
Fungi in aquatic habitats near St Andrews in Scotland

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The species composition and diversity of wood inhabiting fungi was compared in three aquatic habitats, a marine, an estuarine and a freshwater site, in order to determine whether the species present in these areas are cosmopolitan, or if there is a distinct separation between the sites correlated with the change in water conditions. Both molecular and morphological analysis were used for identification purposes. In total 35 species were observed, and three of these species, *Fusarium* sp. 1, *Orbilia* sp. and *Neonectria lucida*, were found at more than one site. Seventeen species were observed in both the freshwater and estuarine sites (Kinness Burn and Guardbridge, respectively), but only four were present at Kinkell Rocks, the marine site. The Shannon Wiener Diversity Index was highest for the freshwater Kinness Burn site (2.8) and lowest for the marine Kinkell Rocks (1.33). The Sorensen's Index of Similarity confirmed that there were no species in common between the marine site, and either of the other sites. The Index between the estuarine and freshwater sites (0.18) suggest that there is some overlap in species composition, but there was still a wide variation in species identity between the two sites. The diversity of the mycota inhabiting the aquatic environments decreased in more saline habitats. The ratio of anamorphs to teleomorphs decreased with a reduction in salinity. Ascomycetes were the most prevalent group of fungi on the wood substrate in the freshwater and estuarine sites.

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Introduction

The diversity and taxonomy of aquatic fungi is gaining increased attention with the recent realisation of their importance. Not only are they important in the decomposition of plant substrates in aquatic environments (Hyde et al. 1998), including on man made structures (Kohlmeyer & Kohlmeyer 1979, Hyde et al. 1998), but they have been also identified as a source of unique bioactive compounds (Jones 1998, Miller 2000, Raghukumar 2008). Aquatic fungi are found in a variety of habitats ranging from saline environments such as

marine, including the hypersaline waters of the Dead Sea, to the freshwater habitats of lakes and streams (Buchalo et al. 1998).

This study focuses on fungi associated with submerged (lignocellulose) substrates in aquatic habitats. Fungal diversity in these habitats has been summarised by Shearer et al. (2007). It has been noted that basidiomycetes, zygomycetes and lichens rarely occur as aquatic fungi, and the remainder of fungi in aquatic habitats include oomycetes, chytridiomycetes, meiosporic and anamorphic ascomycetes (with coelomycetes representing very few of the

latter group) (Shearer et al. 2007). There is, however, some evidence that basidiomycetous fungi, in the form of yeasts, dominate mycoplankton communities (Jones et al. 2009, Gao et al. 2010). Approximately 450 obligate marine ascomycetes (meiosporic) have been described (Shearer et al. 2007, Jones et al. 2009).

Marine fungi are not considered as a taxonomic, but instead an ecological grouping. Kohlmeyer & Kohlmeyer (1979) described obligate marine fungi as "those that grow and sporulate exclusively in a marine or estuarine habitat" and facultative marine fungi as "those from freshwater and terrestrial milieus able to grow and possibly sporulate in the marine environment". Most of these species (over 80%) are ascomycetes adapted to aquatic life with small fruiting bodies, appendaged spores for dispersal and the ability to survive fluctuations in salinity (Hyde et al. 2000). Freshwater fungi are described as species which rely on free water for all or part of their life cycle (Thomas 1996). There are approximately 550 species of ascomycetes described from freshwater habitats (Shearer et al. 2007) with many more Chytridiomycota, aquatic hyphomycetes (about 300 species *vide* Kirk et al. 2001), aeroaquatic anamorphic fungi (about 80 species) and other anamorphic freshwater species (over 400 species, not including aeroaquatic and aquatic hyphomycetes) (Shearer et al. 2007).

The ecology of aquatic fungi affects their distribution both locally and globally, and the factors influencing the fungi are complex, and vary depending on the aquatic environment. What governs the distribution of freshwater fungi is difficult to determine as many areas are under-collected, although some species appear to be more common either in temperate or tropical regions (Shearer et al. 2007, Raja et al. 2009). However, it has been suggested that there is a limitation on distribution, due to temperature, especially in tropical species (Wong et al. 1998). As more studies are undertaken species considered as tropical are also being found in temperate areas (Raja et al. 2004). The type of lignocellulosic debris and plants as sources of debris in streams is also considered to influence the fungi colonising it, as is the pH

of the habitat (Raja et al. 2009). Ristanovic & Miller (1968) studied the tolerance of fungi to differing salt concentrations and demonstrated that freshwater strains of fungi grew best at low salt concentrations, while marine strains tolerated higher concentrations. Estuarine strains were observed to be more like freshwater strains, in that optimal growth occurred at low NaCl concentrations, although salinity primarily governs their distribution, with temperature being a secondary influence (Shearer 1972). Several studies have now demonstrated a change in the composition of fungi upstream compared to in an estuary (Johnson & Sparrow 1961, Shearer 1972, Tsui & Hyde 2004). Salt marshes and mangroves constitute other estuarine habitats, and it is noteworthy that mangroves are considered to be biodiversity 'hotspots' for aquatic fungi with about 200 of the 625 fungi recorded from mangrove habitats considered to be obligate marine species (Shearer et al. 2007). This is particularly true of mangroves with high salinity levels (Jones 2000). Reasons for the great diversity include factors such as a diverse number of substrates and a range of environments in terms of salinity and immersion (Shearer et al. 2007).

