Stable isotopes of Lithosiini and lichens in Hong Kong show the biodiindicator potential of lichenivorous moths

Caren P. Shin, Abby Hoffman, Wanyi Lee, Roger C. Kendrick, David M. Baker, Timothy C. Bonebrake

Abstract

Urban landscapes provide unique environments for a wide variety of plants and animals, but their suitability may be limited by anthropogenic impacts such as pollution. We examined the potential utility of lichen and lichen-feeding moths as biodiindicators of air pollution in Hong Kong by comparing carbon (C) and nitrogen (N) stable isotope values in lichens, lichenivorous and non-lichenivorous moths (Lepidoptera: Erebidae) and a moth outgroup (Lepidoptera: Geometridae). Our results show that stable isotope values for C and N were similar for lichens and lichen feeding moths, while non-lichen feeding moths formed a distinct group. In addition, we found consistent $\delta^{13}C$ and $\delta^{15}N$ values across moth body parts, indicating that any portion of the specimen is suitable for isotopic fingerprinting. Our results highlight that lichen feeding moths may be useful for integrating signals of atmospheric nitrogen pollution and could therefore have utility in monitoring and quantifying air quality over time and space.

Introduction

Tropical biodiversity has been increasingly threatened by anthropogenic environmental change (e.g. Seto et al., 2012). The ecological footprint of rapid urbanization may be assessed by its impacts on the organisms living in cities. In particular, lichens have been well-documented as important biological indicators of atmospheric pollution in urban and industrial areas (e.g. Skye, 1979; Conti and Cecchetti, 2001). As slow growing and relatively long-lived organisms, lichens are able to record yearly perturbations in environmental conditions, as they are reliant on their surroundings for water, minerals and nutrients. Consequently, variations in lichen distribution, species richness and degree of physical and physiological damage may indicate changes in environmental conditions and especially air quality (Pescott et al., 2015).

$SO_2$ and $NO_2$ have been identified as air pollutants that have negative influences on species diversity (e.g. lichens in Gilbert, 1971; Purvis et al., 2003; Giordani, 2007). Climatic factors like humidity may also play a role in determining lichen distribution (Frahm, 2003). Surveys of lichen diversity can differentiate dissimilar zones of lichen communities, which can correspond to certain levels of air pollution (Astà et al., 2002). For example, a high level of atmospheric pollutants may be inferred where lichens are absent or only crustose growth forms are found (e.g. Lecanora spp.), whereas areas with foliose lichen growths (e.g. Lobaria spp.) may indicate better air quality (Hawksworth and Rose, 1970). Other than lichens, ubiquitous urban dwellers including members of the Lepidoptera (e.g. McGeoch and Chown, 1997; Corke, 1999), other arthropods (e.g. Gilbert, 1971; André et al., 1982; Zvereva and Kozlov, 2010) and even birds (Blair, 1999) have been found to be similarly sensitive animal indicators of general ecological health. The occurrence, survival, diversity and behavior of certain organisms may serve as initial markers of recent environmental changes, complementing existing biological and mechanical pollution monitoring systems.

Using stable isotopes, the effect of environmental change on an ecosystem can be traced (Peterson and Fry, 1987; Gannes et al., 1997). In particular, nitrogen (N) is a key nutrient for plants and N deposition has a high likelihood of directly influencing plant growth and competition (e.g. Bobbink et al., 2010) and associated insect communities (e.g. Alstad et al., 1982). The Lepidoptera are associated with a wide range of plants, including lichens, which may be directly used as hosts when in their larval phase (Sigal, 1984) or indirectly for camouflage, mimicry or shelter (Gerson, 1973). Increased levels of atmospheric...
pollutants may negatively influence host plants, which may then indirectly affect Lepidoptera. Indeed, as adult Lepidopterans have organs formed using larval and adult derived nutrients, isotopic differences in individuals (e.g. Molleman and Midgely, 2009) may potentially provide insight into the impact of pollutants on different developmental stages. Though improved air quality has led to a recovery in lichens in some locations (e.g. Rose and Hawksworth, 1981) and even lichen feeding Lepidoptera (Pescott et al., 2015), it is still unclear how, and the extent to which these species will be impacted in the future. A rise in N deposition is predicted to have marked effects on both ecological (e.g. Throop and Lerdau, 2004; Liu et al., 2013) and human health (e.g. Raaschou-Nielsen et al., 2011). Animal indicators could therefore serve as important early warnings of environmental impacts (Niemi and McDonald, 2004).

