

Mortality or more-tile-ity: Tiling response to temperature and humidity extremes in *Agrotis infusa*

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Abstract

The bogong moth (*Agrotis infusa*) is one of Australia's most famous insects, due in part to its spectacular tiling behaviour. The moths have great social and historical significance to Indigenous Australians from large portions of south-eastern Australia, and have been adopted as an icon by many modern groups and artists. These moths aestivate in rocky environments over summer, but often interact with human structures, bringing them spectacularly to the attention of the general public. Despite this, the exact mechanisms driving bogong moth tiling are unknown. The migratory lifestyle of the bogong moth enables moths to avoid the worst of temperature and humidity extremes during the Australian summer. However, conditions in the alpine and subalpine caves in which the moths prefer to aestivate are still intensely warm and dry, but with nights much cooler than the lower altitudes that the moths have left. While the purpose of the moths' tiling behaviour is currently unknown, given these conditions it has been proposed that moths may tile to conserve moisture, heat or both. We exposed groups of moths to temperature extremes, while measuring tiling percentage and effectiveness, and also exposed moths either singly or in groups to extreme humidity, while measuring the same. Contrary to predictions, moths demonstrated both higher temperatures and higher moisture loss when tiling.

Introduction

The bogong moth (*Agrotis infusa*) exhibits tiling behaviour during aestivation. The precise drivers for this behaviour are currently unknown, but could possibly be related to temperature, time of year, relative humidity, diet, elevation, migration triggers, social responses, diurnal cues or predation responses (Common 1954).

Moths aestivate in caves or crevices in boulder fields, generally in alpine or subalpine locations. As aestivation is an energy-intensive process (moths generally have between 55 per cent and 60 per cent body fat before entering aestivation (Common 1954)), moths will need to conserve resources wherever possible. With this in mind, the tiling behaviour may possibly be an adaptation intended to conserve moisture by creating a more humid environment, to minimise water loss via gas exchange.

As the preferred moth aestivation sites are in alpine environments, night and day temperatures vary immensely, especially compared to the more stable environments in which they overwinter. The tiling behaviour may possibly be an adaptation that regulates temperatures during alpine thermal extremes. The unreliability of water access to aestivating moths, and the potential for parasitism by nematodes from available water sources (Welch 1963), means that water conservation is also an important factor.

With warming trends persisting globally, the temperature and humidity profile of the Australian Alps is in danger of dramatic change. If the tiling behaviour of bogong moths functions to stabilise temperature or humidity in aestivating groups, conditions may change rapidly enough that the tiling behaviour becomes useless or, worse, a liability. Moths generally aestivate in caves with a temperature of approximately 9°C (Common 1954), so much higher temperatures may be disruptive. As the bogong moth is of immense cultural and ecological value—for example, ‘perhaps the only native insect in Australia to have been accorded notoriety’ (New 2007) with regards to its spectacular massing on public buildings—and it is also an essential food source for the endangered mountain pygmy possum, *Burramys parvus*, its absence from the alpine environment will be widely detrimental.

Tiling moths are predicted to retain moisture more effectively than isolated moths, and tiling is also predicted to be a response to cooler nights or cave conditions in alpine environments. By analysing the tiling response of moths under temperature and humidity extremes, one should

be able to assess the impact of changing conditions on moth populations. Tiling response to temperature and humidity change is predicted to be conservative, in the sense of preserving both temperature and resources.

Method

Moths were captured overnight in light traps supplied by the NSW Parks and Wildlife Service. These light traps were placed in a boulder field by the road at Charlotte Pass where bogong moths had previously been identified, and were checked for two consecutive nights in mid-December. Moths captured each night were removed from the traps and were kept in dark containers with other moths at 25°C and ambient humidity.

Once sufficient specimens were collected (>110 was deemed sufficient), containers were briefly cooled to 4°C to enable non-traumatic moth handling, and captured moths of species other than *A. infusa* were released.

