this difference (*viz.* substratum, recruitment and predation) are explored.

Pyura stolonifera, which is commonly known as "red-bait" in South Africa, is a large, solitary species, with a tough cellulose test that encloses and protects the animal against predation and wave action (Day 1974b, Branch and Branch 1981). The species is abundant along the coasts of South-Eastern Australia (Fairweather 1991) and southern Africa, from Namibia to Moçambique (Day 1974a). It is found on rocky reefs from the low littoral to a depth of about 15 m, usually forming dense beds where wave action or currents are strong (Klumpp 1984). Such beds provide a habitat for a wide range of associated invertebrate species (Van Driel 1978, Fielding *et al*. 1994).

P. stolonifera is collected for bait by fishers (Jackson 1976, Fairweather 1991, Fielding 1995), and for food by the local people of the coastal areas of Transkei (Bigalke 1973) and Maputaland (Bruton 1980, Fielding 1995, Kyle *et al.* 1997). In the process of harvesting, clumps of *P. stolonifera* are sometimes hacked off the rocks, leaving denuded patches, ranging in size from approximately 0.02 to 0.25 m^2 (Fielding *et al.* 1994). Such harvesting may have a prolonged effect on the productivity of red-bait beds (Fielding *et al*. 1994).

Past studies on *P. stolonifera* have concentrated on ecology (Morgans 1959, Day 1974a), morphology and functional anatomy (Day 1974b), larval development (Griffiths 1976), biomass and production (Van Driel 1978, Field *et al*. 1980, Berry 1982, Fielding 1995), nutritional ecology (Klumpp 1984, Stuart and Klumpp 1984, Seiderer and Newell 1988), harvesting and population dynamics (Fairweather 1991) and associated macroinvertebrate communities (Fielding *et al*. 1994).

The role of disease in controlling marine invertebrate populations is poorly documented in comparison with that of other biotic interactions, such as predation and competition. This study reports on the dramatic impact of a bacterial infection on the ascidian *P. stolonifera.*

In early May 1991, tests of numerous *P. stolonifera* in the Tsitsikamma National Park (Fig.1a) were infected by a thin, white microbial growth and showed signs of atrophication. During the ensuing three months there was a mass mortality of *P. stolonifera*. Fortuitously, a series of recolonization experiments had been initiated in the area seven years before the die-off. This study reports on the composition of a *P. stolonifera* community experimentally cleared from a

* South African National Parks, P.O. Box 176, Sedgefield 6573, South Africa. E-mail: nickh@parks-sa.co.za
† Formerly University of Cape Town; now KwaZulu-Natal Nature Conservation Service, Private Bag X3, Congella, Durban 4013, South Africa. E-mail: jmharris@iafrica.com
‡ Department of Zoology, University of Cape Town, Rondebosch 7700, South Africa. E-mail: gmbranch@uctvms.uct.ac.za
§ Tsitsikamma National Park, P.O. Storms River 6308, South Africa

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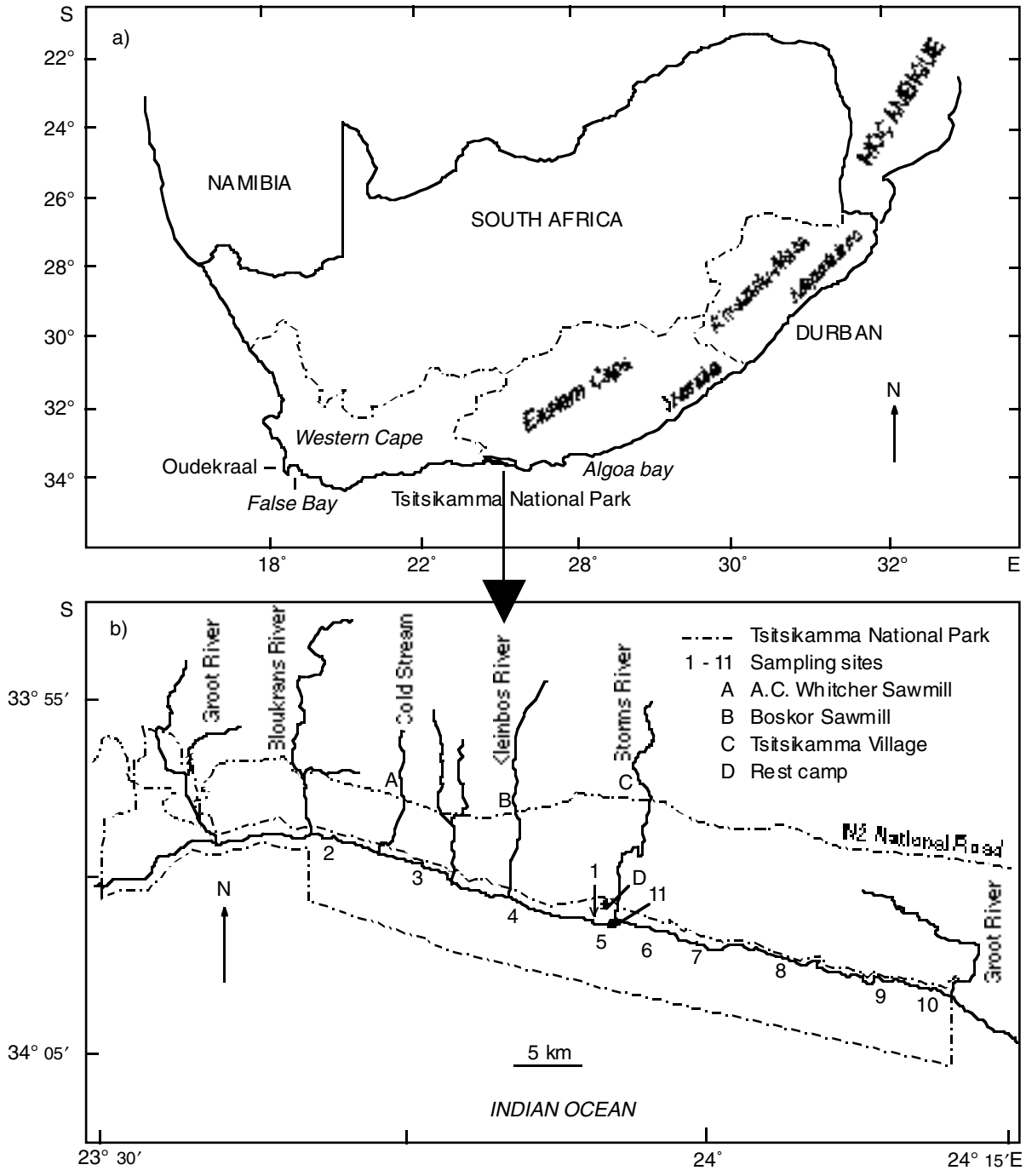

Fig. 1: Location of sampling sites in (a) southern Africa and (b) the Tsitsikamma National Park

series of sampling plots in 1984, the recolonization rates of those cleared plots and those denuded by mass mortality in 1991, the extent of the 1991 die-off, and the nature of microbial populations observed on, and isolated from, infected and healthy *P. stolonifera.*

MATERIALS AND METHODS

Study area

The Tsitsikamma National Park (34°00´S, 23°30´E – 34°04´S 24°12´E) is situated on the coast of the Southern Cape (Fig. 1a). It has a narrow, rocky shoreline at the base of a steep coastal escarpment. The shore comprises vertical ridges and interlying troughs, which run parallel to the coast. These rocky ridges, formed of interlayered sandstone and shale, extend into the subtidal and quickly reach depths in excess of 20 m. The major *P. stolonifera* beds are found on shallow, high-profile subtidal reefs (<15 m deep), which are subject to turbulent water movement. Wave action along this coastline is extremely strong and the uppermost (or intertidal) extensions of the red-bait beds are only exposed at spring low tides and on exceptionally calm days.

