Surveying the threatened species *Thaumatoperla flaveola* across the Mount Buller–Mount Stirling massif

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Surveying the threatened species *Thaumatoperla flaveola* across the Mount Buller–Mount Stirling massif

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The Murray–Darling Freshwater Research Centre offices are located on the land of the Latje Latje and Wiradjuri peoples. We undertake work throughout the Murray–Darling Basin and acknowledge the traditional owners of this land and water. We pay respect to Elders past, present and future.

Contents

Executive summary 1

1 Introduction 3

1.1 Project actions and objectives 3

1.1.1 Conduct survey to confirm existing records and determine abundance and extent 3

1.1.2 Undertake genetic research 4

1.1.3 Map habitat 4

1.1.4 Undertake threat monitoring 5

1.1.5 Project objectives 5

2 Methods 6

2.1 Sites 6

2.2 Surveys and Genetic methods 7

2.2.1 Sampling 7

2.2.2 Molecular methods 8

2.2.3 Genetic analysis 8

2.2.4 Statistical analysis 9

2.3 Mapping 9

3 Results 12

3.1 Surveys 12

3.2 Genetics 13

3.2.1 Species accumulation curves 14

3.3 Mapping 15

4 Discussion 17

4.1 Identified outcomes and future project actions 17

4.1.1 Conduct ongoing surveying 17

4.1.2 Map climate change impacts 17

4.1.3 Threat reassessment 18

4.1.4 Outcomes 18

4.1.5 Future project actions 18

References 20

Appendices 21

Appendix A List of sites investigated during the project or where previous records of *T. flaveola* exist. Numbers of specimens recorded in 2016 and in previous years also included. 21

Appendix B Site attributes generated in ArcGIS for all *T. alpina* occurrence points (surveys start and end points used for data generation) 25

List of tables

Table 1. Sites listed as project specific sites where *T. flaveola* has been recorded...6

Table 2. Additional sites were *T. flaveola* has been recorded 7

Table 3. Recorded occurrences of *T. flaveola* during the 2016 surveys. 12

Table 4. Specimen data for sites were *T. flaveola* has been recorded during the 2016 surveying. 13

Table 5: Overview of site attributes generated in ArcGIS for *T. flaveola* occurrence points. 15

List of figures

Figure 1: Map of Mt Buller-Mt Stirling massif showing elevation and sites of occurrence for *T. flaveola*: (A) listed sites; (B) previous sites of occurrence sites..4

Figure 2. Possible dispersal mechanisms of *T. flaveola*: using stream connectivity as larvae or terrestrial movement by adults. 5

Figure 3. Example of clipped cerci preserved in ethanol and an in-situ specimen of *T. flaveola* post-clipping.. ...8

Figure 4: Map of Mount Buller-Mount Stirling massif showing elevations above 900 m 10

Figure 5: Map of Mount Buller-Mount Stirling massif showing elevations above 900 m with hydrological features shown 11

Figure 6. Haplotype networks for *Thaumatoperla flaveola* by site and by catchment. 14

Figure 7. Species (haplotype) accumulation curve for *T. flaveola* (2015 and 2016 samples)..............................14

Figure 8: Map of the Mount Buller-Mount Stirling area showing elevations, hydrological network and sites of presence records...16

Executive summary

The **objectives** of the project were to:

- conduct a survey to determine the abundance and/or extent of *Thaumatoperla flaveola*
- conduct a survey to confirm existing records of *T. flaveola*
- map the known habitat of *T. flaveola* across its distribution range and identify areas of potential habitable areas
- investigate the population genetics of *T. flaveola*
- undertake threat monitoring for previously identified threats and re-assess the threats to *T. flaveola.*

Results of surveys:

- Surveys recorded 28 individuals observed at six sites within the Delatite, King and Howqua catchments.
- Genetic results suggest that populations are not separated by catchment, with the Delatite and Howqua catchments sharing two haplotypes. This suggests that dispersal of *T. flaveola* may be via the terrestrial adult life-stage. However, the limited sample size available for this study means this hypothesis will need to be tested with ongoing sampling.
- Focussing surveys for *T. flaveola* on the adult life stage is a cost-effective and fairly efficient approach that provides downstream benefits, such as the collection of genetic material.

Habitat mapping of T. flaveola across its distribution range:

- No obvious preference of slope or aspect was detected for *T. flaveola* occurrences.
- Distribution range within the King, Delatite and Howqua catchments needs to be assessed to develop an understanding of the full distribution of *T. flaveola*. The project results suggest a non-synchronous emergence pattern for *T. flaveola,* with adults recorded around the Mount Buller and No 3 Mount area, but not the Mount Stirling area.

Threat monitoring for T. flaveola:

- Previously identified threats included introduced species, ski resort development, forestry, grazing, climate change, fire, degradation of the riparian area, habitat fragmentation, and changes in physico-chemical parameters.
- Previous surveys conducted post-fire showed the continued presence of *T. flaveola* at recorded sites, suggesting that fire did not negatively impact on the species.
- Forestry, grazing and degradation of the riparian area have the potential to change riverine environments, for example, by reducing the shade over the channel, and increasing nutrient runoff and sedimentation. These types of biological impacts on *T. flaveola* populations remain unknown.
- Ski resort development — *T. flaveola* continues to be present in the Mount Buller summit area which has numerous ski runs. Resort runoff has been suggested to potentially impact stream physico-chemical conditions, but this has not yet been demonstrated to directly impact on *T. flaveola*. Knowing the full distribution, water quality requirements and mode of dispersal of *T. flaveola* will aide in establishing the overall threats and management implications to the species, and identify potential threats to certain populations.
- Trout are potentially the most influential introduced species for *T. flaveola*. The impact of trout on *T. flaveola* was not able to be assessed as fish surveys were outside the scope of this study. Mapping of the distribution of trout species in conjunction with the distribution of *T. flaveola* would provide insight into whether these species co-occur and would identify sites to survey to determine if trout directly impact upon *T. flaveola* populations.

- The major threat for *T. flaveola* will be climate change. The impacts of climate change across the alpine region have the potential to heavily impact alpine-adapted insects. The effects of climate change impacts will need to be assessed for both riverine networks and the associated riparian vegetation, and for the species, *T. flaveola.* Species distribution modelling for *T. flaveola* needs to be further assessed under various climate change scenarios.
- Habitat fragmentation has been suggested as a threat due to the land uses throughout the occurrence area, such as forestry, tourism and grazing. Habitat for *T. flaveola* may be further fragmented due to climate change and its associated effects on the riverine network and alpine vegetation. It is important that the mode of dispersal for the species is understood to be able to focus efforts on the possible prevention of fragmentation between the populations.

Identifying and managing potential threatening processes on a specific species requires a basic understanding of the biological and ecological requirements of that species. The limited data we have on *Thaumatoperla flaveola* creates a significant limitation on our ability to understand the threatening or impacting processes on the species.

This project focussed on increasing our understanding of the distribution of *T. flaveola* and identifying the dispersal mechanisms used by *T. flaveola*. Without continued improvements to baseline knowledge, our understanding of the potential threats to the overall species and to individual populations will remain limited.

Future focus needs to be on:

- the distribution and genetic diversity of *T. flaveola* within and between the Delatite, King and Howqua catchments
- the emergence cycles of adult *T. flaveola* across the Mount Buller–Mount Stirling–Mount Winstanley area, the longevity of the adults and the distance adults can travel
- impacts of various climate change scenarios overlaid for the species, performed via species distribution modelling
- understanding the life history of the larvae. Specifically, do they have a multi-year larval stage? How low in altitude do *T. flaveola* occur? How sensitive are they to changes in the physico-chemical conditions of streams?

1 Introduction

Identifying and managing the potential threatening processes on a species requires a baseline understanding of the biological and ecological requirements for that species. Currently, there is a significant limitation on our ability to understand the threatening or impacting processes on the alpine stonefly species, *Thaumatoperla flaveola*. Previous surveys were undertaken in 1994–95 (Doeg 1999) and 2008 (Crowther, Lyon & Papas 2008), which confirmed the presence of *T. flaveola* at previously recorded and new sites. Doeg (1999) provided an insight into the restricted distribution of *T. flaveola*, which was suggested as being approximately a 12 km by 10 km area around the Mount Buller, Mount Stirling and Mount Winstanley massif. It also discussed the conservation status of the species and referred to possible impacts (threats) to the species and/or populations. The 2008 report (Crowther, Lyon & Papas 2008) occurred after the severe alpine bushfires of 2003 and 2006 and was undertaken to survey the impact that this large-scale natural disaster had on the species. The report found no significant impact of fire on *T. flaveola* as the species was present at various burnt and unburnt sites; however, the conclusion contained some uncertainties relating to the potential impact on the physico-chemical conditions of streams due to changes in the runoff post-fire. Other threats listed for *T. flaveola* included habitat fragmentation by various land uses (timber harvesting and alpine resort management), grazing impacts in headwater systems, both long and short term drying conditions, and predation by trout (Doeg 1999; Crowther, Lyon & Papas 2008).