The distribution of marine fungi is governed by several factors with water temperature (Hughes 1974) and salinity (Booth & Kenkel 1986) being dominant, but available habitats and substrates and many other combined elements also have an influence (Jones 2000). Some marine fungi may grow at varying salinities, but will not form fruiting bodies in the absence of salt water (Jones & Jennings 1964). Anamorphic fungi are more likely to be associated with the low saline, freshwater dominated areas of estuarine habitats, while the higher salinity areas are more likely to have teleomorphs present (Shearer 1972).

It has been observed that terrestrial fungi can persist in the aquatic environment, making it difficult to determine if a species is part of the regular mycota, or a brief inhabitant washed in from nearby terrestrial environments (Shearer 1972). Those considered to have inadvertently arrived in an aquatic habitat are considered *transients*, whereas those not collected outside, and undergo their entire life

cycle in aquatic environments, are considered as *residents* (Shearer et al. 2007). However, as Jones et al. (2009) indicated, it is possible that marine isolates considered as terrestrial species are in fact marine counterparts and represent separate species. There are instances in the marine environment where terrestrial species are thought to be rapidly replaced by marine species, as suggested by in vitro studies (Jones & Jennings 1964). This is the case for sediment or soil derived fungi (Miller & Whitney 1981) and has also been demonstrated for mangrove leaves, but not for wood substrates (Pointing et al. 2000). Recently, Pang & Mitchell (2005) noted that many more fungi are recovered when molecular methods are used compared to conventional incubation or culture studies, and more precise ecological information is being generated, especially when the various methods are combined.

The present study investigated the trends observed in wood inhabiting fungi in aquatic environments that varied in salinity. Both morphological and molecular techniques (using the sequences of the ITS region (internal transcribed spacers, ITS1 and ITS2, with the 5.8S gene of the nuclear rDNA), were used to identify the species present in the habitats, and the findings were broadly related to the environmental conditions.

Methods

Collection and incubation

Samples were collected on 11 February 2006 at three sites in the St Andrews area of Fife. Submerged or trapped wood in the intertidal zone was collected at the three sites, which varied in salinity. Kinkell Rocks (marine) (NO 522 150 GB Grid), Guardbridge (estuarine) (NO 452 188 GB Grid) and Kinness Burn (freshwater) (NO 505 163 GB Grid) were selected for the availability of wood samples. They had salinities of 37.6, 8.4 and 0.75 and pH readings of 7.94, 6.61 and 6.64, respectively. Salinities and pH were measured using a Jenway 3010 pH meter. Natural substrates were used instead of baits in this study because they are more heterogeneous, consisting of pieces of wood in various stages of decay, which could support a

higher diversity of fungi (Tsui et al. 2001a). At least fifty samples were collected from each site. They were washed to remove contaminating material such as nematode worms and mud before each sample was stored in a separate plastic bag to avoid contamination from other samples (Lamore & Goos 1978, Tsui et al. 2003). Each sample was taken from separate pieces of wood so as to avoid the potential bias of pseudoreplication. Samples were kept moist using water from the same site thus limiting the growth of terrestrial species/contaminants which could inhibit the slower growing aquatic fungal species (Yuen et al. 1999). Keeping the samples moist also meant that later successional species would also be observed as it is predicted that there is an increase in fungal diversity between 2 weeks and 6 months of incubation (Hyde & Goh 1998).

Examination and culture

Single spore isolations from wood samples were attempted and spores were incubated on either cornmeal agar (1.7% cornmeal agar in distilled water), ii) soyabean meal (1.7% soyabean meal and 1.2% agar in distilled water) and iii) yeast extract agar (1% yeast extract, 1.2% agar, 2% peptone and 2% glucose in distilled water). Seawater, which had been left in the fridge for at least a week to age, was used instead of distilled water for those specimens taken from Kinkell Rocks (Vrijmoed 2000). Antibiotics were used to limit bacterial infections. Either a solution of carbenicillin in distilled water (50 µg per mL agar), or a combination of streptomycin sulphate at a concentration of 90 µg per 300 mL of agar and ampicillin (30 µg per 300 mL agar) was used, with the latter being the more effective. After incubation at room temperature, fungal colonies were sub-cultured in nutrient broths (1.3% nutrient broth in distilled water) of 50 ml in 250 ml conical flasks. These were closed with a cotton wool stopper and incubated at room temperature until growth was adequate for DNA studies. Morphological identification was achieved using texts by Carmichael et al. (1980), Ellis (1971, 1976), Hanlin (1992, 1998), Hyde & Pointing (2000) and Tsui & Hyde (2003).

Table 1. Percentage occurrence and number of wood samples on which a given fungal species was recorded at freshwater (Kinness Burn), estuarine (Guardbridge) and marine (Kinkell Rocks) sites.

