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MULTISPECIES ASSOCIATIONS OF SYMBIONTS ON SHALLOW WATER CRINOIDS OF THE CENTRAL GREAT BARRIER REEF

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Abstract. This paper examines the various structuring mechanisms which control the distribution of symbiont taxa on crinoid individuals. We identify 3 classes of factors which might be pertinent. These are:
1. Physical and global environmental factors,
2. Host specific factors,
3. Inter- and intra-specific interactions among the symbiont species.
Forty-six taxa belonging to the classes Gastropoda, Polychaeta, Myzostomata, Ophiuroidea and Crustacea were identified as symbiotic associates of shallow water crinoids from the central great Barrier Reef. The spatial distribution of the symbiont communities was correlated with host abundance. However, none of the common symbiont taxa showed specificity for a single host species. Stout body morphology and nocturnal behaviour were correlated with higher numbers of associates. Crinoids of the family Comasteraceae had more symbionts than other crinoids.

Introduction

Crinoids, a class of echinoderms, are subject to extensive inhabitation by a suite of epizoic and endozoic invertebrates which range from "accidental visitors to harmful parasites and include a wide variety of taxonomic groups" (Clark 1921). The fossil record for gastropods and myzostomes on crinoids show that inter-species associations have a long evolutionary history. Gastropods, polychaetes, myzostomes, ophiurids and numerous crustaceans (including copepods, amphipods, isopods and decapods) form small communities on extant comatulid crinoids.

Little information exists concerning the nature of relationships between the different symbiont species and their hosts; many shrimp species are regarded as obligate symbionts, while ophiurids and some of the gastropods are facultative since they may also be found living free. Many of the inhabitants feed on the planktonic particles caught by the crinoids (Zmarzly 1984a, Meglitsch 1972) but other, including some gastropods and myzostomes suck the body tissue of their host (Hyman 1955). Some of the rarer associates may even have been accidental visitors rather than true symbionts. Because knowledge of the nature of the different associations is limited and several different kinds of association are represented, we use the term 'symbiosis' following the original definition of de Bary (1879) as redefined by Starr (1975). This defines symbiosis as "the living together of dissimilarly named organisms".

List of species associated with crinoids have been published previously (Potts 1915, Fishelson 1974, Zmarzly 1984a, Türkey *et al.* 1990). In contrast to these descriptive accounts, the present study examines the pattern of association between symbionts and hosts in more detail. Three sets of external and internal parameters, each at its own scale, were tested for their significance in structuring the relationship.

1. The first question concerns whether, and to what degree, the spatial distribution of symbionts within the central Great Barrier Reef is influenced by their physical and biotic environment. Essentially this means asking if the distribution is primarily correlated with the distribution of the crinoids themselves, or if other environmental factors have primacy or at least a significant additional effect.
2. The second question concerns the possibility of host specificity of the symbionts, that is the extent to which the community composition is determined by the morphological characteristics of the hosts.
3. The third question addresses the interactions of the symbionts. By examining the pattern of co-occurrences on the same hosts, we can investigate the degree to which community composition is determined by inter- and intra-specific interactions of the symbionts themselves.

The analytic methods used herein are essentially exploratory rather than confirmatory. It is doubtful if the data conform to the requirements of standard ANOVA techniques. Errors are unlikely to be normally distributed, and relationships unlikely to be linear, or even isotonic. Furthermore we can expect outlying and high leverage points to be present and least squares methods of analysis are known to lack robustness under these conditions. In the absence, at the time of analysis, of programs for general additive modelling, and lacking sufficient samples for comprehensive cross-validation by permutation and bootstrap techniques, exploratory methods would seem more appropriate.

Study sites and methods

A total of 46 sites were sampled on 12 reefs in the central Great Barrier Reef between lat. 18° 50' and 15° 10' S. Half of the samples were taken from a single mid-shelf reef - Davies Reef. Among the other samples mid-shelf and outer reefs were about equally represented. Few sites were studied on near-shore reefs. All crinoids, together with their attached symbionts, were collected from 1m^2 quadrats at 10m depth using SCUBA, and put in separate plastic bags.

The living posture of each crinoid individual was recorded using the following categories

1. cryptic - less than 20% of the arms visible
2. protected - at least the central disc of the animal hidden
3. free-sitting - centro-dorsal disc visible but animal protected from at least one side
4. exposed - animal visible from all directions; generally perched on top of substrate.

In addition the colour and minimum and maximum arm lengths were recorded. The "armlength" variable used in the analyses is the mid-range of the arm lengths. "Body Volume" represents an index visually integrating number of arms, armlength and body stoutness.

Immediately after collection, the symbionts were isolated by placing the crinoid in fresh water for about 10 secs and then shaking it in a bath. This caused most of the symbionts to detach from the host and drop to the bottom of the container. Microscopic checks indicated that this procedure worked effectively for all groups except gastropods. These last held firmly to the host and are therefore not quantitatively collected by this method. Excluding gastropods, less than 5% of the epizoics remained attached to the host. No attempt was made to collect the numerous symbiotic copepods.

A total of 46 symbiont taxa from 5 invertebrate classes were identified in the samples, although the taxonomic level varied. An identification of gastropods and myzostomes to species level could not be obtained before carrying out the analyses, so these were lumped into single taxa. The few polychaete species known to live on crinoids, and the ophiuroids are facultative associates (Guille *et al.* 1986, Hanley 1984, Eldredge 1972), and these were also lumped. Identification of specimens was made using the following keys:

commensal shrimps: Bruce (1982), Banner and Banner (1975);
galatheids: Baba (1977,1979);
isopods: Bruce (1986);
crabs: Sérène (1957), Svevcic (1988), Sakai (1976);
comasterid cironoids: Rowe, Hoggett, Birtles & Vail (1986);
other crinoids: Clark & Rowe (1971).

Exploratory analyses were performed mainly using the TAXON package (Ross *et al.* 1986). The dissimilarity measures used were the Bray-Curtis intersection measure (Bray *et al.* 1957), and the contrast measures of Manhattan (L_1) distance (Williams, 1976) and Gower's metric (Gower, 1971)[1]. Preliminary investigation was made using sequential agglomerative hierarchical nonoverlapping classification (SAHN, Lance et al. 1967) and minimal spanning trees (Gower and Ross 1969), together with ordination by Principal Coordinates Analysis (Gower 1966) with interpretation facilitated by correlating the first five axes with the data (Williams et al. 1968). The dissimilarity measures differ in the stress accorded aspects of dominance and commonness/rarity in the data.

The combination of classification and ordination methods based on different similarity measures was chosen in order to test the robustness of the patterns found in the data. Here we consider only those data structures sufficiently reliable to emerge repeatedly, and only a few exemplifying analyses depicting these consistent results are presented. We regard the results as showing potentially interesting relationships; while correlation is not proof of causality, it remains true that "founded almost implication" in the sense of Havránek (1981a,b) is a good basis for induction of initial hypotheses, which may then be subjected to formal confirmatory testing in an experimental study.

