Doubling the estimate of invertebrate biomass in a rainforest canopy

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Forest canopies represent the functional interface between 90% of the Earth's terrestrial biomass and the atmosphere[1] and include some of the most threatened of all terrestrial ecosystems[2]. However, we lack even a basic understanding of how the biomass of plants and animals is distributed throughout forest canopies, even though this information is vital for estimating energy flow, carbon cycling, resource use and the transfer of materials within this ecosystem[3,4]. Here we measure the biomass of invertebrates living in a common rainforest epiphyte, describe a striking relationship between fern size and the biomass of animals within the ferns, and reveal that one large epiphyte may contain an invertebrate biomass similar to that found in the whole of the rest of the tree crown on which it is growing. Using these data, we show that including the fauna of these epiphytes—a neglected component in rainforest ecosystems—can more than double our estimate of the total invertebrate biomass in an entire rainforest canopy.

Epiphytes are known to be important to the structure and function of rainforest canopies, but there have been no detailed studies of how the animals living within epiphytes might contribute to the canopy ecosystem. The invertebrate biomass contained within bromeliads has been estimated for selected forests on an island in the Caribbean[5], but no previous work has estimated the contribution of an epiphyte fauna to the invertebrate biomass of the canopy as a whole. Here, we chose the bird's nest fern, *Asplenium nidus* (Fig. 1a) as our model epiphyte, because it is abundant[6–8], it occurs at all levels within the canopy, it can grow to exceptionally large sizes (~200 kg fresh weight) and it is widespread within the palaeotropics[9].

We collected the entire fauna (arthropods, gastropods, annelids, amphibians, reptiles and mammals) from 35 ferns of a range of sizes (1–200 kg fresh weight) and vertical heights (2–52 m) from 7 ha of lowland dipterocarp rainforest in Borneo (see Methods). We took the ferns down and sampled the whole of each fern—not just the litter trapped within it. There was a striking relationship between fern size and the biomass of invertebrates within the ferns (Fig. 1b); smaller ferns contained just a few grams of animal biomass, but this increased exponentially in ferns with more than 30 leaves, reaching 158 g in the largest fern.

Because the larger ferns contained disproportionately more animal biomass, these ferns probably make a greater contribution to the canopy biomass as a whole. We therefore looked at large ferns in more detail and selected five specimens, each of which was growing in a separate crown of the dipterocarp *Parashorea tomentella* that contained no other large epiphytes. We sampled the tree crowns by fogging them with insecticide[10]. Because fogging does not collect vertebrate animals, we limited our attention to the invertebrates. A total of 205,428 invertebrate animals were collected from the five ferns, representing an average total biomass of 88 ± 14 g (dry weight) per fern. The invertebrate biomass within the ferns was made up of a diverse range of taxa (Fig. 2a). The ferns contained a total of 27 orders compared with a total of 26 orders in the crowns. However, the bulk of the invertebrate biomass in the ferns was made up of fewer taxa, compared with the more even biomass distribution among the taxa in the crowns (Fig. 2b).

We directly compared the invertebrate biomass in the ferns and crowns using a precision-fogging technique that sampled each fern and an equivalent area of crown just above it[11]. The invertebrate biomass was two orders of magnitude higher in the fern samples than in the crown samples ($42 \pm 6\,g\,m^{-2}$ versus $0.35 \pm 0.04\,g\,m^{-2}$; paired *t*-test: $t = 7.31$, $P = 0.005$, $n = 5$). The higher biomass of invertebrates in the ferns was not only due to larger numbers of individuals, but to a combination of increased abundance and increased body size: the invertebrates in the ferns were both more abundant and larger than those in the crowns. Of the ten largest-bodied orders, eight contained individuals that were significantly heavier in the ferns than those in the crowns (Fig. 3). This direct comparison of the ferns with the crowns also enabled us to estimate the effectiveness of fogging as a method of sampling the fern fauna; fogging sampled 53 ± 8% of the invertebrate biomass within the ferns.