This current project focussed on identifying the distribution of *Thaumatoperla flaveola* and using genetic methods to gain insight into the structure of the populations. Understanding the distribution and dispersal patterns within a population is fundamental to understanding the threatening process that may affect that species/population. Fragmentation within a population, especially so for a threatened species, could lead to significant impacts on the species and needs to be assessed with a population approach, rather than as a complete species range approach. Threatening processes may be consistent across the entire range, or may be restricted to certain populations or a specific life stage. Understanding the population connectivity within *T. flaveola* and the dispersal mechanisms they use will provide greater insight into the potential threatening processes that the species may encounter.

1.1 Project actions and objectives

Seven specific sites were identified for the project: two sites on Boggy Creek, two tributaries to the Delatite River, a site on Mine Creek, a site on South Buller and a site on Whisky Creek (Table 1).

For these specific sites, the project actions were categorised as data collection, analysis and data management, and were to be addressed by the following activities:

1.1.1 Conduct survey to confirm existing records and determine abundance and extent

To confirm existing records, the study involved sampling:

- the specific project sites (Table 1; Figure 1 (A))
- other sites not listed, but where *T. flaveola* had been observed (Table 2; Figure 1 (B)).

To determine abundance and extent, surveys:

- focused on the adult life stage, which is synchronous in emergence and more visible than larvae
- marked start and end points for future comparison for abundance comparisons.

Previous projects had focussed on surveying the larval life stage, with the 2008 survey reporting low numbers of individuals at sites (32 recorded across the 10 sites, with the maximum recorded being 5 at 3 sites). Doeg (1999) does not state the number of individuals recorded during the surveys conducted. Both of these studies mention surveying the riparian vegetation for the presence of adults, but neither study reported observing any adults. The adults of *Thaumatoperla flaveola* are thought to emerge in a highly synchronous manner during the February–March period; however, sightings have been reported much later in the year (April; Vinnie Antony (Mount Stirling Resort Management) pers. comm.). For this project, the target was the adult life stage.

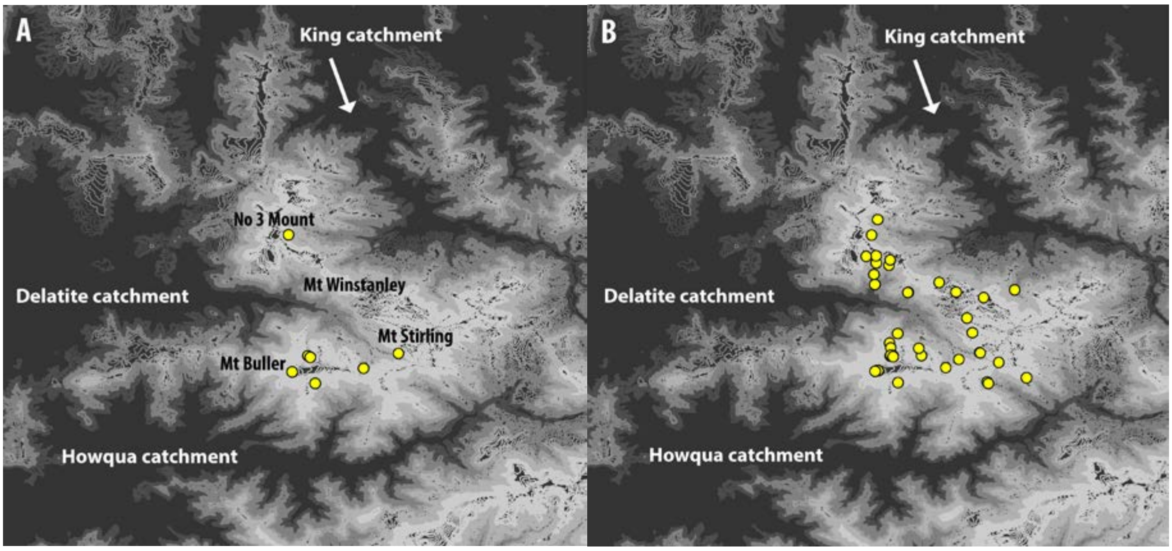

Figure 1. Map of Mount Buller–Mount Stirling massif showing elevation (the paler colour indicates areas above 850 m in altitude and dark grey indicates areas of 750–850 m) and sites of occurrence for *T. flaveola*: (A) listed sites to be re-assessed during this project; (B) previous occurrence sites with locations from Doeg (1999) shown by approximating geo-references.

1.1.2 Undertake genetic research

Targeting the adult life stage during surveys was also a key component of undertaking genetic research for the species. As larvae are known to undergo a multi-year development, using this life stage would introduce the potential for individuals to be re-sampled. By focusing on the adult life stage, each year would provide a new cohort and so enable new genetic data to be collected in future years. The genetic research would provide insight into (1) the distribution of the species across the Mount Buller–Mount Stirling massif, and (2) the genetic diversity within the populations and across the species. These sites were prioritised by:

- confirming the presence of *T. flaveola* within the three catchments: Delatite, Howqua and King
- focusing on sites between catchments that occur close together (i.e. on either side of a ridge-line) — this was informative for genetic analyses to potentially identify how *T. flaveola* disperse either terrestrially as adults or aquatically as larvae (Figure 2)
- focusing on sites in sub-catchments in between known locations — locating additional sites assisted in the understanding of the overall distribution of *T. flaveola* and provided insight into possible threats.

1.1.3 Map habitat

Crowther, Lyon and Papas (2008) suggested that stream substrate (often a result of the underlying geology) was an important requirement for *T. flaveola* and may potentially be a driver of population distribution, and that by mapping the geological distribution across the populations it would be possible to determine if there were any affinities to certain geological areas. This would provide insight into the possible identification of potential areas where the species may occur. The current project used this suggestion as a basis to perform GIS mapping to highlight possible areas of occurrence, and to provide a basis for future surveys or mapping work.

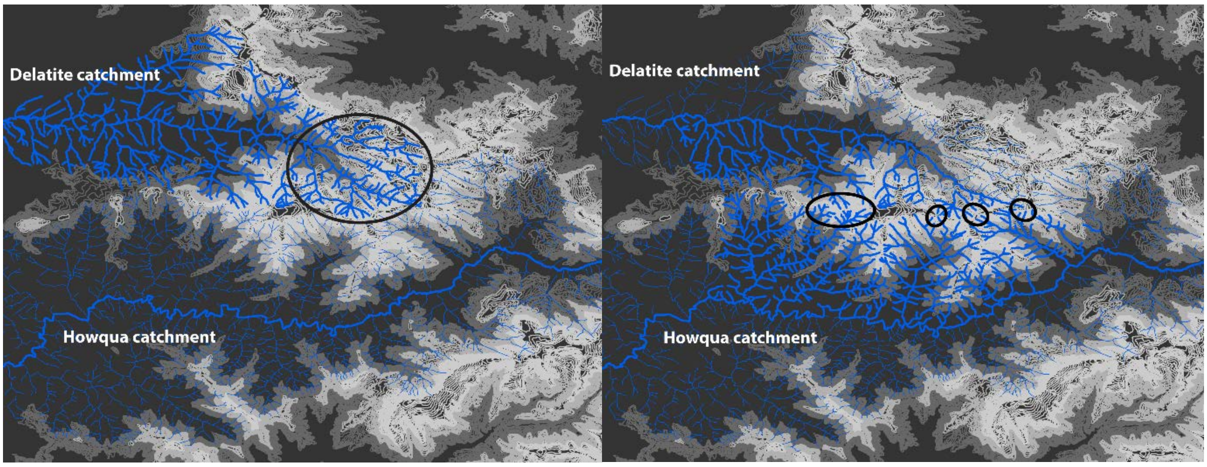

Figure 2. Possible dispersal mechanisms of *T. flaveola* with black circles indicating areas of similarity: left image depicts using stream (i.e. aquatic) movement as larvae, while the right image depicts using terrestrial movement as adults.