To study the inter-relationships between the spatial distribution of the symbionts and other characteristics, we performed Canonical Correlation Analysis on the Principal Coordinates vectors derived from the several dissimilarity matrices (Gittins 1985, Poore 1980). By

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[1] Intersection measures are derived from the amount of each attribute which is shared, contrast measures are derived from the differences. Thus given 2 values x_{ip},x_{jp} for attribute p and items i,j, the Bray-Curtis measures is based on functions of $\mathrm{Inf}(x_{ip},x_{jp})$ while the other 2 measures are functions of $\mathrm{abs}(x_{ip}-x_{jp})$

using these vectors we can expect to reduce the "noise" level somewhat, since only "important" vectors need be included in the analysis. Where classifications were available from several sets of data, comparison could be made by the Friedman-Rafsky test (Friedman *et al.* 1979) This provides a reasonably. powerful, non-parametric test applicable to high dimensional non-normal data. Indeed its power increases with dimensionality and the test can be easily extended to provide a confirmatory test suitable for many ecological purposes.

Results

In total, 1689 symbiont specimens were collected from 1114 crinoid individuals. 56% of these individuals were inhabited by epizoic specimens. The average number (± standard deviation) of symbionts on a crinoid individual was 1.51 ± 1.32 overall, but increased to 2.66 ± 2.08 when only the inhabited crinoids were considered. As many as 30 symbiont individuals belonging to several different taxa were found on single host specimens. The number of symbiont individuals of the same taxon on any host individual averaged 1.12.

Table 1 lists the symbiont taxa found in the samples. A large number of shrimp species were found, many of which are strictly specialized to live on crinoids (Bruce 1982, 1992). The shrimps *Synalpheus stimpsoni* and *Periclimenes* spp., together with the polychaetes and the myzostomes, were the most abundant taxa at a majority of the sites. Other decapod symbionts were brachiuran crabs of the families Majidae and Brachyrhynchidae and the anomuran Galatheidae. Species of the genera *Galathea* and *Allogalathea*, which live preferentially between the cirri on the underside of the crinoids, were found mainly on crinoids with large and stout cirri, such as *Himerometra, Tropiometra* and *Stephanometra*, and not on species without cirri. *Carteolana integra* is the only isopod associated with crinoids on the Great Barrier Reef (Bruce 1986, Potts 1915). It was found on 16 different crinoid species, mostly on those of the genera *Oxycomanthus, Clarkcomanthus, Comanthus* and *Comaster* (Table 2). The polychaetes were represented by the two species *Holoepidella nigropunctata* and *Adyte crinoidicola* (Hanley, pers. comm). Of the ophiuroid associates, only *Ophiomaza cacaotica* is thought to live exclusively on crinoids (Guille *et al.* 1986) but it occurred only in very low numbers in our samples. The other ophiuroids are either facultative symbionts on a variety of hosts, or free living.

Table 1. List of the crinoid associates from the central Great Barrier Reef, giving the total number of specimens occurring in the samples

Polychaeata	286
Myzostomata	340
Crustacea	
Amphipoda	
sp. 1	2
sp. 2	3
Isopoda	
Carteolana integra	127
Decapoda	
Natantia	
Periclimenes affinis	119
Periclimenes albolineata	1
Periclimenes amboinensis	2
Periclimenes attenuatus	2
Periclimenes commensalis	62
Periclimenes tenuis	29
Parapontonia nudirostris	2
Palaemonella pottsi	4
Pontoniopsis comanthi	6
Athanas ornithorhynchos	1
Synalpheus carinatus	7
Synalpheus demani	2
Synalpheus stimpsoni	231
Synalpheus tropidodactylus	3
(unidentified shrimps)	28
Anomura: Galatheidae	
Allogalathea elegans	49
Galathea sp. 2	8
Galathea sp. 3	4
Galathea sp. 4	2
(unidentified Galatheids)	17
Brachiura: Majidae	
Ceratocarcinus longimanus	5
Brachiura: Brachirhyncha	
Harrovia albolineata	22
Gastropoda	82
Annulobalcis sp.	1
Bittium sp.	5
Chrysallida sp.	1
Columbellidae sp.	1
Curveulima spp. 1 - 11	54
Pyrene sp.	2
Stiliferina sp.	1
Triphora sp.	1
Triphoridae sp.	1
Turridae	1
Ophiuroidea	212

1) Spatial distributions of symbionts and crinoids

The distribution patterns of the symbiont taxa over the different types of reef habitats followed largely that of the crinoids (Fabricius 1989). Highest densities, (average numbers per unit area) of most of the symbiont taxa were found in centers of highest crinoid densities as would be expected. The mid-shelf reefs had the highest symbiont densities, with near-shore and outer-shelf reefs being considerably less dense. Clustering consistently separated midshelf reefs from reefs of the edges of the continental shelf, and from lagoon sites. The data on crinoid distribution and its relation to en-

Table 2. Contribution of the symbiont taxa to the distinction of near-shore, mid-shelf and outer shelf reef sites in classifications: Cramer values based on Flexible-Sorting on Bray-Curtis transformed data on seven-groups level. Mean Cramer value for 27 symbiont taxa: 0.48

Symbiont Taxon	No of Specimens	Cramer Value
Myzostomes	340	0.86
Synalpheus stimpsoni	231	0.82
Polychaetes	286	0.73
Periclimenes albolineata	1	0.70
Periclimenes affinis	119	0.67
Isopods	127	0.66
Ophiuroids	212	0.66
Allogalathea elegans	49	0.52

Mean Cramer value for 27 symbiont taxa: 0.48

vironmental factors will be presented elsewhere in detail (Fabricius, in prep.).

All common symbiont taxa discriminate between the site groups (Table 2). Only the isopods, which showed much higher densities on Davies Reef than on any other, together with a few rare taxa, showed aberrant distributions, distinct from the general pattern described above.

Canonical correlation analyses were carried out to compare the distribution patterns of the crinoids to those of the symbionts, and to compare the correlations of abiotic and biotic environmental attributes to both crinoids and symbionts. Again the distribution of both groups of animals was highly correlated. The relationship of the symbionts to the environmental parameters was slightly stronger than that of the distribution of crinoids. The canonical correlation for symbiont/environment was $r = 0.806$ to the first vector pair, and was slightly larger than the correlation between the crinoid/environmental ($r = 0.723$). Correlation to the second vector pair in the symbiont/ environment data was 0.575, and to the third vector pair was 0.428. Chi-square tests on the relatedness of the species densities to the environmental factors were highly significant: $p < 0.005$ for the symbionts, and $p < 0.006$ for the crinoids. However, given the statistical problems, these probabilities should be regarded as indicative only. The dominant environmental variables linked to the first vector, were (negatively) "sedimentation" (-0.821), and "low current" (-0.495) and (positively) "crevices" (0.676) and "live coral" (0.651)

As sampling occurred over a period of six months, including winter and spring, ANOVAs of the abundance of several common symbiont taxa were used to test whether some of the patterns in the data were due to seasonality. No such temporal or seasonal fluctuations were identified.