To assess the relative contribution of the fern fauna biomass, we compared the invertebrate biomass inside the large ferns with that inside the entire crowns of the trees on which the ferns were growing. Before removing the ferns, we fogged $21\,m^2$ of the crown of each tree and measured the horizontal cross-sectional area of each crown. The product of the invertebrate biomass per square metre in each fog (mean $= 0.21 \pm 0.04\,g\,m^{-2}$, $n = 5$) and the estimated surface area of each crown (mean $= 361 \pm 28\,m^2$, $n = 5$) gave us a measure of the total invertebrate biomass—

a

b

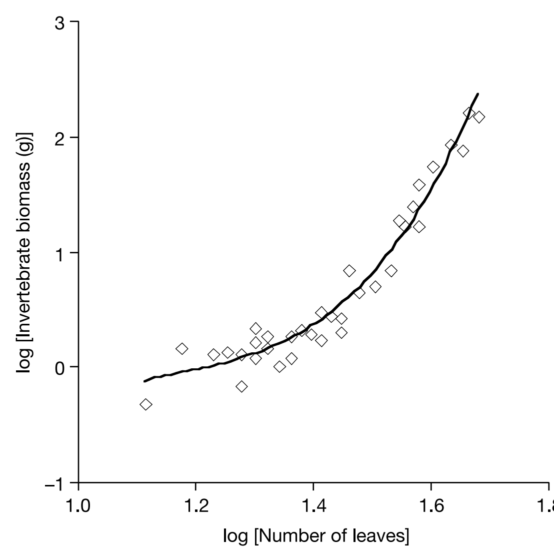

Figure 1 Invertebrate biomass in bird's nest ferns. **a**, The bird's nest fern (*A. nidus*) with leaves reaching up to 2 m in length. **b**, Relationship between the total invertebrate biomass found inside 35 ferns and the number of leaves on each of the ferns ($y = 13.9x^3 - 48.8x^2 + 58.1x - 23.5$, $r^2 = 0.96$).

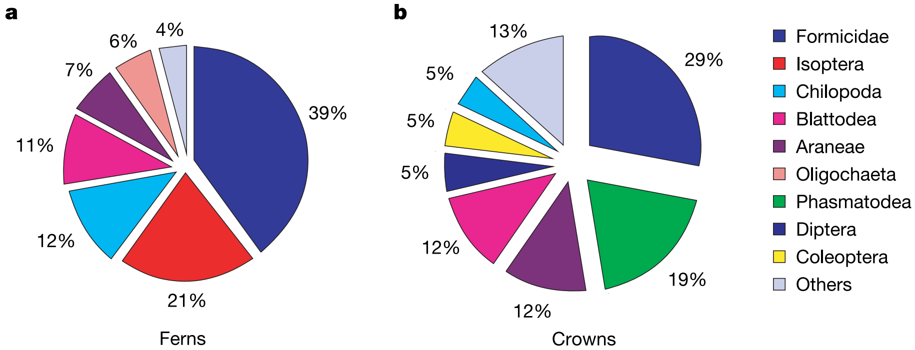

Figure 2 Contributions of various taxa to overall invertebrate biomass in ferns and crowns. **a**, Proportional contributions of the taxa that contributed most to the invertebrate biomass in five large *Asplenium* ferns (22–26 kg dry weight) (total invertebrate biomass = 439 g). **b**, Proportional contributions of the taxa that contributed most to the invertebrate biomass in five tree crowns of *P. tomentella* (total invertebrate biomass = 21 g).

excluding the fern fauna—in the crown of each tree. This figure (86 ± 18 g) is of the same order of magnitude as the total mean invertebrate biomass in a single large fern (88 ± 14 g). Fogging will, of course, sample only a proportion of the invertebrates in the tree crowns. If this proportion is the same as that for the ferns (53%), then the true total in the crowns would be about 160 g, but this is likely to be an overestimate, because the crowns—unlike the ferns—are not designed to capture and retain plant and animal matter. Our results therefore suggest that there is almost as much invertebrate biomass in a single fern as in the whole of the rest of the tree crown on which it is growing.