Original community

During March 1984, some seven years prior to the mass mortality of *P. stolonifera*, nine replicate 0.1 m^2 plots were sampled in densely populated sections of an intertidal red-bait bed at Site 1 (Fig. 1b). The percentage cover of the dominant organisms in each plot was assessed using a 0.1 m^2 quadrat, divided into 49 (20.4 cm^2) squares. Plots were then cleared of all organisms using a hammer and cold-chisels and scraped to bare rock. If less than half of a specimen occurred inside the quadrat, it was removed, but excluded from the analysis. Samples were frozen until analysed. They were then defrosted and washed; specimens were separated in a basin. The residue was passed through a 2-mm mesh sieve, and not the generally used 0.5-mm mesh employed for macrobenthos (McIntyre 1971). The capture of small specimens, especially Isopoda, Amphipoda and Polychaeta, was therefore less efficient, but the loss in biomass was probably very small. Macroinvertebrates and algae collected were sorted into taxa, identified according to Day (1967, 1974a), Kensley (1978), Simons (1976), Branch and Branch (1981) and Richards (1987), and counted. Because many invertebrates shelter beneath red-bait pods and are often only partially visible, their abundance was expressed in terms of numbers. However, values of percentage cover were used for seaweeds and the dwarf barnacle *Notomegabalanus algicola*, as well as to supplement the abundance evaluations of the dominant macroinvertebrates. The shell-free dry masses of the various species were also determined. Shells of molluscs were removed manually or, where necessary, with 5–10% HCl. In the case of barnacles and echinoderms, subsamples were taken and boiled in 10% NaOH for 1 h to dissolve the flesh (Crisp 1971). Samples and subsamples were oven-dried (100°C for 24 h), cooled in desiccators and weighed (nearest 0.01 g).

Recolonization of cleared plots

Five of the cleared plots were selected for recolonization studies, and their positions were marked by chipping grooves into the rock surface. Estimates of percentage cover and counts of organisms colonizing the sites were done *in situ* after four months and then approximately annually until March 1990. Only values of species dominant in either the pre-cleared or post-cleared populations, were used. The mean abundance values for the five plots were determined and compared with those of the initial pre-clearance values for these plots.

No undisturbed control plots were monitored during the study period. However, in 1985 and May 1991 (when mortalities were first observed), numbers of medium to large *P. stolonifera* (length >50 mm) were determined in a series (n = 7–11) of 0.1m^2 plots, randomly selected in undisturbed sections of Site 1. Small pods were not counted because they were often hidden below the canopy layer. These densities were compared with those from the original pre-cleared population using the Kruskal-Wallis test (Zar 1984) and the software program STATGRAPHICS (Statistical Graphics Corporation 1989).

Recolonization of naturally denuded plots

In August 1991, after the mass mortality of *P. stolonifera*, five 0.1 m^2 plots were selected and marked in naturally denuded areas at Site 1. Estimates of percentage cover and counts of the biota in the plots were done approximately annually until September 1994. The means of the five sites were determined and compared to the original pre-denuded populations of 1984. Additional measurements were done at five 0.25 m^2 plots on a shallow (c. 3 m deep), subtidal reef (Site 11), which had been densely populated by *P. stolo-*

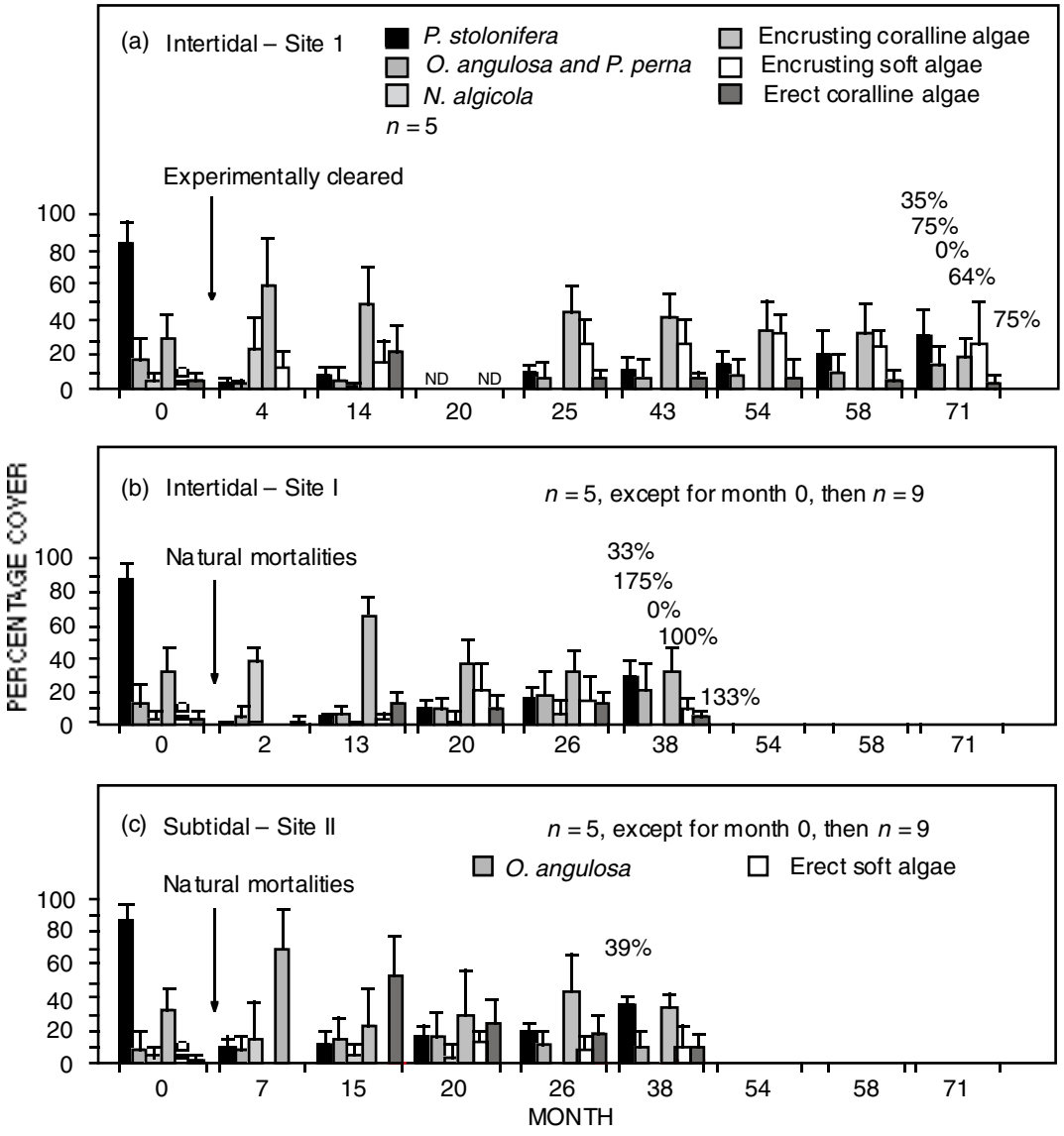

Fig. 2: Changes in the mean percentage (+1*SD*) cover of the fauna and flora in (a) the experimentally denuded and (b) the naturally denuded areas at intertidal Site 1, as well as (c) the naturally denuded areas at Site 11 in the subtidal region, with the extent of the recovery (relative to the undisturbed 1984 population) at Site 1 shown above the last sample

nifera prior to 1991. No pre-mortality samples were taken, but the original *P. stolonifera* densities would have approximated those of Site 1, and those values were used.