2) *Crinoid - symbiont associations*

a) Lack of host specificity of the common symbiont species. Forty-three crinoid species from six different families were represented in this study with the Comasterid family clearly dominant. Fig. 1 displays the taxa and numbers of epizoic specimens found on the 17 most common crinoid species, permitting a comparison of the crinoids as hosts. Differences are apparent both in the degree of infestation and in the compostition of the average symbiont association on the different host species. However, host specificity could be excluded for all symbionts which were found in sufficient numbers and could be identified to species level. These are the isopod *Carteolana integra*, the two polychaete species, the crabs, the galatheids and several of the *Periclimenes* and *Synalpheus* shrimp species.

Although no strict specificity on a single host species existed for any of these symbiont species, nonrandom distribution of the symbionts over the hosts is obvious. For example the *Periclimenes* shrimps were found in particularly high numbers on the *Himerometra* species and *Oxycomanthus benetti*; the Synalpheidae were found to be especially common on *Comanthina schlegeli, Comanthus parvicirrus* and *Capillaster multiradiatus*, but rarely found on the Himerometridae. *Comatula purpurea* and the *Himerometra* species were particularly prone to infestations with myzostomes and polychaetes, while the galatheids were found particularly commonly on *Himerometra* with its strong and stout cirri.

No symbionts were found on 9 of the 43 crinoid species. All except one of these were relatively rare, non-comasterid crinoids (see Table 3). These species with no symbiont inhabitants were excluded from consideration prior to calculating the crinoid species similarities. Classification and ordination analyses were performed to compare crinoid species according to their symbiont communities. Results from these analyses consistently separated a group of ten to twelve crinoid species with similar average associations of symbionts. The patterns identified in these various analyses are here represented by a single dendrogram based on a Flexible Sorting classification (Fig. 2).

The crinoid species separated as group "A" were all comasterids, and represented members of the genera *Comanthus, Comaster,* and *Clarkcomanthus*, together with one *Oxycomanthus* species and one *Comanthina* species. In contrast to the other host species, this group had high numbers of polychaetes, myzostomes, and the shrimp species *Periclimenes affinis* and *Synalpheus stimpsoni*. However, the difference in the symbiont community of species of group A, compared to group "B", was essentially a QUANTITATIVE one. No unique and distinctly different symbiont community characterized this separate group of crinoid species.

None of the species of the family Comasteridae were found without symbionts, and the species with

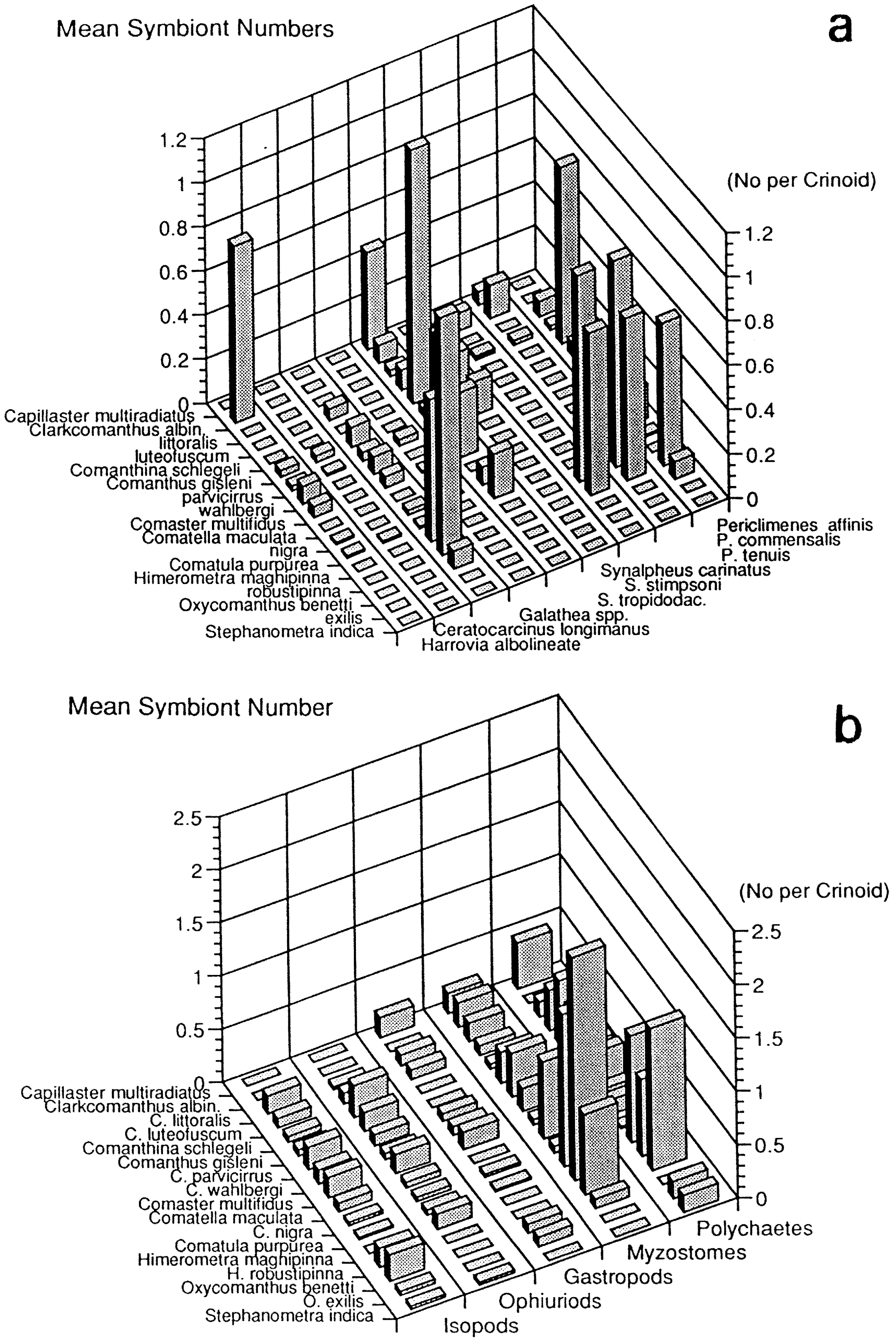

Figure 1. Frequency of a) the more common decapods, and b) some of the remaining symbiont taxa on the 17 most common crinoid species.

Table 3. Morphological and behavioural characteristics of the crinoid species, and degree of symbiosis. N = number of specimens investigated. Listed are: mean armlength; average number of symbionts per crinoid individual; and percentage of individuals with cyrptic, protected, free and exposed living habit at the time of sampling.

