

## Willow leaf and periphyton chemical composition, and the feeding preferences of *Olinga feredayi* (Trichoptera: Conoesucidae)

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**Abstract** The chemical composition of autumn-shed willow (*Salix fragilis*) leaves was examined over a 56-day period of immersion in a stream, and compared with the chemical composition of periphyton. Much of the carbohydrates, phenolic compounds, and tannins were leached from the leaves within the first week. Nitrogen and chlorophyll *a* concentrations increased as a percentage of ash-free dry mass throughout the study period, whereas protein concentration remained relatively constant. In feeding preference studies the larvae of the trichopteran *Olinga feredayi* preferred 56-day willow leaves to periphyton, 7-day, and 28-day-incubated leaves. The most notable difference between 56-day leaves and the other leaves was their lower phenolic content. The preference for 56-day leaves over periphyton may be related to the high ash content of the periphyton from this stream. We conclude that leaves of introduced willow trees can provide a preferred food source for New Zealand stream macroinvertebrates.

**Keywords** *Salix fragilis*; periphyton; chemical composition; *Olinga feredayi*; feeding preferences; streams

### INTRODUCTION

Willows were introduced to New Zealand late last century. Since then they have been extensively used in river training and bank stabilisation, and they are now the second most common exotic tree in New Zealand (Van Kraayenoord 1974). Latta (1974) calculated that a willow tree with a diameter of 0.5 m would shed 25.5 kg (dry weight) of leaves in autumn. A stream with willows spaced at 10 m intervals on both banks would thus receive over 5000 kg km<sup>-1</sup> on the bank and in the stream. Little is known of the effects these leaves have on New Zealand stream ecosystems.

Once leaves are in a stream, chemicals are often leached rapidly from them (Gessner & Schwoerbel 1989). Leaf leachates contain reducing sugars, amino acids, phenolic substances, and important nutrients such as nitrogen (N) and phosphorus (P) (Iversen 1974; Suberkropp et al. 1976). Chemicals leached from leaves can be absorbed rapidly by the benthic microbial community (Lock & Hynes 1976; for a review see Dahm 1981). Other chemical components of leaves, such as structural carbohydrates, are not so readily leached and take longer to decompose (Suberkropp et al. 1976). Some fungi have enzymes capable of breaking down cellulose (Suberkropp et al. 1983), and appear to be primarily responsible for nutritional improvements in the leaves for detritivores (Barlocher 1985).

Detritivores are also directly influenced by leaf chemistry. Leaf consumption and growth by invertebrates have been shown to be positively associated with either the N content of leaves (Iversen 1974; Irons et al. 1988), or their protein content (McMahon et al. 1974; Shepard & Minshall 1981). Tannins and other phenolic compounds have been shown to be negatively associated with

invertebrate leaf consumption and growth (Tahvanainen et al. 1985; Ostrofsky & Zettler 1986; Irons et al. 1988). These chemicals may also reduce fungal cellulase activity (Serrano & Boon 1991). Phenolic chemicals have been found in high concentrations in leaves of the European willow species *Salix viminalis*, *Salix phylicifolia*, *Salix myrsinifolia*, and *Salix aquatica* (Julkunen-Tiitto 1985).

Periphyton is generally assumed to be preferred by aquatic insects over leaves and leaf detritus, and it is considered to be of higher food quality, as it produces significantly higher growth rates (Ward & Cummins 1979; Bird & Kaushik 1984).

Our aims were to determine how some chemical components of willow leaves change with time while in a stream, to compare the chemical composition of leaves with that of a sample of periphyton, and to examine the feeding preferences of *Olinga feredayi* (Trichoptera: Conoesucidae) larva, which are facultative shredder-browsers (Ryder & Scott 1988) when offered a choice between the periphyton and leaves that had been incubated for different periods of time in a stream.

