Surveying the threatened species *Thaumatoperla alpina* across the Bogong High Plains

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Surveying the threatened species *Thaumatoperla alpina* across the Bogong High Plains

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Cover Image: *Thaumatoperla alpina* on *Bossia* spp.

Photographer: Julia Mynott

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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.

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Executive summary

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

- conduct a survey of *Thaumatoperla alpina* across the Bogong High Plains to confirm existing records and identify new locations
- map the known habitat of *T. alpina* across its distribution range and identify areas of potential habitable areas
- undertake threat monitoring for previously identified threats and re-assess the threats to *T. alpina.*

Conducting surveys on *T. alpina*:

- The presence of *T. alpina* within the East Kiewa, West Kiewa and Mitta Mitta catchments was confirmed. Survey results recorded 233 individuals at 15 sites in the East Kiewa catchment, 13 adults at Jaithmathang Creek in the West Kiewa catchment and 24 adults in a tributary of Whiterock Creek in the Mitta Mitta catchment.
- Genetic diversity within the population: results suggest that populations are separated by catchment, with the West Kiewa, East Kiewa and Mitta Mitta catchments not sharing any haplotypes. This suggests that *T. alpina* disperse via the riverine network as larvae.
- Focusing surveys for *T. alpina* on the adult life stage is an efficient and cost-effective approach, which provides added benefits, such as the collection of genetic material.

Habitat mapping of *T. alpina* across its distribution range:

- *Thaumatoperla alpina* showed no preference toward a particular slope or aspect.
- The distribution range of *T. alpina* within the West Kiewa and Mitta Mitta catchments needs to be assessed to develop an understanding of the full distribution of the species. The results from this project suggest a non-synchronous emergence pattern for *T. alpina,* with adults appearing in the West Kiewa and East Kiewa catchments from February–March, and then in the Mitta Mitta catchment during April.

Threat monitoring for *T. alpina*:

- Previously identified threats included introduced species, ski resort development, forestry, grazing, climate change, fire, degradation of the riparian area, and changes in physico-chemical parameters.
- Previous surveys conducted post-fire showed the continued presence of *T. alpina* 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. These types of biological impacts on *T. alpina* populations remain unknown.
- Trout are potentially the most influential introduced species for *T. alpina*. The impact of trout on *T. alpina* was not able to be assessed as it was not possible to identify fish observed in the streams in situ. Mapping of the distribution of trout species in conjunction with the distribution of *T. alpina* would provide insight into whether these species co-occur and would identify sites to survey to determine if trout directly impact upon *T. alpina* populations.
- Ski resort development — *T. alpina* continues to be present in the Summit area above the village of Falls Creek, which has numerous ski runs. It has been suggested that resort runoff potentially impacts upon stream physico-chemical parameters, but this has not been directly shown to affect *T. alpina*. Knowing the full distribution of *T. alpina* will aid in establishing the overall threats to the species and identify potential threats to certain populations.

- The major threat for *T. alpina* 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. alpina.*

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

This project focused on increasing our understanding of the distribution of *T. alpina* and identifying the dispersal mechanisms used by *T. alpina*. 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 of *T. alpina* in the West Kiewa and Mitta Mitta catchments
- the emergence cycles of adult *T. alpina* across the Bogong High Plains, the longevity of the adults and the distance adults can travel
- impacts of various climate change scenarios on the distribution of *T. alpina* performed via species distribution modelling
- understanding the life history of the larvae: do they have a multi-year larval stage? Are they burrowers? How far down (in altitude) do *T. alpina* occur? How sensitive are they to changes in the physico-chemical parameters of streams?

1 Introduction

Identifying and managing potential threatening processes on specific species requires a basic understanding of the biological and ecological requirements of that species. The limited data we have on *Thaumatoperla alpina* creates a significant limitation on our ability to understand the threatening or impacting processes on the species. Previous surveys were undertaken in 2005 and 2008 after major alpine bushfires, and these surveys confirmed the presence of *T. alpina* at both previously recorded and new sites (McKay, Bryce & Papas 2005; Crowther, Lyon & Papas 2008). However, while these reports confirmed the species' presence and provided some habitat variables for aquatic systems, the ability to infer the threats to the species was still limited. As such, the 2005 and 2008 reports listed generalised alpine threats to the species: introduced species (e.g. brown and rainbow trout), ski resort development, forestry, grazing, climate change, degradation of the riparian area, fire and changes in stream physico-chemical parameters (McKay, Bryce & Papas 2005; Crowther, Lyon & Papas 2008).

This current project focussed on identifying the distribution of *Thaumatoperla alpina* and using genetic methods to gain insight into the structure of the populations. Understanding dispersal within a population is fundamental to understanding the threatening process that may affect that species/population. Fragmentation within a population, especially so for an endangered species, could lead to significant impacts on the species and needs to be assessed with a population by population approach, rather than as a complete species over its range. 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. alpina* 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

The 13 specific project sites that were identified for the project are shown in 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

To confirm existing records, a survey was proposed to include:

- the specific project sites (Table 1; Figure 1 (A))
- other sites not listed, but where *T. alpina* had been observed (Table 2; Figure 1 (B))
- additional sites where *T. alpina* might be expected to occur.

Previous projects had focussed on surveying larvae, with both the 2005 and 2008 surveys reporting low numbers (six at 5 sites and eleven at 6 sites, respectively). The larvae are known to burrow and are often collected underneath boulders. The burrowing habit of larvae makes it difficult to collect them, and they are not commonly collected in traditional sweep/kick net aquatic sampling. The 2008 survey reported the occurrence of approximately 50 adults at one site (McKay_UPR), suggesting that *T. alpina* has a highly synchronous emergence period. For this reason, it was decided to target the adult life stage in this study.

The additional sites sampled for this project would provide insight into (1) the distribution of the species across the Bogong High Plains and (2) the genetic diversity within the populations and across the species. These sites would be prioritised by:

- Confirming the presence of *T. alpina* within the West Kiewa and Mitta Mitta catchments — currently there is a single record in both catchments, Big Hill in the West Kiewa (1 larva) and Whiterock Creek in the Mitta Mitta (2 adults).
- Comparing sites between catchments that occur close together (i.e. on either side of a ridge) — this will be informative for genetic analyses to potentially identify if *T. alpina* disperse either terrestrially as adults or aquatically as larvae (Figure 2).
- Identifying sites in sub-catchments between known locations — locating additional sites will assist in the understanding of the overall distribution of *T. alpina* and will provide insight into possible threats.

1.1.2 Map habitat

A previous report (Crowther, Lyon & Papas 2008) suggested that stream substrate (often a result of the underlying geology) was an important requirement for *T. alpina*, and may potentially be a driver of population distribution. Crowther, Lyon and Papas (2008) suggested that mapping the geological distribution across the populations would make it 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 base for future surveys or mapping work.

1.1.3 Undertake threat monitoring

To assess threats to the known populations of *T. alpina* that had previously been identified:

- introduced species
- ski resort development
- forestry
- grazing
- climate change
- degradation of the riparian area
- physico-chemical parameters.

Previously, it was suggested that these threats be assessed while performing population surveys. This project would seek to re-assess the threats for the populations and species.

1.1.4 Project objectives

The objectives of this project were to:

1. undertake a broad survey of the species *T. alpina* both at previously listed sites and at potential sites for the species
2. confirm the presence of *T. alpina* in the West Kiewa and Mitta Mitta catchments as well as throughout the East Kiewa catchment (where most of the listed sites occur)
3. 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
4. re-assess the currently identified threats to *T. alpina* as well as any potential threats to the species.