Crinoid Species	NC	Mean Armlgth	Symb. per Crin	Emergence crypt.	protec.	free	expos.
Amphimetra laevipinna	4	3.75	0	.25	.0	.0	.75
Antedonid sp.b	1	7.0	1.00	.0	1.0	.0	.0
Capillaster multiradiatus	16	8.18	1.25	.06	.31	.12	.50
Cenometra bella	1	3.0	0	.0	.0	.0	1.0
Clarkcomanthus							
albinotus	13	6.46	0.69	.17	.48	.17	.17
littoralis	29	6.06	0.62	.48	.37	.06	.06
luteofuscum	57	5.29	1.21	.28	.56	.15	.0
Comanthina schlegeli	20	9.90	2.90	.05	.35	.30	.28
Comanthus							
alternans	5	10.80	2.80	.0	.0	.20	.80
gisleni	34	7.82	1.29	.61	.35	.02	.0
parvicirrus	73	8.85	1.78	.35	.44	.14	.06
wahlbergi	53	5.75	1.02	.56	.34	.05	.03
Comaster							
brevicirrus	1	4.0	0	.0	1.0	.0	.0
gracilis	4	7.0	1.25	.25	.75	.0	.0
multifidus	61	7.44	1.58	.27	.58	.08	.05
pulcher	1	8.0	1.00	.0	.0	1.0	.0
Comatella							
maculata	40	8.12	0.75	.82	.17	.0	.0
nigra	89	7.12	1.65	.30	.50	.13	.05
sp. a	5	6.80	0.75	.40	.20	.40	.0
stelligera	16	6.81	0.75	.25	.68	.0	.06
Comatula							
pectinata	8	8.12	0.63	.87	.12	.0	.0
purpurea	26	5.72	0.44	.78	.19	.01	.0
Decametra							
sp.a	3	5.66	0	.0	.33	.0	.66
laevipinna	2	4.50	0	.0	.0	.50	.50
Dorometra nana	4	4.25	0	.50	.50	.0	.0
Euantedon polytes	3	5.66	1.67	.33	.33	.0	.33
Himerometra							
bartschi	1	5.0	1.00	.0	.0	.0	1.0
magnipinna	14	10.64	3.14	.0	.0	.12	.87
robustipinna	11	12.72	7.36	.0	.18	.0	.81
Iconometra intermedia	1	6.0	0	1.0	.0	.0	.0
Liparometra articulata	1	4.0	0	.0	1.0	.0	.0
Oligometra serripinna	1	5.0	0	.0	.0	.0	1.0
Oxycomanthus							
bennetti	12	8.50	4.17	.50	.08	.0	.41
comanthipinna	14	7.07	0.64	.50	.35	.14	.0
exilis	23	5.87	0.26	.73	.21	.04	.0
mirus	4	10.0	2.25	.25	.75	.0	.0
perplexum	1	8.0	3.00	.0	.0	.0	.0
Petasometra clarae	8	5.75	0.13	.0	..0	.0	1.0
Stephanometra							
indica	31	5.45	0.26	.19	.06	.09	.64
oxyacantha	5	12.20	3.80	.0	.20	.80	.0
spicata	1	10.0	1.00	.0	1.0	.0	.0
spinipinna	4	8.50	3.25	.50	.25	.25	.0
Tropiometra afra	3	15.33	3.33	.0	.0	.0	1.0
Total no of indiv	1117		1689				
Mean		[illegible]	1.51	.41	.39	.08	.10
SD		1.56					

both the highest diversity and the highest number of symbionts belonged to this family. Yet the degree of inhabitation also varied considerably among the comasterids: for example 68% of the individuals of *Comanthus parvicirrus* (N = 273) carried one or more epizoic individuals, whereas only 20% of another very common species, *Comatula purpurea* (N = 126), carried symbionts.

Patterns in the crinoid species - symbiont matrix could have been caused by variable or patchy distribution over the sampling sites of either of the animal groups. To test this the data matrices 'crinoid densities versus sites' and 'symbiont densities versus sites' were co-analyzed with canonical correlation analyses. The analyses proved that the crinoid distribution and crinoid-symbiont associations were unrelated patterns. All sites showed similarly low correlations to the main canonical variates in both data sets. This finding was also confirmed by a Friedman-Rafsky test (Friedman *et al.* 1979): the groups of crinoids determined from their spatial distributions showed no relationship to the groups of crinoids determined from their symbionts.

b) Symbiont numbers, crinoid morphology and crinoid behaviour. The lack of discrete host specificity for a majority of the symbiont taxa inidcates that factors other than host taxonomic identity determine the patterns of the crinoid-symbiont relationship. We therefore sought morphological and behavioural characteristics of the hosts to better predict the associations. Since the abundances of the different symbiont taxa were highly correlated with each other, the following analyses were based on total numbers of symbionts to simplify the recognition of general patterns.

Arm length, body volume, and the degree of daytime emergence of the individuals were tested for their influence on inhabitation rates. Table 3 summarizes data on mean arm length of the individual crinoid species, their emergence during the day, and their mean number of symbionts.

The mean average arm length in the crinoid community was 7.5 cm. The frequency of arm lengths was normally distributed (Kolmogorov-Smirnov test; p > 0.999), both for the whole crinoid community and for the three most common species *Comanthus parvicirrus, Comaster multifidus* and *Comatula purpurea.* A size-dependent transition from a cryptic to a more exposed day-time living mode of the crinoids was apparent. In many species, with the exception of some non-comasterids, there was a trend for cryptic specimens to be small, whereas all size categories were found in the groups of protected and free living animals (Table 4).

Size distribution was bimodal for exposed specimens; they were very large or very small. The small exposed specimens were mainly non-comasterids (*Petasometra clarae, Amphimetra laevipinna,*

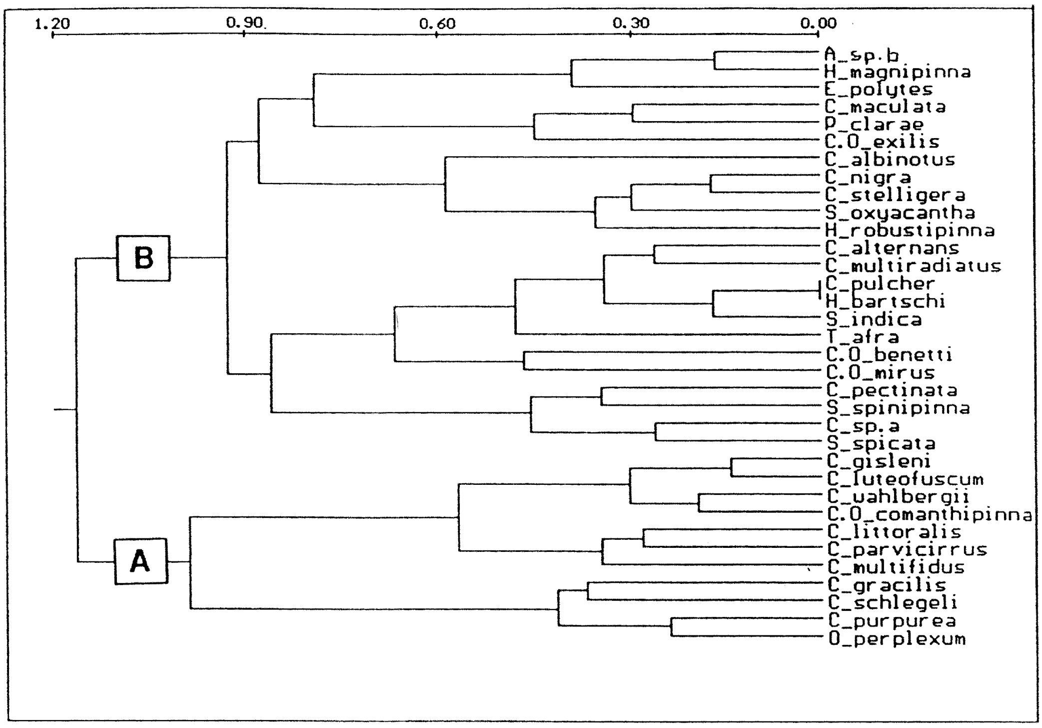

Figure 2. Crinoid species classified by their symbiont inhabitation. Flexible- sorting classification on data transformed with the Manhattan dissimilarity measure. For the species names: see Table 3, for symbiont taxa determining the two major groups A and B: see text.