A tropical metropolis situated in Southeast Asia, Hong Kong has a considerably rich and representative lichen diversity for its limited land area (Aptroot and Seaward, 1999). Pressured with seasonally poor regional air quality (Fig. 1), coal power, automobile and shipping emissions, Hong Kong provides an ideal opportunity to study the early warning systems of atmospheric pollution: bioindicators. This study examines the suitability of using stable isotopes to evaluate the relationship of moths with a lichenivorous larval phase to lichens and their environment, with implications for understanding air pollution impacts on ecosystems. We compared the δ13C and δ15N values of three moth species with putative lichenivorous larvae (Lithosiini: Arctiinae; Erebidae) to three closely-related moth species that have non-lichenivorous larvae (Lithosiini: Arctiinae; Erebidae) in Hong Kong. These two groups were also compared with members of the Geometridae as an outgroup. We hypothesized that 1.) both δ13C and δ15N values between the three study moth groups (lichenivorous, non-lichenivorous, outgroup) would vary from each other as a result of their different host plant preferences. Furthermore, to demonstrate the specific larval-host plant association between lichenivorous moths and lichens, 2.) similar δ13C and δ15N values for lichenivorous moths and lichens would validate this association, which would contrast with different isotopic values for the non-lichenivorous and outgroup Lepidopterans. Finally, we hypothesized 3.) δ13C and δ15N isotopic differences might exist across the moth body, due to the assimilation of nutrients by individual parts at different developmental stages.

Materials and methods

Study areas and sampling

Three main locations in Hong Kong (Fig. 1; Kowloon Tong, Pok Fu Lam, Lamma Island) were chosen for the duration of this study from October 2013 to March 2014. Kowloon Tong and Pok Fu Lam are densely populated, well-developed sites compared to Lamma Island, a rural, small outlying community. Kowloon Tong features primarily residential buildings, with a few small urban parks that are largely covered by anthropogenic surfaces. Pok Fu Lam contains more compactly-built residential and commercial buildings, intermixed with small urban parks, though it borders Pok Fu Lam Country Park, a protected natural area. In contrast, Lamma Island is largely undeveloped, with few low-rise residential buildings. We targeted six common moth species for collection during the study period: three moths with reputed lichenivorous larvae (Brunia antica Walker, Schistophleps bipuncta Hampson and Daniélithosia sp. cf. tigroides [Lithosiini: Arctiinae; Erebidae]), compared with three species with non-lichenivorous larvae (Creatonotos transiens Walker, Nyctemera adversata Schaller [Arctiini; Arctiinae;...
Erebidae) and *Syntomoides imaon* Cramer (Symptomini; Arctiinae; Erebidae). Members of the Geometridae were sampled when possible to constitute a comparison group.

Individuals of these three groups were collected on suitable nights (6:30–9:00 pm, dry and mild, cool and cloudy weather) at the three study sites. Moths were collected opportunistically by walking around each study site and inspecting resting areas for about 30 min (e.g. sides of buildings, around planted vegetation). We also used Robinson style light traps (see Kendrick, 2002 for details) on several nights. To supplement the moth collection, single trips to other locations were made (Pak Tam Chung and Admiralty during the study period, and Hong Kong International Airport at the end of the wet season in 2015 and 2016). Moths from Hong Kong International Airport were collected using Pennsylvania style light traps.

Lichen samples were collected around and in the Lung Fu Shan Country Park surrounding Pok Fu Lam, where most targeted moths were found. Additional single trips to Pok Fu Lam and similar locations were made in June to July 2015 to supplement the lichen collection (Fig. 1; Tai Po Kau, Sai Kung, Ma On Shan, Wilson Trail, Lantau, Lion Rock). We collected lichen opportunistically by scraping off the lichen from the rock, bark, or other substrates on which they were found, with care taken to avoid including the substrate in the sample. Lichen species were not definitively identified, but each replicate resembled one species on observation (e.g. by growth form).

**Sample preparation and analysis**

Moth specimens were dried at 40–60 °C and dissected into five main sections (head, thorax, abdomen, legs, wings) to investigate possible differences in carbon and nitrogen isotope values from those organs formed before and after eclosion, when an adult emerges from its pupal case (e.g. Molleman and Midgely, 2009). Lepidopteran wings are formed during the pupal stage, indicating sources of dietary C and N consumed as a larvae, whereas other body parts would be composed of both larval and adult derived C (De Niro and Epstein, 1978; Tieszen et al., 1983) and N dietary sources (De Niro and Epstein, 1981, for amino acid turnover in O'Brien et al., 2002 and O'Brien et al., 2003, carbon allocation in O'Brien et al., 2004). Lichen samples were also dried at 40–60 °C. Some lichen samples were ground with a mortar and pestle for homogenization. Each sample was weighed on a microbalance and placed in Sn capsules. The δ13C and δ15N values were determined by a Nu Instruments Perspective Stable Isotope Ratio Mass Spectrometer (SIRMS) coupled to a Eurovector EA3028 elemental analyzer via an open-split interface at the University of Hong Kong. These isotope ratios are expressed in standard δ-unit notation, defined as: δX = [(Rsample/Rstandard)-1] *1000, where X = 13C or 15N, R is either the 13C/12C ratio for carbon or 15N/14N ratio for nitrogen. Isotope values were normalized to international standards (VPDB for carbon and Air for nitrogen) using a certified internal acetanilide standard acquired from Indiana University (10% N, 70% C; δ13C = −29.5‰, δ15N = 1.2‰). Both δ13C and δ15N standards were precise to ± 0.2‰.