2 Methods

2.1 Sites

Sites sampled during the project included: the 13 identified by Department Environment, Land, Water and Planning (DELWP) (Table 1, Figure 1(A)); 4 non-DELWP-listed sites where *T. alpina* has been previously recorded (Table 2, Figure 1 (B)); and 28 additional sites, 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. alpina* is unknown and could involve dispersal by larvae in the riverine networks, or dispersal by the flightless adults overland. *Thaumatoperla alpina* has previously been recorded in the Mitta Mitta, East Kiewa and West Kiewa catchments, with the majority of records in the East Kiewa catchment. Within the East Kiewa catchment, there is a further split into two major sub-catchments: Pretty Valley and Rocky Valley. Sites along the physical ridge-lines between these catchments and sub-catchments were identified for investigating the dispersal mechanisms of *T. alpina*. These were The Fainters (separates the West Kiewa catchment from the East Kiewa catchment), The Summit (separates Pretty Valley and Rocky Valley catchments) and Spion Kopje (separates East Kiewa and Mitta Mitta catchments). A full list of sites sampled during this project is presented in Appendix A.

Table 1. Listed project sites for *Thaumatoperla alpina.*

Site Code	Site Name	Catchment	Latitude	Longitude
WKIE2	Big Hill Creek	West Kiewa	-36.77449	147.18502
MCK_MID	McKay Creek	Pretty Valley	-36.86627	147.25042
MCK_UPR	McKay Creek (upper)	Pretty Valley	-36.87057	147.26240
PVCK	Pretty Valley Creek and tributaries	Pretty Valley	-36.86408	147.22716
MCKAY1	Tributary of McKay Creek	Pretty Valley	-36.87095	147.25655
TURN2	Turntable Creek	Pretty Valley	-36.84870	147.24243
FAIN1	Tributary of Fainter Creek	Pretty Valley	-36.86367	147.20887
MCKAY3	Tributary of McKay Creek	Pretty Valley	-36.86384	147.24779
PVCK1	Tributary of Pretty Valley Creek	Pretty Valley	-36.81149	147.22434
PVT3	Tributary of Pretty Valley Creek below Pretty Valley Pondage	Pretty Valley	-36.88113	147.24954
RVT1	Tributary of Rocky Valley Creek, Bogong High Plains Road near Howmans Gap	Rocky Valley	-36.84551	147.25745
TURN1	Tributary of Turntable Creek	Pretty Valley	-36.85307	147.24652
ARTH1	Unnamed tributary on Little Arthur fire track	Rocky Valley	-36.79268	147.24621

Table 2. Sites not listed as project-specific sites, but where *T. alpina* has been recorded.

Site Code	Site name	Catchment	Latitude	Longitude
PVT_#s (numerous occurrence points in the same vicinity)	Tributary of Pretty Valley Creek below pondage off Pretty Valley Road	Pretty Valley	-36.88040 -36.88056 -36.88113 -36.88123 -36.88270 -36.88343	147.24983 147.25019 147.24954 147.24963 147.25124 147.25138
WROCK_TRIB	Unnamed tributary of Whiterock Creek, between Howmans Gap and Falls Creek	Mitta Mitta	-36.81056	147.3137
ROCK1	Tributary of Rocky Valley Storage around Rocky Knobs area	Rocky Valley	-36.89190	147.28970
McKay2	Tributary of McKay Creek below Mt McKay Road	Pretty Valley	-36.86992	147.25631

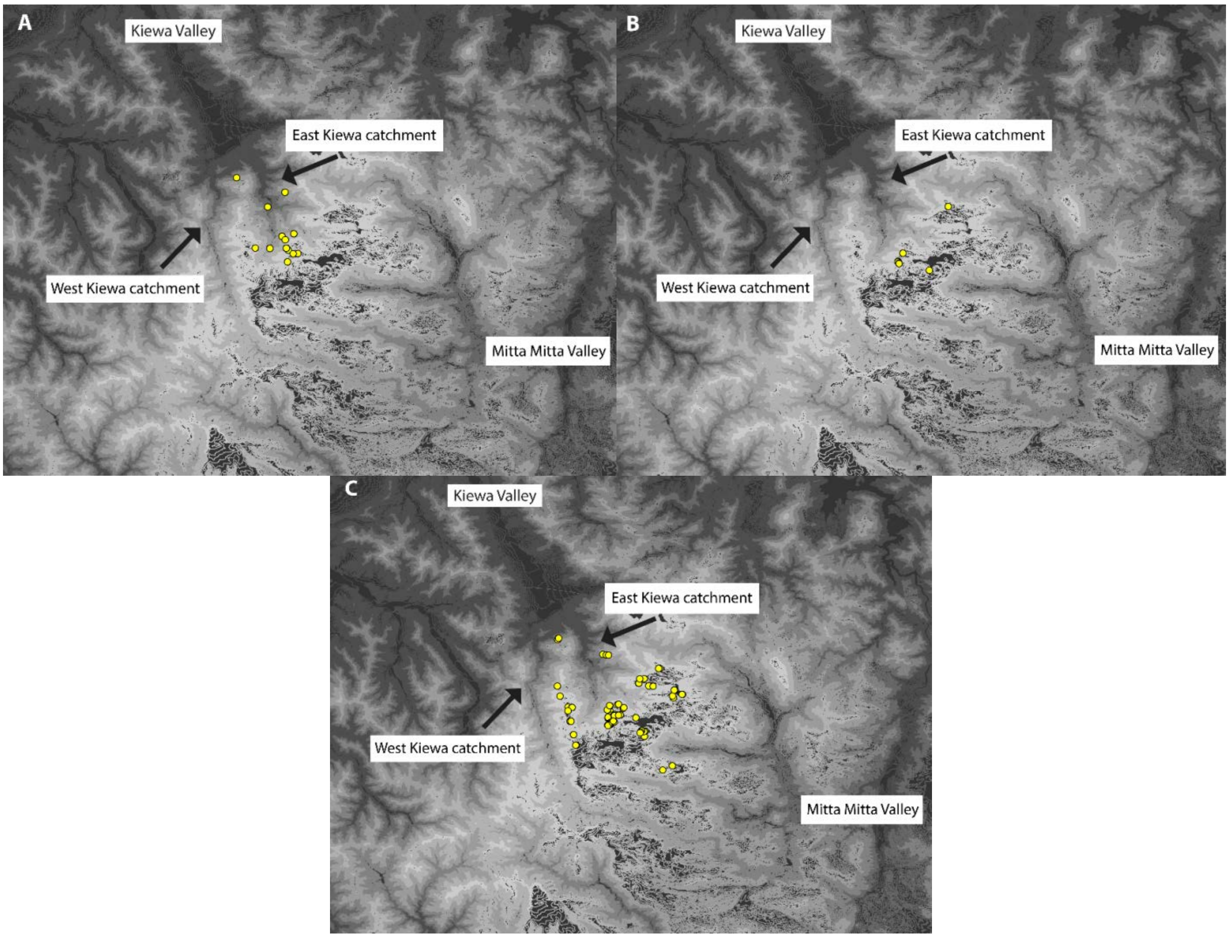

Figure 1. The distribution of sites where *T. alpina* was recorded: (A) 13 DELWP sites, (B) other pre-2016 sites, (C) all sampled sites in 2016.

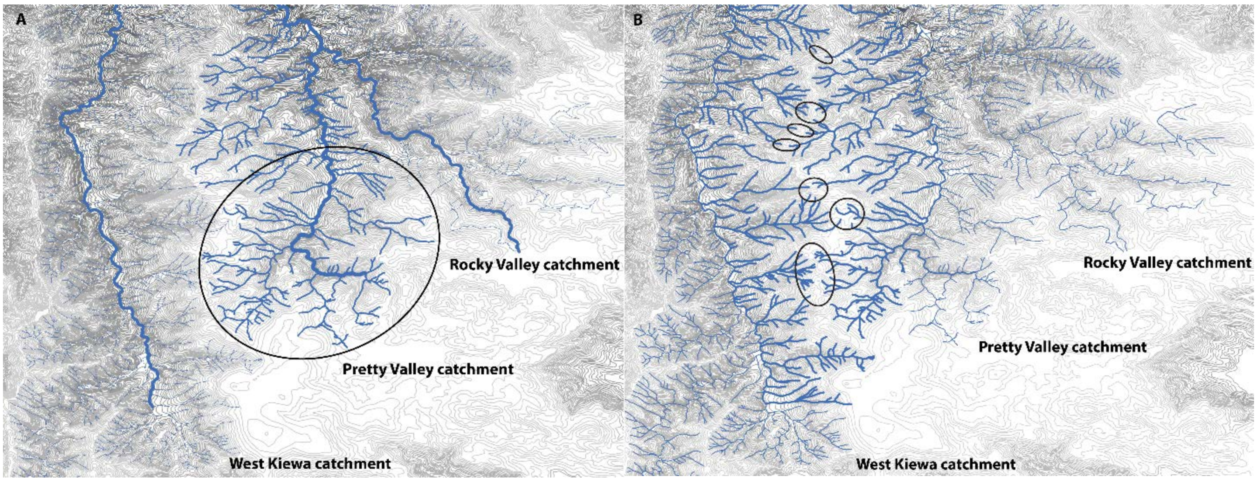

Figure 2. Comparison of expected genetic similarity based on modes of dispersal: (A) larval — within a catchment and (B) terrestrial — between catchments. Highlighted circles denote areas of expected similarity.