Species	Kinness Burn (freshwater)		Guardbridge (estuary)		Kinkell Rocks (marine)	
	No. of wood samples	% occurrence	No. of wood samples	% occurrence	No. of wood samples	% occurrence
<i>Acremomium strictum</i>			1	1.85		
Apiosporous ascomycete	1	1.89				
<i>Ascotaiwania</i> sp.	1	1.89				
Basidiomycete sp. 1			1	1.85		
Basidiomycete sp. 2	1	1.89				
Bionectriaceae sp. 1			1	1.85		
Bionectriaceae sp. 2	1	1.89				
Bitunicate ascomycete	1	1.89				
<i>Cosmospora vilior</i>			2	3.70		
Discomycete sp. 1	1	1.89				
Discomycete sp. 2			2	3.70		
<i>Fusarium</i> sp. 1	2	3.77	1	1.85		
<i>Fusarium</i> sp. 2			1	1.85		
<i>Kirschsteiniiothelia</i> sp.	1	1.89				
<i>Leptosphaeria</i> sp.			1	1.85		
<i>Nectria lugdunensis</i>			1	1.85		
<i>Nectria</i> sp.	1	1.89				
<i>Neonectria lucida</i>	1	1.89	4	7.41		
<i>Nodulisporium</i> sp.			1	1.85		
<i>Orbilia</i> sp.	1	1.89	1	1.85		
<i>Peziza</i> sp.	1	1.89				
<i>Scolecobasidium salinum</i>					1	2
<i>Sporidesmium</i> sp.			1	1.85		
<i>Torula</i> sp.			1	1.85		
<i>Trichoderma</i> sp.	1	1.89				
Unidentified hypho sp. 1					2	4
Unidentified hypho sp. 2					1	2
Unidentified hypho sp. 3	2	3.77				
Unidentified hypho sp. 4			1	1.85		
Undetermined	2	3.77			1	2
Unitunicate ascomycete	1	1.89				
<i>Xylaria</i> sp.			4	7.41		
<i>Zopfiella</i> sp.			1	1.85		

DNA preparation

Fungal hyphae were removed from the nutrient broth and washed with sterile distilled water and then dried, weighed, wrapped in aluminium foil and stored in liquid nitrogen. The fungal cells were disrupted using a pestle and mortar before the DNA was isolated using a DNeasy Plant Mini Kit made by Qiagen. The protocol followed was as detailed in the manufacturer's booklet with the optional step of centrifuging the lysate for 5 minutes at 14000 rpm after AP2 had been added being undertaken. The resulting solution was placed

on a 1% agarose gel (50 ml TAE buffer, 0.5 g agarose and 2.5 µl ethidium bromide) and run against a λ marker. Primers specific to the ITS region (ITS1 and ITS4) of the rRNA genes were used, ITS 1 – 5'-TCC GTA GGT GAA CCT GCG -3', ITS4 – 5' -TCC TCC GCT TAT TGA TAT GC -3'. The PCR solution contained 100 ng/mL of DNA, a 10x Buffer (White et al. 1990), 10 mM dNTP, the two primers mentioned above, sterilised distilled water and Taq polymerase at a concentration of 5 µl/ml. The PCR settings used to amplify the DNA were an initial denaturation at 94°C for 4

minutes, followed by 35 cycles of 35 seconds of denaturation at 94°C, 30 seconds of annealing at 55°C and 1 minute extension at 72°C. After the 35 cycles a final extension of 4 minutes at 72°C was inserted. The resulting solution was run in a 2% agarose gel against a 100bp ladder. DNA was purified using Rapid PCR purification (Maligen Biosciences) carrying out the protocol outlined in the handbook and the DNA was sent to Dundee University for sequencing. Sequences from both primers were aligned and placed in a nucleotide–nucleotide BLAST program run by NCBI and the results used to produce a phylogenetic tree using the neighbour joining method.

Statistics

Percentage occurrence (Eq. 1) was calculated for each species in each habitat while similarity between sites was examined using the Sorensen's Index of Similarity (S) (Eq. 2).

$$\frac{\text{Number of wood samples a given species occurred on}}{\text{Number of samples examined}} \times 100 \quad \text{Eq. 1}$$

$$S = \frac{2c}{a+b} \quad \text{Eq. 2}$$

a = total number of species at site 1

b = total number of species at site 2

c = number of species common at both sites

Diversity was calculated using the Shannon Wiener Index (Eq. 3) (Abdel-Raheem 2004)

$$H = - \sum p_i \ln(p_i) \quad \text{Eq. 3}$$

H = index of species diversity

p_i = the proportion of the i th species out of the total population

Results

In total 35 species were observed but only three of those species were found at more than one site. Seventeen species were observed in both the freshwater and estuarine sites (Kinness Burn and Guardbridge, respectively) but only four were present at Kinkell Rocks, the marine site (Table 1).