## METHODS

### Food collection and incubation

Willow (*Salix fragilis*) leaves were obtained from several trees during autumn leaf fall by shaking tree branches, and then collecting the freshly fallen leaves. Leaves were incubated in Silverstream (45°70'S, 174°10'E), a second-order willow-lined stream. They were loosely packed in nylon bags (30 cm × 15 cm, 3 mm mesh) which allowed entry of macroinvertebrates that would normally be associated with decomposing leaves. The incubation protocol of Collier & Winterbourn (1986) was followed. Thus leaves were incubated for 7, 28, and 56 days, with the 56-day treatment beginning immediately after leaf collection. The 7- and 28-day treatment leaves were frozen until use, and these treatments were begun 7 and 28 days before the completion of the 56-day experiment. Mean water conditions during incubation were: temperature 6.5°C, pH 7.3, total N 350 µg l<sup>-1</sup>, and total P 15.5 µg l<sup>-1</sup>. In each treatment, three replicate bags were tied to submerged logs on the edge of a riffle. After incubation, a sub-sample of leaves was frozen for chlorophyll *a* extraction, another sub-sample was dried in an incubator at 30°C for chemical analysis, and the remainder was used for feeding preference

experiments. Leaves for food preference experiments were macerated in a Sorvall omni-mixer, to reduce them to periphyton-sized particles (0.5–1 mm in diameter), thus eliminating the possibility of the animals using leaves as habitat.

Periphyton and larvae of *O. feredayi* were collected from Styx Creek (45°20'S, 170°10'E). The periphyton was made up mainly of diatoms, with some filamentous algae. Stones were washed in water which was passed through a sieve on which the periphyton was collected. The periphyton sample was subdivided in the same way as the willow leaf samples.

### Periphyton and leaf chemistry

Leaves and periphyton were dried to constant weight in an incubator at 30°C. They were then finely ground using a mortar and pestle. Sub-samples from each treatment were combusted at 550°C to determine percentage ash. Results are presented as percentages of ash-free dry mass (AFDM). Protein was determined by a dye binding method (Bradford 1976), with two extractions (Pick 1987), and the addition of activated carbon to reduce interference from phenols (Shepard & Minshall 1981). Carbohydrate was determined by the method of Dubois et al. (1956), phenolic compounds and condensed tannins as described by Julkunen-Tiitto (1985), total N by Kjeldahl wet digestion (Nelson & Sommers 1980), and chlorophyll *a* using the method of Lorenzen (1967) with the modifications suggested by Nusch (1980). Four replicate samples were used in most analyses. Sample sizes were approximately 3 mg for protein, 15 mg for carbohydrate and N, 150 mg for phenolics and condensed tannins, and 500 mg for chlorophyll *a* and ash.

### Food preference experiments

Larvae of *O. feredayi* were maintained in the laboratory in stream water at temperatures of about 15°C. They were kept without food for 24 h before the beginning of an experiment.

All experiments were conducted in 6 cm diam. plastic containers. Foods were tested in pairs; 0.80 ± 0.05 g (wet weight) of one food was added to one half of the container, and the same amount of another food was added to the other half. The foods were leaves that had been incubated in the stream for 7, 28, and 56 days; and periphyton. All possible pairs of the four foods were examined, with four replicates of each pair.

After the food was added, four *Olinga* were placed randomly into each container, and left for a period of 1 h to acclimatise and detect any differences in food quality. The animals' locations were determined photographically using a Canon F-1 camera with a motordrive, and infra-red film and flash. The camera was programmed with the aid of a Unitron 386SX-20 computer and a program written in Turbo Pascal 6.0, to take one picture randomly within every consecutive 40-min period for 24 h.

The experiment was run twice, in two consecutive 24-h periods using different animals.

Preliminary observations indicated that the animals moved freely and fed more or less continuously while in containers. We are thus confident that our results were not biased by *Olinga* becoming satiated, or failing to move from their initial positions.

Results of the preference tests were analysed with the Bradley-Terry model of paired comparisons (Bradley 1976), as recommended by David (1988). When all possible paired combinations are observed, as they were in the present study, this model derives a probability value for each food which predicts the choice made in the presence of all potential foods. To test the null hypothesis of equal probability among all foods, the likelihood ratio  $\chi^2$  was calculated and tested against the appropriate  $\chi^2$  statistic (Bradley 1976).

## RESULTS

### Periphyton and leaf chemistry

The willow leaves had an ash component of  $10\% \pm 3.5\%$  (mean  $\pm$  SE,  $n = 4$ ) dry weight. The ash component of the periphyton sample was substantially higher, at  $65\% \pm 3.8\%$  ( $n = 4$ ) dry weight.