2.2 Surveys and genetic methods

2.2.1 Sampling

Previous studies of *T. alpina* have focussed on the larval stage and have recorded very low numbers at sites. This could be due to either the ecology of large predators having low abundances or the burrowing nature, into the hyporheic area, of the species. While the larval stage of *Thaumatoperla* can be challenging to collect, as previously mentioned, the adult life stage is relatively easy to collect. The adults are flightless, and are suggested 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, on the top of riparian vegetation, as well as on trunks, stems and leaves of Snow Gum (*Eucalyptus pauciflora*) patches. A GPS reading was taken at the start of the transect (which usually began at the transition of the bog/pool system to the heathland system with stream flow), with the transect finished either after at least 10 specimens were sampled for DNA, no further specimens were found, or the terrain became too difficult to access. A GPS reading was then taken at the end of each transect and a range of habitat variables were estimated for the reach sampled, including: stream width, stream depth, substrate composition, riparian vegetation class, maximum height of vegetation and presence of weeds (low, medium, high).

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 (new individuals emerge each year). A small 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. alpina* post-clipping (right picture). Note the ball of liquid at the clipped site.

2.2.2 Molecular methods

Genetic techniques were used to assess the population structure within the species *Thaumatoperla alpina* and to determine the mode of dispersal used by *T. alpina*. 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 ridge.

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 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 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 Bogong High Plains 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. alpina* 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. alpina* using water network (water connectivity) and elevation (*T. alpina* have been recorded predominantly above 800 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.

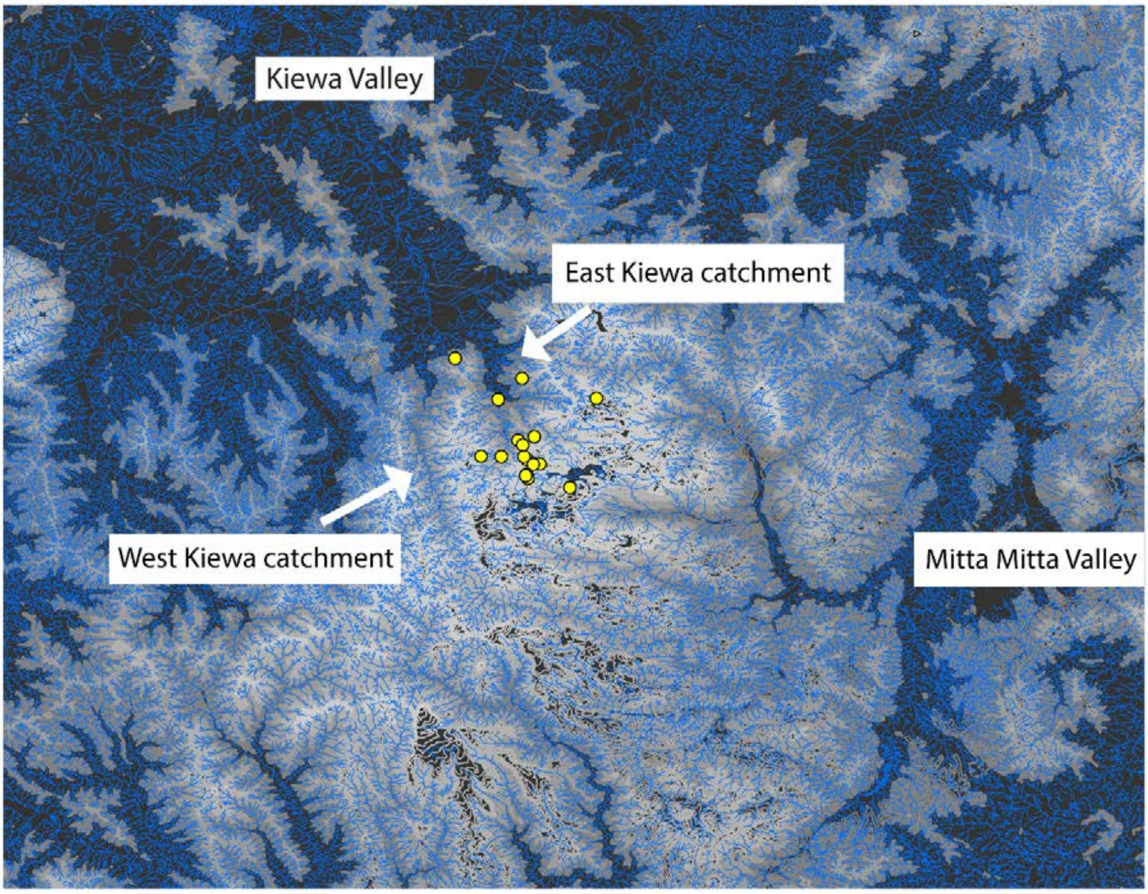

Figure 4. Map of Bogong High Plains showing elevations above 750 m (paler shades) and the hydrological network. Sites where *Thaumatoperla alpina* have been recorded are shown in yellow.

3 Results

3.1 Surveys

Forty-three sites were assessed during a four week period in February 2016, with 30 being surveyed for *T. alpina*, 9 visually assessed from the roadside and 4 not sampled (sites shown in Appendix A). Once the presence of *T. alpina* was confirmed and genetic samples obtained, the site was not revisited. Of the 33 sites sampled, *T. alpina* was recorded at 17 sites. It was found that surveys conducted above the tree-line were more efficient due to better accessibility, as the riparian vegetation was easier to navigate and survey for adults, improving efficiency and increasing the chances of a positive outcome. *Thaumatoperla alpina* were considered present in a system if they were recorded in the headwaters, for example, the Turntable Creek sites TURN2 and TURN1 (at 1120 m and 1320 m, respectively) had no records during this study but 6 adults were recorded in the headwaters at the site SUM2 (1620m).

Of the 13 listed sites provided by DELWP, 9 were resampled and had adults collected within the same system. The only listed systems that did not have *T. alpina* recorded in this study were Big Hill Creek in the West Kiewa catchment and an unnamed tributary on Little Arthur Fire Track. These sites were problematic to sample for adults for the reasons listed above, as they were located in shrubby dry forest vegetation at an elevation of 750 m (WKIE2) or in montane damp forest (ARTH1). A visual inspection of these sites from the roadside did not observe any *T. alpina*. Therefore, to confirm the presence in the West Kiewa catchment, surveys were carried out in accessible areas above the tree-line along the Fainters ridge-line and around Mount Jaithmathang. A survey of Jaithmathang Creek resulted in the collection of 13 adults and the confirmation of *T. alpina* within the West Kiewa catchment. Similarly in the Mitta Mitta catchment, the listed site, a tributary to Whiterock Creek, was surveyed in February 2016 with no *T. alpina* recorded. An additional sampling trip was undertaken in April 2016 to re-survey the site with the subsequent collection trip recording 24 *T. alpina* adults, confirming the presence of *T. alpina* in the Mitta Mitta catchment. Within the East Kiewa catchment, 233 specimens were recorded at 15 sites, with 185 specimens having tissue taken for genetic analysis.