1.1.4 Undertake threat monitoring

Threats that had previously been identified for populations of *T. flaveola* included:

- forestry
- ski resort development
- grazing
- trout
- climate change
- degradation of the riparian area
- habitat fragmentation
- physico-chemical conditions.

Crowther, Lyon and Papas (2008) studied the effect of fire on *T. flaveola* and found no significant impact between fire-affected areas and non-fire-affected areas. However, the conclusion contained some uncertainties relating to the potential impact of fire and post-fire conditions on streams (i.e. physico-chemical conditions due to changes in the runoff post-fire). Other threats listed for *T. flaveola* included habitat fragmentation by various land uses (timber harvesting and alpine resort management), grazing impacts in headwater systems, drying conditions, and predation by trout (Doeg 1999; Crowther, Lyon & Papas 2008).

This project seeked to re-assess the threats for the populations and species.

1.1.5 Project objectives

The objectives of this project were to:

1. undertake a broad survey of the species *T. flaveola* both at previously listed sites and at potential sites for the species
2. collect genetic material to assess the genetic diversity within the species and populations and to gain an insight into the dispersal mechanism for the species, either terrestrially as adults or stream-based as larvae
3. re-assess the currently identified threats to *T. flaveola* and identify any other potential threats to the species.

2 Methods

2.1 Sites

Sites sampled during the project were focused on the seven identified by the Department of Environment, Land, Water and Planning (DELWP) (Table 1, Figure 1(A)) and additional accessible sites where *T. flaveola* had been recorded (Table 2, Figure 1 (B)). Additional sites were chosen in areas close to locations already reported that would provide insight into the possible dispersal mechanisms (shown in Figure 2). Currently, the mode of dispersal for *T. flaveola* is unknown, and could involve movement by larvae in the riverine networks or movement by the wingless adults overland. *Thaumatoperla flaveola* has previously been recorded in the King, Delatite and Howqua catchments, with the majority of records in the Delatite catchment. Sites along the ridges between these catchments were identified for investigating the dispersal mechanisms of *T. flaveola*. These were the Mount Buller summit (separating the Delatite catchment from the Howqua catchment), the Mount Stirling–Mount Winstanley ridge (separating the King and Delatite catchments) and Corn Hill Track area around Howqua Gap (separating the Delatite and Howqua catchments). A full list of sites sampled during this project is presented in Appendix A.

Table 1. Sites listed as project specific sites where *T. flaveola* has been recorded.

Site code	Site name	Catchment	Latitude	Longitude
BOGGYBR	Boggy Creek	Delatite	-37.13975	146.43425
BOGGY1	Boggy Creek tributary	Delatite	-37.14046	146.43545
DEL1	Delatite River tributary	Delatite	-37.13841	146.48213
DEL2	Delatite River tributary	Delatite	-37.14636	146.46371
MINE1	Mine Creek tributary	King	-37.06745	146.42760
STHBULL	South Buller Creek	Howqua	-37.14802	146.42672
WHISKY	Whisky Creek	Howqua	-37.1516	146.43587

Table 2. Additional sites were *T. flaveola* has been recorded; sites denoted with (*) are approximated geo-references from Doeg (1999).

Site code	Site name	Catchment	Latitude	Longitude
BROWN	Brown Creek upstream of Circuit Road	Delatite	-37.10613	146.44346
CORN1	Northern tributary of Corn Hill Creek upstream of Corn Hill Logging Track	Howqua	-37.15471	146.48631
CURR1	Tributary to Currajung Creek upstream of old log track opposite Bus Huts off No. 3 Road	Delatite	-37.08869	146.43399
CURR_LWR	Currajung Creek crossing Mount Stirling Road	Delatite	-37.10177	146.42576
CURRUPR	Currajung Creek upper western branch upstream of Pinnacle Road	Delatite	-37.08665	146.42111
FORK1	Fork Creek crossing Fork Creek Trail	King	-37.10886	146.48392
FORK2	Fork Creek at Circuit Road	King	-37.10065	146.46014
Unnamed Creek*	Unnamed stream upstream of Circuit Road	King	-37.10461	146.50047
Unnamed Creek 1*	Unnamed Creek downstream of Corn Hill Logging Track	Howqua	-37.15404	146.48573
STAN*	Stanley Creek crossing Circuit Road	Howqua	-37.15170	146.50680
Chalet Ck1*	Chalet Creek	Delatite	-37.13990	146.45067
Chalet Ck2*	Chalet Creek	Delatite	-37.13597	146.44921
BLUFF*	Bluff Creek off Circuit Road	Delatite	-37.12770	146.47788
BALDY*	Baldy Creek upstream of Telephone Junction	Delatite	-37.11978	146.47496
FALLS*	Falls Creek off Circuit Road	Delatite	-37.10587	146.46907

2.2 Surveys and genetic methods

2.2.1 Sampling

Previous studies of *T. flaveola* have focussed on the larval stage and have recorded very low abundances at sites. This could be due to either the ecology of large predators having low abundances or the burrowing nature of the species. While the larval stage of *Thaumatoperla* can be challenging to collect, as previously mentioned, the adult life stage has been suggested to be relatively easy to collect. The adults are flightless, and are thought to be highly synchronous in their emergence (February–April), and usually occur within the riparian area. By targeting the adult life stage, it would be possible to gather important information for previous knowledge gaps, particularly distribution and abundance.

Sampling for adult specimens was conducted by a visual survey along a transect of stream length. Visual surveys were conducted by two people in the riparian area, searching near the stream edge for newly emerged specimens and in the riparian vegetation on trunks, stems and leaves of vegetation. A GPS reading was taken at the start and end of each transect, with the transect finished either after no further specimens were found or the terrain became too difficult to access.

Genetic data were collected by clipping one of the two posterior abdominal filaments (cerci) from adult specimens. Adult life stages were targeted for genetic data due to their synchronised emergence periods, visible nature within the riparian area and short-lived adult life. A section of cerci was clipped from each specimen and placed in a tube of 100% ethanol for DNA to be extracted in the laboratory (Figure 3). The sex, distance from stream and vegetation type collected from, and height on the vegetation were also noted for each specimen, before returning the specimen to its point of collection.

Figure 3. Example of clipped cerci preserved in ethanol (left picture) and an in situ specimen of *T. flaveola* post-clipping (right picture). The female underneath has a clipped cerci (from a previous days sampling) whereas the male on top has not yet been clipped.

2.2.2 Molecular methods

Genetic techniques were used to assess the population structure within the species, *Thaumatoperla flaveola,* and to determine the mode of dispersal used by *T. flaveola*. The molecular data generated in this study were from mitochondrial DNA that was extracted from a clipped piece of cerci using a Qiagen DNeasy blood and tissue kit following standard protocols (Qiagen Handbook 2006). A region of the cytochrome *c* oxidase subunit 1 gene (*COI*) was amplified using Folmer primers (HCO2198 and LCO1490; Folmer et al. 1994). All primers were M13-tailed to facilitate sequencing. Polymerase chain reaction (PCR) conditions for the *COI* fragment used the following protocol: 60 seconds at 94 ^{0}C; 5 cycles of: 60 seconds at 94 ^{0}C, 90 seconds at 45 ^{0}C, 90 seconds at 72 ^{0}C; 35 cycles of: 60 seconds at 94 ^{0}C, 60 seconds at 50 ^{0}C, 60 seconds at 72 ^{0}C; a final cycle of 4 minutes at 72 ^{0}C. PCR preparations of 40 µL were made either with: 4 µL buffer reagent, 2 µL 50 mM $MgCl_2$, 0.8 µL of each primer, 0.1 µL Platenium taq polymerase (Invitrogen, Melbourne), 1 µL of DNA template, and 13.3 µL of ddH_2O, or with: 20 µL Taq mastermix (Qiagen), 1 µL DNA template, 0.8 µL of each primer and 17.4 µL of RNA-free water (Qiagen). PCR products were sent to Macrogen Inc. (Seoul, Republic of Korea) for purification and sequencing.

2.2.3 Genetic analysis

Sequence data were assembled in DnaBaser version 2.91.5 (Heracle BioSoft SRL Romania, www.DnaBaser.com) with mismatches, if present, assessed visually. Alignments were generated using MUSCLE (Edgar 2004) in MEGA version 5.2 (Tamura et al. 2011) and translated to protein sequences to check for stop codons. Nexus files were exported from MEGA and imported into the haplotype mapping program PopArt (Population Analysis with Reticulate Trees, http://popart.otago.ac.nz) with haplotype networks generated using the TCS algorithm (Clement, Posada & Crandall 2000). Sequence data were assigned traits based on catchment and site.