Abiotic factors

Temperature measurements were taken near the Storms River mouth close to Site 1 between 1982

and 1992 (Fig. 1b). Surface sea temperature was measured daily at 08:00 using either a mercury or alcohol thermometer. The extremes in daily air temperature were determined using maximum and minimum thermometers at a Weather Bureau station until April 1991, and thereafter from thermistors of an automatic weather station. Except for sea temperatures, which were measured to the nearest 0.5°C after 1990, all other temperature measurements were to the nearest 0.1°C. Temperatures during 1991 were compared with the mean monthly values for the period 1982–1990. Similar comparisons were done with rainfall data measured at Storms River Forest Station (Weather Bureau records).

P. stolonifera mortalities

On 13 May 1991, the proportion of *P. stolonifera* specimens infected with a white microbial growth was determined in 40 randomly selected 0.1 m^2 plots along 60 m of an intertidal reef at Site 1 (Fig. 1b).

The loss of red-bait from nine subtidal red-bait beds (Sites 2 – 10, Fig. 1b) was assessed between October 1991 and January 1992 by scuba-diving. The beds were situated approximately 50–550 m offshore and at depths ranging from 3 to 15 m. Transects, which extended along the length of reef, were done on the ridge, seaward edge and shoreward edge of each red-bait bed. On each transect, a 0.25 m^2 gridded quadrat was placed contiguous to itself 20 times but, where necessary, allowance was made for discontinuities in reef structure. Numbers of quadrats lacking *P. stolonifera* and recently colonized by the short-lived (<8 months) pioneering barnacle species, *Notomegabalanus algicola* (Bokenham and Stephenson 1938, Millard 1952, Fricke *et al*. 1982), were counted to determine loss of *P. stolonifera* cover. Six replicate transects were done on the ridge and edges of each red-bait bed and the mean values were determined.

Bacterial analyses

SCANNING ELECTRON MICROSCOPY

Samples of tests from three healthy and three infected *P. stolonifera* individuals were collected in May 1991, immediately fixed in 2.5% gluteraldehyde (in 0.2-µm filtered seawater) and stored at 4°C. These samples were desalinated (75, 50, 25 and 0% filtered seawater/distilled water), dehydrated (10, 20, 30, 50, 70, 90, 96 – 100% ethanol) and subsequently dried in a Polaron Critical Point Drier using CO_2 as a transitional fluid. The tests were then mounted on stubs using a glue-graphite mixture, sputtercoated with gold paladium, and viewed with a Cambridge S200 scanning electron microscope.

EPIFLUORESCENT MICROSCOPY

Five samples of tests from both healthy and infected individuals were placed in 5 ml of 4% formalin (in 0.2-µm filtered seawater) and stored in the dark at 4°C. The tests were later homogenized, diluted in tetrasodium pyrophosphate (×10 for healthy samples, ×100 for infected samples), sonicated for five minutes to detach cells from the tissue (Ellery and Schleyer 1984, Velji and Albright 1986) and stained with 4´,6-diamidinophylindole (DAPI, Porter and Feig 1980) at a final concentration of 5 $\mu g \cdot ml^{-1}$ DAPI (Schallenberger *et al.* 1989) for 20 minutes. A 2 mℓ aliquot was then filtered onto an irgalon black-stained 0.2 µm nucleopore filter (Parsons *et al*. 1984) and examined under oil by means of a fluorescent microscope. Bacteria on the filters were counted in randomly selected fields at 1 000× magnification using a Nikon Labophot-2 microscope, Nikon DM400 filter block and Nikon Neofluor 100/30 oil objective. At least 20 fields or 200 bacteria were counted per slide. Densities were expressed as numbers of $bacteria \cdot mm^{-2}$ surface area of test. Cell size and shape for the different categories of bacteria were measured at 1 000× magnification, and confirmed by scanning electron microscope views.

API tests

Three samples from both healthy and infected red-bait specimens were collected, homogenized in 5 mℓ of sterile seawater and diluted to 10^{-1}, 10^{-2} and 10^{-3}. Three 200 µℓ aliquots of each solution were spread onto 1% peptone seawater agar plates (Harris *et al*. 1991). These plates were incubated for seven days at room temperature (c. 20°C). The first 16 colonies encountered in a clockwise direction were systematically picked off each of the healthy and infected plate collections, after first drawing a line randomly across the plate. Pure colonies were obtained by streaking at least a further three times. Of the initial collection of 16 colonies, eight were selected from each of the healthy and infected sources to cover the full suite of morphologically distinct isolates. Cell shape and colony colour were recorded, and the isolates were tested for the following properties or activities to allow classification to genera (Krieg 1984): gram stain, motility, catalase (3% H_2O_2), oxidase (oxidase reagent) and fermentation of glucose (tetrazolium indicator plates, Bochner and Savageau 1977). For the

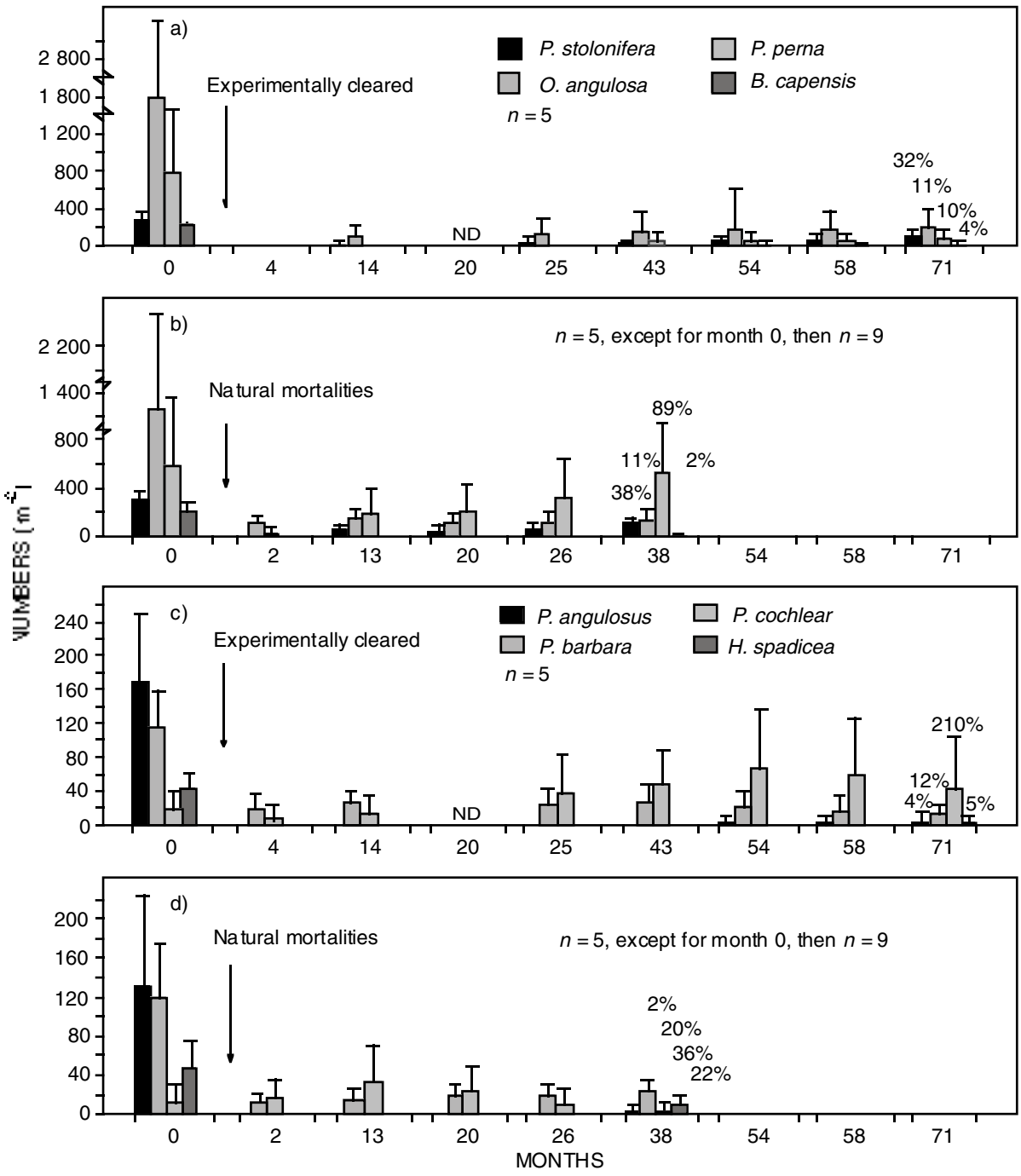

Fig. 3: Changes in the abundance (numbers·m^{-2}, +1*SD*) of (a–b) the dominant sessile and (c–d) mobile invertebrate species in the experimentally and naturally denuded areas at intertidal Site 1, with the extent of the recovery (relative to the undisturbed 1984 population) shown above the last sample

identification of non-enteric gram-negative rods, the API 20 NE system was used (API 20 NE Analytical Profile, Bio Merieux).