To quantify the overall contribution of these epiphytes to the invertebrate biomass of the entire rainforest canopy, we obtained independent estimates on a hectare basis of the biomass contained within the ferns and within the rest of the crowns. To measure the fern component, we needed to estimate the number and size of the ferns in a hectare and the invertebrate biomass within each fern. We used our collection of 35 ferns of different sizes to produce a regression equation that described the total invertebrate biomass in a fern on the basis of its number of leaves (Fig. 1b). Using this equation and the results from intensive fern surveys in 7 ha of forest, we estimated that the total amount of invertebrate biomass contained within the ferns is 3776 ± 275 g ha^{-1} ($n = 7$). To estimate the invertebrate biomass per hectare of tree crowns, we fogged the canopies of five trees of *P. tomentella* using 21 trays of 1 m^2 each per tree. We extrapolated these measurements to a hectare basis to provide an estimate of tree crown invertebrate biomass of 2,026 ± 373 g ha^{-1} ($n = 5$). If we make the rather conservative assumption that fogging extracts the same proportion of invertebrate biomass from tree crowns as it does from ferns—that is, 53%—then the invertebrate biomass within the tree crowns could be as high as 3,822 ± 703 g ha^{-1}.

Although we fogged only one species of tree, the numbers and biomass of animals that we collected are in agreement with data from other studies of canopy arthropods in southeast-Asian lowland-tropical rainforest and Amazonia[12–15]. One of the most comprehensive fogging studies of canopy invertebrates was carried out in the neighbouring island of Sulawesi[12], which sampled a range of tree species from a large area of lowland forest, using a total of 960 trays (each 1 m^2). This study gave a mean abundance of 213 animals (range 64–461) per square metre, compared with our mean abundance measure of 160 (range 78–553). The Sulawesi study did not give data on biomass, but by using our data on the mean lengths of individuals of canopy invertebrates, we can estimate that the mean biomass in the Sulawesi study was 210 (range 46–563) mg m^{-2} compared with the figure from the current study of 214 (range 34–618).

Our results show that this one species of epiphyte can contain half the entire invertebrate biomass within a hectare of rainforest canopy. This indicates that a substantial proportion of the energy and nutrients converted into animal biomass in this rainforest canopy is being funnelled through this one species of fern. We have almost certainly underestimated the general role of large epiphytes in the canopy ecosystem. *A. nidus* is just one species of large epiphyte; in this part of Borneo alone there are another 12 species of *Asplenium*[7] and some of these are equal in size to *A. nidus*. Other large ferns such as the stag's horn fern (*Platycerium* spp.) or flowering epiphytes of the Pandanaceae could well contain similar amounts of invertebrate biomass. It is worth noting that trash-basket ferns of the genus *Asplenium* are widespread and abundant in rainforests throughout the palaeotropics. Because of their size and abundance, it is likely that these particular ferns have a marked effect on invertebrate biomass distribution in rainforest canopies over a large geographical area.

To our knowledge, this is the first estimate of the contribution of epiphytes to the total invertebrate biomass of an entire rainforest canopy. Our results indicate that epiphytes have a more substantial impact on the animal communities in tropical rainforests than was previously imagined and challenge our current understanding of

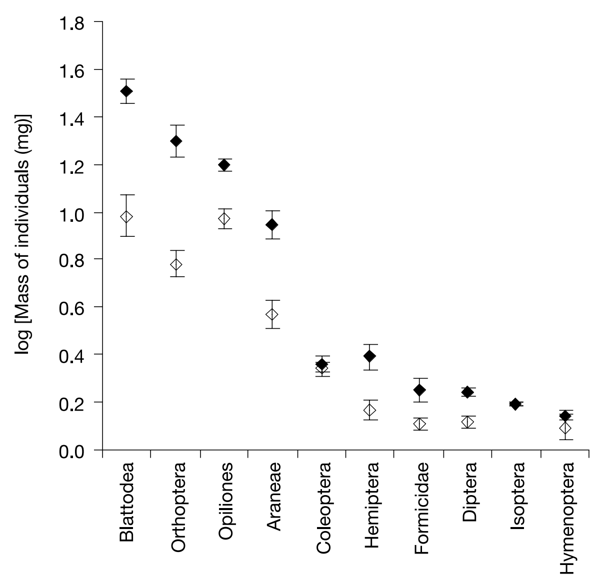

Figure 3 Individual biomass. The mean mass of individual animals from eight out of ten of the largest-bodied taxa was greater in the five large ferns (closed symbols) than in the corresponding host crowns (open symbols) ($F_{9,80} = 59.3$, $P < 0.001$). There was no significant interaction between individual mass and habitat ($F_{9,80} = 1.49$, $P = 0.172$).