Moths destined for the humidity treatment were placed in rectangular clear plastic containers, in groups of 10 or 11. Solo enclosures were constructed for the humidity treatment: made of cardboard, fine mesh and clothes pegs, of a size designed to allow insertion of a single moth and moth movement without escape or actual flight.

The containers for the humidity treatment were filled with a layer of orange silica desiccant balls and were weighed. Moths were added (either 10 or 11 moths, depending on box and on number of moths available) and the container weighed again. Weight of solo enclosures with and without a moth was also determined, and then the moths were placed in a dark room (in order to control for the possibility of light/dark cues for tiling behaviours) at 25°C. Moth mass loss was recorded after 24 hours, for both groups and isolated moths. All mass loss was considered to be water loss, as other routes for mass loss (e.g. frass) were negligible. Moth mass loss was considered a more reliable method of measuring water transfer than silica mass gain, as net gain in silica mass could easily have been atmospheric, from the air within the container, or incomplete.

Moths destined for the temperature treatment were placed in rectangular clear plastic containers, randomly allocated to either hot or cold, with each treatment further containing four groups, comprising 3, 5, 8 or 10, and 13 moths. No replications were possible due to low number of available moths.

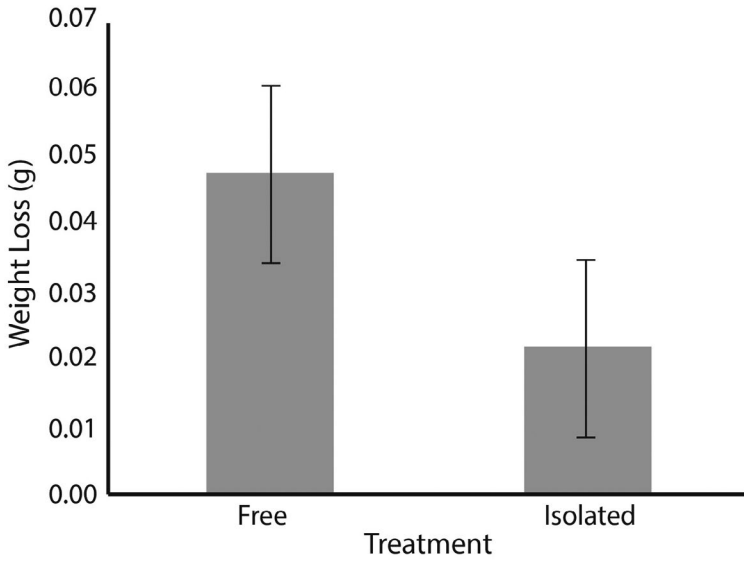


Figure 1: Weight loss (g) of free and isolated moths kept in low humidity (n=5).

Note: Error bars are standard deviation.

Source: Ming-Dao Chia.

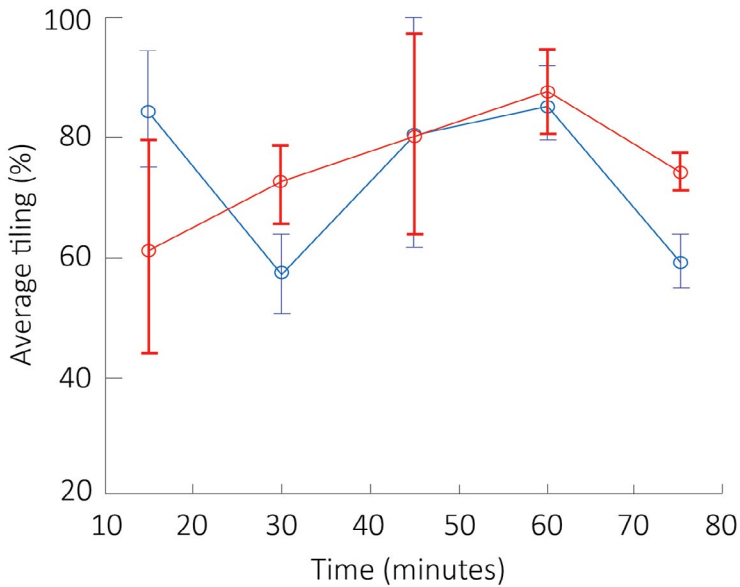


Figure 2: Moth tiling (as percentage) in hot (red) and cold (blue) treatments vs time in minutes.