RESULTS

Original community

The mean percentage cover and population density of *P. stolonifera* recorded from the nine plots cleared at Site 1 were 86 (± 10) % and 295 (± 63) individuals·m^{-2} respectively (Figs 2b, 3b). Some 67% of the *P. stolonifera* specimens had lengths >45 mm, the size at which *P. stolonifera* reaches sexual maturity on the warmer KwaZulu-Natal coast (Schleyer and Fielding 1992). Associated with the *P. stolonifera* was a diverse assemblage of fauna. Excluding the Porifera, Amphipoda and Bryozoa, a total of 47 macroinvertebrate species was recorded. The most dominant invertebrate species were the barnacle *Octomeris angulosa*, the brown mussel *Perna perna* and the anemone *Bunodosoma capensis,* which constituted 26, 12 and 11% of the total shell-free biomass (560 g·m^{-2}) of the associated fauna respectively. These species were often partially or totally hidden beneath the red-bait pods, and the percentage cover determined for *O. angulosa* and *P. perna* in the pre-cleared plots underrated their abundance (Fig. 2a–c). Estimates of algal cover were more accurate, because algae were usually epiphytic on the upper surfaces of red-bait pods and exposed barnacles. The encrusting corallines were the most dominant algae (Fig. 2a–c).

Recolonization of experimentally and naturally denuded areas

During the recolonization studies of experimentally cleared plots, no major changes were observed in the abundance of red-bait in undisturbed areas at Site 1. The population density of *P. stolonifera* (length >50 mm) in the pre-cleared plots (1984) was similar to those recorded in adjacent undisturbed areas in 1985 and 1991 (Kruskal – Wallis test: $H = 0.90$, $n = 9, 7, 10$, $p > 0.6$).

Plots denuded by the mortality of *P. stolonifera* differed from experimentally cleared plots in that some of the associated fauna remained. At Site 1, substantial numbers of the sessile barnacle *O. angulosa* (100 ± 34 individuals·m^{-2}) and brown mussel *P. perna* (22 ± 34 individuals·m^{-2}) were recorded in the plots just two months after the die-off of *P. stolonifera* (Fig. 3b), and these individuals were remnants of the original community. The status of mobile herbivores, such as *Patella barbara* and *Patella cochlear,* was more difficult to assess, because their numbers after two months were comparable with those recorded four months after complete clearing of experimental plots (Fig. 3c, d).

The recolonization process of plots cleared experimentally and those denuded by the mass mortality at Site 1 were generally similar. Major patterns observed were as follows:

(i) Some 2–4 months after denudation the dwarf barnacle *Notomegabalanus algicola* dominated the invertebrate fauna, although a large proportion of these barnacles had already died. After 13–14 months, virtually all the *N. algicola* were dead (Fig. 2a, b). The attached shells of the dead *N. algicola* provided a suitably rough substratum for the development of encrusting coralline algae and, more especially in the subtidal areas, erect coralline algae (Fig. 2a–c). These coralline algae became dominant during the early stages (4–14 months) of recolonization. Thereafter, their abundance declined steadily, whereas that of encrusting soft algae tended to increase (Fig. 2a–c).

(ii) Recolonization by sessile invertebrates such as *P. stolonifera*, *O. angulosa* (filter-feeders) and *B. capensis* (sedentary carnivore) was slow. This was especially apparent in the experimentally cleared plots, where their numbers after 71 months were <35% of their original pre-cleared values (Fig. 3a). Much faster rates of recovery were recorded in the naturally denuded areas, particularly for *P. perna* and *P. stolonifera* ($U=2.3$, $n=5.5$, $p < 0.03$), with their numbers exceeding 35% of the original density after only 38 months (Fig. 3b).

(iii) In the case of mobile herbivores, numbers of *Parechinus angulosus*, *Haliotis spadicea* and *P. barbara* in the experimental and naturally denuded areas remained depressed (<30% of the original undisturbed densities) throughout the study (Fig. 3c, d). The abundance of *P. cochlear* varied markedly. In the experimentally cleared plots, numbers of *P. cochlear* increased steadily to more than three times the pre-cleared value, before declining. A weak (Spearman ranked) correlation ($r_s=0.49$, $n=6$, $p < 0.08$) was recorded between the numbers of *P. cochlear* and the percentage cover of encrusting coralline algae, a group commonly associated with this limpet. In the naturally denuded areas, numbers of *P. cochlear* peaked much earlier (after 13 months, cf. 54 months).

At Site 11 in the subtidal region *P. perna* was absent, and most of the *O. angulosa* cover (8 ± 8%) recorded