Survey transects were, on average, 99.39 m long, but ranged from 11.5 m to 265.37 m (Appendix A). Habitat variables (summarised in Appendix B) were biased by the change to above tree-line sampling only. It appeared that specimens were located closer to the water body when *T. alpina* began to emerge from the stream; however, it is not possible to further analyse this as no sites were re-sampled over time. It is interesting to note from Table 3 that the Whiterock Creek samples were located within an average of 0.25 m of the stream in April (having been not recorded 5 weeks prior) and the Jaithmathang Creek specimens were recorded at an average of 9.15 m in February. This may suggest a staggered emergence across the Bogong High Plains. Individuals were sexed prior to clipping, and across all sites there were 107 females and 104 males recorded. Specific sites did not show a 1:1 ratio, with Whiterock Creek recording 7 females and 17 males (Table 3).

Table 3. Specimen data for individuals that had genetic samples collected.

Site code	Males (#)	Females (#)	Paired (#)	Found on (most common)	Distance from stream (m)			Height from ground (m)		
					Max	Min	Avg	Max	Min	Avg
FAIN_STH	6	6	1	*Leucopogon* spp.	5	0	1.03	0.6	0.1	0.29
MCK_UPR	4	6	0	*Leucopogon* spp.	6	0	1.92	1.2	0.2	0.58
Mount McKay Summit Road	4	5	0	*Oleria* spp.	3	0.3	0.98	1.2	0.2	0.6
McKay2	6	5	3	*Podolobium* spp.	5	0.3	1.99	2	0.2	0.8
JAITH1	6	7	4	Snow Gum	20	2	9.15	4	1	1.82
Nth_Nelse3	13	5	2	Epacrid spp.	6	0	1.67	0.25	0.1	0.15
PVT3	3	7	0	Epacrid spp.	0.4	0	0.2	1.2	0.15	0.62
PV Valley 2	3	8	0	*Bossia* spp.	8	0.05	1.53	1.3	0.5	0.97
FAIN3	4	7	3	Snow gum	5	0	2.75	2.4	0.4	1.41
FAIN2	4	6	0	*Leucopogon* spp.	6	0	4.9	1	0.1	0.46
Nth_Nelse4	7	11	4	*Baekea* spp.	5	1	1.97	1.8	0.6	1.24
Nth_Nelse1	6	4	0	Epacrid spp.	25	1.2	8.22	1.5	0.15	0.46
SUM1	6	5	2	*Bossia* spp.	2	0.5	1.4	1.3	0.4	0.81
SUM3	14	13	2	*Tasmannia* spp.	15	0	5.79	1.5	0.2	0.87
SUM2	1	5	0	Snow Gum	10	0	1.88	3.2	1	1.93
WROCK_TRIB	17	7	0	Epacrid spp.	1.5	0	0.25	1	0	0.27

3.2 Genetics

At the 17 sites where *T. alpina* were recorded, a total of 270 individuals were observed, with material collected from 222 specimens for genetic analyses. Of the 222 specimens sampled, 24 were from the Mitta Mitta catchment (MM), 13 from the West Kiewa catchment (WK) and 185 from the East Kiewa catchment (with 90 from the Pretty Valley sub-catchment (PV) and 95 from the Rocky Valley sub-catchment (RV)). Of these samples, sequence data were generated for 206 specimens, and 25 haplotypes were identified across the population. Haplotype network maps indicated that the WK site and the MM site were distinct in the haplotypes located within these sites (WK = 2 haplotypes (with 4–5 base-pair (bp) difference) and MM = 2 haplotypes (with 1–2 bp difference)). The rest of the haplotypes were shared within the East Kiewa catchment, with a large number sharing the same haplotype (52.4%). There was an indication from the haplotype network that the Rocky Valley and Pretty Valley sub-catchments showed a difference between the unique haplotypes that were recorded in this study. Different traits were applied to the dataset: catchment and ridge-line. Ridge-line was investigated to see 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 ridge-lines allocated were Fainters (between West Kiewa catchment and Pretty Valley), Spion Kopje (between Mitta Mitta catchment and Rocky Valley), Summit (between Rocky Valley and Pretty Valley) and McKay (between the Pretty Valley sub-catchment and McKay Creek sub-catchment). The haplotype network based on ridges did not show an indication that movement occurs across the ridge-lines.

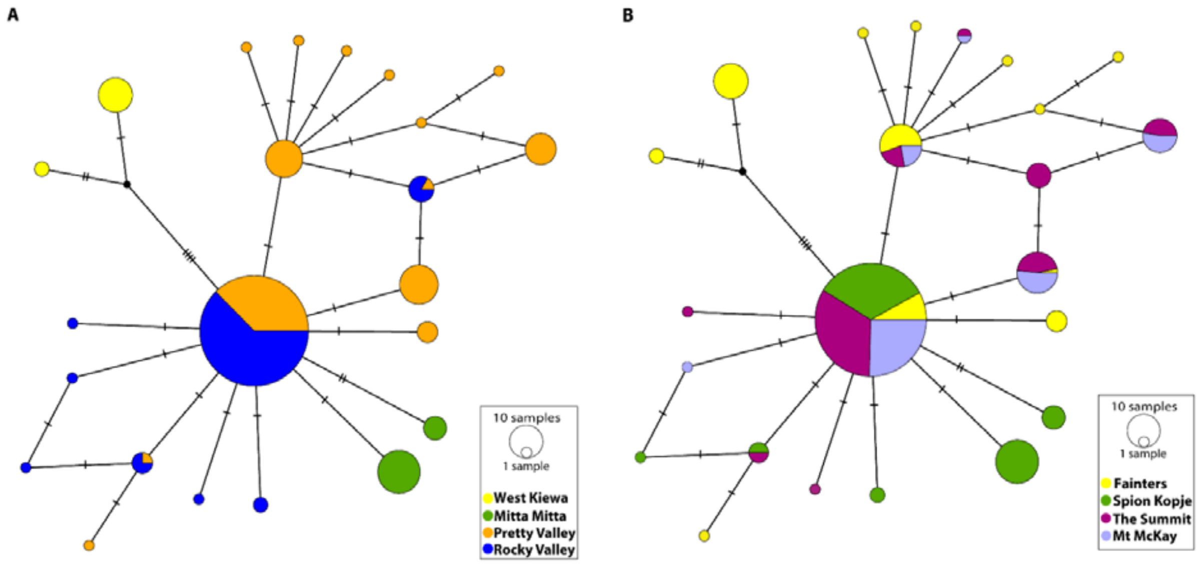

Figure 5. (A) Haplotype network for *Thaumatoperla alpina* by catchment; yellow — West Kiewa, green — Mitta Mitta, orange — Pretty Valley, blue — Rocky Valley. (B) Haplotype network by ridge-line (i.e. West Kiewa site and Fainters sites are separated by the Fainter ridge-line); yellow — Fainter ridge, green — Spion Kopje, purple — The Summit, lavender — Mount McKay.

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 samples had not yet plateaued either for the species or within the East Kiewa catchment (Figures 6 and 7). The Chao1 extrapolator did, however, show an asymptote within the East Kiewa catchment (Figure 7), which suggests that this catchment has been relatively well-sampled genetically. The species as a whole did not quite reach an asymptote (Figure 6), which would be expected with the limited sampling within the West Kiewa and Mitta Mitta catchments. This suggests that the project design was robust and that the West Kiewa and Mitta Mitta catchments would require at least 12 sites each to adequately represent the genetic diversity within these catchments.