Table 4. Correlations between body volume of the individual crinoid, rates of inhabitation, and percentage of specimens with cryptic, protected, free and exposed daytime habit. N = number of specimens; Arml. = average armlength; mean = mean number of symbionts, SD = standard deviation, min, max = minimum and maximum number of inhabitants per host individual; crypt. = cryptic, prot. = protected habit. Total number of cases: 1114.

Body-volume	N	Arm-length (cm)	Mean No. symbionts	Daytime Emergence [%]			
				crypt.	protec.	free	exposed
1	58	2.914	0.107	0.793	0.121	0.052	0.034
2	158	4.861	0.285	0.532	0.272	0.025	0.171
3	265	5.989	0.822	0.449	0.408	0.091	0.049
4	231	7.372	1.104	0.442	0.429	0.087	0.039
5	215	8.651	2.159	0.344	0.437	0.135	0.051
6	129	10.395	3.008	0.155	0.457	0.109	0.116
7	43	13.581	3.651	0.023	0.465	0.326	0.093
8	18	17.000	7.778	0.000	0.111	0.167	0.667

Oligometra serripinna, Decametra laevipinna and *Euantedon polytes*.) but small individuals of *Stephanometra indica* and of the comasterids *Capillaster multiradiatus* and *Comaster multifidus* were sometimes found exposed during the day. The mean symbiont number of these specimens was very low (0.37 ± 0.39) and well below the overall mean.

Large exposed crinoids were mostly *Himerometra* spp. and *Tropiometra afra*, but also included comasterids such as *Oxycomanthus benetti, Comathina schlegli* and *Clarkcomanthus albinotus*. Among the large exposed crinoids, the mean symbiont number was significantly higher (3.25 ± 1.69) than in the group of the cyptic and protected crinoid individuals. The Himerometridae have, in common with *Oxycomanthus* spp. and *Comanthina schlegeli* , large size, long, stout cirri and exposed living posture. All three genera bore similarly high numbers of polychaetes, *Periclimenes* spp, galatheidae and myzostomes, but very low numbers of Synalpheidea and no crabs (Fig. 1)

The habit of small individuals was predominantly cryptic during the day. The combination of small size, mostly crypic habit, and low symbiont numbers (0.97 ± 1.67) characterized in particular members of the families Colobometridae and Antedonidae, and the

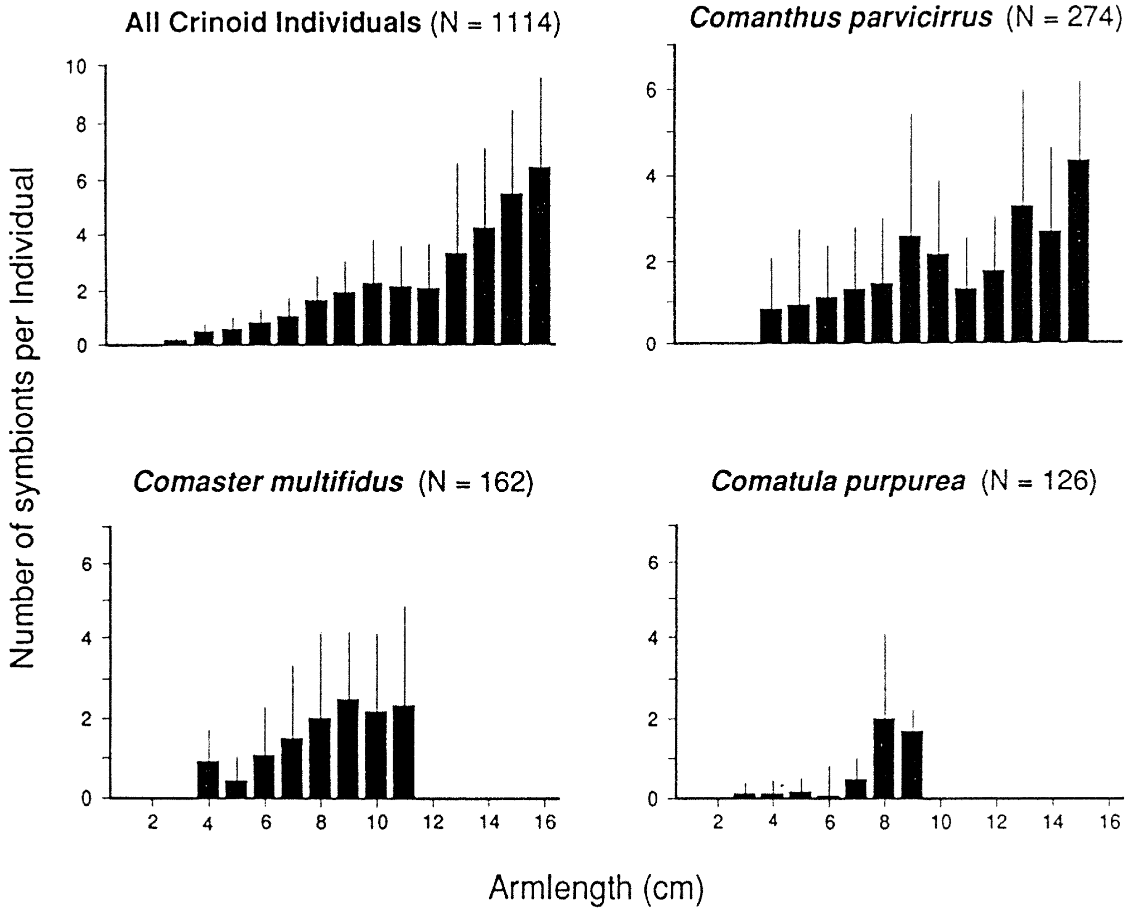

Figure 3. Average symbiont numbers on crinoids as a function of crinoid arm length. N = Number of individuals. The bars indicate standard deviations. a) all species pooled (linear correlation coefficient r = 0.7, p < 0.0001) b) - d) the three most common crinoid species: *Comanthus parvicirrus* (r = 0.61), *Comaster multifidus* (r = 0.59), and *Comatula purpurea* (r ± =0.38). Level of significance for each < 0.001.

comasterid genus *Comatula,* as well as a wide range of small individuals of other species.