Unidentified hyphomycete sp. 1 was observed on two samples (4%) in the marine site while the other three species were observed on only one sample (2%) each (Table 1). On average there were 0.1 specimens of fungi per sample. Only one species, KR40, was identified to species level at the marine site as a result of DNA sequencing and morphological identification (Ellis 1976). The BLAST results showed that an anamorphic ascomycete, *Scolecobasidium salinum* (G.K. Sutherl.) M.B. Ellis, was the closest relative of the analysed sample (Table 2). Twenty-five specimens of fungi were found at the estuarine Guardbridge site (Table 1). *Neonectria lucida* (Höhn.) Samuels & Brayford and a *Xylaria* sp. were found on 4 samples (7.4%) and were the most frequently found species at Guardbridge. On average 0.46 specimens of fungi were found on each sample and this was the highest result obtained for any of the habitats. Broth cultures were obtained for six species from Guardbridge. One of the species was believed to be a contaminant after being morphologically identified as a *Xylaria* sp. while the DNA sequencing suggested it was *Aspergillus fumigatus* Fresen., a fast growing opportunist. DNA analysis of sample GB13 produced a 100% sequence comparison between the sample and *Acremonium strictum* W. Gams, which was also the closest species when the phylogenetic tree was drawn. The DNA sequence analysis for GB13W was not as conclusive as it produced three species (*Cylindrocarpon obtusisporum* (Cooke & Harkn.) Wollenw., *Neonectria lucida* and *Nectria lugdunensis* J. Webster) with 100% sequence comparison. The phylogenetic tree placed *Cylindrocarpon obtusisporum* as the closest species, but this was not conclusive due to short branch lengths. After analysis using both DNA and morphological data, *Nectria lugdunensis* was believed to be the closest species to the sample. DNA sequencing of GB14 and GB20 both produced 99% comparison matches between the samples and *Cosmospora vilior* (Starbäck) Rossman & Samuels, which is a parasite of other fungal tissue and does not use the wood substrate. The DNA sequencing and phylogenetic tree produced *Neonectria lucida* as the closest species to the sample GB15.

The same number of species were found at the freshwater Kinness Burn site, as at

Table 2. BLAST results from GenBank for fungi isolated.

Specimen number	GenBank accession number	Species identification
GB14	GU726751	<i>Cosmospora vilior</i>
GB13W	GU726752	<i>Nectria lugdunensis</i>
GB13	GU726753	<i>Acremonium strictum</i>
GB15	GU726754	<i>Neonectria lucida</i>
GB20	GU726755	<i>Cosmospora vilior</i>
KR40	GU726756	<i>Scolecobasidium salinum</i>

estuarine Guardbridge, although there were only 19 specimens observed on the samples (Table 1). On average therefore 0.32 specimens of fungi were found on a sample. Unidentified hyphomycete sp. 3 and *Fusarium* sp. 1 were most frequently found being recorded on 3.7% of the samples. The highest result from the Shannon Wiener Index was obtained at the freshwater Kinness Burn site (2.80). This was slightly higher than the diversity index obtained at the estuarine Guardbridge site, which gave a result of 2.66. The marine site Kinkell Rocks had the lowest diversity out of the three sites with the Shannon Wiener Diversity Index giving a result of only 1.33. The Sorensen's Index of Similarity confirmed that there were no species in common between the marine site Kinkell Rocks and either Guardbridge or Kinness Burn with results from the Index both being 0. The sites at Guardbridge and Kinness Burn were found to have three species in common. This gave 0.18 for the Sorensen's Index of Similarity, which suggests that there is some overlap in species composition but there was still a wide variation in species identity between the two sites. Only six specimens were successfully cultured in the nutrient broth (Table 2). It was observed that species were often slow growing in culture especially with the marine species which led to problems with contaminants such as *Aspergillus fumigatus* and bacteria which out-compete the slower growing aquatic species.

It was found that the ratio between (not necessarily related) anamorphs and teleomorphs declined with reduction in salinity (Fig. 1). There appeared to be a large decrease in the proportion of anamorphs between the marine site and the other two sites. This result though may be an artefact of the low sample number or the sampling regime. Meiosporic ascomycetes were found to be the most dominant group with no

species being found at the marine Kinkell Rocks site and 11 and 8 being found at Kinness Burn and Guardbridge, respectively (Fig. 2).

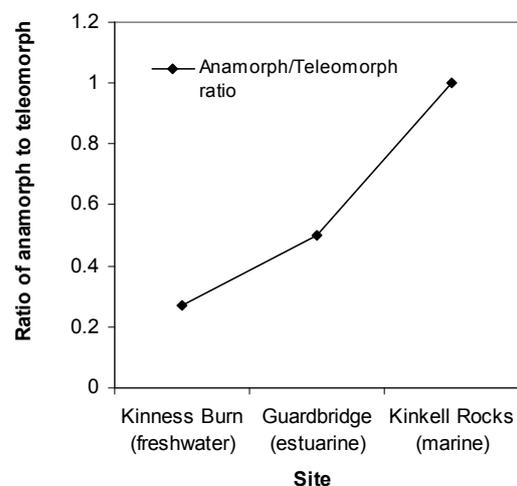


Fig. 1. The ratio of anamorphs to teleomorphs.