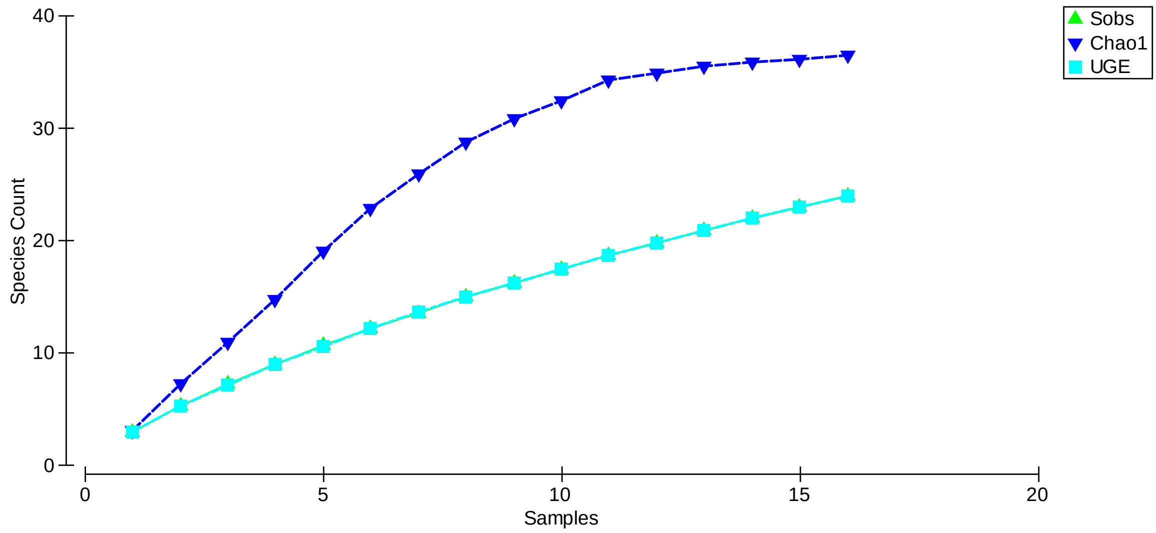

Figure 6. Species (haplotype) accumulation graph of all sites. Chao1 does not show an asymptote, but begins to plateau. This would be due to the limited number of sites in the West Kiewa and Mitta Mitta catchments.

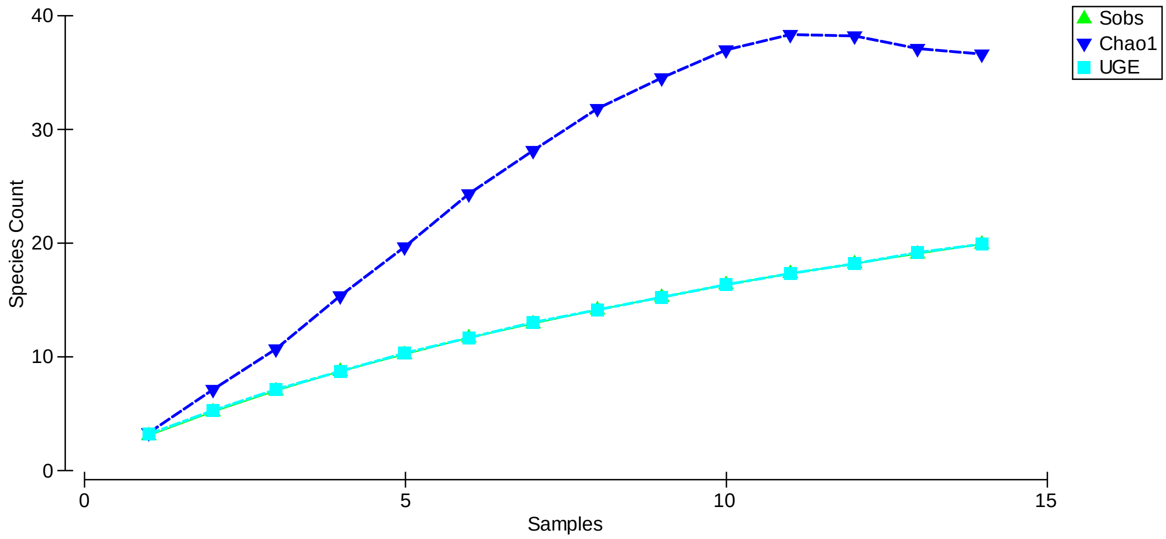

Figure 7. Species (haplotype) accumulation graph excluding sites 7 (West Kiewa) and 16 (Mitta Mitta). Chao1 shows an asymptote, indicating that the genetic diversity was well-sampled within the East Kiewa catchment.

3.3 Mapping

Occurrence data, including previous occurrence records, were entered into GIS (shown in Figure 8). 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 C). Aspect, when grouped into North, North-East, East, South-East, South, South-West, West and North-West (Table 4), showed sites in every sector with the exception of South-East. Slope, when grouped into bins of 5°, showed coverage from 0° to 40° (minimum recording 1.21° at MCK_UPR and maximum recording 36.99° at MCK_MID, Table 4) with the majority of sites within the range 5–10° (n=10) and 20–25° (n=9). EVC classes were extracted and most sites grouped into the various levels of sub-alpine woodland (n=15); sub-alpine grasslands, shrublands and woodlands (n=26); shrublands or grasslands (n=13) and woodlands (n=15) (Table 4). The EVCs would have been biased by the surveys conducted in 2016.

Table 4. Overview of site attributes generated in ArcGIS for *T. alpina* occurrence points (surveys start and end points used for data generation).

Aspect	No.	Slope (°)	No.	EVC Group	No.	EVC subgroup	No.
N (330°–30°)	9	0–5	2	Dry forest	1	Sheltered and/or high altitude	1
NE (30°–60°)	1	5–10	10	Sub-alpine grasslands, shrublands or woodlands	26	Shrublands or grasslands	13
E (60°–120°)	7	10–15	7	Montane grasslands, shrublands or woodlands	2	Woodlands	15
SE (120°–15 °)	0	15–20	6	Wet or damp forest	3	Wet	1
S (150°–210°)	9	20–25	9	Heathlands	9	Sub-alpine	9
SW (210°–240°)	2	25–30	5	Riparian scrubs or swampy scrubs and woodlands	1	Damp	2
W (240°–300°)	10	30–35	2				
NW (300°–330°)	4	35–40	1				

