The effect of incubation on the occurrence of marine fungi on randomly collected lignocellulose samples

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Random wood samples were collected from five diverse marine habitats in Brunei and examined for fungi, within 1-2 weeks and after six months incubation. This paper highlights the differences obtained during these two periods, including percentage occurrence and numbers of fungi identified, with incubation being more favourable to samples from beach, lake and rocky shoreline sites, than to those from mangrove sites. Thus incubation should be another parameter to be considered when interpreting ecological results of studies in the marine environment. A standardized approach, such as examination at several incubation periods and/or baiting is considered suitable for future studies.

Keywords: incubation, marine fungi, percentage occurrence, standardized techniques.

Techniques used for the study of fungal communities in the marine environment include random collection of wood samples, followed by incubation in moist chambers and laboratory examination after one to several weeks or months (Jones & Hyde, 1988). Identification depends on the presence of fruiting bodies and critics have suggested that many fungi may be overlooked as they may not yet be sporulating. However, since a large number of random samples are usually studied, wood will be in various stages of decay and therefore one might expect to identify many of the fungi involved in the decay of that host. Unfortunately, young decaying samples are not usually collected and early colonizers are often absent in host lists (Tan & al., 1989; Hyde, 1990a; Leong & al., 1991).

The period of incubation is also quite variable and may affect results. Collections of mangrove wood from Ranong, Thailand were examined within one week (Hyde & al., 1990), whereas collections from Brunei were examined within one to two weeks and after six months incubation (Hyde, 1989). The differences in occurrence following these periods of incubation for Brunei are reported in this paper.

Material and methods

Anduki Beach (sand–buried wood and logs), Anduki Lake (intertidal branches, roots of *Casuarina* sp. and logs), Tungit Pungit (drift-

Tab. 1.– Percentage occurrence of marine fungi on wood samples before and after incubation.

	Anduki Beach			Anduki Lake			Tg. Pungit Headland			Kpg. Serasa Mangrove			Kpg. Seria Mangrove		
	1–2 weeks	6 months	Difference	1–2 weeks	6 months	Difference	1–2 weeks	6 months	Difference	1–2 weeks	6 months	Difference	1–2 weeks	6 months	Difference
No. of species identified	15	16	+1	20	27	+7	16	21	+5	40	33	–7	23	21	–2
Total No. of fungal identifications	103	125	+22	82	108	+26	67	164	+97	132	81	–51	92	102	+10
New species occurring following incubation		2			8			9			6			0	
Species not occurring following incubation		1			1			4			13			2	

Tab. 2.– Selected examples of the percentage occurrence of marine fungi on wood samples before and after incubation.

	Anduki Beach			Anduki Lake			Tg. Pungit Headland			Kpg. Serasa Mangrove			Kpg. Seria Mangrove		
	1–2 weeks	6 months	Difference	1–2 weeks	6 months	Difference	1–2 weeks	6 months	Difference	1–2 weeks	6 months	Difference	1–2 weeks	6 months	Difference
Acrocordiopsis patilii Borse & Hyde	–	–	–	–	–	–	–	–	–	5	1	–4	–	–	–
Corollospora pulchella Kohlm., I. Schmidt & Nair	36	48	+12	4	1	–3	3	17	+14	–	1	+1	–	–	–

Halocyphina villosa Kohlm. & Kohlm.	–	–	–	–	–	–	–	–	–	14	3	–11	5	5	–
Halosarpheia marina (Cribb & Cribb) Kohlm.	–	–	–	17	4	–13	1	1	–	10	6	+4	3	1	–2
H. salina (Meyers) Kohlm.	12	13	+1	6	22	+16	10	55	+45	2	–	–2	–	–	–
Humicola alopallonella Meyer & Moore	–	–	–	–	6	+6	6	22	+16	–	1	+1	–	–	–
Leptosphaeria australiensis (Cribb & Cribb) G.C. Hughes	2	2	–	9	8	–1	–	–	–	10	3	–7	2	1	–1
Lulworthia grandispora Meyers	–	–	–	7	1	–6	–	–	–	8	1	–7	17	17	–
Savoryella appendiculata Hyde & Jones	19	33	+14	3	7	+4	1	9	+8	–	–	–	1	1	–
Torpedospora radiata Meyers	–	–	–	–	–	–	5	13	+8	–	–	–	–	–	–