Discussion

It is noticeable from the results of the present study shown in Table 1, that the fungi recorded were not necessarily typical of either marine or estuarine sites. There are several possible reasons for this. Firstly, not all fungi were identified to species, and it is possible that particular representative species have therefore been overlooked, for instance marine species in the estuarine site. Also due to a lack of identification to the species level it was impossible to determine whether the species were aquatic species or terrestrial species that had survived for a period of time in the aquatic environment. An unidentified species in the freshwater site (KB39) was believed to be an aquatic species due to the helical arrangement of the spore allowing for better floatation.

The location of the collecting sites may also be influential. Hyde & Goh (1998) and Tsui et al. (2001a,b) suggested that pollution

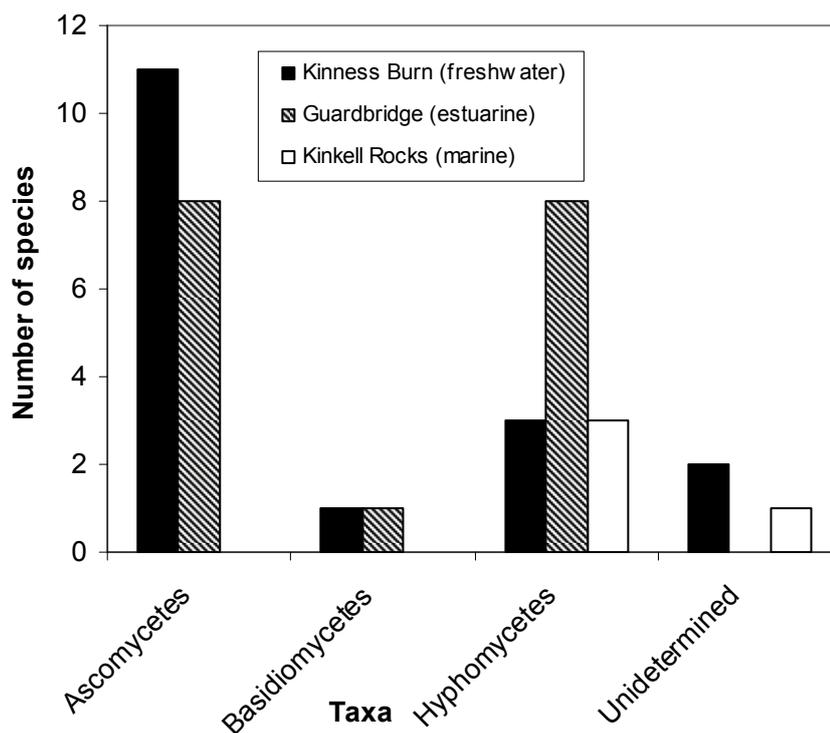


Fig. 2. Number of species found in different taxonomic groups.

of the aquatic environment could have an influence on fungal diversity, affecting the composition of fungal communities and increasing diversity. In the current study pollution was not measured, but as St Andrews is in an agricultural area and the study site for the marine environment was in the broad vicinity of a sewage outflow pipe, pollution may have an effect on the diversity observed (Anon. 2006). Comparisons with sites that were less affected by agricultural practices would need to be undertaken to gain an appreciation of the effect pollution was having on the fungal diversity.

Furthermore, incubation of the samples was limited to 3 weeks. To observe the full spectrum of species growing on the samples Hyde & Goh (1998) suggested an incubation time of up to 6 months in order to allow slow growing species to emerge. Diversity increases with time of incubation and thus in the current study the complete fungal diversity may not have been realised (Prasannarai & Sridhar 1997, Hyde & Goh 1998).

There was a negative relationship between the salinity of the environment and the diversity of fungi present. A similar trend was observed by Shearer (1972) in Chesapeake Bay with similar results for

species richness being found in both studies. However, again this could also be as a result of the short incubation time (Hyde & Goh 1998, Prasannarai & Sridhar 1997) or, particularly for the marine site, the paucity of substrates and the exposed nature of the site (Jones 2000).

Three taxa were found in common between the freshwater and estuarine sites (*Fusarium* sp. 1, *Orbilina* sp. and *Neonectria lucida*) while no taxa were found in common between the marine site and either the freshwater or estuarine sites. Many studies have demonstrated that there is little overlap between marine and freshwater sites (Shearer et al. 2007), and that freshwater and estuarine sites had more taxa in common than either did to marine ones (Ristanovic & Miller 1968, Shearer 1972, Shearer et al. 2007). However, mangrove sites, which are essentially estuarine, have been shown to harbour many obligate marine species (Tsui & Hyde 2004, Shearer et al. 2007) and to have a quite different fungal community compared to adjacent freshwater sites (Tsui & Hyde 2004).

The observed differences in species composition in the habitats could be due to the negative effects of saline conditions on non-marine species (Jones & Jennings 1964). The negative effect on non-marine species may not

be down to the salinity in general, but the toxicity of specific ions as suggested by Descals & Moralejo (2001). Marine species are not found in low saline conditions, not because they cannot grow vegetatively, but because the production of fruiting bodies can not occur out of seawater. This was demonstrated by Jones & Jennings (1964) using *Lulworthia floridana* Meyers where perithecia did not form in the absence of seawater. Marine species have been shown to be sensitive to the composition and balance of cations in the environment, which may also limit the distribution of species (Jones & Jennings 1964).