Note: Error bars are standard deviation.

Source: Ming-Dao Chia.

Moths undergoing temperature treatment were kept at either 25°C (hot treatment) or 4°C (cold treatment) and had temperature and number tiling measured at 15-minute intervals for a total of 75 minutes. These temperatures were selected in order to provide extremes compared to the standard aestivation conditions of bogong moths, in caves ranging between 9°C and 14°C (Common 1954). Temperature was measured both of the ambient air and of the tiling moths. Temperature measurement was taken by a Fluke 568 infrared thermometer, aimed at a piece of cardboard attached to the box and then aimed at any groups of tiling moths. Number of moths tiling was calculated from photographs taken at the same time as the temperature measurements.

After 24 hours for the humidity moths and 75 minutes for the temperature moths, all specimens still alive were released into the boulder field from which they were initially captured.

Results

Data were collected on average moth water loss, calculated by subtracting the final weight of the container and silica without moths from the initial weight of the container with moths, and then dividing the result by the number of moths. Average moth weight loss was calculated (rather than total weight loss per box) in order to compare more accurately to weight loss in the isolated moths. There was no statistically significant relationship between group presence and weight loss, although there was a trend towards lower weight loss in isolated moths (Figure 1).

As moth tiling generally occurs at approximately 9°C (Common 1954), temperature extremes of 25°C and 4°C were selected. Unfortunately, measurement was restricted to a 75-minute time period, as moths in smaller-sized groups began to exhibit high mortality rates. Dead moths were not removed, and were considered as tiling/non-tiling based on their location at death. Mortality occurred only in the heat treatment groups, and then only in the heat treatment groups with five moths or fewer. Moth mortality is thus not accounted for in calculations of tiling. Over the given time period of 75 minutes, no statistically significant relationship between moth percentage tiling and temperature was demonstrated (Figure 2).

Discussion

The results show no definite statistical relationship, but do indicate that trends exist. Free moths lost a near-significant amount of body mass (as water weight) when compared to isolated moths. This was contrary to hypothesis, and requires further investigation. Tiling moths only showed a significant difference between temperature groups at the 75-minute mark, with moths tiling much more in warmer groups than in cold. It is important to consider that moths may have taken longer to tile than expected, and that the duration of observations was too short. Neither temperature is outside the natural range of moths, though it is outside their range of aestivation temperatures. This result corresponds to the hypothesis, considering that tiling moths may attempt to be conserving water. However, considering the water loss in tiling moths was greater, rather than less, increased tiling under warm conditions may serve a different purpose.

Replication trials were impossible to conduct due to the difficulty of sustainable moth collection, and temperature regulation relied on equipment not designed for the purpose. With these limitations taken into consideration, the hypothesis may require refinement rather than rejection.

While the existing literature, though limited, indicates a potential positive relationship between tiling percentage and survival of extreme temperature and dryness conditions (Common 1954), nothing in the current work indicates definitively any kind of relationship between tiling and moth survival under either set of conditions. The absence of statistical significance in any direction indicates that either the trigger for tiling behaviours is something else entirely, or that the method was flawed. Either way, there is no demonstrable causal relationship between either humidity levels and tiling or temperature and tiling. Given that animal behaviour is rarely simple, and rarely stems from a single cause, attributing the tiling behaviour to one cause, or even to two, seems to be a flawed response in and of itself. Possibly the lack of statistical significance was due to a failure to observe many of the other potential factors. As several standard measures of circadian rhythm (for example, eye pigmentation; Common 1954) indicate that the trigger for tiling behaviours is unrelated to day length, that is less likely to be a cause, but there are still several other potential factors left unaccounted for.