Total carbohydrate concentration in leaves decreased from about 75% AFDM to 25% in the first week of incubation, and thereafter remained relatively constant. Mean total carbohydrate concentration of periphyton was 34% AFDM (Fig. 1). Protein concentration of leaves decreased slightly during the course of the study from 4 to 2%, whereas N content approximately doubled from 1 to 2%. Periphyton had approximately twice this proportion of protein, and a higher N content than leaves (Fig. 1). The phenolic content of leaves was high, initially about 12% of the leaf AFDM. This was mainly made up of tannins which were leached

or decomposed rapidly over the first 7 days, and then more slowly until day 56 when almost no phenolics or tannins remained. Periphyton contained about 1.5% phenolic compounds, but no tannins (Fig. 1). The amount of chlorophyll *a* associated with leaves increased progressively during incubation, to about one-sixth of that in periphyton, as a result of colonisation by algae.

### Food preferences

*Olinga* larvae showed a clear preference for 56-day-incubated leaves over all other foods presented. There was little evidence of any preferences among periphyton, and 7-day and 28-day leaves (Fig. 2). The Bradley-Terry model predicted that if *Olinga* were presented a choice of all foods tested in this study, it would spend 78.5% of the time feeding on 56-day leaves, 8.7% on periphyton, 6.4% on 28-day leaves, and 6.4% on 7-day leaves. The likelihood ratio  $\lambda$  was calculated and resulted in:

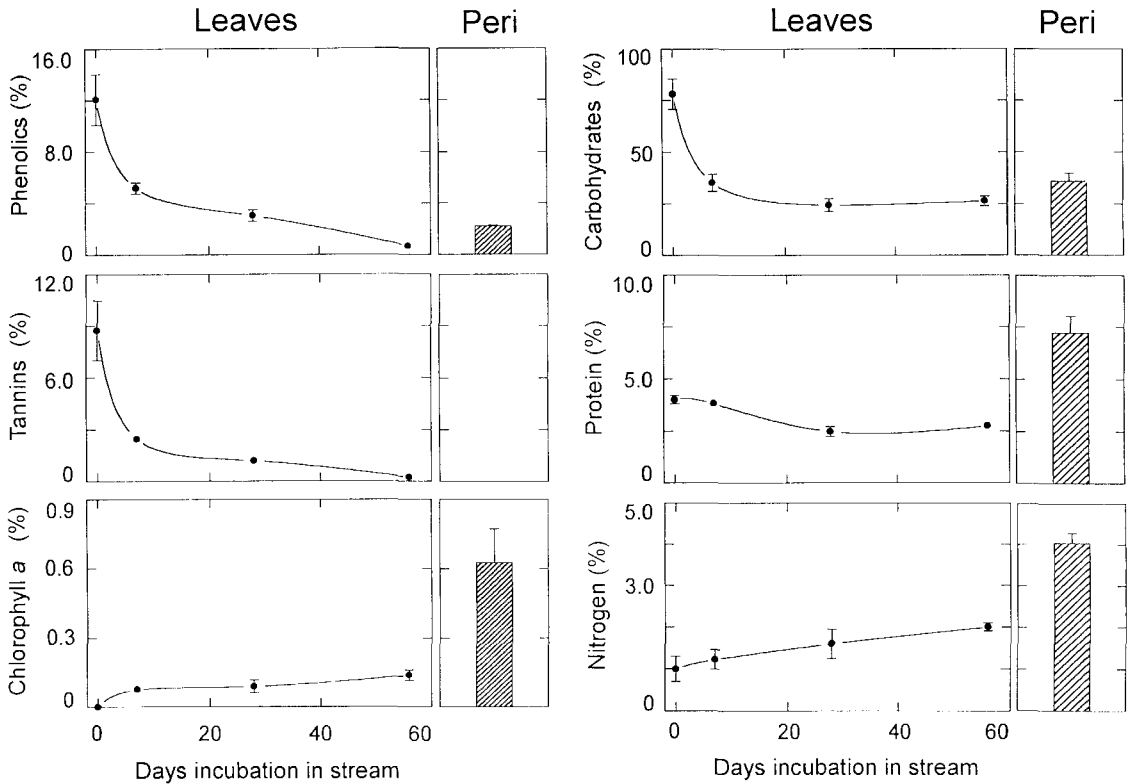
$$\lambda = 9.95, \chi_3^2 \text{ statistic} = 7.82 \text{ (for } P = 0.05\text{)}.$$

Hence we rejected the hypothesis that all foods had an equal probability of being eaten.