Salinity was also expected to affect the ratio between anamorphs and teleomorphs, with teleomorphs expected to dominate in high saline conditions and anamorphs in areas of lower salinity (Shearer 1972). No reliable trend was observed in the current study. Teleomorphic fungi are documented to occur at lower temperatures, while anamorphic growth have a wider, often higher temperature range (Chatmala et al. 2002). The short incubation time in this study of only 3 weeks means it is possible that insufficient time was allowed for complex ascomata to develop.

Several studies have been undertaken on the diversity of fungi inhabiting submerged wood in UK freshwater sites by Willoughby & Archer (1973) and Hyde & Goh (1999), plus other studies of aquatic fungi associated with submerged test blocks of wood e.g. Kane et al. (2002). Twenty five species of fungi were found to colonise wood substrates in the River Coln, England during a single collection in the month of September (Hyde & Goh 1999) while Willoughby & Archer (1973) identified 41 species during a year round study on Smooth Beck, Westmoorland, England. Both these studies reported species in the same genera as the present study. Both Hyde & Goh (1999) and the current study identified a *Leptosphaeria* sp. although because neither study was able to identify the fungus to species level any further comparisons were impossible. *Ascotaiwania pallida* K.D. Hyde & Goh was identified by Hyde & Goh (1999) while an *Ascotaiwania* sp. was observed in this study. *Fusarium* spp. were found in both the present study and the one by Willoughby & Archer (1973) and *Nectria lugdunensis* was identified

from both studies as well. *Leptosphaeria*, *Ascotaiwania* and *Nectria* are common genera with several species found in freshwater environments and at least one in the marine environment (Ranghoo et al. 2000, Sivichai & Jones 2003). *Scolecobasidium salinum* has been recorded in the UK in marine habitats previously (Ellis 1976).

Genera observed in the current study were also observed in tropical regions e.g. *Leptosphaeria*, *Nectria* and *Torula* (Tsui et al. 2001a) and *Kirschsteiniotelia* and *Ascotaiwania* (Hyde & Goh 1998), however, the distribution of aquatic fungi, particularly freshwater fungi, is difficult to determine due to the uneven geographic range of collection efforts (Cai et al. 2003, Raja et al. 2009).

Basidiomycetes are rarely found in aquatic habitats, but in the current study one specimen was found at Guardbridge and the other at Kinness Burn. These basidiomycetes could have been in wood recently deposited into the aquatic environment and survived to develop during the incubation in the plastic bags. There are though exceptions, such as *Crucella subtilis* Marvanová & Suberkr., which is an aquatic basidiomycete, and the dominance of basidiomycetes in mycoplankton (Gao et al. 2010). As the basidiomycetes were not fully identified it was not possible to determine whether they were of terrestrial or aquatic origin (Goh & Hyde 1996).

Cosmospora vilior, a fungicolous species which lives on *Xylariaceae*, was found to be present on two samples and this may suggest that *Xylaria* were present on more samples than they were observed on (Rossman et al. 1999).

The surrounding environment also influences the fungal diversity. The riparian environment is especially important. Wood samples differ in their resistance to fungal attack with hardwoods, such as mahogany (*Swietenia mahagoni*) rarely colonised by fungi, while softwoods are commonly colonised (Kohlmeyer & Kohlmeyer 1979). In the current study the riparian environment was limited, but a relatively diverse collection of samples of wood were made which were in different states of decay. This might provide more niches for the fungi to exist in and a higher possible fungal diversity (Tsui et al.

2001a).

This study followed the basic principles of Tsui et al. (2001a), so that comparisons could be made, but instead of storing the samples in plastic boxes the samples were sealed in plastic bags. Another deviation from the methods of Tsui et al. (2001a) was that the samples were kept moist using different methods. Tsui et al. (2001a) used dampened tissues while the current study kept the samples moist by pipetting water onto the wood pieces, but despite these differences the two studies should be comparable. Révay & Gönczöl (1990) used a different method of incubation as their samples were stored in aerated water filled boxes. These different methods of culture could affect the results especially in the presence /absence of hyphomycetes which differed substantially between the two studies. Although these differences could be down to variations in water chemistry the possibility of the methodology affecting the results has to be considered.

This study has shown that there is a general trend towards the reduction of diversity with increasing salinity and that there is some overlap between freshwater and estuarine species, but none between either and marine species. The incubation time was thought to be insufficient for the full diversity of species to be recorded, but a snapshot of the diversity of these habitats has been obtained. Both morphological identification and DNA sequencing were used and this increased the number of species that could be identified; using both methods minimised the problems associated with each method.