wood trapped in rocks), Kampong Serasa (intertidal driftwood, mangrove roots and branches) and Seria mangroves (intertidal driftwood, roots and branches) were selected as sites as they represented a diverse range of habitats. Samples were returned to the laboratory in sterile plastic bags and examined within one to two weeks microscopically for the presence of marine fungi. Examined material was then incubated for a further six months in alcohol sterilised sandwich boxes containing moist filter paper at room temperature and reexamined. All samples were kept under similar conditions, so that temperature, light regime, and humidity were relatively constant for all collections. Fungi were identified from fruiting structures in sporocarps or superficial conidia in the case of Hyphomycetes.

Results and discussion

In Tab. 1 the number of species of marine fungi identified and the total number of identifications made, before and after long incubation are given for the five sites investigated by Hyde (1989). The number of additional fungal species identified and those absent after long incubation are also given. Selective examples for individual fungi are given in Tab. 2. The greatest number of new fungal species after long incubation (9) occurred on samples from Tungit Pungit, a rocky headland and Anduki lake (8), an inland sea water lake. The number of taxa were also greater following long incubation of samples from these sites (97 and 26). In marked contrast fewer fungi were present on Serasa mangrove samples, with 13 species not being present after long incubation and 51 fewer identifications. This may be due to true succession (ie. dying out or being replaced by others (Tan & al., 1989). The common practice of incubating material affects the percentage occurrence of fungi found on samples. Incubation time is therefore a further parameter that must be considered when studying marine fungal ecology.

Examination of specific examples reveal the differences to be particularly noteworthy. At Tungit Pungit headland *Halosphaeria salina* was found on 10% of samples before incubation and 55% of samples following incubation. *Corollospora pulchella* (+14%) and *Humicola alopallonella* (+16%) also increased with incubation period. In contrast, at Serasa mangrove, *Halocyphina villosa* occurred on 14% of samples before and 3% following incubation. Similar decreases occurred in *Leptosphaeria australiensis* (-7%) and *Lulworthia grandispora* (-7%).

Incubation is thought to promote fructification of fungi developing on wood (Jones & Hyde, 1988) and these results confirm this to a certain extent. Samples from headland, lakes and beach sites have a greater occurrence of fungi after extended incubation. However,

the fungal community alters during incubation and some fungi can no longer be detected, thus the change of environment in the moist chamber selectively promotes the growth and sporulation of certain fungi.

Incubation of mangrove wood samples, over a long period, does not appear to promote sporulation, as the number of species and identifications (Kampong Serasa) declined, although some new fungi were identified. The moist chamber is probably a less favourable environment than the mangrove itself, which has been suggested to be ideally suited for the development of fungi (Jones & Hyde, 1988).

Incubation is also thought to promote the sporulation and occurrence of Hyphomycetes (Hyde & Jones, 1988). In this study this was only partly true, and was dependent on the site from which the samples were collected. At Tungit Pungit headland *Humicola alopallonella* increased from 6 to 22%. Other hyphomycetes were uncommon. At Anduki lake chlamydospores became common on samples (+16%) after incubation, while the occurrence of *Cirrenalia pygmea* (+6%) and *Humicola alopallonella* (+6%) also increased. At Serasa and Seria mangroves there was little increase in Hyphomycetes.

The results of this study indicate that caution must be used when interpreting results of percentage occurrence gained through examination of randomly collected samples. In the mangrove, occurrence depends on many factors, such as salinity, length of submersion, depth in the mangrove, host and type of sample (Hyde & Jones, 1988; Tan & al., 1989; Hyde, 1990a, b). The length of incubation must also be considered. Perhaps future studies should adopt a standard incubation period, or more practically, samples should be examined as soon as possible and then at regular intervals. Baiting is also an appropriate technique, since samples are examined at various periods during the decay of the wood (Jones & Hyde, 1988; Tan & al., 1989). However, fewer species are identified from baits than from randomly collected samples (Shearer & Webster, 1985; Jones & Hyde, 1988). It should be noted that these results apply to a tropical situation and that the situation with collections from temperate regions may be quite different.

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