Stable isotope values for each body part of the six target species were tested for normality with the Shapiro-Wilk Normality test and Levene’s test for homogeneity of variances. Those groups that satisfied both tests were tested with one-way analysis of variance (ANOVA). In the event the data had unequal variances among groups, the non-parametric Kruskal-Wallis one-way ANOVA was used. If significant differences were found from ANOVA, appropriate post-hoc tests were conducted.

**Results**

Two lichenivorous (*B. antica, Danielithosia sp. cf. tigrioides*) and two non-lichenivorous (*C. transiens, N. adversa*) moth species were collected, while no individuals of the other lichenivorous (*S. bipuncta*) and non-lichenivorous moth (*S. imaon*) were found (Table A.1, in supporting information). Five individuals of the geometrid outgroup were collected (*H. talaca* Walker, *A. carissima* Butler, *C. olearia* Guenée,
Stable isotope values between moth body parts

There were different degrees of variation but no significant differences found for δ13C and δ15N values across different moth body parts (head, thorax, abdomen, legs, wings, Table 1), which refutes our third hypothesis. This overall similarity in δ13C and δ15N values regardless of selected body part was true for both the lichenivorous (all at approximately δ13C = −27‰, δ15N = −5‰) and non-lichenivorous group (all at approximately δ13C = −30‰, δ15N = 5‰). Most δ13C and δ15N values for all species’ body parts were normally distributed (with the exception of δ13C from the non-lichenivorous C. transiens’ legs and wings, Shapiro-Wilk test, both P ≈ 0.04) and homoscedastic (P > 0.05). Results of one-way ANOVA were not significant for all target species’ body parts, whether for δ13C or δ15N values (P > 0.05).

Table 1

<table>
<thead>
<tr>
<th>Lichens as host plants</th>
<th>Abdomen</th>
<th>Legs</th>
<th>Wings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bruna antica</td>
<td>3</td>
<td>δ13C: −27.4 ± 3.6</td>
<td>−26.6 ± 2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>δ15N: −5.5 ± 6.2</td>
<td>−4.8 ± 6.2</td>
</tr>
<tr>
<td>Danielithosia sp. cf. tigrioides</td>
<td>5®</td>
<td>δ13C: −26.8 ± 0.5</td>
<td>−26.3 ± 1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>δ15N: −5.7 ± 3.0</td>
<td>−6.2 ± 3.6</td>
</tr>
<tr>
<td>Other host plants</td>
<td>4®</td>
<td>δ13C: −28.4 ± 5.7</td>
<td>−29.7 ± 6.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>δ15N: 4.7 ± 3.8</td>
<td>4.4 ± 4.1</td>
</tr>
<tr>
<td>Nycetemera adversata</td>
<td>5®</td>
<td>δ13C: −30.1 ± 1.4</td>
<td>−29.9 ± 1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>δ15N: 4.3 ± 1.2</td>
<td>3.7 ± 1.8</td>
</tr>
</tbody>
</table>

* Though 5 individuals were analyzed, only 3 adequate readings were found for the abdomen and none for the head, legs or wings.
® There were only 4 adequate readings for the abdomen.

Discussion

Our results illustrate that δ13C and δ15N isotopes can differentiate moths by host plant preference, affirm larval-host plant associations, and use of any main body part will yield congruous results. Moths with lichen-feeding larvae (Lithosiini) have distinctly lower δ15N values when compared to related tribes in the same subfamily (Arctiinae: Arctiini & Syntomini), outgroup (Geometridae) and other Lepidopteran examples (Table 2). These low δ15N values strongly resemble atmospheric sources of nitrogen (e.g. Fogel et al., 2008). Considerable variation in moth isotopic signatures was observed, such as those δ15N values from by B. antica (among all body parts, −12.4−7.8‰), although they were predominantly negative overall. Additionally, isotopic variability may be attributed to the limited sampling, though supplemental collection was attempted after the initial study period. Nonetheless, the lichenivorous moths’ δ15N values fall within the isotopic range for lichens in this study, among others (Table 2). The marked isotopic signature indicates the potential of lichenivorous moths as a suitable additional biodiagnostic of atmospheric pollution alongside lichens.