2.2.4 Statistical analysis

Statistical analyses were performed using PRIMER version 6 (PRIMER-E Ltd, Plymouth). To find natural groupings of samples and to detect sites more similar to each other based on haplotype, the CLUSTER routine was used. This is a hierarchical cluster method using group-average linking combined with the SIMPROF routine, which looks for statistical significance of genuine clusters in samples using 999 permutations to determine if the dendrogram branches are significant and showing genuine groups. Nonmetric multidimensional scaling (nMDS) was used to determine if the samples were different. nMDS plots were generated using 50 random starts from a Bray Curtis similarity matrix using non-transformed sample data. The samples were compared using river system, catchment and ridge-line as factors. The similarity of samples determined using CLUSTER, was superimposed on the nMDS groupings to show the relative similarity of each group.

A two-way analysis of similarity (ANOSIM) was performed to test the null hypothesis of no community differences between sites. ANOSIM calculates a test statistic (Global R between 0–1), which provides a comparative measure of the degree of separation of pre-defined sampling groups and its probability of occurring by chance using the default number of 999 permutations (Clarke and Warwick 2001). The higher the Global R value, the larger the differences between samples, and if all permuted statistics are greater than the Global R then the null hypothesis can be rejected (Clarke and Warwick 2001).

Haplotype accumulation curves were generated using the SPECIES ACCUMULATION PLOT routine using the default number of 999 permutations. The routine attempts to predict the number of taxa (haplotypes) that would be observed as the number of samples increases and tends to an asymptote, which enables an estimate of the increased information to be gained from using a greater number of samples.

2.3 Mapping

Mapping during the project was performed using a geographical information system (GIS) (ArcGIS version 10.2.2, ESRI Inc.). The Mount Buller, Mount Stirling and Mount Winstanley study area was delineated according to a 30 km buffer extending from the previously listed points of occurrence. All current locations of occurrence for *T. flaveola* were marked as points within this study area extent. For each point, location characteristics including slope, aspect, elevation, vegetation type and associated watercourse were identified. The study area was overlayed on a digital elevation model (DEM) for eastern Victoria (10 x 10 m cell) from which slope and aspect were modelled. Elevation was extracted from the 1:25 000 scale GIS dataset providing contours for Victoria (El_Contour; DELWP 2016a), while water connectivity was derived from the GIS dataset of the Victorian watercourse network at the same scale (HY_WATERCOURSE; DELWP 2016b). These two key factors were used to form a base distribution potential for *T. flaveola* using water network (water connectivity) and elevation (*T. flaveola* have been recorded predominantly above 1100 m, shown in Figure 4). Vegetation characteristics at each occurrence point were derived from a spatial dataset that estimates the 2005 extent of native plant communities classified by Ecological Vegetation Class (EVC) (NV2005_EVCBCS; DELWP 2008). EVCs incorporate community composition, structure and associated environmental factors (DSE 2002). Such spatial information provides a potential proxy for various stream attributes and/or environmental variables that are not available over such a large extent and/or on such a fine scale.

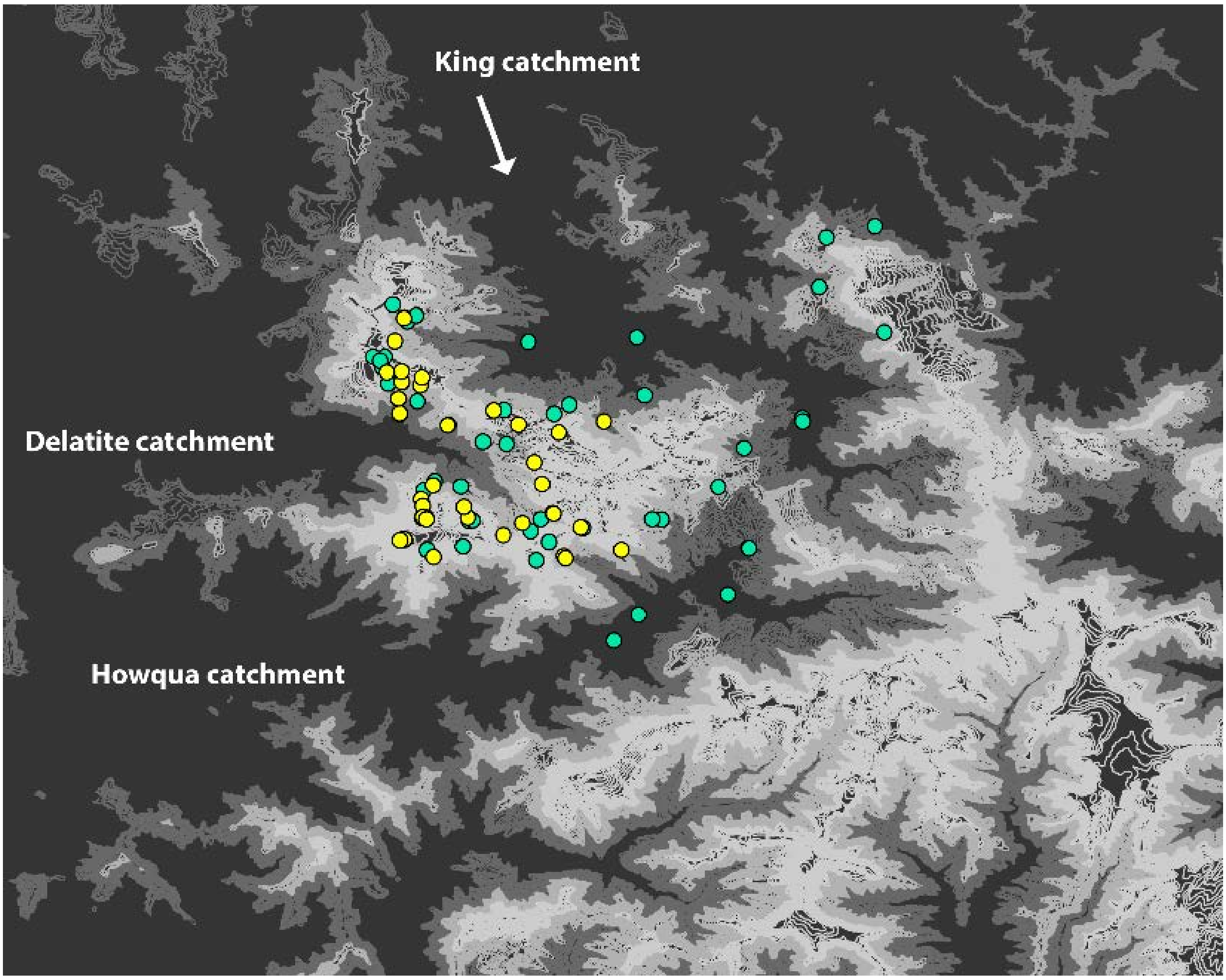

Figure 4. Map of Mount Buller–Mount Stirling massif showing elevations above 900 m (dark grey indicates areas of 900–1000 m altitude, and paler grey indicates areas above 1000 m). Sites of presence records of *Thaumatoperla flaveola* are shown in yellow, while recorded absences are shown in green.