Tiling may be a social response, as indicated in some observations by Common (1954). Moths form aggregations around centres, although it is still unclear whether this is based on condition optimality or on some kind of herd behaviour. Without anthropomorphising too greatly, this 'social response' may also be defence against predation. *Agrotis infusa* already possesses false eyes, but a massing display may be perceived as a threat to predators, or perhaps the moths are merely finding safety in numbers.

Without rearing moths in the laboratory or testing temperatures in the field, it is impossible to be entirely sure that mortality and tiling patterns are not responses to procedure-based drama (handling, exposure to humans, etc.). The box transition process in particular was incredibly distressing to moths, with some requiring repeated heating to room temperature and then cooling to below handling temperature before they were transferred to their final container. While this would certainly explain mortality rates in handled moths, some moth trauma was unfortunately unavoidable.

Given the short time, low n-value and lack of replications, however, these results may indicate nothing more than that the method was flawed. The lack of replications make proper statistical analysis impossible, and the lack of any trend may be indicative of a lack of relationship, or it may be that non-trend results were simply anomalous and poorly accounted for.

The humidity treatment was hard on the moths, and exposing moths to extremely dehydrated conditions may have destroyed their mechanisms for coping with the mildly dehydrated conditions any humidity response to tiling may protect against. Future research should focus on providing humidity gradients closer to those found in the field, over longer periods of time, rather than relying on extremes to provide accurate data for conditions occurring in the middle.

The limited time made it impossible to determine whether or not mortality rates were in fact due to temperature or to tiling percentage. Running the temperature tests for longer would have given some indication of whether stabilisation would happen and a trend be visible in groups.

Even if tiling is effective, it might be so within such a limited range that the admittedly brute-force techniques used in this trial would have been insufficient to observe any trends. Rarely do alpine rock shelters in the shade reach 25°C, for example, so any warming effect of tiling may be of more use to moths at cold temperatures, and a completely different tiling response may have been seen in moths kept at a theoretical 18°C. A

greater range of temperature tests would allow observation of any range of tiling behaviours, lending some support to the notion that tiling serves a thermal purpose.

Tiling may also serve jointly, creating higher humidity while also increasing temperature. Perhaps under natural conditions, ambient temperature and humidity are such that the moths run very little risk of heat-based mortality, while using tiling to maintain safe humidity conditions.

Examination of a greater range of temperatures, a greater and more stable range of humidity, using more field-like containers and specimens exposed to less trauma may still indicate some relationship between tiling and heat or humidity. Should that fail, examination of other social and environmental factors may prove key to explaining the peculiar tiling behaviour. CO₂ knockout procedures would allow handling to proceed more smoothly and would reduce moth trauma, making for more reliable results. The construction of moth solo enclosures of fine, breathable plastic mesh would be simpler, perhaps shaped something like a pit trap for initial entry, with the ingress then removed.

While very little can be done to prevent moth tiling (according to Common (1954), moths will tile on any available, sufficiently shaded surface once they reach their preferred aestivation sites) should it be detrimental to moth survival under warmer conditions, the provision of more appropriate locations for moth tiling may become a necessity should tiling behaviour act effectively to conserve moisture.

Isolated moths tended towards losing less weight, which may have been to do with mass and energy conservation as a stress response. If a moth is alone, excess movement could quite easily attract predators. Further, with no need to move towards tiling groups, the solo moths may have expended less energy. A trade-off between the energy needed to tile and the energy saved by tiling may exist, but have not been present under these conditions due to the humidity extremes to which moths were subjected.

Despite the prevalence of tiling behaviour in dry conditions, bogong moth tiling appears to have no significant effect on preventing water loss. Tiling is not demonstrably a response to either heat or cold, and in fact causes higher mortality rates at warm temperatures. The method used in this experiment was unsuitable for determination of any potential relationship between tiling behaviour and either heat or humidity.

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