the distribution and functional significance of animals in the world's rainforest canopies. Two further kinds of investigation are required to establish the broader significance of these findings. First, is the occurrence of substantial animal biomass in the epiphytes of rainforest canopies a widespread phenomenon? Do other large epiphytes of the high canopy, both in southeast Asia and in other tropical regions, contribute significantly to the total animal biomass of the canopy? The only way to answer this involves the challenging and laborious process of bringing these epiphytes down from the canopy and sampling them completely. Second, what role does this animal biomass play in the functioning of the canopy ecosystem as a whole? What function do the animals have in carbon and nutrient cycling, in decomposition and in the maintenance of biodiversity in the canopy? This can only be solved by detailed sampling and by conducting experiments within the canopy itself.

There will undoubtedly be other, overlooked concentrations of invertebrates in the canopy, living in a wide range of habitats other than epiphytes[16,17]. As further studies are made of the animals both in epiphytes and elsewhere in the canopy, the estimates of total invertebrate biomass will increase and the relative importance of epiphytes may need to be revised. This paper is the first step in this process and shows that the inclusion of one species of epiphyte can have dramatic effects on the estimates of invertebrate biomass in the canopy. □

Methods

Fogging

We used Pybuthrin 33, a synthetic pyrethroid insecticide with low mammalian toxicity, in a Swing Fog SN 50 fogging machine. Circular trays ($1\ m^2$) made of waterproof material and aluminium hoops were used to collect falling invertebrates. Fogging began at daybreak and lasted approximately 12 minutes. To determine the horizontal cross-sectional area of each crown we measured the distance at 20° intervals from the base of the trunk to the tips of the branches.

Fern collecting

Ferns were collected from undisturbed lowland dipterocarp rainforest in Danum Valley, Sabah, Malaysian Borneo (4° 58′ N, 117° 48′ E). Fern collection took place in approximately 100 ha of forest adjacent to the Danum Valley field centre and the Segama river. Each fern was netted before removal and carried back to the laboratory, where it was carefully dissected and the animals extracted using conventional methods[11,18,19]. Plant material was hand-sorted after the animals had been extracted. Animal material was then examined using binocular dissecting microscopes capable of distinguishing animals larger than 50 μm. We were thus able to identify all arthropods, including small animals such as mites and springtails.

Fern surveys

The seven 1-ha plots used for the fern surveys were located in the same 100 ha of undisturbed forest from where we collected the ferns. Using binoculars, we performed 10-m transects at ground level and treetop surveys from an emergent tree in the centre of each plot.

Biomass estimation

Animal biomass was calculated from length measurements of individual animals[5,20,21]. We established the relationship between leaf number and animal biomass (Fig. 1b) using 35 ferns collected from the same 100 ha of undisturbed forest mentioned above. From this relationship, after counting the number of ferns in each hectare and the number of leaves on each of the ferns, we were able to estimate the total animal biomass in the ferns of 1 ha of rainforest. The estimate for the crowns was based on the mean animal biomass in 21 trays of $1\ m^2$ each in the crown fogs from each of five trees, multiplied up to a hectare basis.

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Unusually dynamic sex roles in a fish

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Sex roles are typically thought of as being fixed for a given species. In most animals males compete for females, whereas the females are more reluctant to mate. Therefore sexual selection usually acts most strongly on males[1,2]. This is explained by males having a higher potential reproductive rate than females, leading to more males being sexually active (a male-biased operational sex ratio)[3,4]. However, what determines sex roles and the strength of sexual selection is a controversial and much debated question[3,5–10]. In this large-scale field study, we show a striking temporal plasticity in the mating competition of a fish (two-spotted goby, *Gobiusculus flavescens*). Over the short breeding season fierce male–male competition and intensive courtship behaviour in males were replaced by female–female competition and actively courting females. Hence, sex role reversal occurred rapidly. This is the first time that a shift in sex roles has been