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Nestedness in assemblages of metazoan ecto- and endoparasites of marine fish

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Abstract

Assemblages of metazoan ectoparasites of 79 species and gastrointestinal helminths of eight species of marine fishes were analysed to examine whether nestedness is related to sample size, abundance, species richness, and prevalence of infection, and whether the use of z-scores or Monte Carlo simulations yields different results. No significant differences in the number of nested assemblages were found with the two methods, and neither sample size nor abundance, but prevalence of infection of ectoparasites was correlated with nestedness. Species richness was significantly correlated with nestedness only when fish species with fewer than three parasite species were not excluded. Differential colonisation probabilities are the most likely cause of nestedness.

Key words: Nestedness; Parasites; Marine fish; Community ecology

An important ecological question is whether parasites live in structured communities or in unstructured assemblages. One method that has recently been introduced into parasite community ecology to study such questions is nestedness analysis [1–6]. While frequently used in ecology as a test for community structure (see Worthen [7] for a review), this method is still infrequent in parasite community ecology in spite of its great heuristic value

when used at the parasite infracommunity level. Spurred by a conflict between two related papers, the one by Guégan and Hugueny [1] and the other one by Worthen and Rohde [4], and the technical reply made by two of us on the use of Monte-Carlo simulations in community ecology [5], we decided to examine this question in the present report.

Communities are said to have a nested structure “if the species comprising depauperate assemblages represent non-random subsets of progressively richer communities” [4]. Worthen and Rohde [4] found nestedness in 13 communities of metazoan ectoparasites of marine fishes ($P < 0.05$, based on individual values), but only in one of 38 com-

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munities after conservative Bonferroni corrections for multiple comparisons, confirming previous conclusions that ectoparasites of marine fish may live in largely random, unstructured assemblages. Hugueny and Guégan [5] criticised the results of Worthen and Rohde, since they used *z*-scores to calculate significance and obtained an insignificant result, whereas Guégan and Hugueny [1] found significant nestedness in their study. These authors proposed the use of a method which does not make assumptions on statistical distributions (as does the use of *z*-scores). They also pointed out that communities not included in Worthen and Rohde's [4] analysis because of highly skewed distributions of simulation scores can be analysed with a distribution-free method.

In this paper, we analyse data on 79 assemblages of metazoan ectoparasites of marine fish (including 33 analysed by Worthen and Rohde [4]), and eight assemblages of gastrointestinal helminths of marine fish for nestedness. We also examine the question of whether nestedness is related to sample size, abundance, species richness, or prevalence of infection.

Nestedness scores, *N*, were generated by determining the richness of the most depauperate assemblages for each species, and by calculating the sum of instances in which parasite species were absent from a richer assemblage. The scores were compared with the *N* values of 1000 randomly generated presence-absence matrices, obtained by a modified RANDOM1 program of Patterson and Atmar [8]. *P*-values are the proportion of simulated *N* values lower than or equal to the observed *N* [5]. Scores were calculated separately for ectoparasites (Table 1) and endoparasites (Table 2).

Twenty-nine fish species had fewer than three parasite species, so nestedness scores are irrelevant (Tables 1 and 2, "non-sense"). Of the remaining 50 species, 15 were nested with a probability level of $P < 0.05$ (range of species richness from 3 to 13), and seven of these species with $P < 0.01$ (range of species richness from three to 22). None of the Antarctic and only two of the tropical endoparasite assemblages were nested (Table 2). After correcting for multiple contrasts using a sequential Bonferroni correction [9], only the three species with a *P*-value of 0.001 remained significant.