The correlation between the number of associates and crinoid size, for all species investigated over all sites, is highly significant ($r = 0.70$, $p > 0.001$, Fig 3a). This relationship was also highly significant when calculated for three common species (Fig. 3b-d).

The number of symbionts for classes of identical arm length of the two most common crinoid species, *Comanthus parvicirrus* and *Comaster mulitifidus,* were not statistically different. In contrast, the number of symbionts was significantly lower on *Comatula purpurea* ($p < 0.001$) than on identical arm length classes of *Comanthus parvicirrus* and *Comaster multifidus.* No such distinction between the species existed when a measure of body volume, which integrated arm number, body stoutness and arm length on a ranked scale, was used instead of arm length. The difference may be explained by the small body volume relative to arm length of *Comatula purpurea*; the species of the genus *Comatula* are characterized by a maximum of ten arms, whereas, for example, the genera *Comanthus* and especially *Comaster* may have up to 60 arms per individual and are more bulky animals.

Canonical correlation analyses for single common symbiont taxa showed that the occurrence of most taxa, and particularly of large *Synalpheus stimpsoni* and the ophiuriods, was highly correlated with the host attributes 'big size', and 'free' or 'protected' living habit during the day. Therefore the presence and abundance of single symbiont taxa, as well as the total number of associates, can be explained by host size, and prevalent day-time emergence.

3. Interactions between the symbionts

Observed frequencies of co-occurrence of symbionts on any host were compared with the frequencies expected for random distributions. Fisher's exact tests were used on pairs of the most common symbiont taxa found on the same host individual; the analyses were performed ignoring the host species. Table 5 lists the probability that the symbiont taxa are randomly distributed among the hosts, and the type of association. (No probabilities can be given for cases with zero values).

The very large pistol shrimp *Synalpheus stimpsoni* showed the highest number of negative associations. It shared the same host less frequently than expected with the shrimp *Periclimenes commensalis* and the galatheids, and is never found with *Periclimenes tenuis. Periclimenes affinis* and the galatheids were often mutually exclusive, while *Harrovia albonineata* was never found together with any galatheid, or with *Periclimenes tenuis.*

Cases of more frequent co-occurrence than expected were also common. The polychaetes, myzostomes, and some decapods, showed significantly postitive associations among each other, and with several other taxa. *Periclimenes affinis* shared frequently its host with *Synalpheus stimpsoni* and with *Periclimenes tenuis.*

Table 5. Co-occurrence of pairs of symbiont taxa on the same host individuals. Significance values for randomness of distribution are based on Fisher's Exact Tests. 0 = no cases of co-occurrence; + = species co-occur more frequently, and - = species co-occur less frequently than expected by chance. * = p < 0.001; ns = no significant deviation from expected values. For the names of the symbiont taxa: see Table 1. N = number of specimens in the samples.

Co-occurrence of Pairs of Symbionts

		Pol	Myz	Iso	Paf	Pco	Pte	Sst	Gal	Hal	Gas
Polychaetes	(N = 286)										
Myzostomes	(N = 340)	+ **									
Isopods	(N = 127)	+ **	- **								
P. affinis	(N = 119)	+ *	+ **	ns							
P. commensalis	(N = 62)	+ **	+ **	ns	ns						
P. tenuis	(N = 29)	+ **	+ **	ns	+ **	ns					
S. stimpsoni	(N = 231)	ns	- *	- *	+ **	- **	0				
Galatheids	(N = 82)	ns	ns	ns	- *	ns	+ **	- **			
H. albolineata	(N = 22)	ns	ns	0	ns	ns	0	ns	0		
Gastropods	(N = 82)	ns	ns	ns	ns	+ *	0	+ **	ns	ns	
Ophiuroids	(N = 212)	+ **	ns	ns	- *	+ **	+ **	ns	+ **	- **	ns

0 = no cases of co-occurrence
- = co-occurrence lower than expected by chance
+ = co-occurrence higher than expected by chance
ns = no significant deviation from expected values
** = p < 0.001
* = p < 0.01

Discussion

1. Spatial distribution of the symbionts

The main feature of the crinoid distribution across the continental shelf lie in density differences (Fabricius 1989), and the symbiont communities largely follow this pattern. The independence of the number of symbionts per crinoid specimen from the crinoid density at each site, and the high correlation between crinoid and symbiont distribution suggests that "amount of inhabitable crinoids" represents a limiting resource. Abele (1976) came to the same conclusion for decapod crustaceans living in association with coral heads. In contrast, Preston *et al.* (1990) found clear cross-shelf differences in communities of decapod shrimps on the hard coral *Pocillopora verrucosa* and concluded there was a limitation of these communities by replenishment, either in the form of recruitment or of migration. Gotelli *et al.* (1983) also recorded within-reef differences in inhabitation rates of *Pocillopora damicornis* by decapods.

For the crinoid-associates, the crinoids represent the immediate environment for the symbionts except at times of dispersal. The distribution of these microhabitats seems to override the influence of physical or other environmental attributes. The only exception is the isopod *Carteolana integra,* with an average density more than twice as high on any particular host at Davies Reef than in any other study area.

2. *Crinoids as Hosts*

Morphological and behavioural attributes of the crinoids, rather than species identity, were found to be the main determinant of the level of occupation by symbionts. Shelter provided by the hosts for the epizoic animals may be an important factor influencing the numbers of symbionts per host individual. The oral disk of large bulky specimens is well protected by a fairly impenetrable thicket of numerous arms. Such animals, as well as the smaller but predominantly hidden specimens, show the highest inhabitation rates. In contrast, small, exposed living non-comasterid species provide little shelter and are almost free from symbionts.

In this study, a significant correlation was shown between day-time emergence and the size of the (mainly comasterid) crinoids, independent of taxon. Vail (1987) was the first to discuss a general size dependence in the degree of day-time emergence, whereas previously patterns of day-time emergence were assumed to be purely taxon-specific (*e.g.* Meyer *et al.* 1980). Avoidance of predation by fish may explain this correlation, although many crinoid species are thought to be protected against predation by distasteful or toxic chemical compounds in their body tissue (Rideout *et al.* 1979).

Symbionts living on the outside of crinoids are often well camouflaged. Shrimps, polychaetes and, to a lesser extent, the myzostomes, match the high colour variability of the crinoids by an often striking colour resemblance to their individual host (pers. obs., Potts 1915). Only the isopods, with their cryptic living mode, are conspicuously white, but even with these the widths and darkness of a few longitudinal black or purple stripes allow an adaptation to their hosts colour.

Meyer *et al.* (1984) have documented an attach by a saddled butterfly fish (*Chaetodon ephippium*) on a crinoid. They interpreted this as a potential attack on some of the symbionts. This observation, as well as the extent of camouflage found among the inhabitants, supports the hypothesis that shelter may be a crucial factor for the distribution of the symbionts. It means that the crinoids might not only suffer loss of food to the associates; they may also suffer the additional cost of being damaged by fish intent on feeding on symbionts.