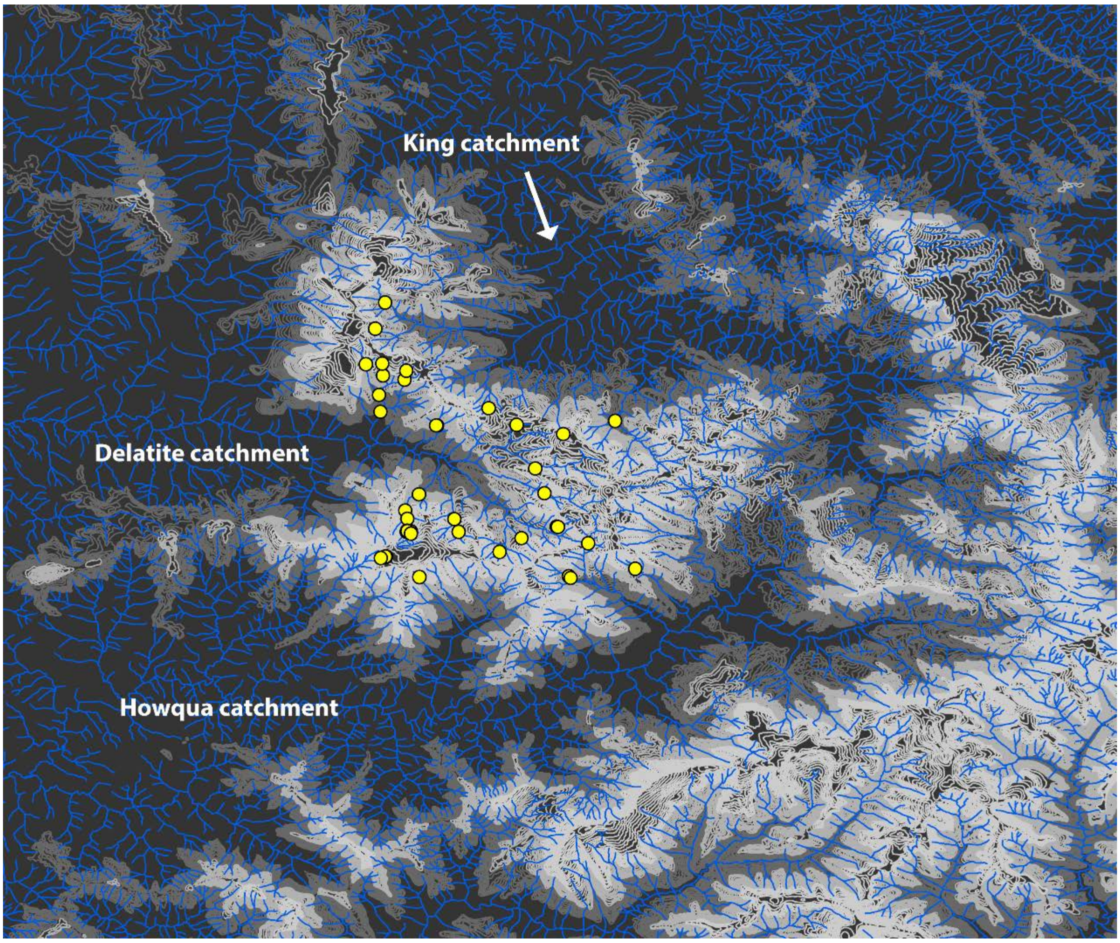

Figure 5. Map of Mount Buller–Mount Stirling massif showing elevations above 900 m with hydrological features shown (dark grey indicates areas of 900–1000 m altitude, and paler grey indicates areas above 1000 m). Sites of presence records of *Thaumatoperla flaveola* shown in yellow.

3 Results

3.1 Surveys

Three sampling trips were conducted from late March 2016 to April 2016 to survey *Thaumatoperla flaveola*. Twenty-two sites were assessed, with 13 being surveyed for *T. flaveola* and nine being visually assessed from the roadside (Appendix A). The majority of sites were sampled on each of the three sampling events as very low numbers were being recorded. With this in mind, focus was placed on the project listed sites. Of the 13 sites surveyed, 28 individuals of *T. flaveola* were recorded at six sites (Table 3). *Thaumatoperla flaveola* was recorded at five of the seven listed sites provided by DELWP, with the sites DEL1 and DEL2 not having any adults observed. However, a project trial in 2015 by the author did record *T. flaveola* at these two listed sites, with a total of 13 individuals from four sites that year (Table 3). The additional site at which *T. flaveola* was recorded was Mine Creek.

Survey transects were, on average, 65.83 m long but ranged from 20.70 m to 224.48 m (Appendix A). Adults were more difficult to locate than had been expected, with many adults observed moving over the ground or fallen logs. South Buller Creek was the only site to record *T. flaveola* on each sampling trip (Table 3) with a few clipped individuals seen on the following sampling occasion. The numbers observed across the sampling trips (Table 3) suggest that the emergence of *T. flaveola* is not synchronous across the Mount Buller–Mount Stirling area, and that the adults may not live for up to two months, as has been reported previously. Trip 1 was conducted from 21–23 March, Trip 2 from 4–6 April and Trip 3 from 11–13 April. Individuals that had been clipped during Trip 1 were not seen during Trip 2, whereas a single clipped female was seen during Trip 3 in a similar location to where a female had been clipped during Trip 2.

Some sites were problematic to sample due to thick riparian vegetation, weeds (i.e. blackberry, stinging nettle), steep slopes or unstable boulder fields. A visual survey was still conducted from the roadside or as far as was accessible.

Table 3. Recorded occurrences of *T. flaveola* during the 2016 surveys shown per site and sex ratios for each trip. Sites in close proximity have been pooled (e.g. MINE and MINE1 are Mine Creek; BOGGYBR and BOGGY1 are Boggy Creek).

Site	Trip 1	Trip 2	Trip 3	2016 total	2015 trip
South Buller	4	10	3	17	2
Boggy Creek	1	1	6	8	6
Mine Creek	0	2	0	2	-
Whisky Creek	1	0	0	1	0
Delatite	0	0	0	0	2
Fork Creek	0	0	0	0	3
Males	4	4	3	11	5
Females	2	9	6	17	8

Table 4. Specimen data for sites where *T. flaveola* has been recorded during the 2016 surveying.

Site code	Males (#)	Females (#)	Paired (#)	Found on (most common)	Distance from stream (m)			Height from ground (m)		
					Max	**Min**	**Average**	**Max**	**Min**	**Average**
BOGGYBR	2	1	0	Bridge	1	0.2	0.73	1.4	0.8	1.13
BOGGY1	1	4	1	*Leptospermum* spp.	1.5	0.5	1.1	2	1	1.8
MINE	0	1	0	Log	-	0	-	-	1.3	-
STHBULL	6	11	3	Snow Gum	10	0	3.71	1.7	0	1
WHISKY	1	0	0	Rock	-	0	-	-	0	-
MINE1	1	0	0	*Acacia* spp.	-	0.5	-	-	2	-

3.2 Genetics

At the 6 sites where *T. flaveola* were recorded a total of 28 individuals were observed with material collected from all specimens for genetic analyses. A further 10 individuals from the 2015 trial project had genetic material collected. Of the 38 specimens genetically sampled 4 were from the King catchment, 14 from the Delatite catchment and 20 from the Howqua catchment. Of these samples, sequence data were generated for 36 specimens and 15 haplotypes were identified across the species. Haplotype network maps indicated that the King catchment sites were distinct in the haplotypes located within this catchment by a 1-4 base pair (bp) difference (Figure 6). The rest of the haplotypes were shared across the Howqua and Delatite catchments sites, with the majority of sequences from the South Buller site (Howqua) and the Boggy Creek sites (Delatite). These two sites shared two haplotypes, but also showed a range of genetic diversity within the site (six haplotypes were recorded at both sites).Traits were examined at both a catchment level and site level to investigate if there was a genetic pattern between catchments that were close to each other (i.e. shared a common ridge-line), which may indicate whether terrestrial movement occurs in the species. The haplotype network based on catchments suggested that movement of adults may occur across the ridge-lines (Figure 6).

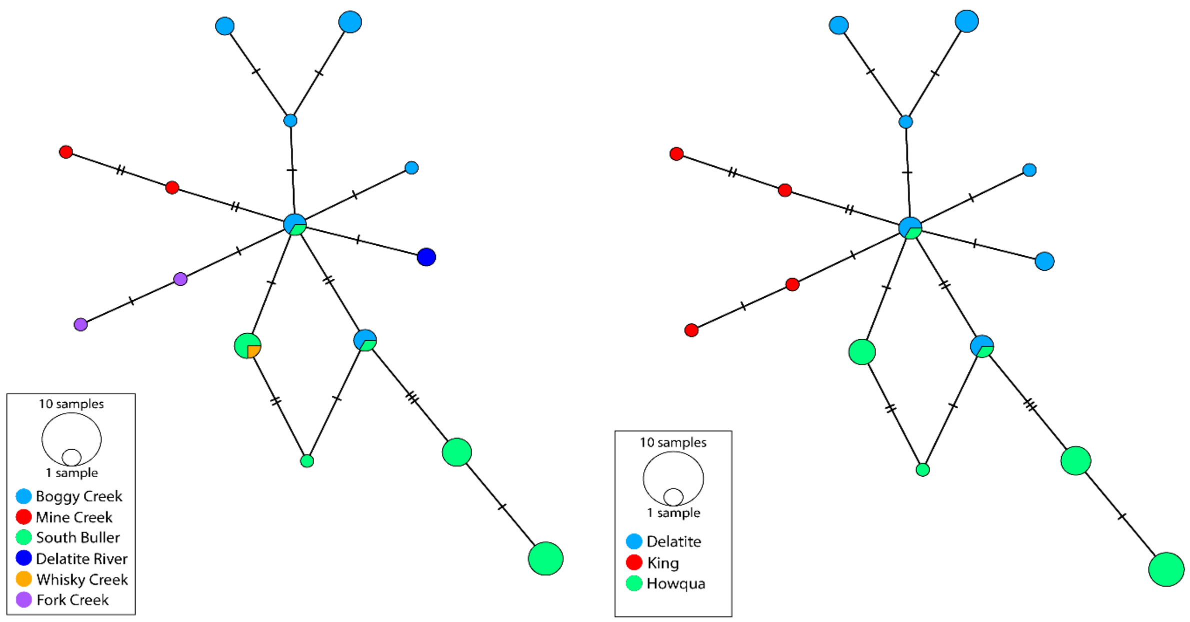

Figure 6. Haplotype networks for *Thaumatoperla flaveola*. The figure on the left shows haplotypes by site (pale blue — Boggy Creek and tributary, red — Mine Creek and tributary, green — South Buller Creek, dark blue — Delatite River tributaries, gold — Whisky Creek and purple — Fork Creek); the figure on the right shows haplotypes by catchment (blue — Delatite, green — Howqua, red — King).