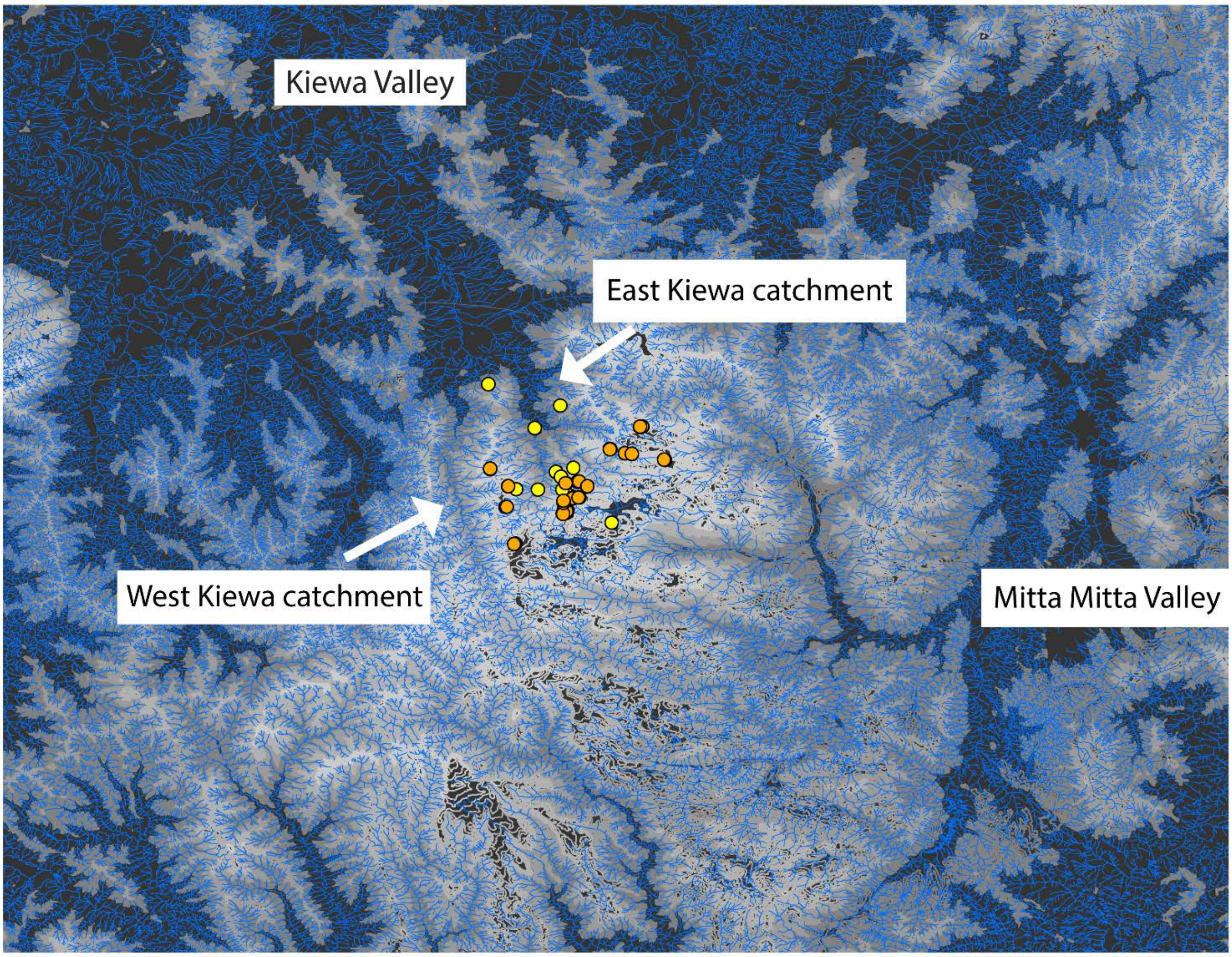

Figure 8. Map of Bogong High Plains showing elevations above 750 m (paler shades) and the hydrological network. Sites where *Thaumatoperla alpina* have been recorded are shown in yellow (pre-2016) and orange (2016).

4 Discussion

This project was able to make a significant contribution to the knowledge of the endangered species *Thaumatoperla alpina*. The overall project design, surveying the terrestrial adults during the emergence period either for presence/absence or genetic data, was shown to be extremely efficient and successful.

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 survey to confirm existing records
- map habitat
- undertake threat monitoring.

4.1.1 Conduct ongoing surveying

Thaumatoperla alpina was observed to be present in a number of known and previously unknown locations. It is recommended that continued surveying following the methods in this project is conducted to establish the full distribution of *T. alpina*, focusing on the distribution within the West Kiewa and Mitta Mitta catchments. The continued use of measured transects would enable an estimate of abundance and, if used in subsequent surveys, could give an estimate of population change over time (including the potential impact of climate change). Ongoing surveys would also provide insights into the emergence period of *T. alpina,* which had been regarded as synchronous across the Bogong High Plains, but seems to involve a gradual emergence from one side of the Bogong High Plains to the other with adults potentially only living for a couple of weeks. Monitoring over the emergence period at sites would provide valuable information on the life cycle of the species and additionally provide important information for land managers across the Bogong High Plains.

4.1.2 Map climate change impacts

Mapping of *Thaumatoperla alpina* under various climate change scenarios should be undertaken. Changes in the connectivity of waterways due to climate-related changes could be a major threat to the dispersal within the populations, especially within the East Kiewa catchment. A restriction in the possible distribution of *Thaumatoperla alpina* 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.

- Fire, which was listed as a possible threat to the species, appears to not have affected *T. alpina* (continued presence after the 2003 and 2006 bushfires). It needs to be determined whether the impact of these fires was withstood due to the species' multi-year larval stage (i.e. if one year's adult population was impacted there still will be adults the next year). Understanding the impact of fire to both life stages *T. alpina* would provide insight into whether this is a significant threat to the species. We also need to develop an understanding of whether adults survive by delaying their emergence or due to larvae having multi-year life cycles (i.e. larval survival is able to sustain the populations if a season of adults is impacted). Does the increase in nutrients after a fire event affect *T. alpina*? Do adult *T. alpina* become more vulnerable to predation after fire?

- Impacts of water storages and waterway re-alignments (i.e. aqua ducts) need to be assessed: these may be contributing to the species' absence from the Watchbed Creek sub-catchment (i.e. the Rocky Valley storage).
- The impact of trout was difficult to assess, but it does not seem to have a detrimental effect on *T. alpina*. To have a better understanding of where trout occur throughout the Bogong High Plains' watercourses would be beneficial in understanding whether these species co-occur or not. The larval stage of *Thaumatoperla alpina* is known to burrow, which may be an important avoidance strategy against trout predation.

4.1.4 Outcomes

- The presence of *Thaumatoperla alpina* was confirmed within the East Kiewa, West Kiewa and Mitta Mitta catchments.
- The populations within the catchments were shown to be genetically distinct and suggestive of dispersal predominantly via the aquatic network. The priority to further support this would involve the collection of genetic material in the Mitta Mitta and West Kiewa catchments. This project suggests that the majority of genetic variation within these catchments should be detectable in approximately 12 sites within each catchment.
- Surveys focusing on the adult life stage are efficient and effective, especially above the tree-line. Future surveys should adopt this technique.
- Climate change will be the major threat to *T. alpina* in the foreseeable future, with impacts likely to result in higher water temperature, reduced snow occurrence (water recharge/runoff), higher air temperatures and changes to riparian vegetation, which will subsequently affect stream nutrients and chemistry as well as shading of the water body.

4.1.5 Future project actions

- Assess the distribution of *T. alpina* in the West Kiewa and Mitta Mitta catchments.
- Survey the emergence patterns and longevity of *T. alpina* across the Bogong High Plains. This has potential to guide land managers within the Bogong High Plains 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. alpina* or not. If the species are not co-occurring, investigate the possible predation of *T. alpina* by trout.
- Assess the impact of hydrological modification to *T. alpina*. A record of *T. alpina* in the Rocky Knobs area suggests that either *T. alpina* larvae can cross Rocky Valley storage or that *T. alpina* adults are able to move into the area from the Mitta Mitta catchment. Further understanding of the impacts that previous hydrological modifications have had on *T. alpina* will provide a framework for predicting future impacts should further hydrological modifications be required within the Bogong High Plains.

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Appendices

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

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				
WKIE2	Big Hill Creek	-36.77449	147.18502	-	-	Surveyed from road side	0	-	1 (2008)
WKIE_BH2	Big Hill Creek	-36.77281	147.18358	-	-	Surveyed from road side	0	-	-
MCK_MID	McKay Creek	-36.86638	147.25098	-36.86640	147.25163	57.99	0		1 (2005)
MCK_UPR	McKay Creek (upper)	-36.87053	147.26147	-36.87056	147.26444	265.37	10	10	1 (2005) 3 (2008) + approx. 50 adults
PVCK	Pretty Valley Creek and tributaries	-36.86408	147.22716	-	-	Not sampled	-	-	Unknown number — adult life stage (2001)
MCKAY1	Tributary of McKay Creek	-36.87050	147.25643	-36.87132	147.25687	118.68	13	11 (2016) 6 (2015)	8 (2015), 2 (2008)
TURN2	Turntable Creek	-36.84870	147.24243	-	-	Surveyed from road side	0	-	Unknown number — larvae (2001)