Large, stiffened and spiny proximal pinnules of Indo-Pacific crinoid genera have been interpreted as defence mechanisms against either predation or inhabitation of symbionts (Meyer *et al.* 1977). The symbiont populations found on *Stephanometra*, a crinoid genus which is well armed with a rigid pallisade of pinnules around the oral disk, suggests that this morphological characteristic is not a deterrent to associated polychaetes and myzostomes, which can move freely between the spines. But large decapods and above all the Synalphidae, which are specialized for a life on the central calyx of crinoids, are not found on animals of this genus. The Pontoniinae shrimps also only rarely inhabit them.

Comasterid crinoids were found to be more likely to carry symbionts than the other comatulid crinoid families. The reason for this difference is not immediately obvious. However, a majority of the symbiont taxa feed on the strings of mucus and plankton particles which are transported along the crinoid arms in the ambulacral grooves towards the crinoids mouth (Potts 1915, Hyman 1955). One major difference between the comasterids and the other families is that the comasterids are unable to cover the ambulacral grooves of their arms, whereas all other families are able to cover these grooves either with small plates or by bending the edges together so that the grooves almost become tubes (Hyman 1955). This easier accessibility of food on the comasterids compared to the non-comasterids may partly explain the higher susceptibility of the comasterids to symbionts.

Since several factors are confounded with size, colonisation and relocation experiments are required to clarify which aspects of the host size may influence the inhabitation rates. Besides the provision of shelter by the crinoid host, space competition between the symbionts on the host may influence the inhabitation rates: on large animals with many arms there is obviously more space for symbionts than on small hosts, and more species with similar site preferences may coexist. A additional group of reasons relate to the probabilities of colonization. Large and exposed living individuals are more likely to be found by colonizing symbionts. Again, larger crinoid individuals may be older than the small ones of the same species, which means a longer time has been available for possible colonisation by symbionts on larger individuals.

The multispecies associations on crinoids from the Great Barrier Reef are an example of a highly non-specific symbiosis. All of the common symbionts which were identified to species level were found on more than one host species. Fishelson (1974) has recorded host-specifity for some symbiotic gastropods and myzostome species from the northern Red Sea. Specificity for these taxa may exist at the species level on the Great Barrier Reef, but the present study was limited by identification difficulties; only higher taxonomic levels were distinguishable in these groups. The number of shrimp specimens found by Zmarzly (1984b) on a single host species may have been an artefact of the low numbers of investigated crinoid individuals. It certainly could not be confirmed in the present study for any of the more common shrimp taxa.

3. *Interactions Between and Among Symbiont Taxa*

Very little is known about the biology and ecology of most of the associates. The descriptive approach of analyzing frequencies of co-occurrence of taxa used here does not purport to identify uniquely the underlying mechanisms for patterns of co-occurrence between different taxa. Behavioural and biological studies are required in order to determine whether, and to what extent, antagonistic or neutral behaviour, or related or dissimilar host preferences, could have determined the frequency with which any two taxa were found sharing a particular host (Freeland 1986, Holmes 1973, 1986). A first simple model could be developed from the theories of island biogeography, regarding crinoids as islands and the symbiont species as (meta-)populations inhabiting them.

Predator - prey relationships between some of the symbionts are suspected, with several of the shrimps feeding on symbiotic copepods and smaller scaleworms (Fishelson 1974, Zmarzly 1984a). Among other possible reasons, co-existence seems to be assisted by resource partitioning, with species forming guilds. In an investigation of feeding types among seven closely related symbiotic pontoniine shrimps from Marshall Islands, four different feeding types could be distinguished. Only two species, which belonged to the same feeding types, excluded each other, all other species were able to coexist (Zmarzly 1984b).

Coexisting symbionts also seem to avoid competiton by occupying niches separated in space, although presumably this reflects other more profound and proximal causes. For example, most of the different *Periclimenes* species are found on the proximal or middle arm sectors, the Synalphidae are found on the central calyx, the polychaetes on the more distal arm sectors, and the Galatheidae on the underside between the crinoid cirri (own observations, Potts 1915, Fishelson 1969, Zmarzly 1984a).

The interactions are not restricted to interspecific relationships. The synalphid shrimps are stated to be one of the few cases of monogamous invertebrates which live mostly as male-female couples on crinoids (pers. observ., Potts 1915, Zmarzly 1984a). Active intra-specific competition are territorial defense may be the reason that more than two specimens were only rarely found on a crinoid host.

In this study the term symbiosis has been used to describe a very wide range of relationships. However, in spite of this diversity of relationships, for most symbionts various attributes of the substrate organism, such as shelter and accessability of food, were the main determinants for inhabitation rates, and host specialisation played only a subordinate role.

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REFERENCES

Abele, L. G. 1976. Comparative species composition and relative abundance of decapod crustaceans in marine habitats of Panama. Mar. Biol. 38: 263-278.

Baba, K. 1977. Biological results of the Snellius Expedition XXVIII. Zoologische Mededelingen, Rijksmuseum Leiden 50, No 15.

Baba, K. 1979. Expedition Rumphius II, Galatheid crustaceans (Decapoda, Anomura). Bull. Mus. natn, Hist. nat. Paris, 4th Ser. 1 Sect. A: 643-657.

Banner, D. M. & Banner, A. H. 1975. The Alpheid Shrimps of Australia, Part 2: The Genus Synalpheus. Rec. Austral. Mus. Vol. 29.

Bray, R. J. & Curtis J. T. 1957. An ordination of the upland forest communities of southern Wisconsin. Ecol. Monogr. 27: 325-349.

Bruce, A.J. 1982. The shrimps associated with Indo-West Pacific echinoderms. Australian Museum Memoires No. 16.

Bruce, A. J. 1992. Two new species of *Periclimenes* (Crustacea: Decapoda: Palaemonidae) from Lizard Island, Queensland, with notes on some related taxa. Rec. Austral. Mus. 44: 45-84.

Bruce, N. L. 1986. Cirolinidae (Crustacea, Isopoda). Rec. Austral. Mus. 1986, Suppl. 6.

Clark, A. H. 1921. *Monograph of the existing Crinoids: Part 2.* Bull. United States National Mus. 82(1).

Clark, A. M. & Rowe, F. W. E. 1971. *Monograph of shallow-water Indo-West Pacific Echinoderms.* Trustees of the Bristish Museum (National History), Bath.

Critchlow, D. E. 1980. *Metric methods for analyzing partially ranked data.* Lecture Notes in Statistics 34., Springer Verlag, Berlin.

De Bary, A. 1879 *Die Erscheinung der Symbiose.* Verlag KL Trubner, Strassburg.

Elredge L. G. 1972. Associates of Acanthaster planci. In: R. T. Tsuda (compiler): *Proceedings of the University of Guam Trust Territory Acanthaster planci Workshop.* University of Guam, Marine Laboratory, UOG Station, Mangilao, Guam.

Fabricius, K. E. 1989. Distribution and Ecology of Tropical Shallow Water Crinoids and their Associated Fauna. Unpublished Thesis, University of Munich, 134 pp.

Fishelson, L. 1974. Ecology of the northern Red Sea crinoids and their epi- and endozoic fauna. Mar. Biol. 26: 183-192.