3.2.1 Species accumulation curves

For species accumulation curves, Chao1 was selected as the extrapolator as it uses count data rather than presence/absence data and takes into account rare haplotypes in the pooled samples (a haplotype that has only one or two occurrences in the pooled dataset). The UGE and Sobs accumulation graphs indicated that sampling had not yet plateaued for the species (Figure 7). The Chao1 extrapolator did not show an asymptote for the species, which suggests that the species was under-sampled genetically. This would be expected due to the low numbers of specimens collected within each of the three catchments. This suggests that future surveys within all catchments will be required.

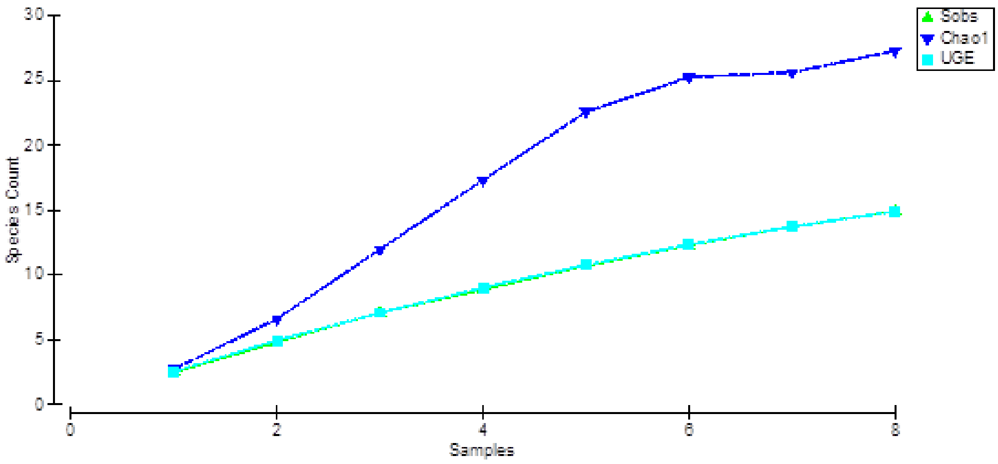

Figure 7. Species (haplotype) accumulation curve for *T. flaveola* (2015 and 2016 samples). The Chao1 extrapolator shows that the species was genetically undersampled.

3.3 Mapping

Occurrence data, including previous occurrence records (shown in Figure 8), were inputted into GIS. Data for site attributes were extracted for record points (survey start and end points were used for data extraction). Sites showed no indication of a preference to aspect or slope (data shown in Appendix B). Aspect, when grouped into North, North-East, East, South-East, South, South-West, West and North-West (Table 4) showed sites in every sector. Slope, when grouped into bins of 5° showed coverage from 5° to 35° (minimum recording 6.87° at Falls Creek and maximum recording 33.35° at Baldy Creek, Table 4) with the majority of sites within the range 10–15° (n=9) and 25–30° (n=8). EVC classes were extracted and most sites grouped into the various levels of montane dry woodland (n=19); montane damp forest (n=6); sub-alpine treeless vegetation (n=5) and sub-alpine woodland (n=4) (Table 4).

Table 5. Overview of site attributes generated in ArcGIS for *T. flaveola* occurrence points (surveys start and end points used for data generation).

Aspect	**No.**	**Slope (°)**	**No.**	**EVC category**	**No.**	**EVC subgroup**	**No.**
N (330°–30°)	5	0–5	0	Montane dry Woodland	19	Woodlands	23
NE (30°–60°)	2	5–10	3	Montane damp forest	6	Damp	7
E (60°–120°)	4	10–15	9	Sub-alpine treeless vegetation	5	Shrublands or grasslands	5
SE (120°–150 °)	1	15–20	6	Sub-alpine woodland	4		
S (150°–210°)	9	20–25	5	Damp forest	1		
SW (210°–240°)	3	25–30	8				
W (240°–300°)	7	30–35	4				
NW (300°–330°)	1	35–40	0				

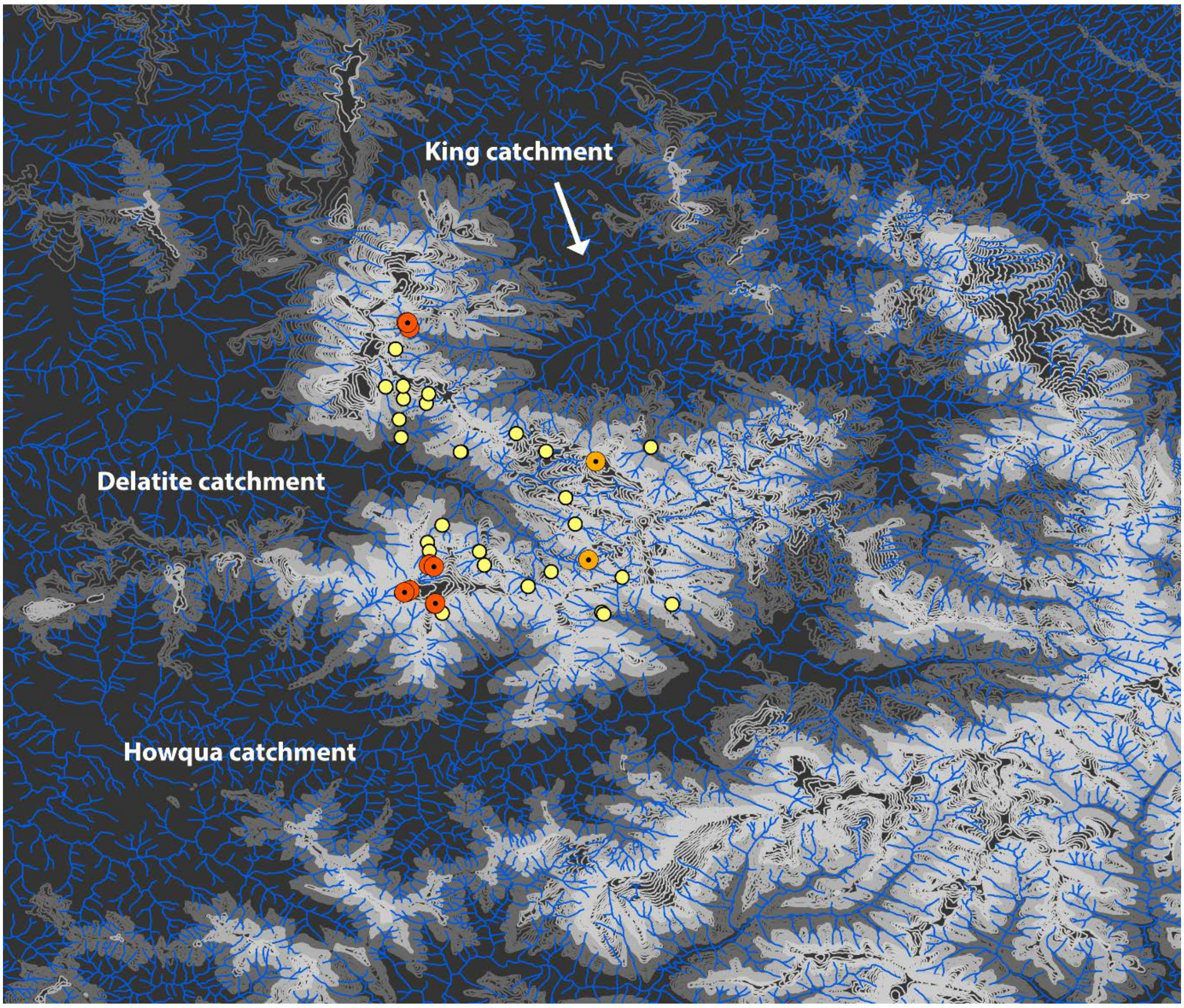

Figure 8. Map of the Mount Buller–Mount Stirling massif elevations above 900 m and the hydrological network (dark grey indicates areas of 900–1000 m altitude, and paler grey indicates areas above 1000 m). Sites of presence records of *Thaumatoperla flaveola* are shown in yellow (pre-2015), orange (2015) and red (2016).