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References

- Abdel-Raheem AM 2004 – Study of the effect of different techniques on diversity of freshwater hyphomycetes in the River Nile (Upper Egypt). *Mycopathologia* 157, 59–72.
- Anon. 2006 – Scottish Bathing Waters 2006. http://www.sepa.org.uk/water/water_publications/bathing_water.aspx checked 25/04/2006.
- Booth T, Kenkel NC 1986 – Ecological studies of lignicolous marine fungi: a distribution model based on ordination and classification. In: *The Biology of Marine Fungi* (ed ST Moss). Cambridge University Press, Cambridge, UK, 297–310.
- Buchalo AS, Nevo E, Wasser SP, Oren A, Molitoris HP 1998 – Fungal life in the extremely hypersaline water of the Dead Sea: First records. *Proceedings of the Royal Society London B*. 265, 1461–1463.
- Cai L, Zhang KQ, Hyde KD 2003 – Freshwater ascomycetes. In *Freshwater Mycology*. (eds CKM Tsui, KD Hyde). Fungal Diversity Research Series 10, 275–326.
- Carmichael JW, Kendrick WB, Connors IL, Sigler L 1980 – *Genera of Hyphomycetes*. The University of Alberta Press, Alberta, Canada.
- Chatmala I, Valyasevi R, Tanticharoen M 2002 – Anamorph/teleomorph connections of marine Ascomycota. In: *Fungi in Marine Environments* (ed. KD Hyde). Fungal Diversity Research Series 7, 59–68.
- Descals E, Moralejo E 2001 – Water and asexual reproduction in the Ingoldian fungi. *Botanica Complutensis* 25, 13–71.
- Ellis MB 1971 – *Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew, Surrey, England.
- Ellis MB 1976 – *More Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew, Surrey, England.
- Gao Z, Johnson ZI, Wang G – 2010 Molecular characterization of the spatial diversity and novel lineages of mycoplankton in Hawaiian coastal waters. *The ISME Journal* 4, 111–120.
- Goh TK, Hyde KD 1996 – Biodiversity of freshwater fungi. *Journal of Industrial Microbiology* 17, 328–345.
- Hanlin RT 1992 – *Illustrated Genera of*

- Ascomycetes. APS Press, Minnesota, USA.
- Hanlin RT 1998 – Illustrated Genera of Ascomycetes, Volume, II. APS Press, Minnesota, USA.
- Hughes GC 1974 – Geographical distribution of the higher marine fungi. *Veröffentlichungen des Instituts fuer Meeresforschung Bremerhaven Suppl* 5, 419–441.
- Hyde KD, Goh TK 1998 – Fungi on submerged wood in Lake Barrine, north Queensland, Australia. *Mycological Research* 102, 739–749.
- Hyde KD, Goh TK 1999 – Fungi on submerged wood from the River Coln, England. *Mycological Research* 103, 1561–1574.
- Hyde KD, Jones EBG, Leano E, Pointing SB, Poonyth AD, Vrijmoed LLP 1998 – Role of fungi in marine ecosystems. *Biodiversity and Conservation* 7, 1147–1161.
- Hyde KD, Pointing SB 2000 – Marine Mycology – A Practical Approach. (eds KD Hyde, SB Pointing). *Fungal Diversity Research Series* 1, 1–377.
- Hyde KD, Sarma VV, Jones EBG 2000 – Morphology and taxonomy of higher marine fungi. In: *Marine Mycology – A Practical Approach* (eds KD Hyde, SB Pointing). *Fungal Diversity Research Series* 1, 172–204.
- Johnson TW, Sparrow FK 1961 – Fungi in Oceans and Estuaries. J. Cramer, Weinheim.
- Jones, EBG 1998 – Des champignons dans l’océan. *Biofutur* 179, 18–20.
- Jones EBG 2000 – Marine fungi: some factors influencing biodiversity. *Fungal Diversity* 4, 53–73.
- Jones EBG, Jennings DH 1964 – The effect of salinity on the growth of marine fungi in comparison with non-marine species. *Transactions of the British Mycological Society* 47, 619–625.
- Jones EBG, Sakayaroj J, Suetrong S, Somrithipol S, Pang KL 2009 – Classification of marine Ascomycota, anamorphic taxa and Basidiomycota. *Fungal Diversity* 35, 1–187.
- Kane DF, Tarn WY, Jones EBG 2002 – Fungi colonising and sporulating on submerged wood in the River Severn, UK. In: *Fungal Succession* (eds KD Hyde, EBG Jones). *Fungal Diversity* 10, 45–55.
- Kirk PM, Cannon PF, David JC, Staplers JA 2001 – Ainsworth and Bisby’s Dictionary of the Fungi. C.A.B. International, Wallingford, UK.
- Kohlmeyer J, Kohlmeyer E 1979 – *Marine Mycology: The Higher Fungi*. Academic Press Inc., London.
- Lamore BJ, Goos RD 1978 – Wood inhabiting fungi of a freshwater stream in Rhode Island. *Mycologia* 70, 1025–1034.
- Miller JD 2000 – Screening for secondary metabolites. In: *Marine Mycology – A Practical Approach* (eds KD Hyde, SB Pointing). *Fungal Diversity Research Series* 1, 158–171.
- Miller JD, Whitney NJ 1981 – Fungi of the Bay of Fundy III. Geofungi in the marine environment. *Marine Biology* 65, 61–68.
- Pang K-L, Mitchell JI 2005 – Molecular approaches for assessing fungal diversity in marine substrata. *Botanica Marina* 48, 332–347.
- Pointing SB, Fryar SC, Hyde KD, Mouzouras R 2000 – Assessment of wood decay, fungal interactions and colonization. In: *Marine Mycology – A Practical Approach* (eds KD Hyde, SB Pointing). *Fungal Diversity Research Series* 1, 113–136.
- Prasannarai K, Sridhar KR 1997 – Effect of incubation period of driftwood on the occurrence of marine fungi. *Indian Journal of Marine Sciences* 26, 380–382.
- Raghukumar C 2008 – Marine fungal biotechnology: an ecological perspective. *Fungal Diversity* 31, 19–35.
- Raja HA, Campbell J, Shearer CA 2004 – Additional reports of freshwater lignicolous meiosporic and mitosporic euascomycetes from the Great Smoky Mountains National Park. Annual Meeting of the Mycological Society of America, Asheville, North Carolina. *Inoculum* 55, 32.
- Raja HA, Schmit JP, Shearer CA 2009 – Latitudinal, habitat and substrate distribution patterns of freshwater ascomycetes in the Florida Peninsula. *Biodiversity and Conservation* 18, 419–455.