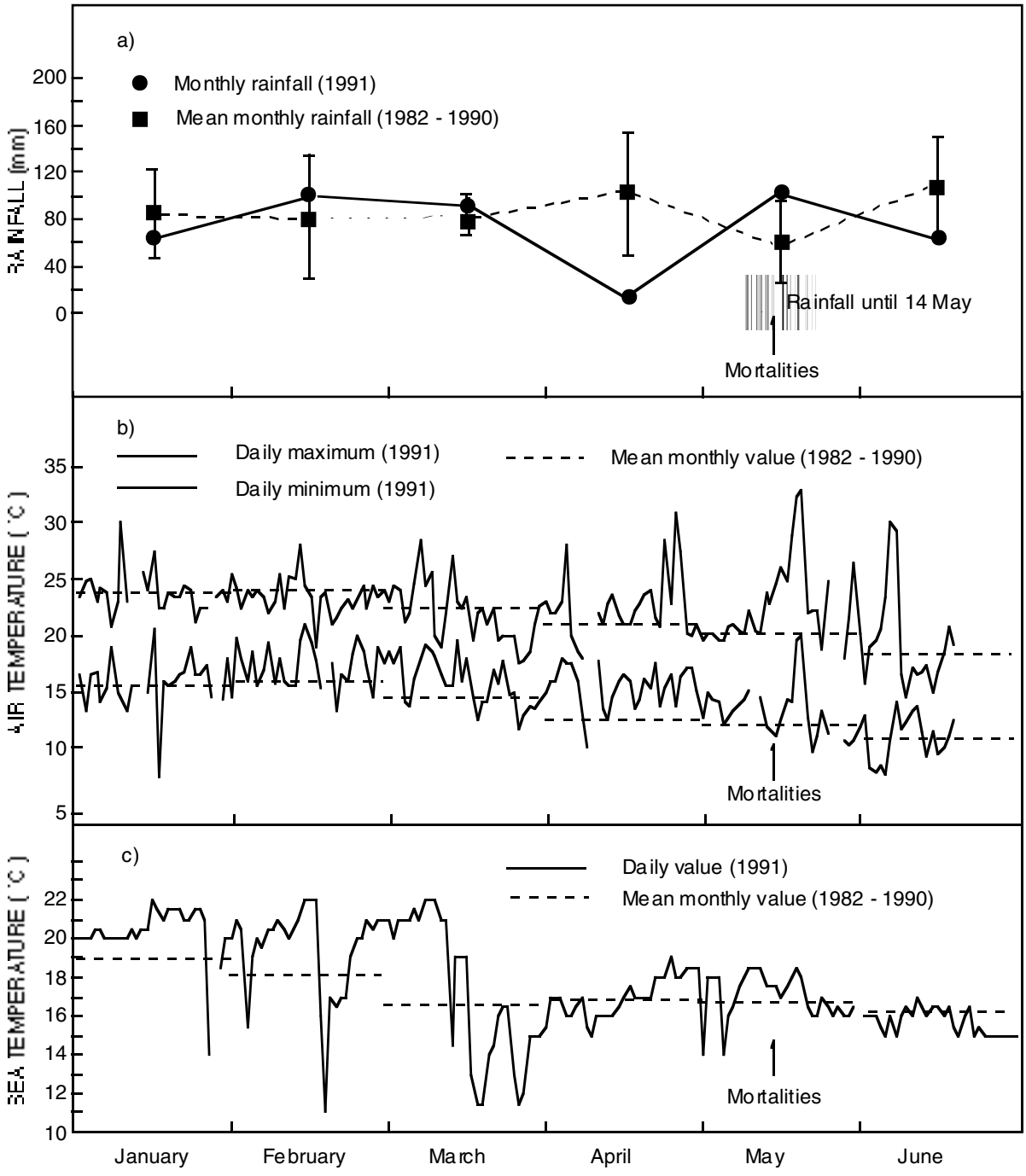

Fig. 4: Variations in (a) rainfall (±1*SD*), (b) air temperature and (c) sea temperature just prior to *P. stolonifera* mortalities

Table I: Mean and standard deviation (*SD*) of percentage surface cover lacking healthy *P. stolonifera* and showing signs of recent colonization by *Notomegabalanus algicola* measured in six replicate transects at nine subtidal sites (2–10) and in three replicate transects at an intertidal site (1)

Site	Ridge top		Seaward slope		Landward slope	
	Mean depth 7 ± 3 m		Mean depth 9 ± 3 m		Mean depth 9 ± 3 m	
	% Cover		% Cover		% Cover	
	Mean	*SD*	Mean	*SD*	Mean	*SD*
Subtidal	42	17	20	9	17*	8*
Intertidal	74	6	–	–	–	–

* Based on measurements taken from only eight sites

in the monitoring plots seven months after the *P. stolonifera* mortalities were remnants of the original population (Fig. 2c). The overall pattern of recolonization at Site 11 appeared similar to that of intertidal Site 1, except that erect coralline algae rather encrusting coralline algae were dominant in the early stages of recolonization (Fig. 2c).

Abiotic factors and *P. stolonifera* mortalities

No signs of oil pollution, chemical pollution or bloom of harmful algae were observed in the park during the two months preceding the mass mortality of *P. stolonifera*. However, there were sudden and prolonged declines in surface sea temperatures in March 1991, followed by unusually high air temperatures and low rainfall conditions (Fig. 4a–c). These conditions may have adversely affected *P. stolonifera* and created conditions favourable for a microbial epidemic.

On 13 May 1991, 50% of the 709 *P. stolonifera* investigated at intertidal Site 1 were either infected with a microbial growth or already atrophying. Some five weeks later, turbulent seas ripped most of the infected red-bait pods from the rocks, and in early July 1991 an estimated 35 000 red-bait pods washed ashore in a cove near the Groot River (Fig. 1b). By late August 1991, 74% of the *P. stolonifera* at Site 1 had been removed (Table I).

Observations prior to the mortality suggested that most of the subtidal red-bait beds in the study area had a dense cover of red-bait, and at two sites the mean percentage covers of *P. stolonifera* were 82 (±13) and 99 (±1)% (Hanekom and Coetzee 1990). It was estimated that approximately one-quarter of the *P. stolonifera* population at subtidal Sites 2–10 was lost (Table I). The loss of *P. stolonifera* cover was significantly greater (nonparametric multiple comparison, Dunn test, Zar 1984: $Q = 2.8$, $k = 3$, $p > 0.02$) on the shallow (7 ± 3 m deep) crests of the reefs (42% loss) than on the deeper (9 ± 3 m) landward edges, where 17% was lost. This difference may be partly attributed to the greater water turbulence experienced on the shallower reef crests, which may have ripped loose healthy specimens that had lost the protective support of neighbouring individuals.

Bacterial analyses

SCANNING ELECTRON MICROSCOPY

Microscopic investigation indicated that the test rather than the flesh of *P. stolonifera* was the main site of microbial infection. Inspection of the surface of tests of healthy individuals revealed a thin covering of colonizing microbiota, which included a diverse assemblage of diatoms, rod and coccoid bacteria, as well as thin filamentous chains of bacteria (20–40 µm long chains, cells 1–2 µm long, 0.6–0.7 µm wide, Fig. 5a). However, infected tests were heavily colonized by microbes, which were dominated by chains of thick (1–1.2 µm long, 1.2–1.5 µm wide cells) and thin (1–2 µm long, 0.6–0.7 µm wide cells) bacteria and large, fat curved rods or vibrios (3–3.5 µm long, 1–1.2 µm wide, Fig. 5b). Large and small cocci were also present, but less abundant.

EPIFLUORESCENT MICROSCOPY

Bacterial densities on infected tests were high, with the numbers of cocci, rods and chains being markedly greater on infected tests ($64.3 \pm 5.5 \times 10^6$ bacteria·mm^{-2}) than on healthy ones ($5.1 \pm 0.1 \times 10^6$ bacteria·mm^{-2}, Fig. 6). The bacterial communities on healthy tests, which were dominated numerically by small and large cocci (58%), did not include fat curved rod bacteria and thick chains of bacteria, forms that accounted for more than 45% of the bacteria on infected tests (Fig. 6). Thin curved rods, present in low numbers on healthy tests, were absent from infected tests.

API-TESTS

In terms of overall colony appearance, the initial plating of bacteria from tests of healthy and infected specimens yielded 5–8 and 2 morphologically different strains of bacteria respectively. After these colonies were streaked for purity, the eight isolates from the healthy tests and seven from the infected tests were