4 Discussion

This project was able to make a significant contribution to the knowledge of the vulnerably-listed species, *Thaumatoperla flaveola*. The overall project approach of surveying the terrestrial adults during the emergence period either for presence/absence or genetic data, was shown to be a practical method, although no more successful than previous surveys that focussed on the larval stage. For genetic surveys, it is a good surveying strategy to implement as the adults have an annual emergence unlike the multi-year larvae. The success of the method would be improved with a greater understanding of the specific environmental cues for the species. Emergence periods for *Thaumatoperla flaveola* have been regarded as synchronous with the summer–autumn period and generally occur over February–May. The specific environmental cues for the emergence of the adults are not known, but specimens have been observed in higher abundances after the first heave frost, particularly in the Mount Stirling area (Vinnie Antony pers. comm.). The 2015 sampling event was conducted after the first heave frost (approximately two weeks after the frost), whereas the 2016 sampling events did not occur after a heave frost (a warm and extended summer period had extended well into April). This may potentially explain the lack of sightings in the Mount Stirling area. More information is required on the emergence cues of *Thaumatoperla flaveola* in order to better understand the emergence patterns across the species and to better inform possible impacts on the adult life stage of this species.

The major points from this project have been summarised below.

4.1 Identified outcomes and future project actions

For these sites, the identified categories for actions were data collection, analysis and data management, which were to be addressed by the following actions:

- conduct surveys to confirm existing records
- map habitat
- undertake genetic research
- undertake threat monitoring.

4.1.1 Conduct ongoing surveying

Thaumatoperla flaveola was observed to be present in a number of known locations, but in low abundances. It is recommended that continued surveying following that of this project is conducted to establish the full distribution and genetic diversity of *T. flaveola*, especially within the three catchments. Ongoing surveys would also provide insights into the emergence periods of *T. flaveola*, which had previously been regarded as synchronous across the Mount Buller, Mount Stirling and Mount Winstanley area, but now appear to be quite varied across these areas with adults potentially only living for a couple of weeks. Monitoring over the emergence period at sites would provide valuable information on the species and additionally provide important information for land managers across the Mount Buller, Mount Stirling and Mount Winstanley area.

4.1.2 Map climate change impacts

Mapping of the distribution of *Thaumatoperla flaveola* under various climate change scenarios should be undertaken. The species has not been recorded below 1100 m and changes due to climate-related events could be a major threat to the populations. A restriction in the possible distribution or fragmentation of *Thaumatoperla flaveola* could have detrimental impacts on the species as a whole.

4.1.3 Threat reassessment

Our understanding of the continued threats to the species still needs to be developed.

- Forestry and grazing has been suggested to impact on the riparian area of the riverine system and, in turn, the physico-chemical conditions of the riverine network. A policy for streamside protection zones for the species has been in place since 2001 (Crowther, Lyon & Papas 2008). The adherence, benefits or impacts have not been assessed; however, the species has still been recorded at sites within State Forest areas where grazing does occur. The impact of these potential threats will need to be re-assessed when more detailed baseline knowledge has been generated for the species.
- Ski resort development — *T. flaveola* continues to be present within the Mount Buller–Mount Stirling alpine resort land. Degradation of the riparian area and runoff from the resort has been suggested to potentially impact stream physico-chemical conditions, but this has not been directly assessed in this study, nor been shown to impact on *T. flaveola* in previous studies. The impacts of water storages and waterway re-alignments (such as aqueducts) need to be assessed as these have the ability to significantly impact the hydrology and ecology downstream and the effect on *T. flaveola* is unknown. Knowing the full distribution, mode of dispersal and longevity of *T. flaveola* will aide in establishing the overall threats to the species and guide management for the species.
- The impact of trout was difficult to assess without having a better understanding of where trout occur throughout the Mount Buller–Mount Stirling watercourses. This knowledge would be beneficial in understanding whether these species co-occur or not. The larval stage of *Thaumatoperla flaveola* is known to burrow, which may be an important avoidance strategy against trout predation.
- Climate change will be the major threat to *T. flaveola* in the foreseeable future, with the known distribution of *T. flaveola* very restricted and never been recorded below 1100 m. This alpine distribution will invariably be impacted by climate change and it will be important to know the biological and ecological restraints of the species in order to understand how it will respond to the detrimental impacts of climate change.

4.1.4 Outcomes

- The presence of *Thaumatoperla flaveola* was confirmed within the King, Howqua and Delatite catchments.
- The populations within the King catchment were shown to be genetically distinct; however, the Delatite and Howqua catchments showed shared haplotypes, which is suggestive of dispersal being undertaken terrestrially by the adults. Ongoing surveying should continue to collect genetic data so that the variation within each catchment/population can be assessed.
- Surveys focussing on the adult life stage are as effective as surveying larvae, with the addition of being able to collect genetic data from an annual cohort. Future surveys should adopt this technique to develop the knowledge of the species.
- Climate change will be the major threat to *T. flaveola* in the foreseeable future with impacts likely to result in higher water temperatures, reduced snow occurrence (water recharge/water runoff), higher air temperatures and changes to riparian vegetation, which will affect stream nutrients and chemistry as well as shading of the water body.

4.1.5 Future project actions

- Assess the distribution of *T. flaveola* and genetic diversity within and across all three major catchments.
- Survey the emergence patterns and longevity of *T. flaveola* across the Mount Buller, Mount Stirling and Mount Winstanley area. This has potential to guide land managers within the area as

to what impacts they may have on the population during the adult emergence period (i.e. spraying for weed management; trimming vegetation for ski run maintenance).

- Use GIS mapping to establish whether trout are co-occurring with *T. flaveola* or not. If the species' are not co-occurring, investigate the possible impacts trout are having on *T. flaveola*.
- Undertake species distribution modelling to assess the potential impacts of climate change and develop an understanding of the biological and ecological requirements of the larvae.
- Assess the impact of hydrological modification to *T. flaveola*. Further understanding of the impacts that the historically hydrological modifications have had on *T. flaveola* will provide a framework for predicting future impacts if hydrological modifications are required within the Mount Buller, Mount Stirling and Mount Winstanley area.

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Appendices

Appendix A: List of sites investigated during the project or where previous records of *T. flaveola* exist. Numbers of specimens recorded in 2016 and in previous years also included. Sites denoted with (*) are approximated geo-references from Doeg (1999).

Site code	Site name	Start point		End point		Survey distance (m)	Number recorded at site	Number of genetic samples	Number previously recorded
		Latitude	Longitude	Latitude	Longitude				
BOGGYBR	Boggy Creek at bridge on Klingsporn Trail	-37.13964	146.43422	-37.13993	146.43402	36.76	3	3 (2016) 4 (2015)	Unknown number — larvae life stage (1999) 2 larvae (2008) 6 adults (2015)
BOGGY1	Boggy Creek tributary	-37.14046	146.43545	-	-	Sampled from track	5	5	5 larvae (2008)
BOGGYUPR	Boggy Creek at base of Grimus lift	-37.14143	146.43245 Mid-point	-37.14127 -37.14110	146.43323 146.43303	Sampled two streams	0	-	Unknown number — larvae life stage (1999)
DEL1	Delatite River tributary	-37.13841	146.48213	-	-	Sampled from road	0	2 (2015)	Unknown number — larvae life stage (1999) 4 larvae (2008) 2 (2015)
DEL2	Delatite River tributary	-37.14636	146.46371	-	-	Not sampled	0	-	Unknown number — larvae life stage (1999) 1 larva (2008)
MINE1	Mine Creek tributary	-37.06885	146.42825	-	-	Sampled from road	1	1	2 larvae (2008)