Freeland, B. 1986. Arms races and convenants: the evolution of parasite communities. In: J. Kikkawa & D. J. Anderson (eds.) *Community Ecology.* Blackwell Scientific Publications, London.

Friedman, J. & Rafsky, L. C. 1979. Multivariate generalizations of the Wald-Wolfowitz and Smirnow two-sample tests. Annals Statistics 7: 697-717.

Gittins, R. 1985. *Canonical Analysis - A review with applications in Ecology.* Springer Verlag, Heidelberg.

Gotelli, N. J. & Abele, L. G. 1983. Community patterns of coral associated decapods. Mar. Ecol. Prog. Ser. 48: 131-139.

Gower, J.C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53: 325-338.

Gower, J.C. 1971. A general coefficient of similarity and some of its properties Biometrics 27: 857-874.

Gower, J. C. & Ross, G. J. S. 1969. Minimum spanning trees and single linkage cluster analysis. Appl. Statist. 18: 54-64.

Guille, A., Laboute, P, & Menou, J. L. 1986. *Guide des étoiles de mer, oursins et autres echinodermes du lagon de Nouvelle-Caledonie.* Editions de ORSTOM, Collection Faune tropicale no. XXV, Paris.

Hanley, J. R. 1984. New host and locality records of the commensal *Adyte crinoidicola* (Polychaeta, Polynoidae). The Beagle 1(1).

Havránek, T. 1981a. The present state of the GUHA software. Int. J. Man-Machine Stud. 15: 253-264.

Havránek, T. 1981b. Formal systems for mechanized statistical inference Int. J. Man-Machine Stud. 15: 333-350.

Holmes, J.C. 1973 Site selection by parasitic helminthes: interspecific interactions, site segregation and their importance to the development of helminth communities. Can. J. Zool. 51: 333-347.

Holmes, J. C. & Price, P. W. 1986. Communities of parasites, In: J. Kikkawa & D. J. Anderson (eds.): *Community Ecology*, Blackwell, London.

Hyman, L. H. 1955. Crinoidea, In: The Invertebrates, Vol. IV, Echinodermata. New York.

Lance, G. N. & Williams, W. T. 1967. A general theory of classificatory sorting strategies I: Hierarchical systems. Computer J. 9: 373-380.

Megalitsch, P. A. 1972. *Invertebrate Zoology.* Univ. Press New York.

Meyer, D. L. & Meyer, D. B. 1977. Adaptive radiation of the comatulid crinoids. Palaebiology 3: 74-82.

Meyer, D. L., LaHaye, C. A., Holland, N. D., Arneson, A. C. & Strickler, J. R. 1984. Time lapse cinematography of feather stars (Echinodermata, Crinoidea) on the Great Barrier Reef, Australia: demonstration of posture changes, locomotion, spawning and possible predation by fish. Mar. Biol. 78: 179-184.

Meyer, D.L. & Macurda, D.B. Jr. 1980. Ecology and distribution of the shallow-water crinoids of Palau and Guam. Micronesia 16: 59-99.

Poore, G.C.B. & Mobley, M.C. 1980. Canonical correlation analysis of marine macrobenthos survey data, J. exp. mar. Biol. Ecol. 45: 37-50.

Potts, F.A. 1915. The fauna associated with the crinoids of a tropical coral reef: With especial reference to its colour variations. Carnegie Publications No. 212, Washington.

Preston, N.P. & Doherty, P.J. 1990. Cross-shelf patterns in the community structure of coral-dwelling Crustacea in the central region of the Great Barrier Reef. I. Agile shrimps. Mar. Ecol. Prog. Ser. 66: 47-61.

Rideout, J.A., Smith, N.B. & Sutherland, M.D. 1979. Chemical defense of crinoids by polyketide sulphates. Experiments 35(10): 1273-1274, Basel.

Ross, D., Dale, M.B., Shields, K. & Hulett, C. 1986. *TAXON users manual, Edition P4* CSIRONET Reference Manual 6, CSIRONET, Black Mountain, Canberra ACT, Australia.

Rowe, F.W.E,. Hoggett, A.K., Birtles, R.A & Vail, L.L. 1986. Revision of some comasterid genera from Australia, with descriptions of two new genera and nine new species. Zool. J. Linnean Soc. 86: 197-277.

Sakai, T. 1976. *Crabs of Japan and the adjacent seas.* Kodansha, Tokyo.

Serene R, Tran Van Duc & Nguyen Van Luom. 1957. Eumedoninae du Viet-Nam. Treubia 24 (2).

Starr, M.P. 1975. A generalized scheme for classifying organismic assocations. Symposia of the Society for Experimental Biology. No XXIX: Symbiosis, Cambridge University Press.

Svevcic, Z., Castro, P. & Gore, R.H. 1988. Re-establishment of the family Eumedonidae. J. Nat. Hist. 22: 1301-1324.

Turkey, M. & Gothel, H. 1990. Garnelen als Untermieter von Haarsternen. Natur und Museum 120(12): 401-404.

Vail, L. 1987. Diel patterns of emergence of crinoids from within a reef at Lizard Island, Great Barrier Reef. Mar. Biol. 93, 551-560.

Williams, W.T. 1976. *Pattern Analysis in Agricultural Science.* Elsevier Scientific Publishing Co., Melbourne. 331 pp.

Williams, W.T. & Lance, G.N. 1968. Choice of strategy in analysis of complex data. Statistician 18: 31-44.

Zmarzly, D. 1984a. Distribution and ecology of shallow-water crinoids at Enewetak Atoll, Marshall Islands, with an annotated checklist of their symbionts. Pacific Sci. 38 (2):105-122

Zmarzly, D.L. 1984b. Resource partitioning and coexistence of Pontoniinae shrimps associated with crinoids at Enewetak Atoll, Marshall Islands. Am. Zool. 24: 41.

Appendix

Friedman-Rafsky Test. This test provides a simple robust non-parametric multivariate test analogous to a runs test but with greater power as the dimensionality increases. Several generalisations are possible but we shall describe here only the simplest procedure.

Given n observations from sample A, and m from sample B,

1) Calculate the dissimilarity between each pair of the (n + m) observations.

2) Calculate from these the minimum spanning tree. This links similar observations by edges to form a tree connecting all the observations, and which is as short as possible as measured by the dissimilarity.

3) Count the number of links in the tree between observations from different samples; that is the link has an A sample at one end and a B sample at the other.

The expected number of such links under the assumption that the two samples do not differ, and the variance of this estimate, can be obtained from n and m. A correction factor is necessary since a single observation could be linked to many others. In our case if the two samples differ we expect a low number of linkages between observations from different samples. Our observed number of such linkages can be compared to the expected number under the null hypothesis to determine its probability.

Friedman-Rafsky also indicate a test for differences in variance, and recommend the use of several spanning trees to increase efficiency. Critchlow (1980) has extended the procedure and shown that ANY (hyper)graph linking the observations may be used. There are other possibilities for analyses with three or more samples, so that the approach of the test permits a flexible analysis of a variety of data.

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