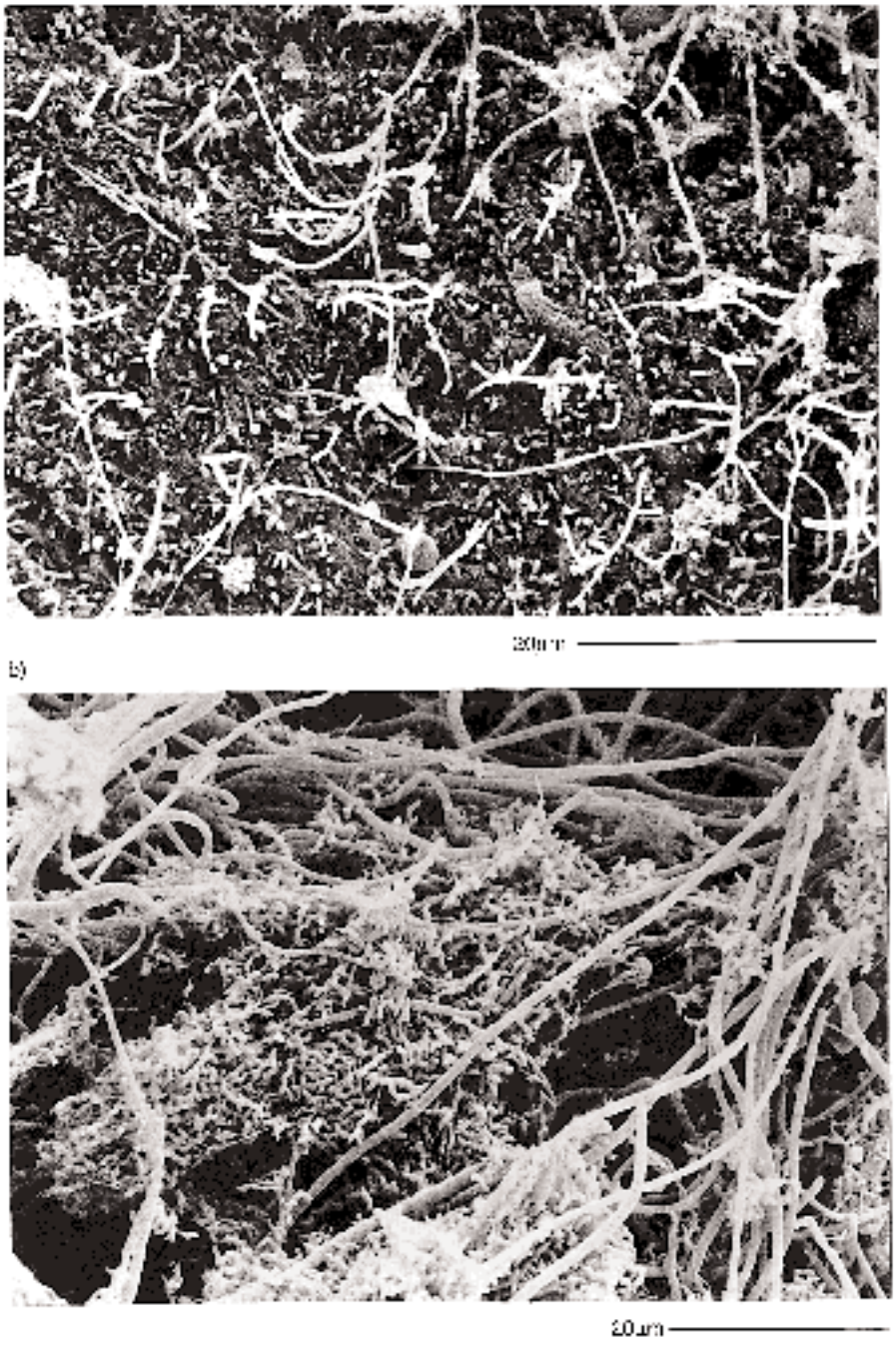

Fig. 5: Photographs of (a) cocci, rods and fungal threads on a healthy *P. stolonifera* test, and (b) fat curved rods and thick chains on an infected tes

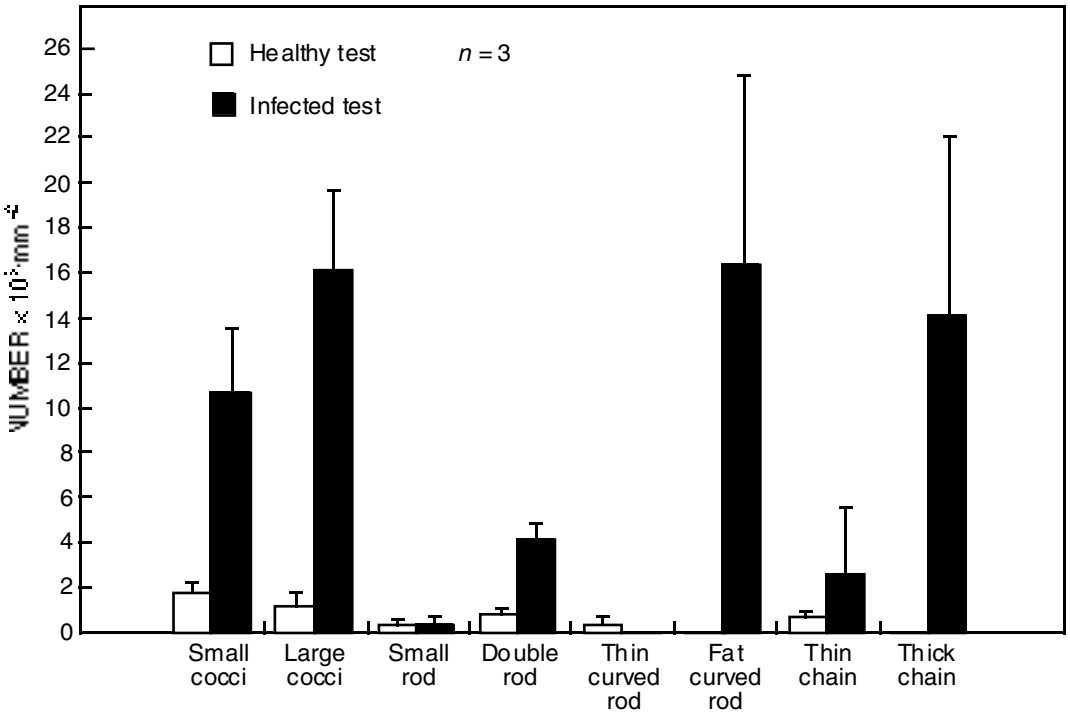

Fig. 6: Mean densities (+1*SD*) of the various microbes recorded per millimetre of healthy and infected tests of *P. stolonifera* using epiflourescent microscopy

all found to be gram negative and oxidase positive. The isolates from healthy tests were diverse, containing representatives of the genera *Vibrio*, *Aeromonas*, *Pseudomonas* and the *Flavobacterium-Cytophaga* complex. All seven isolates from infected tests were large curved rods belonging to the genus *Vibrio*, and were motile, fermentative (glucose), and TCBS- and catalase-positive. Of these, two isolates were *Vibrio vulnificus* (Table II). The API 20NE method gave good discrimination identification of *Vibrio vulnificus* and *Pseudomonas vesicularis*, but poorer discrimination identification of the *Aeromonas* strains as *Aeromonas salmonicida masouoida/achromogenes*.

DISCUSSION

Prior to the mass mortality of *P. stolonifera*, the mean shell-free dry mass of its associated fauna at Site 1 (560 g·m^{-2}) was intermediate between that determined for an intertidal (366 g·m^{-2}) and a subtidal (670 g·m^{-2}) site in KwaZulu-Natal (Fielding *et al.* 1994). Much lower biomasses (68–168 g·m^{-2}) were recorded by Van Driel (1978) from five subtidal sites in Algoa Bay, but his analysis excluded all the Porifera, Hydrozoa, Isopoda, Amphipoda and Bryozoa and, therefore, was not comparable with the above studies. The invertebrate species monitored in the recolonization experiment constituted >70% of the total number and biomass of the associated fauna in the red-bait beds at Site 1, and are common species on exposed shores of the Southern Cape (Coetzee and Munnick 1988, Coetzee and Zoutendyk 1993). Most of these species (viz. *O. angulosa*, *B. capensis*, *P. angulosus, P. barbara* and *H. spadicea*) were very slow to re-occupy areas cleared in the red-bait beds, and their rates of recovery were lower than that of *P. stolonifera* (Fig. 3a–d). This suggests that red-bait beds, like mussel beds and kelp holdfasts, may play an important role as a shelter from wave action (Ojeda and Dearborn 1989) and a refuge from predators (Branch *et al.* 1987, Ojeda and Dearborn 1991). Therefore, harvesting methods for *P. stolonifera* should avoid breaking