Sample size was not significantly correlated with RANDOM1 probabilities (fish species with fewer than three parasite species excluded, $n = 50$, infected hosts only, $\text{adj.}-r^2 = 0.000$, $F = 0.05$; infected plus uninfected hosts, $r^2 = 0.000$, $F = 0.92$; all fish species included, $n = 79$, infected hosts only, $\text{adj.}-r^2 = 0.022$, $F = 2.79$; infected plus uninfected hosts, $\text{adj.}-r^2 = 0.000$, $F = 0.18$). We conclude that increase in sample size does not significantly bias values of nestedness. Abundance (total number of parasites of all species/total number of hosts examined for a given host species) also was not significantly correlated with RANDOM1 probabilities (fish species with less than three parasite species excluded, $n = 50$, $r^2 = 0.016$, $F = 1.81$; all fish species included, $n = 79$, $\text{adj.}-r^2 = 0.036$, $F = 3.92$). Species richness of ectoparasites, i.e. number of parasite species per host species, was significantly correlated with RANDOM1 probabilities ($n = 79$, $\text{adj.}-r^2 = 0.192$, $F = 19.54$, $P < 0.001$): as parasite species richness increases in the community, so the probability that a nested hierarchy might exist rises, but when fish species with fewer than three parasite species were removed ($n = 50$), then $\text{adj.}-r^2 = 0.011$. Additionally, the prevalence of infected hosts (percent of hosts infected with any parasite species) was similarly correlated with RANDOM1 probabilities ($n = 79$, $\text{adj.}-r^2 = 0.445$, $F = 63.48$, $P < 0.0001$). This indicates that an increase in prevalence of infection increases nestedness. After removal of fish species with fewer than three parasite species, regressions yielded similar results ($n = 50$, $\text{adj.}-r^2 = 0.268$, $P = 0.0001$), thus confirming the effect of prevalence on nested hierarchy patterns. Moreover, multiple regression analysis suggests that prevalence, i.e. the percent of infected hosts, and perhaps species richness, i.e. the number of parasite species (just not significant with two-tailed statistics!), contribute to nestedness patterns observed across hosts (Table 3). After removal of fish species with fewer than three parasite species, the species richness independent variable was excluded from multiple regression procedure ($n = 50$, $\text{d.f.}_{(2,47)}$, t -ratio = 0.181, $P = 0.857$). All these findings confirm the fact that poor assemblages of parasites as observed in these marine fish have a low probability of being found nested. This confirms that significance is easier to resolve in larger matrices [10]. Concerning the

Table 1
Nestness scores for metazoan ectoparasite communities infecting marine fishes

Site/Species	n_1	n_2	sp.	N	P	Comment
Papua New Guinea (26–28°C)						
<i>Megalaspis cordyla</i>	13	0	5	4	0.177	not nested
<i>Scomerooides tol</i>	23	0	6	11	0.001	nested
Great Barrier Reef (20–28°C)						
<i>Lethrinus miniatus</i>	41	0	22	265	0.001	nested
Eden, Southern NSW						
<i>Scomber australasicus</i>	348	105	10	1461	0.739	not nested
Brazil (19–26°C)						
<i>Paralanchurus brasiliensis</i>	10	40	2	0	1	non-sense
<i>Scomber japonicus</i>	55	43	6	93	1	not nested
<i>Trichiurus lepturus</i>	46	14	4	3	0.018	nested
Coffs Harbour NSW (18–25°C)						
<i>Atractoscion aequidens</i>	16	10	5	4	0.241	not nested
<i>Bathygobius cocosensis</i>	0	50	0	—	—	—
<i>Bodianus vulpinus</i>	31	0	6	21	0.006	nested
<i>Girella tricuspidata</i>	22	22	6	5	0.712	not nested
<i>Herklotsichthys castelnaui</i>	38	80	4	8	1	not nested
<i>Lepidotrigla argus</i>	3	41	2	0	1	non-sense
<i>Monodactylus argenteus</i>	34	1	3	0	0.035	nested
<i>Pempheris compressa</i>	41	1	3	1	0.017	nested
<i>Pomatomus saltatrix</i>	64	1	5	2	0.031	nested
<i>Rhabdosargus sarba</i>	52	25	5	42	0.465	not nested
<i>Seriola lalandi</i>	21	0	4	8	0.831	not nested
<i>Sillago ciliata</i>	12	15	6	17	0.972	not nested
<i>S. flindersi</i>	89	3	4	13	0.001	nested
New Zealand (13–18°C)						
<i>Aldrichetta forsteri</i>	28	19	2	0	1	non-sense
Argentina (9–18°C)						
<i>Micropogon furnieri</i>	24	7	5	12	0.022	nested
<i>Patagonotothen ramsayi</i>	11	20	2	0	1	non-sense
<i>Percophis brasiliensis</i>	92	9	2	0	1	non-sense
<i>Prionotus nudigula</i>	45	21	4	22	1	not nested
<i>Scomber japonicus</i>	3	57	1	0	1	non-sense
North Sea (5–16°C)						
<i>Gadus morhua</i>	34	10	3	1	0.509	not nested
<i>Scomber scombrus</i>	62	21	3	4	0.007	nested
<i>Trachurus trachurus</i>	10	67	3	0	1	not nested
<i>Trigla lucerna</i>	25	3	4	17	0.220	not nested
Pacific Canada (5–14°C)						
<i>Anoplopoma fimbria</i>	23	9	2	0	1	non-sense
<i>Atheresthes stomias</i>	31	6	3	0	0.279	not nested
<i>Aulorhynchus flavidus</i>	11	11	2	0	1	non-sense
<i>Citharichthys sordidus</i>	11	10	2	0	1	non-sense
<i>Clupea harengus pallasii</i>	24	1	3	0	0.069	not nested