## DISCUSSION

Initial concentrations of carbohydrate, protein, and N in leaves were similar to those reported for leaves of other *Salix* species by Shepard & Minshall (1981), and phenol concentrations were similar to those found by Julkunen-Tiitto (1985) in several *Salix* species. Most of the phenols and carbohydrates were leached within the first week. The remaining phenols were leached more slowly, whereas carbohydrates remained at a relatively constant proportion of AFDM for the remainder of the study. A similar pattern has been observed for phenol and carbohydrate concentrations in other leaf species in streams (Suberkropp et al. 1976). After incubation of leaves for 56 days, the proportion of AFDM represented by carbohydrates was similar to that found in periphyton. However, it is likely that the carbohydrates in the dead leaves were mainly structural carbohydrates such as cellulose, whereas carbohydrates in the living periphyton cells may have been largely non-structural.

Nitrogen and chlorophyll *a* were the only two variables observed to increase in proportion during the course of the experiment. A similar pattern has been found for N in leaves elsewhere (Iversen 1973; Gessner & Schwoerbel 1989). This pattern



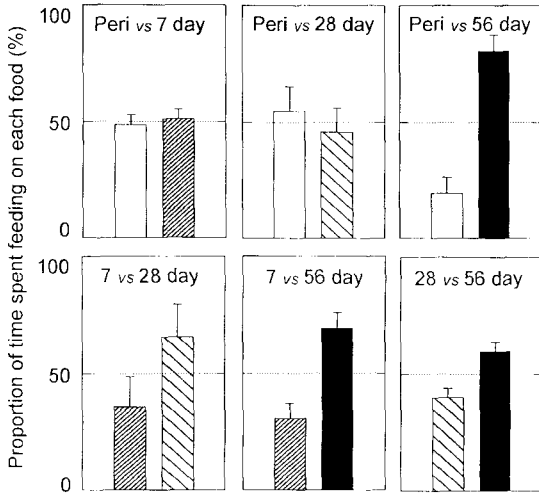
**Fig. 1** Total carbohydrates, protein, nitrogen, phenolic compounds, tannins, and chlorophyll *a* as percentage ash-free dry mass in leaves from leaf-bags incubated in Silverstream after 0, 7, 28 and 56 days, with corresponding figures for periphyton from Styx Creek. Bars indicate 1 standard error. Leaves, willow leaves; Peri, periphyton.

may result from non-nitrogenous components being lost by leaching, leaving N-rich compounds such as lignins (Webster & Benfield 1986), and/or from colonisation by micro-organisms (e.g., Buckley & Triska 1978). After 56 days, the leaves had about half the N of periphyton.

In contrast to the increase in N, protein levels decreased slightly in leaves during the experiment. It is possible that more leaching or degradation of leaf protein did occur, only to be hidden by the addition of microbial protein. The amount of protein in leaves after incubation for 56 days was just under half of that observed in the periphyton. An input of 5000 kg km<sup>-1</sup> of leaves on the banks and in the water, calculated from Latta (1974), would represent 3800 kg km<sup>-1</sup> of carbohydrates, 215 kg km<sup>-1</sup> of protein, 55 kg km<sup>-1</sup> of N, and 600 kg km<sup>-1</sup> of phenolics. Willow leaves are thus likely to contribute large amounts of nutrients to streams, but they also contribute large amounts of phenolic

compounds which may be inhibitory to stream flora and fauna.