Table II: Results of API 20 NE identification tests

Parameter										
Isolate	Gram	Motility	Catalase	Oxidase	Glucose ferment	TCBS	Cell shape	Cell Size	Colour	Species
Healthy										
1	––	+	+	+	+	+	c–r	l	cr	*Vibro vulnificus*
2	–	–	–	+	+	–	c–r	m	cr	*Aeromonas salmonicidia**
3	–	+	+	+	–	–	c–r/r	m	cr	*Pseudomonas vesicularis*
4	–	+	+	+	–	+	r	l	cr	*Vibrio* sp.*?*
5	–	–	–	+	–	–	co	s	t	?
6	–	–	–	+	+	–	?	m	cr/p	*Aeromonas salmonicidia**
7	–	+	–	+	–	–	r	m	yy	*Flavobacterium/Cytophaga*
8	–	–	–	+	+	–	co	s	cr	*Aeromonas salmonicidia**
Infected										
1	–	+	+	+	+	+	c–r	l	cr	*Vibrio* sp.*?*
2	–	+	+	+	+	+	c–r	l	cr	*Vibrio* sp.*?*
3	–	+	+	+	+	+	c–r	l	cr	*Vibrio* sp.*?*
4	–	+	+	+	+	+	c–r	l	cr	*Vibrio vulnificus*
5	–	+	+	+	+	+	c–r	l	cr/y	*Vibrio vulnificus*
6	–	+	+	+	?	+	c–r	l	cr	*Vibrio* sp.*?*
7	–	+	+	+	+	+	c–r	l	cr	*Vibrio* sp.*?*

c–r = curved rods, r = rod, co = cocci
cr = cream, y = yellow, t = tan, p = pink, yy = bright yellow
l = large (3 – 4 µm), m = medium (2 – 3 µm), s = small (0.5 – 1 µm)
* *Aeromonas salmonicidia = Aeromonas salmonicidia mascoucidia/achromogenes*

down the protection afforded by the remaining sections of red-bait beds.

Areas denuded by both experimental clearing and the mass mortality of *P. stolonifera* were quickly and extensively colonized by the dwarf barnacle *N. algicola* (Figures 2a–c). This rapid colonization of new or freshly cleared substrata by *N. algicola* and its short-lived dominance has also been noted in the Western Cape (Bokenham and Stephenson 1938, Millard 1952, Fricke *et al.* 1982). However, foliar algae (*Ulva*, *Enteromorpha* and *Hypnea*), which are generally pioneers in recolonization of littoral reefs in KwaZulu-Natal (Jackson 1975, cited in Berry 1982), were not common at Site 1 (Fig. 2a–c).

In KwaZulu-Natal, *P. stolonifera* is apparently slow to re-occupy cleared areas that have been colonized by an algal turf, unless residual individuals remain (Jackson 1975, cited in Berry 1982). Despite the limited cover of turf-like algae (<25%) in the experimentally cleared plots at Site 1, recolonization by *P. stolonifera* was slow (11–13% after 3 years). Unfortunately, experimental clearing was done at only one site and in one season (late summer), and no control plots were regularly monitored. However, in most of the other studies of *P. stolonifera,* the recolonization of cleared plots (varying in size from 0.25 to 4 m^{-2}) has also been poor (Table III). Slow recovery rates have likewise been recorded for the brown mussel *P. perna* in experimentally cleared plots in mussel beds in southern KwaZulu-Natal (Lambert and Steinke 1986) and Transkei (Dye 1992). In the Transkei, where adult mussel stocks are severely depleted, the poor recovery appears to be coupled with a very small and intermittent supply of larvae (Harris *et al.* 1998). *P. stolonifera* stocks, which are situated in turbulent infratidal and subtidal areas, are far more protected from exploitation than those of the brown mussel. High densities of *P. stolonifera* (expressed as numbers·m^{-2}) were recorded at sampling sites in KwaZulu-Natal (186–288, Fielding *et al.* 1994), Algoa Bay (246–774, Van Driel 1978), Storms River (295, this study) and to a lesser extent False Bay (74–105, De Villiers and Tarr 1987), and spawner stock is unlikely to be a limiting factor in the recolonization experiments.

In the study of De Villiers and Tarr (1987), young *P. stolonifera* seemed to settle well and regularly on navigational buoys in False Bay, but not in cleared 4 m^2 plots on two nearby subtidal reefs. Recruitment in the experimentally cleared plots at Site 1 also appeared to be extremely poor, despite the presence of large neighbouring *P. stolonifera* populations. Young recruits (c. 8–25 mm) were usually found attached or adjacent to adult individuals (Fairweather 1991, Schleyer and Fielding 1992, NH, pers. obs.). Reoccupation by *P. stolonifera* was, therefore, mainly by encroachment from expansion of the surrounding

Table III: Mean recovery rates of *P. stolonifera* in experimentally and naturally denuded plots

Locality	Tidal region	Plot size (m^2)	Plot number	Animals removed	Time span (years)	% Recovery (number)		% Recovery (cover)		Data source
						Mean	*SD*	Mean	*SD*	
Experimentally cleared										
Australia	Inter	0.25	32	*Pyura*	2	c. 0	c. 0	c. 0	c. 0	Fairwater (1991)
Algoa Bay	Sub	0.25	–	All	2	c. 0	c. 0	c. 0	c. 0	Van Driel (1978)
False Bay	Sub	4.0	2	*Pyura*	3	3	3	–	–	De Villiers and Tarr (1987)
Oudekraal	Sub	4.0	1	*Pyura*	3	24	–	–	–	De Villiers and Tarr (1987)
TNP	Inter	0.1	5	All	3.5	11	7	13	7	This study
KwaZulu-Natal	Inter	1.0	3	All	3	–	–	4–6	6–8	Fielding (1995)
KwaZulu-Natal	Inter	0.25	3	All	3	–	–	5	4	Fielding (1995)
KwaZulu-Natal	Sub	1.0	3	All	3	–	–	c. 5	c 7	Fielding (1995)
Naturally cleared										
TNP	Inter	0.10	5	*Pyura*	3	38	13	33	12	This study
TNP	Sub	0.25	5	*Pyura*	3	c. 32	7	c. 39	5	This study

TNP = Tsitsikamma National Park

colony and, to a lesser extent, cluster formation on vacant space, apparently as a result of enhanced survival of individuals that settle near established pods (De Villiers and Tarr 1987, NH pers. obs.). In the case of the brown mussel, the presence of adult conspecifics also enhances the settlement and survival of larval recruits (Harris *et al*. 1998). Despite this pattern, the rate of recolonization of entire red-bait beds (Sites 1 and 11) denuded by the mass mortality was approximately three times faster than that of experimentally cleared plots in a healthy red-bait colony (c. 35 v. 12% after three years, Table III). Remnant barnacles (*O. angulosa)* and mussels (*P. perna*) in naturally denuded areas may have assisted the recolonization process by providing a protected habitat for the settlement and survival of *P. stolonifera* larvae. However, in experiments in False Bay and Australia (Table III), where plots were cleared by merely decapitating the *P. stolonifera* pods and leaving the attached portion of the pods to decay with the associated fauna unharmed, the rate of recovery by *P. stolonifera* was very slow, less than that of completely denuded experimental plots in this survey (Table III). Therefore, other factors were assumed to be important.

Gut analyses of three species of solitary ascidians (*Chelyosoma productum*, *Pyura haustor* and *Ascidia callosa*) have shown that ascidian larvae are consumed by mature individuals (Bingham and Walters 1989). However, capture efficiencies appear to be low, and larval numbers collected from the surface of living aggregations of those ascidians were similar to those from mimic aggregations (Bingham and Walters 1989). The brief planktonic stage of *P. stolonifera*, probably less than one day in optimum conditions (Griffiths 1976), would further reduce the vulnerability of their larvae to this form of predation.