Table 1 (continued)
 Nestedness scores for metazoan ectoparasite communities infecting marine fishes

Site/Species	n_1	n_2	sp.	N	P	Comment
<i>Cymatogaster aggregata</i>	37	3	4	20	0.020	nested
<i>Damalichthys vacca</i>	20	1	5	4	0.155	not nested
<i>Eopsetta jordani</i>	13	8	3	0	0.379	not nested
<i>Gadus macrocephalus</i>	16	4	4	2	0.075	not nested
<i>Glyptocephalus zachirus</i>	3	17	2	0	1	non-sense
<i>Hemilepidotus spinosus</i>	1	19	1	0	1	non-sense
<i>Hippoglossoides elassodon</i>	25	22	4	10	1	not nested
<i>Hippoglossus stenolepis</i>	29	10	2	0	1	non-sense
<i>Lepidopsetta bilineata</i>	27	11	6	24	0.047	nested
<i>Microstomus pacificus</i>	10	17	2	0	1	non-sense
<i>Parophrys vetulus</i>	19	9	4	9	0.262	not nested
<i>Pleuronichthys decurrens</i>	20	0	3	2	1	not nested
<i>Sebastes alutus</i>	32	0	4	9	0.002	nested
<i>S. flavidus</i>	8	14	3	1	1	not nested
<i>S. maliger</i>	28	0	4	0	1	not nested
<i>S. pinniger</i>	22	2	4	9	0.029	nested
<i>Sebastolobus alascanus</i>	26	4	4	2	0.221	not nested
<i>Syngnathus griseolineatus</i>	61	42	4	28	1	not nested
<i>Theragra chalcogramma</i>	10	12	3	1	0.510	not nested
<i>Mallotus villosus</i>	0	26	0	—	—	—
<i>Thaleichthys pacificus</i>	0	28	0	—	—	—
"Deep water" NSW (4–15°C)						
<i>Allocyttus verrucosus</i>	0	32	0	—	—	—
<i>Atypichthys strigatus</i>	10	54	5	3	1	not nested
<i>Azygopus pinnifasciatus</i>	0	22	0	—	—	—
<i>Bassanago bulbiceps</i>	0	23	0	—	—	—
<i>Centroberyx affinis</i>	19	18	3	2	0.228	not nested
<i>Ceratoscopelus warmingii</i>	6	101	1	0	1	non-sense
<i>Chauliodus sloani</i>	0	21	0	—	—	—
<i>Chlorophthalmus nigripinnis</i>	14	20	5	11	0.358	not nested
<i>C. sp. 2</i>	2	23	1	0	1	non-sense
<i>Coelorinchus sp. C</i>	9	20	2	0	1	non-sense
<i>Coryphaenoides serrulatus</i>	6	14	3	0	1	not nested
<i>Cyttus mccullochi</i>	0	34	0	—	—	—
<i>Enoplosus armatus</i>	13	48	2	0	1	non-sense
<i>Epigonus denticulatus</i>	0	49	0	—	—	—
<i>Genypterus blacodes</i>	20	5	3	7	0.12	not nested
<i>Helicolenus papillosus</i>	17	12	4	6	1	not nested
<i>Hoplichthys haswelli</i>	22	4	4	1	0.009	nested
<i>Hoplostethus atlanticus</i>	11	33	2	0	1	non-sense
<i>H. intermedius</i>	5	15	1	0	1	non-sense
<i>H. mediterraneus</i>	0	32	0	—	—	—
<i>Hyperoglyphe antarctica</i>	14	6	3	0	1	not nested
<i>Lepidion microcephalus</i>	28	1	2	0	1	non-sense
<i>Lepidorhynchus denticulatus</i>	5	35	2	0	1	non-sense
<i>Mesobius antipodum</i>	0	28	0	—	—	—
<i>Myctophum phengodes</i>	11	86	2	0	1	non-sense
<i>Notoscopelus resplendens</i>	2	32	1	0	1	non-sense
<i>Rouleina sp.</i>	0	30	0	—	—	—
<i>Symbolophorus barnardi</i>	0	26	0	—	—	—
<i>Tripteroptychys gilchristi</i>	8	27	2	0	1	non-sense
<i>Zeus faber</i>	13	9	3	1	0.435	not nested