The detritivore examined in this study, *Olinga*, showed a strong preference for leaves that had been incubated for 56 days, and little preference among leaves that had been incubated for 7 and 28 days, and periphyton. One likely factor contributing to this finding was the high ash content of the periphyton in this study, which resulted in there being less organic material in periphyton than in leaves. For equivalent dry weights, 56-day willow leaves contained approximately three times as much N and carbohydrate as periphyton, twice as much protein, and similar amounts of chlorophyll *a*. The 7-day and 28-day leaves also had more of these chemicals per unit weight. Ash concentrations approximating our figure of 65% have been noted elsewhere among freshwater algae (see Cummins & Wuycheck 1971), and are not unusual for periphyton (Graham 1990). Our results indicate



**Fig. 2** Proportion of time spent by *Olinga* feeding on pairs of foods in laboratory trials. Bars indicate 1 standard error ( $n = 4$ ). Dotted line shows equal feeding time on each food type. Peri, periphyton; 7 day, 28 day, and 56 day mark willow leaves incubated in a stream for the various periods.

that under such conditions willow leaves can be preferred over periphyton, although the commonly-held view is that algae are a higher-quality food (Ward & Cummins 1979; Bird & Kaushik 1984).

Other researchers have also found that stream invertebrates prefer willow leaves that have been incubated for about 50 days in a stream to those that have been incubated for shorter periods (Mutch & Davies 1984; Collier & Winterbourn 1986), and similar results have been recorded for other leaf species in New Zealand streams (Rounick & Winterbourn 1983). The increase in palatability of leaves from 28 to 56 days might have resulted from leaching of compounds from the leaves, or from an increase in the microbial population associated with the leaves. Foods high in N and P are often preferred (Iversen 1974; Irons et al. 1988), and those high in phenolics and alkaloids often rejected (Irons et al. 1988; Rank 1992). In the present study, 56-day leaves had the highest proportion of N, and the lowest phenolic content of all leaves. N levels, however, increased only slightly between 28 and 56 days, and there was no concomitant increase in protein. Thus the reduction in phenolic compounds to very low levels between 28 and 56 days seems more likely to be responsible for the preference observed.

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## REFERENCES

- Barlocher, F. 1985: The role of fungi in the nutrition of stream invertebrates. *Botanic journal of the Linnean Society* 91: 83–94.
- Bird, G. A.; Kaushik, N. K. 1984: Survival and growth of early instar nymphs of *Ephemera subvaria* fed various diets. *Hydrobiologia* 119: 227–223.
- Bradford, M. M. 1976: A rapid and sensitive method for the quantification of microgram quantities of protein using the principle of protein-dye binding. *Analytical biochemistry* 72: 248–254.
- Bradley, R. A. 1976: Science, statistics, and paired comparisons. *Biometrics* 32: 213–232.
- Buckley, B. B.; Triska, F. J. 1978: Presence and ecological role of nitrogen-fixing bacteria associated with wood decay in streams. *Verhandlungen, Internationale Vereinigung für theoretische und angewandte Limnologie* 20: 1333–1339.
- Collier, K. J.; Winterbourn, M. J. 1986: Processing of willow leaves in two suburban streams in Christchurch, New Zealand. *New Zealand journal of marine and freshwater research* 20: 575–582.
- Cummins, K. W.; Wuycheck, J. C. 1971: Caloric equivalents for investigations in ecological energetics. *Mitteilungen, Internationale Vereinigung für theoretische und angewandte Limnologie* 18: 1–158.
- Dahm, C. N. 1981: Pathways and mechanisms for removal of dissolved organic matter in streams. *Canadian journal of fisheries and aquatic sciences* 38: 68–76.
- David, H. A. 1988: The method of paired comparisons. New York, Oxford University Press. 188 p.
- Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. 1956: Colorimetric method for determination of sugars and related substances. *Analytical chemistry* 28: 350–356.