Disparate isotopic signatures among adult moths with documented different host plants are to be expected, since they have organs formed using larval derived resources. By comparing similar study systems for the expected isotopic difference between diet and tested tissue, or trophic enrichment factor, it is thus possible to confirm generally considered trophic links. Though other host plants have not been studied, the correspondence between stable isotope values found for lichens and those moths with putative lichenivorous larvae is not surprising, and falls within expected ranges of discrimination factors for both δ15N and δ13C (e.g. Caut et al., 2009; Hyodo, 2015; McCutchan Jr et al., 2003). Lichen δ15N and δ13C values spanned a relatively large range, but were predominantly similar among sampled sites. Variation in microenvironmental parameters such as precipitation (Ma et al., 2012), substrate type from which the lichen were collected (Beck and Mayr, 2012), and some sample contamination from the substrate may
have contributed to the range in values detected. While multiple lichen species may have been incorporated in samples, further increasing variation, other studies have reported similar values (Table 2).

Stable isotope analysis may aid in identifying Lepidopteran host plants and corroborating existing records. Of the three targeted lichen-nivorous species, only Brania spp. (formerly Eilema spp.) were found in the HOTS database to feed from host plants including lichens, although Daniellithosia spp. (formerly Tigriones spp.) were noted to also feed on mosses (Robinson et al., 2010). This study supports these database records, as the tested moths (B. antica, D. sp. cf. tigriones) exhibit comparable isotopic values to lichens and mosses, which are also similar (Table 2). HOSTS list reaffirms that the non-lichen-nivorous group and tested outgroup species do not feed on lichens or mosses, but on a range of other host plant families. Indeed, the diverse number of possible host plants for the non-lichen-nivorous and outgroup moths may have influenced their relative separation in isotopic space, a possible example of trophic niche separation.

Similar δ15N and δ13C values across all moth body parts indicate no difference between those formed before or after eclosion. For fruit-feeding butterflies, δ15N was similar between their body and wings (Molleman and Midgely, 2009), with variation suggested to derive from metabolic processes, the transition between larval and adult phase (also demonstrated in other metamorphosing insects, Tibbets et al., 2007), in addition to the potential range of host plants for a particular species. Further investigation may clarify the degree to which, and how these dietary preferences in larval and adult phases influence resource allocation, such as for reproduction, as well as in what form the nutrient takes (e.g. uric acid in termites, Tayasu et al., 2000). Our results indicate there may be no need to dissect moth specimens, or even use the entire individual, if a sufficient sample weight can be obtained for future stable isotope investigations. This is an important consideration where historical collections and museum archives could be subsampled for reconstructions of atmospheric pollution.

As land use and pollution levels have changed with increasing development in Hong Kong, lichen diversity and distributions may also be impacted. Thrower (1980), in a citizen science survey of lichens throughout Hong Kong, found that diversity was lower in sites heavily affected by air pollution. In our surveys we found few areas without lichen growth, but this may be attributed to a rise in resilient species (e.g. Lecanora conizaeoides Nyl. ex Crome) observed on commonly cultivated trees and shrubs. Characteristic lichens used to qualify air pollutant levels (e.g. Thrower, 1980) and the moths chosen for this study (Kendrick, 2002) were not frequently observed or collected. Given our limited sampling, it is still ambiguous how increased atmospheric pollutant concentrations affect organisms, although all lichen had mean %N > 1.0, a proposed indicator of air pollution (Penn et al., 2011). There have been negative (e.g. reduced butterfly abundance, Corke, 1999) and unclear (e.g. stimulated host plant growth without affecting its moth dependent, Osbrink et al., 1987) results. There are plausible cascade effects (e.g. pollution, Scoble, 1992, Corlett, 2001, Knop et al., 2017; and food chain, e.g. bats, birds, small vertebrates, man, Scoble, 1992, Young, 1997, Majerus, 2002) if Lepidopteran populations decline in abundance and diversity from pollution, although the extent of these impacts are difficult to estimate (Pescott et al., 2015).

This application of stable isotope analyses for indicator species can be implemented with other appropriate organisms, and on archived specimens from existing collections. By comparing isotopic values of historical and present day samples in concurrence with other available environmental data, changes over time in climate, population or even extinctions may be better understood. Here we show how moths may be useful in such investigations. In this example, we demonstrated that moths with a lichen-nervorous larval phase (Lithosini: Arctiinae: Erebidae) had stable isotopic values reflecting those of nearby lichens. Increased nitrogen availability from atmospheric pollutants may be a factor in amplifying the extent to which moths discriminate against heavier isotopes, resulting in lower δ15N values in heavily polluted areas. By incorporating suitable bioindicators into environmental assessments, a more extensive appraisal may be obtained.

### Author declaration
The authors declare no conflict of interest in this study.

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### Declarations of interest
None.

### Appendix A. Supplementary data
Supplementary data to this article can be found online at [https://doi.org/10.1016/j.aspen.2018.08.002](https://doi.org/10.1016/j.aspen.2018.08.002).
References