Site code	Site name	Start point		End point		Survey distance (m)	Number recorded at site	Number of genetic samples	Number previously recorded
		Latitude	Longitude	Latitude	Longitude				
MINE	Mine Creek	-37.06745	146.42760	-37.06730	146.42726	35.26	1	1	Unknown number — larvae life stage (1999)
Unnamed3	Unnamed Creek crossing No 3 Road near junction with Circuit Road	-37.10032	146.46378	-37.09985	146.46370	51.96	0	-	-
STHBULL	South Buller Creek	-37.14802	146.42672	-37.14748	146.42828	146.91	19 (2016)	19 (2016) 2 (2015)	Unknown number — larvae life stage (1999) 5 larvae (2008) 2 adults (2015)
WHISKY	Whisky Creek	-37.15160	146.43587	-37.15138	146.43593	24.74	1	1	Unknown number — larva (2001) 5 larvae (2008)
GIN	Gin Creek at bottom of Bull Run	-37.15216	146.44330	-37.15182	146.44313	40.47	0	-	-
COWCAMP	Cow Camp Creek from Village Circuit Track	-37.14816	146.44715	-37.14828	146.44730	20.70	0	-	-
BROWN	Brown Creek upstream of Circuit Road	-37.10613	146.44346	-	-	Not sampled	-	-	Unknown number — larvae life stage (1999) 3 larvae (2008)
CORN1	Northern tributary of Corn Hill Creek upstream of Corn Hill Logging Track	-37.15471	146.48631	-	-	Not sampled	-	-	2 larvae (2008)
CORN2	Tributary of Corn Hill Creek crossing Corn Hill Road	-37.15605	146.47739	-	-	Sampled from road	0	-	-
CORN3	Tributary of Corn Hill Creek crossing Corn Hill Road	-37.15140	146.47782	-	-	Sampled from road	0	-	-

Site code	Site name	Start point		End point		Survey distance (m)	Number recorded at site	Number of genetic samples	Number previously recorded
		Latitude	Longitude	Latitude	Longitude				
CURR1	Tributary to Currajung Creek upstream of old log track opposite Bus Huts off No. 3 Road	-37.08869	146.43399	-	-	Not sampled	-	-	Unknown number — larvae life stage (1999) 3 larvae (2008)
CURR_LWR	Currajung Creek crossing Mount Stirling Road	-37.10177	146.42576	-	-	Sampled from road	0	-	Unknown number — larvae life stage (1999)
CURRUPR	Currajung Creek upper western branch crossing Pinnacle Road	-37.08391	146.41977	37.08192	146.41978	224.48	0	-	Unknown number — larvae life stage (1999) 3 adults (2015)
FORK1	Fork Creek crossing Fork Creek Trail	-37.10919	146.48408	-37.10886	146.48392	40.00	0	2 (2015)	Unknown number — larvae life stage (1999)
FORK2	Fork Creek at Circuit Road	-37.10191	146.48219	-37.10224	146.48223	39.41	0		Unknown number — larvae life stage (1999)
Unnamed Creek*	Unnamed stream upstream of Circuit Road	-37.10461	146.50047	-	-	Not sampled	-	-	Unknown number — larvae life stage (1999)
Unnamed Creek 1*	Unnamed Creek downstream of Corn Hill Logging Track	-37.15404	146.48573	-	-	Not sampled	-	-	Unknown number — larvae life stage (1999)
STAN*	Stanley Creek crossing Circuit Road	-37.13810	146.52622	-	-	Sampled from road	0	-	Unknown number — larvae life stage (1999)
STAN1	Tributary to Stanley Creek crossing Circuit Road	-37.14130	146.51795	-37.14026	146.51749	123.08	0	-	-
KING1	Tributary to King River at junction of Circuit Road and Speculation Track	-37.11414	146.55128	-37.11409	146.55076	47.49	0	-	-

Site code	Site name	Start point		End point		Survey distance (m)	Number recorded at site	Number of genetic samples	Number previously recorded
		Latitude	Longitude	Latitude	Longitude				
Chalet Ck1*	Chalet Creek	-37.13990	146.45067	-	-	Not sampled	-	-	Unknown number — larvae life stage (1999)
Chalet Ck2*	Chalet Creek	-37.13597	146.44921	-	-	Not sampled	-	-	Unknown number — larvae life stage (1999)
BLUFF*	Bluff Creek off Circuit Road	-37.12770	146.47788	-	-	Sampled from road	0	-	Unknown number — larvae life stage (1999)
BALDY*	Baldy Creek upstream of Telephone Junction	-37.11978	146.47496	-	-	Not sampled	-	-	Unknown number — larvae life stage (1999)
FALLS*	Falls Creek off Circuit Road	-37.10587	146.46907	-	-	Not sampled	-	-	Unknown number — larvae life stage (1999)
TIMBER	Timbertop Creek crossing Doughty Road	-37.13838	146.35595	-	-	Sampled from road	0	-	Doeg (1999) mentions a collection made in the 1950s at Mount Timbertop

Appendix B: Site attributes generated in ArcGIS for all *T. flaveola* occurrence points (surveys start and end points used for data generation).

Site code	Site name	Aspect	Slope	EVC name	EVC group name	EVC subgroup name
BOGGYBR	Boggy Creek at bridge on Klingsporn Trail	67.97	20.38	Sub-alpine woodland	Sub-alpine grasslands, shrublands or woodlands	Woodlands
BOGGYBR	Boggy Creek at bridge on Klingsporn Trail	70.19	19.67	Sub-alpine woodland	Sub-alpine grasslands, shrublands or woodlands	Woodlands
BOGGY1	Boggy Creek tributary	13.61	20.82	Sub-alpine woodland	Sub-alpine grasslands, shrublands or woodlands	Woodlands
DEL1	Delatite River tributary	156.24	20.34	Montane dry woodland	Montane grasslands, shrublands or woodlands	Woodlands
DEL2	Delatite River tributary	16.15	15.94	Montane dry woodland	Montane grasslands, shrublands or woodlands	Woodlands
MINE1	Mine Creek tributary	46.40	28.54	Montane dry woodland	Montane grasslands, shrublands or woodlands	Woodlands
MINE	Mine Creek	216.22	13.81	Montane damp forest	Wet or damp forests	Damp
MINE	Mine Creek	113.21	33.03	Montane damp forest	Wet or damp forests	Damp
STHBULL	South Buller Creek	264.84	25.64	Sub-alpine treeless vegetation	Sub-alpine grasslands, shrublands or woodlands	Shrublands or grasslands
STHBULL	South Buller Creek	260.10	17.03	Sub-alpine treeless vegetation	Sub-alpine grasslands, shrublands or woodlands	Shrublands or grasslands
WHISKY	Whisky Creek	179.15	26.38	Sub-alpine treeless vegetation	Sub-alpine grasslands, shrublands or woodlands	Shrublands or grasslands
WHISKY	Whisky Creek	173.03	31.05	Sub-alpine treeless vegetation	Sub-alpine grasslands, shrublands or woodlands	Shrublands or grasslands

Site code	Site name	Aspect	Slope	EVC name	EVC group name	EVC subgroup name
BROWN	Brown Creek upstream of Circuit Road	224.46	13.04	Montane damp forest	Wet or damp forests	Damp
CORN1	Northern tributary of Corn Hill Creek upstream of Corn Hill Logging Track	142.35	13.14	Montane dry woodland	Montane grasslands, shrublands or woodlands	Woodlands
CURR1	Tributary to Currajung Creek upstream of old log track opposite Bus Huts off No. 3 Road	180.19	19.68	Montane damp forest	Wet or damp forests	Damp
FORK1	Fork Creek crossing Fork Creek Trail	284.91	25.35	Sub-alpine woodland	Sub-alpine grasslands, shrublands or woodlands	Woodlands
FORK2	Fork Creek at Circuit Road	25.51	21.95	Montane dry woodland	Montane grasslands, shrublands or woodlands	Woodlands
STAN*	Stanley Creek crossing Circuit Road	183.33	30.49	Montane damp forest	Wet or damp forests	Damp
Chalet Ck1*	Chalet Creek	61.13	25.36	Sub-alpine treeless vegetation	Sub-alpine grasslands, shrublands or woodlands	Shrublands or grasslands
Chalet Ck2*	Chalet Creek	45.73	19.59	Montane dry woodland	Montane grasslands, shrublands or woodlands	Woodlands
BLUFF*	Bluff Creek off Circuit Road	253.12	11.28	Montane dry woodland	Montane grasslands, shrublands or woodlands	Woodlands
BALDY*	Baldy Creek upstream of Telephone Junction	22.97	33.35	Montane damp forest	Wet or damp forests	Damp
FALLS*	Falls Creek off Circuit Road	182.75	6.87	Montane dry woodland	Montane grasslands, shrublands or woodlands	Woodlands