Table 1 (continued)
Nestedness scores for metazoan ectoparasite communities infecting marine fishes

Site/Species	n_1	n_2	sp.	N	P	Comment
Antarctica (0–3°C)						
<i>Champscephalus gunnari</i>	0	21	0	—	—	—
<i>Channichthys rhinoceratus</i>	4	13	1	0	1	non-sense
<i>Lepidonotothen squamifrons</i>	12	34	2	0	1	non-sense
<i>Macrourus holotrachys</i>	13	7	3	3	1	not nested
<i>Pleuragramma antarcticum</i>	0	29	0	—	—	—
<i>Trematomus bernacchii</i>	7	15	2	0	1	non-sense
<i>T. eulepidotus</i>	4	22	1	0	1	non-sense
<i>T. hansonii</i>	4	17	1	0	1	non-sense
<i>Zanclorhynchus spinifer</i>	0	22	0	—	—	—

n_1 : number of infected hosts; n_2 : number of uninfected hosts; sp.: number of parasite species used for analysis; N : observed nestedness score; P : Monte Carlo probability; non-sense: nestedness score invalid because fewer than 3 parasite species present.

Table 2
Nestedness scores for gastrointestinal helminth communities infecting marine fishes

Site/Species	n_1	n_2	sp.	N	P	Comment
Great Barrier Reef (20–28°C)						
<i>Lethrinus miniatus</i>	13	0	13	19	0.013	nested
<i>Siganus doliatus</i>	23	1	7	35	0.600	not nested
<i>S. lineatus</i>	15	0	5	3	0.012	nested
<i>Sillago analis</i>	18	2	11	57	0.452	not nested
Antarctica (0–3°C)						
<i>Champscephalus gunnari</i>	18	1	3	0	0.081	not nested
<i>Lepidonotothen squamifrons</i>	16	18	8	36	0.864	not nested
<i>Macrourus holotrachys</i>	18	0	5	1	0.086	not nested
<i>Pleuragramma antarcticum</i>	2	19	3	0	1	non-sense
<i>Zanclorhynchus spinifer</i>	22	0	4	10	0.279	not nested

n_1 : number of infected hosts; n_2 : number of uninfected hosts; sp.: number of parasite species used for analysis; N : observed nestedness score; P : Monte Carlo probability; non-sense: nestedness score invalid because fewer than 3 parasite species present.

endoparasites, only the two fish species examined in the smallest numbers were nested.