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				
FAIN1 * type location	Tributary of Fainter Creek	-36.86367	147.20887	-	-	Not sampled	-	-	Unknown number — adult life stage
FAIN2	Tributary of Fainter Creek	-36.84623	147.18669	-36.84613	147.18673	11.66	10	10	-
FAIN_STH	Tributary to Tawonga Hut Creek	-36.86083	147.20193	-36.86093	147.20235	39.06	14	10	-
FAIN3	Tributary to Tawonga Hut Creek	-36.87858	147.19971	-36.87865	147.20098	113.49	38	11	-
MCKAY3	Tributary of McKay Creek	-36.86390	147.24770	-	-	Surveyed from road side	0	-	-
PVCK1	Tributary of Pretty Valley Creek	-36.81149	147.22434	-	-	Surveyed from road side	0	-	1 (2005)
PVT3 N.B. all PVT_#s merged into PVT_3	Tributary of Pretty Valley Creek below Pretty Valley Pondage	-36.88168	147.25213	-36.88225	147.25130	97.33	10	10 (2016) 5 (2015)	8 (2015)
RVT1	Tributary of Rocky Valley Creek, Bogong High Plains Road near Howmans Gap	-36.84551	147.25745	-	-	Not sampled	-	-	1 adult (2011)
TURN1	Tributary of Turntable Creek	-36.85307	147.24652	-	-	Not sampled	-	-	1 (2008)
ARTH1	Unnamed tributary on Little Arthur fire track	-36.79268	147.24621	-	-	Surveyed from road side	0	-	2 (2005), 1 (2008)
ARTH2	Unnamed tributary on Little Arthur fire track	-36.79202	147.24195	-	-	Surveyed from road side	0	-	-
ARTH3	Unnamed tributary on Little Arthur fire track	-36.79297	147.24930	-	-	Surveyed from road side	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				
WROCK_TRIB	Unnamed tributary of Whiterock Creek, between Howmans Gap and Falls Creek	-36.81047	147.31562	-36.81040	147.31380	162.59	24	24	1 (2012)
ROCK1	Tributary of Rocky Valley Storage around Rocky Knobs area	-36.89267	147.29050	-36.89077	147.29167	235.11	0	-	1 (2001)
McKay2	Tributary of McKay Creek below Mount McKay Road	-36.86992	147.25631	-	-	Surveyed from road side	-	3 (2015)	3 (2015), 1 (2005), 1 (2008)
PV_McKay Rds Jnc	Tributary of Pretty Valley Creek	-36.88012	147.25192	-36.88062	147.25097	101.25	0	-	-
PV Valley 2	Tributary of Pretty Valley Creek	-36.88402	147.24807	-36.88425	147.24862	55.45	12	11	-
Mount McKay Summit Road	Tributary of McKay Creek near Summit Road turnoff	-36.87485	147.24865	-36.87347	147.24865	153.15	10	9	-
Nth_Nelse1	Tributary to North Nelse Creek off Spion Kopje	-36.83325	147.3062	-36.83350	147.30670	52.52	9	9	-
Nelse1	Tributary to Nelse Creek	-36.84834	147.33280	-36.84593	147.33167	285.87	0	-	-
SUM1	Tributary to Rocky Valley Creek	-36.85715	147.26066	-36.85685	147.26235	154.34	13	11	-
SUM2	Tributary to Turnback Creek	-36.85850	147.25110	-36.85837	147.25050	55.42	6	6	-
BogongJack	Bogong Jack Creek	-36.83313	147.18328	No end point recorded		Approx. 150	0	-	-
BIG1	Tributary to Big River	-36.82478	147.29301	-36.82372	147.29520	228.06	0	-	-
SpKo1	Tributary to Spion Kopje Creek	-36.82394	147.28946	Too steep		-	0	-	-
HOLL1	Tributary to Hollands Creek	-36.84380	147.34433	-36.84416	147.34455	44.51	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				
ROCK2	Rocky Valley tributary — Boomerang XC Track	-36.87988	147.25413	-36.87925	147.25686	253.23	0	-	-
ROCK3	Rocky Valley tributary — Boomerang XC Track	-36.87925	147.25686	-36.87842	147.25538	163.81	0	-	-
Nth_Nelse2	Tributary to North Nelse Creek —Spion Kopje Track	-36.82933	147.28793	-36.82981	147.28803	54.00	11	10	-
Nth_Nelse3	Tributary to North Nelse Creek — Big River Track	-36.83852	147.33500	-36.83831	147.33398	93.92	24	20	-
JAITH1	Tributary to Jaithmathang Creek	-36.90968	147.20835	-36.90983	147.20703	118.81	13	13	-
SUM3	Tributary to Rocky Valley Creek — Valley of the Moon	-36.86116	147.26898	-36.86098	147.26919	27.38	35	27	-
Nth_Nelse4	Tributary to North Nelse Creek near Grey Hills Track	-36.82953	147.28831	-36.82981	147.28803	39.87	18	18	-
Nelse2	Tributary to Nelse Creek at Edmondson Hut	-36.84834	147.33279	-36.84593	147.33167	285.72	0	-	-
FAIN4	Tawong Hut Creek at Tawonga Huts	-36.89612	147.20413	No end point recorded		Approx. 100	0	-	-
FAIN5	Tributary to West Kiewa River off Fainter Track	-36.86523	147.19702	No end point recorded		Approx. 100	0	-	-

Appendix B: Habitat variables collected during survey transects. All measurements are estimates.

Site code	Site name	Stream width (m)			Stream depth (m)			Riparian width (m)			Riparian height (m)			Weed presence	Stream shading (%)
		Max	Min	Avg	Max	Min	Avg	Max	Min	Avg	Max	Min	Avg		
MCK_MID	McKay Creek	3	1.5	2	1	0.15	0.4	30	15	20	4	1.5	2	Medium	95
MCK_UPR	McKay Creek (upper)	3	0.5	1	1.5	0.3	0.5	5	1	2	0.7	0.2	0.35	Low	15
MCKAY1	Tributary of McKay Creek	1.2	0.1	0.3			0.1							Low	
FAIN2	Tributary of Fainter Creek	0.8	0.2	0.7	0.2	0.05	0.1	5	1	3	1.2	0.1	0.6	Low	70
FAIN_STH	Tributary to Tawonga Hut Creek	1	0.2	0.4	0.15	0.05	0.1	5	0.2	1	0.8	0.1	0.45	Low	45
PVT3 N.B. all PVT_#s merged into PVT_3	Tributary of Pretty Valley Creek below Pretty Valley Pondage	1	0.1	0.3	0.15			30	20	25	3	0.1	0.5	Low	
WROCK_TRIB	Unnamed tributary of Whiterock Creek, between Howmans Gap and Falls Creek	1	0.05	0.3	0.4	0.1	0.2	15	5	10	3.5	0.1	0.5	Low	60
PV_McKay Rds Jnc	Tributary of Pretty Valley Creek	0.5	0.05	0.125	0.1	0.02	0.05	20	5	10	1.2	0.5	1	Low	30
PV Valley 2	Tributary of Pretty Valley Creek			1			7.5			5	1	0.5		Low	60
Mount McKay Summit Road	Tributary of McKay Creek near Summit Road turnoff	1	0.05	0.2	0.5	0.05	0.1	20	5	15	0.8	0.4	0.5	Low	85
Nth_Nelse1	Tributary to Nelse Creek off Spion Kopje	1.2	0.1	0.35	0.3	0.02	0.05	10	1	3	1.5	0.05	0.5	Low/Medium	20
Nelse1	Tributary to Nelse Creek	1.2	0.05	0.8	0.4	0.05	0.15	15	1	5	0.6	0.1	0.4	Low	10
SUM1	Tributary to Rocky Valley	0.6	0	0.2	0.2	0.05	0.1	20	5	10	1.5	0.5	0.8	Low	85
SUM2	Tributary to Turnback Creek	1	0.1	0.3	0.1	0.05	0.05	30	5	15	4	0.5	1	Low	80

Site code	Site name	Stream width (m)			Stream depth (m)			Riparian width (m)			Riparian height (m)			Weed presence	Stream shading (%)
		Max	Min	Avg	Max	Min	Avg	Max	Min	Avg	Max	Min	Avg		
Nth_Nelse3	Tributary to North Nelse Creek — Big River Track	5	0.2	0.5	0.3	0.05	0.1	6	0.2	1	0.6	0.1	0.4	Low	50
JAITH1	Tributary to Jaithmathang Creek	1.5	0.1	0.5	0.5	0.2	0.2	15	1	5	4	0.1	0.5	Medium/High	80
SUM3	Tributary to Rocky Valley Creek —Valley of the Moon	1.2	0.2	0.4	0.2	0.05	0.1	50	10	30	1.5	0.5	1	Low	85
Nth_Nelse4	Tributary to North Nelse Creek near Grey Hills Track	5	0.5	0.6	0.2	0.02	0.1	30	15	20	1.5	0.5	0.8	Low	85