Fielding (1995) recorded a high rate of mortality for juvenile (<10 mm) *P. stolonifera* in KwaZulu-Natal, apparently as a result of predators. In the Tsitsikamma area, *P. stolonifera* (presumably mainly small individuals) form a substantial component of the diets of the fish *Sparodon durbanensis* (Buxton and Clarke 1991) and *Diplodus sargus capensis* (Mann and Buxton 1992). Despite this, the fastest rates of recolonization by *P. stolonifera* along the Cape coast have been recorded at sites readily accessible to fish, namely navigational buoys in False Bay, a 4 m^2 cleared plot at Oudekraal (De Villiers and Tarr 1987) and naturally denuded redbait beds in the study area (Table III). Conversely, recovery rates in small (1.0–0.1 m^2) plots surrounded by intact *P. stolonifera* were generally poor (Table III). This trend in *P. stolonifera* colonization may be explained if invertebrate species that are dependent on red-bait as a habitat or shelter were feeding on the newly settled *P. stolonifera* larvae in nearby denuded areas. Predators that prey on juvenile red-bait and grazers that incidentally consume or "bulldoze" newly settled recruits could both have this effect. Schleyer and Fielding (1992) observed subtidal hermit crabs and prosobranch molluscs consuming juvenile *P. stolonifera* in KwaZulu-Natal, but the extent of their effect is unknown. Invertebrate species that are associated with the intertidal and shallow subtidal red-bait beds of the Tsitsikamma study area, and which may influence the survival of newly settled *P. stolonifera*, are the crab *Plagusia chabrus,* the whelk *Nucella squamosa* (through direct predation) and the gastropods

Haliotis spadicea and *Patella barbara* (by dislodging larvae and recruits through their grazing action). However, this explanation must remain speculative until experiments have been undertaken to test this hypothesis.

During 1991, *P. stolonifera* was the only invertebrate species observed in the study area that suffered from a disease and experienced a mass mortality. Tests of infected specimens were covered with a thin film of microbes and showed signs of atrophication. However, the inner animal flesh, despite having a fairly strong aroma, was generally fairly firm and intact. Flocks of kelp gulls *Larus dominicanus* gathered on exposed redbait beds to feed on the infected *P. stolonifera* specimens, ripping open weakened tests and devouring the soft flesh. Fish such as galjoen *Dichistius capensis* also fed ravenously on the flesh of opened specimens.

Industrial activity in the Tsitsikamma region is low and the water quality of the rivers is good (Harrison *et al.* 1996). However, small quantities of pollutants probably enter the study area from effluent outfalls situated between 3 and 10 km upstream from the mouths of the Lottering River (A. C. Whitcher Sawmill), Kleinbos River (Boskor Sawmill) and Storms River (Tzitzikamma Total Village), as well as from sewage filtration ponds in the restcamp of Tsitsikamma National Park (Fig. 1b). Despite this, the mass mortality of *P. stolonifera* appeared to have resulted from natural causes, because: (1) no signs of oil or chemical pollution were noted during April and May of 1991, (2) *P. stolonifera* was the only invertebrate species observed with a microbial infection and/or suffering large mortalities, and (3) infected *P. stolonifera* were recorded intertidally as well as subtidally (to a depth of 15 m) along a 47 km stretch of coastline. Therefore, natural lethal factors, such as red tide (Horstman 1981, Horstman *et al.* 1991), freshwater flooding (Goodbody 1961) and extremes in water temperature (Newell 1979, Gilmore *et al.* 1978) were considered.

No harmful algal blooms were noted in the nearshore region just prior to or during the die-off of *P. stolonifera,* and toxic algal events do not appear to be lethal to *P. stolonifera* (Horstman 1981, Horstman *et al.* 1991, G. C. Pitcher, Sea Fisheries [SF], pers. comm.). Also excluded as a possible factor was freshwater flooding, because rainfall prior to the *P. stolonifera* mortalities was extremely low (Fig. 4a).

Fluctuations (7.5°C within 48 h) and extremes (22.0–11.5°C) in sea temperatures between March and May 1991 were substantial (Fig. 4c). However, temperature oscillations caused by cold upwelling events are common along the Tsitsikamma coast, and very severe declines in surface sea temperatures (>9°C within 48 h) during January of 1981 and 1984 (Bower and Crawford 1981, Hanekom *et al.* 1989) caused no notable mortalities in the *P. stolonifera* populations (P. Joubert, National Parks Board, pers. comm., NH, pers. obs). The large and prolonged declines in surface sea temperatures, attributed to upwelling (Schumann *et al.* 1982, Mitchell-Innes 1988), followed by unusually high air temperatures in April 1991 (Fig. 4b, c), may have created conditions favourable for a microbial epidemic.

Tests of virtually all atrophying *P. stolonifera* observed at Site 1 were infected with a bacterial growth, and colonies cultured from infected tests all comprised *Vibrio* bacteria (Table II). In KwaZulu-Natal, tests of individual *P. stolonifera* have been observed frequently with a covering of a similar whitish film, and these animals invariably died (P. J. Fielding, Oceanographic Research Institute, pers. comm). However, this infection did not spread to adjacent animals and, in some instances, physical damage to the test appeared to initiate the formation of this film. Aquarium-held ascidians are also often attacked by bacteria following injury or decay of epibionts. In these cases, bacterial sheets enter through the siphon and finally kill the ascidian (Monniot 1990). Therefore, it is possible that the bacterial growth observed in this study was not the primary cause of death in *P. stolonifera*, but was a secondary infection. However, the specific nature of the bacteria associated with diseased specimens, the fact that the test appeared to be the major site of infection and decay, and the absence of any other logical cause of death lead to the conclusion that the *Vibrio* bacteria recorded were responsible for the mass mortality of *P. stolonifera*. Moreover, a *Vibrio* bacterium was apparently responsible for a wasting disease and large mortalities in seven species of starfish (Asteroidea) in southern California during the early 1980s. This disease also usually occurred when local sea temperatures were abnormally high (>16°C, Schroeter and Dixon 1988, Richards and Davis 1993, Kushner *et al*. 1995a, b). Diseases have been a source of sizable mortalities in other marine invertebrates, such as corals (Garrett and Ducklow 1975, Gladfelter 1982, Williams and Bunkley-Williams 1990, Bythell and Sheppard 1993, Coles 1994 as cited by Nagelkerken *et al*. 1997) and octocorals (Garzón-Ferreira and Zea 1992, Nagelkerken *et al*. 1997), whereas a wasting "syndrome" (no bacterial or viral agent found) caused a large mortality of black abalone *Haliotis cracherodii* and purple sea urchins *Strongylocentrotus purpuratus* (Richards and Davis 1993, Kushner *et al.* 1995b).

Subsequent to the present study, during the first quarter of 1997 there was again bacterial infection and mortalities of *P. stolonifera* along the Tsitsikamma coast, as well as in the Buffalo Bay area

some 75 km to the west. A similar infection and die-off of *P. stolonifera* was noted at Smitswinkel Bay in False Bay during the early 1960s (L. Hutchings, SF, pers. comm.) This event was localized, with no other sizable mortalities being noted farther than 8 km either side of Smitswinkel Bay. Large mortalities of *P. stolonifera* in the False Bay area have on occasions been reported to Marine and Coastal Management, but the causes of these events were not investigated (R. J. Q. Tarr, MCM, pers. comm.). The scarcity of records suggests that disease-related mass mortalities of *P. stolonifera* are rare and the virulence of the pathogen may be related to particular environmental conditions.

In summary, it appears that the mass mortality of *P. stolonifera* along the Tsitsikamma coast was probably caused by bacterial infection, which led to a degradation of the test and subsequent losses to predation and wave action. While the reasons for the bacterial infection remain unclear, the consequences were dramatic. Large proportions of the *P. stolonifera* stocks, amounting to many thousands of tons, were lost from a considerable stretch of coastline. Dramatic changes took place in the composition of the associated fauna and flora. Recovery was slow, though not as slow as in the small, experimentally cleared areas. In addition to its impact on the target species, a disease-mediated mortality of a habitat-providing species such as *P. stolonifera* also impacts indirectly on the whole community.

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