In conclusion, the use of z-scores discovers about as many cases of nestedness as the assumption-free method: 13 of 38 communities (34%) were found to be nested by Worthen and Rohde [4], 22 of 50 (44%) in this study based on individual P -values; after multiple comparison correction, one significantly nested community was found in the previous study and three in this study. However, not

all of the parasite communities in the two studies were exactly the same: 18 corresponded exactly in the nestedness scores and the numbers of parasite species. If only these were compared, nine were nested at the 0.05 and of these three at the 0.01 level in the previous study [4], and eight were nested at the 0.05 and of these four at the 0.01 level in the present study. Again, there is no significant differences between the number of nested parasite communities found in the two studies. Interestingly,

Table 3

Results of a multiple regression analysis illustrating the influence of both prevalence (percent of infected hosts after arcsin transformation) and species richness (number of ectoparasite species per host species, values kept untransformed) values for explaining the nested hierarchy structure expressed by Monte-Carlo probabilities (after arcsin transformation). Variable to explain: community nestedness significance

Dependent variable	Coefficient	<i>t</i> -ratio	Probability (two-tailed)
	2.009		
Prevalence (in arcsin)	−0.910	6.30	<0.001
Species richness	−0.041	1.88	0.064

Adj.- $r^2 = 0.462$, d.f._(2,76), $F = 34.55$, $P < 0.0001$.

some species which were nested in one study were not nested in the other. For example, *Megalaspis cordyla* had a nested community at the 0.01 level in the first but a non-nested community in the second study, and *Sebastes pinniger* had a non-nested community in the first but a nested community at the 0.03 level in the second study.

Our findings that only some parasite communities in both studies were nested and that a considerable number of host species are parasitised by a single or by two ectoparasite species, strongly suggest that ectoparasite communities live in largely unstructured, non-nested assemblages, supporting earlier conclusions by Rohde [11, 12], Rohde et al. [13, 14], and Worthen and Rohde [4]. The same applies to the endoparasite assemblages examined.

Nestedness has been found in some other studies. Thus, Guégan and Huguény [1] and Huguény and Guégan [5] found nestedness in the infracommunities of ectoparasites of the tropical freshwater fish species, *Labeo coubie*. However, these authors examined fish ranging from very small (110 mm standard length) to large body size (497 mm) in the context of Island Biogeography theory with host individuals considered as islands, and smaller infracommunities considered as proper subsets of the larger ones, whereas most of our fish were adults with a relatively small range of body size (see [14]). It is likely that examination of developing parasite communities will find a greater proportion of nested communities. This is suggested by the

recent study of Kleeman [manuscript "Community ecology of ecto- and endoparasites of a tropical fish species, *Siganus doliatus*"] who did not find nestedness for ectoparasites of *Siganus doliatus*, when only juvenile or only adult fish were considered, but found nestedness when juvenile and adult fish were considered together.

When considering hosts with small size ranges, as in this contribution, the best way to deal with duplicate communities could be to test for parasite co-occurrence patterns across hosts. Of course, when these co-occurrence patterns have been analysed, random patterns have been the rule (for example [14], further references therein). Greater likelihood of nestedness in developing communities suggests a mechanism responsible: differential colonisation probabilities. It seems highly unlikely that all parasite species are acquired entirely at random, but much more likely that different parasites are often acquired in a certain order, depending on changing food and habitat preferences with growth of the fish. Also, older fish have had more time to accumulate the rarer parasite species.

Further studies are needed to show whether freshwater fishes in general differ from marine fishes, and whether certain ecological parameters, such as food or habitat, have an effect on nestedness. However, we wish to stress that nestedness of the parasite communities on some fish does not prove that these communities are structured by interspecific competition, i.e. by interspecific effects on their niches. Several other causes for nestedness exist, of which differential colonisation probabilities seem to be most likely in this case (see above).

Overall, unstructured, depauperate, unpredictable and clumped parasite assemblages in host fish appear to be the rule, and structured, species rich and predictable communities appear to be the exception, at least for fish of small size ranges.

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