Appendix C: Site attributes generated in ArcGIS for all *T. alpina* 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
WKIE_BH2	Big Hill Creek	4.34266	8.75964	Shrubby dry forest	Dry forest	Sheltered and/or high altitude
MCK_MID	McKay Creek	21.3873	36.9901	Sub-alpine shrubland	Sub-alpine grasslands, shrublands or woodlands	Shrublands or grasslands
MCK_MID	McKay Creek	0.836697	34.7463	Montane dry woodland	Montane grasslands, shrublands or woodlands	Woodlands
MCK_UPR	McKay Creek (upper)	247.754	1.2101	Alpine grassland	Sub-alpine grasslands, shrublands or woodlands	Shrublands or grasslands
MCK_UPR	McKay Creek (upper)	198.423	25.2711	Alpine grassland	Sub-alpine grasslands, shrublands or woodlands	Shrublands or grasslands
PVCK	Pretty Valley Creek and tributaries	7.00312	6.10337	Sub-alpine woodland	Sub-alpine grasslands, shrublands or woodlands	Woodlands
MCKAY1	Tributary of McKay Creek	341.523	20.5835	Sub-alpine shrubland	Sub-alpine grasslands, shrublands or woodlands	Shrublands or grasslands
TURN2	Turntable Creek	272.397	12.4229	Wet forest	Wet or damp forest	Wet
FAIN2	Tributary of Fainter Creek	93.2507	19.4356	Alpine grassy heathland	Heathlands	Sub-alpine
FAIN2	Tributary of Fainter Creek	96.5301	17.5612	Alpine grassy heathland	Heathlands	Sub-alpine
FAIN3	Tributary to Tawonga Hut Creek	111.838	16.0575	Sub-alpine woodland	Sub-alpine grasslands, shrublands or woodlands	Woodlands
FAIN_STH	Tributary to Tawonga Hut Creek	196.811	17.0627	Sub-alpine wet heathland/Alpine valley peatland mosaic	Heathlands	Sub-alpine

Site code	Site name	Aspect	Slope	EVC name	EVC group name	EVC subgroup name
FAIN_STH	Tributary to Tawonga Hut Creek	172.673	7.94279	Sub-alpine woodland	Sub-alpine grasslands, shrublands or woodlands	Woodlands
FAIN3	Tributary to Tawonga Hut Creek	85.4353	3.26023	Sub-alpine woodland	Sub-alpine grasslands, shrublands or woodlands	Woodlands
MCKAY3	Tributary of McKay Creek	289.129	9.55893	Sub-alpine woodland	Sub-alpine grasslands, shrublands or woodlands	Woodlands
PVCK1	Tributary of Pretty Valley Creek	83.2917	34.4645	Damp forest	Wet or damp forest	Damp
PVT3	Tributary of Pretty Valley Creek below Pretty Valley Pondage	260.781	20.6073	Sub-alpine woodland	Sub-alpine grasslands, shrublands or woodlands	Woodlands
PVT3	Tributary of Pretty Valley Creek below Pretty Valley Pondage	239.496	21.2756	Sub-alpine woodland	Sub-alpine grasslands, shrublands or woodlands	Woodlands
TURN1	Tributary of Turntable Creek	309.593	25.2924	Montane dry woodland	Montane grasslands, shrublands or woodlands	Woodlands
ARTH1	Unnamed tributary on Little Arthur fire track	186.487	16.8002	Montane damp forest	Wet or damp forest	Damp
WROCK_TRIB	Unnamed tributary of Whiterock Creek, between Howmans Gap and Falls Creek	253.702	11.4525	Sub-alpine woodland	Sub-alpine grasslands, shrublands or woodlands	Woodlands
ROCK1	Tributary of Rocky Valley Storage around Rocky Knobs area	100.969	12.6621	Sub-alpine woodland	Sub-alpine grasslands, shrublands or woodlands	Woodlands

Site code	Site name	Aspect	Slope	EVC name	EVC group name	EVC subgroup name
PV Valley 2	Tributary of Pretty Valley Creek	257.676	6.46651	Sub-alpine wet heathland/Alpine valley peatland mosaic	Heathlands	Sub-alpine
PV Valley 2	Tributary of Pretty Valley Creek	270.582	6.96185	Sub-alpine woodland	Sub-alpine grasslands, shrublands or woodlands	Woodlands
Mount McKay Summit Rd	Tributary of McKay Creek near Summit Road turnoff	331.851	13.5434	Alpine grassy heathland	Heathlands	Sub-alpine
Mount McKay Summit Rd	Tributary of McKay Creek near Summit Road turnoff	333.449	22.0613	Sub-alpine shrubland	Sub-alpine grasslands, shrublands or woodlands	Shrublands or grasslands
Nth_Nelse1	Tributary to North Nelse Creek off Spion Kopje	163.786	20.6077	Sub-alpine shrubland	Sub-alpine grasslands, shrublands or woodlands	Shrublands or grasslands
Nth_Nelse1	Tributary to North Nelse Creek off Spion Kopje	224.996	21.9687	Alpine grassy heathland	Heathlands	Sub-alpine
SUM1	Tributary to Rocky Valley	21.5235	7.14449	Sub-alpine shrubland	Sub-alpine grasslands, shrublands or woodlands	Shrublands or grasslands
SUM1	Tributary to Rocky Valley	11.1933	6.24125	Sub-alpine shrubland	Sub-alpine grasslands, shrublands or woodlands	Shrublands or grasslands
SUM2	Tributary to Turnback Creek	305.633	13.9046	Sub-alpine woodland	Sub-alpine grasslands, shrublands or woodlands	Woodlands
SUM2	Tributary to Turnback Creek	311.357	26.9968	Sub-alpine shrubland	Sub-alpine grasslands, shrublands or woodlands	Shrublands or grasslands
Nth_Nelse3	Tributary to North Nelse Creek — Big River Track	288.522	10.8165	Alpine grassy heathland	Heathlands	Sub-alpine

Site code	Site name	Aspect	Slope	EVC name	EVC group name	EVC subgroup name
Nth_Nelse3	Tributary to North Nelse Creek — Big River Track	324.927	7.74163	Alpine grassy heathland	Heathlands	Sub-alpine
JAITH1	Tributary to Jaithmathang Creek	249.346	18.0822	Sub-alpine woodland	Sub-alpine grasslands, shrublands or woodlands	Woodlands
JAITH1	Tributary to Jaithmathang	271.741	20.202	Alpine grassy heathland	Heathlands	Sub-alpine
SUM3	Tributary to Rocky Valley Creek — Valley of the Moon	49.9402	12.3026	Sub-alpine woodland	Sub-alpine grasslands, shrublands or woodlands	Woodlands
SUM3	Tributary to Rocky Valley Creek — Valley of the Moon	83.4918	8.39918	Alpine grassland	Sub-alpine grasslands, shrublands or woodlands	Shrublands or grasslands
Nth_Nelse4	Tributary to North Nelse Creek near Grey Hills Track	153.568	23.0915	Sub-alpine woodland	Sub-alpine grasslands, shrublands or woodlands	Shrublands or grasslands
Nth_Nelse4	Tributary to North Nelse Creek near Grey Hills Track	178.41	27.8987	Riparian forest/Creekline grassy woodland mosaic	Riparian scrubs or swampy scrubs and woodlands	-
Nth_Nelse2	Tributary to North Nelse Creek — Spion Kopje Track	153.568	23.0915	Sub-alpine woodland	Sub-alpine grasslands, shrublands or woodlands	Shrublands or grasslands
Nth_Nelse2	Tributary to North Nelse Creek — Spion Kopje Track	170.133	25.7981	Late-lying snowpatch herbland	Sub-alpine grasslands, shrublands or woodlands	Shrublands or grasslands