- Ranghoo VM, Hyde KD, Liew ECY 2000 – Molecular techniques and phylogeny. In: Marine Mycology – A Practical Approach (eds KD Hyde, SB Pointing). Fungal Diversity Research Series 1, 308–336.
- Révay Á, Gönczöl J 1990 – Longitudinal distribution and colonization patterns of wood-inhabiting fungi in a mountain stream in Hungary. *Nova Hedwigia* 51, 505–520.
- Ristanovic B, Miller CE 1968 – Salinity tolerances and ecological aspects of some fungi collected from freshwater, estuarine and marine habitats. *Mycopathologia* 37, 273–280.
- Rossmann AY, Samuels GJ, Rogerson CT, Lowen R 1999 – Genera of Bionectriaceae, Hypocreaceae and Nectriaceae (Hypocreales, Ascomycetes). *Studies in Mycology* 42, 1–248.
- Shearer CA 1972 – Fungi of the Chesapeake Bay and its tributaries. III. The distribution of wood inhabiting ascomycetes and *fungi imperfecti* of the Patuxent River. *American Journal of Botany* 59, 961–969.
- Shearer CA, Descals E, Kohlmeyer B, Kohlmeyer J, Marvanova L, Padgett D, Porter D, Raja HA, Schmidt JP, Thornton HA, Voglymayr H 2007 – Fungal biodiversity in aquatic habitats. *Biodiversity and Conservation* 16, 49–67.
- Sivichai S, Jones EBG 2003 – Teleomorphic-anamorphic connections of freshwater fungi. In: *Freshwater Mycology* (eds CKM Tsui, KD Hyde). Fungal Diversity Research Series 10, 259–272.
- Thomas K 1996 – Australian freshwater fungi. In: *Introductory Volume to the Fungi (Part 2). Fungi of Australia, Vol IB.* (ed CA Grgurinovic). Australian Biological Resources Study, Canberra, Australia 1–37.
- Tsui CKM, Hyde KD (eds) 2003 – *Freshwater Mycology. Fungal Diversity Research Series 10*, 1–350.
- Tsui CKM, Hyde KD 2004 – Biodiversity of fungi on submerged wood in a stream and its estuary in the Tai Ho Bay, Hong Kong. *Fungal Diversity* 15, 205–220.
- Tsui CKM, Hyde KD, Hodgkiss IJ 2001a – Colonization patterns of wood inhabiting fungi on baits in Hong Kong rivers, with reference to the effects of organic pollution. *Antonie van Leeuwenhoek* 79, 33–39.
- Tsui CKM, Hyde KD, Hodgkiss IJ 2001b – Longitudinal and temporal distribution of freshwater ascomycetes and dematiaceous hyphomycetes on submerged wood in the Lam Tsuen River, Hong Kong. *Journal of the North American Benthological Society* 20, 533–549.
- Tsui CKM, Hyde KD, Hodgkiss IJ 2003 – Methods for investigating the biodiversity and distribution of freshwater ascomycetes and anamorphic fungi on submerged wood. In: *Freshwater Mycology* (eds CKM Tsui, KD Hyde). Fungal Diversity Research Series 10, 195–209.
- Vrijmoed LLP 2000 – Isolation and culture of higher filamentous fungi. In: *Marine Mycology – A Practical Approach* (eds KD Hyde, SB Pointing). Fungal Diversity Research Series 1, 1–20.
- Willoughby LG, Archer JF 1973 – The fungal spora of a freshwater stream and its colonization pattern on wood. *Freshwater Biology* 3, 219–239.
- Wong MKM, Goh T-K, Hodgkiss IJ, Hyde KD, Ranghoo VM, Tsui CKM, Ho W-H, Wong WSW, Yuen T-K 1998 – Role of fungi in freshwater ecosystems. *Biodiversity and Conservation* 7, 1187–1206.
- Yuen TK, Hyde KD, Hodgkiss IJ 1999 – Interspecific interaction among tropical and subtropical freshwater fungi. *Microbial Ecology* 37, 257–262.