- Gessner, M. O.; Schwoerbel, J. 1989: Leaching kinetics of fresh leaf-litter with implications for the current concept of leaf-processing in streams. *Archiv für Hydrobiologie* 115: 81–90.
- Graham, A. A. 1990: Siltation of stone-surface periphyton in rivers by clay-sized particles from low concentrations in suspension. *Hydrobiologia* 199: 107–105.
- Iversen, T. M. 1973: Decomposition of autumn-shed beech leaves in a spring brook and its significance for the fauna. *Archiv für Hydrobiologie* 72: 305–312.
- Iversen, T. M. 1974: Ingestion and growth in *Sericostoma personatum* (Trichoptera) in relation to the nitrogen content of ingested leaves. *Oikos* 25: 278–282.
- Irons III, J. G.; Oswood, M. W.; Bryant, J. P. 1988: Consumption of leaf detritus by a stream shredder: influence of tree species and nutrient status. *Hydrobiologia* 160: 53–61.
- Julkunen-Tiitto, R. 1985: Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. *Journal of agriculture and food chemistry* 33: 213–217.
- Latta, I. K. 1974: Some effects of willows (*Salix fragilis*) on New Zealand streams and their faunas. Unpublished MSc thesis, University of Otago, Dunedin, New Zealand.
- Lock, M. A.; Hynes, H. B. N. 1976: The fate of 'dissolved' organic carbon derived from autumn-shed maple leaves (*Acer saccharum*) in a temperate hard-water stream. *Limnology and oceanography* 21: 436–443.
- Lorenzen, C. J. 1967: Determination of chlorophyll and pheo-pigments: spectrophotometric equations. *Limnology and oceanography* 12: 343–346.
- McMahon, R. F.; Hunter, R. D.; Russell-Hunter, W. D. 1974: Variation in aufwuchs at six freshwater habitats in terms of carbon and of carbon:nitrogen ratio. *Hydrobiologia* 45: 391–404.
- Mutch, R. A.; Davies, R. W. 1984: Processing of willow leaves in two Alberta Rocky Mountain streams. *Holarctic ecology* 7: 171–176.
- Nelson, D. W.; Sommers, L. E. 1980: Total nitrogen analysis of soil and plant tissues. *Journal of the Association of Official Analytical Chemists* 63: 770–778.
- Nusch, E. A. 1980: Comparison of different methods for chlorophyll and pheopigment determination. In: Rai, H. ed. The measurement of photosynthetic pigments in fresh waters and the standardization of methods. *Archiv für Hydrobiologie, Ergebnisse der Limnologie* 14: 14–36.
- Ostrofsky, M. L.; Zettler, E. R. 1986: Chemical defences in aquatic plants. *Journal of ecology* 74: 279–287.
- Pick, F. R. 1987: Carbohydrate and protein content of lake seston in relation to plankton nutrient deficiency. *Canadian journal of fisheries and aquatic sciences* 44: 2095–2101.
- Rank, N. E. 1992: Host plant preference based on salicylate chemistry in a willow leaf beetle (*Chrysomela aeneicollis*). *Oecologia* 90: 95–101.
- Rounick, J. S.; Winterbourn, M. J. 1983: Leaf processing in two contrasting beech forest streams: effects of physical and biotic factors on litter breakdown. *Archiv für Hydrobiologie* 96: 448–474.
- Ryder, G. I.; Scott, D. 1988: The applicability of the River Continuum Concept to New Zealand Streams. *Verhandlungen, Internationale Vereinigung für theoretische und angewandte Limnologie* 23: 1441–1445.
- Serrano, L.; Boon, P. L. 1991: Effect of polyphenolic compounds on alkaline phosphatase activity: implication for phosphorus regeneration in Australian freshwaters. *Archiv für Hydrobiologie* 123: 1–19.
- Shepard, R. B.; Minshall, G. W. 1981: Nutritional value of lotic insect feces compared with allochthonous materials. *Archiv für Hydrobiologie* 90: 467–488.
- Suberkropp, K.; Arsuffi, T. L.; Anderson, J. P. 1983: Comparison of degradative ability, enzymatic activity, and palatability of aquatic hyphomycetes grown on leaf litter. *Applied and environmental microbiology* 46: 237–244.
- Suberkropp, K.; Godshalk, G. L.; Klug, M. J. 1976: Changes in the chemical composition of leaves during processing in a woodland stream. *Ecology* 57: 720–727.
- Tahvanainen, J.; Helle, E.; Julkunen-Tiitto, R.; Lavola, A. 1985: Phenolic compounds of willow bark as deterrents against feeding by mountain hare. *Oecologia* 65: 319–323.
- Van Kraayenoord, C. W. S. 1974: Willows. *New Zealand's nature heritage* 7: 2730–2737.
- Ward, G. M.; Cummins, K. W. 1979: Effects of food quality on growth of a stream detritivore, *Paratendipes albimanus* (Meigen) (Diptera: Chironomidae). *Ecology* 60: 57–64.
- Webster, J. R.; Benfield, E. F. 1986: Vascular plant breakdown in freshwater ecosystems. *Annual review of ecology and systematics* 17: 567–594.