Experimental warming and precipitation interactively modulate the mortality rate and timing of spring emergence of a gallmaking Tephritid fly

Xinqiang Xi1, Dongbo Li1, Youhong Peng2, Nico Eisenhauer3,4 & Shucun Sun1,2

Global climate change is mostly characterized by temperature increase and fluctuating precipitation events, which may affect the spring phenology and mortality rate of insects. However, the interaction effect of temperature and precipitation on species performance has rarely been examined. Here we studied the response of the gall-making Tephritid fly *Urophora stylata* (Diptera: Tephritidae) to artificial warming, changes in precipitation, and the presence of galls. Our results revealed a significant interaction effect of warming, precipitation, and galls on the life-history traits of the focal species. Specifically, when the galls were intact, warming had no effect on the phenology and increased the mortality of the fly under decreased precipitation, but it significantly advanced the timing of adult emergence and had no effect on the mortality under increased precipitation. When galls were removed, warming significantly advanced the timing of emergence and increased fly mortality, but precipitation showed no effect on the phenology and mortality. In addition, gall removal significantly increased adult fresh mass for both females and males. Our results indicate that the effect of elevated temperature on the performance of species may depend on other environmental conditions, such as variations in precipitation, and species traits like the formation of galls.

Climate change may significantly alter the performance of various species1,2. One well-known response of organisms to climate change is the shift in species phenology, as indicated by the advance of vegetation greening and animal emergence in spring3. This response is particularly important to many herbivorous insect species because spring phenology determines the duration of their growth and development in the following growing season as well as their synchrony with the phenology of host plants (e.g. ref. 4). Another significant effect of climate change is on species mortality rate, which is exemplified by frequent pest outbreaks in several recent reports5,6. In particular, in temperate regions, where the major environmental fluctuations during the transition period from winter to spring often result in high insect mortality rate6, a slight change in spring climate can be crucial to population dynamics of insects. Studies have used historical records7 or laboratory data on thermal tolerance8 to infer the effect of climate change on insect survival and phenology. However, field experiments are still largely lacking (but see ref. 9), which may limit the accuracy of predictions of insect survival and phenology in nature-like conditions8.

Global warming may shift animal spring phenology10 because temperature is critical to animal metabolic rate and activity particularly for ectothermic insects whose physiological processes are largely temperature-dependent11. Spring warming therefore likely advances the emergence of hibernating insects in temperate regions12. Consistently, numerous studies have recorded the advance of spring emergence in various insect species13–15. Furthermore, increasing temperatures may also alter insect mortality16,17 by physiologically
Moreover, warming significantly increased the mortality rate of flies in the decreased and average precipitation (Fig. 1). When galls were intact, the mortality rate decreased with increasing precipitation (Table 1, Fig. 1a).

One of the potential co-determining abiotic factors of species survival and phenology is the change in precipitation. In fact, in addition to shifts in precipitation patterns, extreme precipitation events are likely to become more frequent in many parts of the world.For example, neutral and negative effects of warming on spring phenology (delayed) and survival have also frequently been reported, varying with locations and species. These contrasting results suggest that warming effects might be mediated by other abiotic factors and/or species traits.

Species traits are likely to mediate responses of insect species to global warming. Insects may undergo unfavorable winters in different life stages (e.g. as larvae, pupae, or adults) with different strategies against environmental fluctuations (with none, with ootheca, silk bags or plant galls covered eggs or larvae). For instance, naked larvae should be more sensitive to temperature change than those embedded in galls, which are often produced to prevent parasitoid enemies in many insect species. Galls do not only block light to make insects insensitive to photoperiod but also buffer external temperature fluctuations. Typically, insects can "sense" physical environmental change and emerge from galls unless the galls are moistened by precipitation. Thus, we hypothesize that gall-making species are less likely to be sensitive to changes in temperature than in precipitation.

The Tibetan plateau has experienced pronounced warming during the past decades and is predicted to have a higher than average temperature increase in the near future, and extreme precipitation events occur more frequently than before. Climate change has induced significant changes in plant phenology and primary production on the plateau, and these changes can be ascribed to temperature increase or altered precipitation. Yet, effects of increasing temperature and altered precipitation on insect phenology and survival have rarely been tested in this high altitudinal region. In this study, we investigated the effects of experimental warming and manipulated precipitation on the mortality and spring emergence of the gall-making Tephritid fly . The primary question was whether the warming effect on the life history traits of the studied insect species depends on precipitation. In addition, we also experimentally removed galls to examine the gall effect on species mortality and phenology. The second question was whether the climate effect on species performance is modulated/mediated by the presence of galls.

### Results

#### Mortality rate.

Warming and precipitation as well as precipitation and galls interactively affected the mortality rate of larvae (Table 1). The presence of galls significantly decreased the mortality rate of the maggots (Table 1, Fig. 1). When galls were intact, the mortality rate decreased with increasing precipitation (Table 1, Fig. 1a). Moreover, warming significantly increased the mortality rate of flies in the decreased and average precipitation treatments but did not significantly alter mortality at increased precipitation (significant warming × precipitation interaction; Table 1, Fig. 1a). When the galls were removed, warming consistently increased mortality rates in all of the precipitation treatments, while the precipitation effect was non-significant (Fig. 1b).

#### Timing of adult emergence and adult fresh mass.

Warming, precipitation, and galls interactively affected the timing of adult emergence (significant warming × precipitation × gall interaction; Table 2). When galls were intact, warming delayed the adult emergence for males but not females under average precipitation condition, but it advanced the adult emergence for both sexes under increased precipitation (Fig. 2a).

When galls were removed, warming significantly advanced the adult emergence for both sexes (Fig. 2b), but males emerged significantly earlier than females (3.1 days advance; Table 2). The precipitation effect, however, was not significant (Fig. 2b). Moreover, the effect of galls on the timing of adult emergence is modulated/mediated by the presence of galls.

|            | d.f. | $\chi^2$ | P    |
|------------|------|----------|------|
| Gall       | 1    | 1844     | 0.046|
| Warming    | 1    | 1834     | 0.001|
| Precipitation | 2 | 1583     | <0.001|
| Gall × Warming | 1 | 1583     | 0.670|
| Gall × Precipitation | 2 | 1547     | <0.001|
| Warming × Precipitation | 2 | 1530     | <0.001|
| Gall × Warming × Precipitation | 2 | 1527     | 0.180|
| Residual   | 1333 | 1913     |      |

Table 1. Results of generalized linear model (with binomial errors) showing the effects of warming (ambient, warmed (ambient + 1.9°C)), precipitation (increased (30% increased), average (average of past 53 years), and decreased (30% decreased)), and the presence of galls (intact, removed) as well as their interactions on the mortality rate of larval maggots at the end of the experiment.
Figure 1. Mortality rate of larval maggots with intact (a) and with galls removed (b) as affected by warming (ambient, warmed (ambient +1.9 °C)) and precipitation (30% increased precipitation, average (average of past 53 years), and 30% decreased precipitation). Different letters above the bars denote significant differences among treatments at $P = 0.05$.

|                | Timing of emergence | Adult fresh mass |
|----------------|---------------------|------------------|
|                | d.f. | SS   | F    | P     |                | SS  | F    | P     |
| G              | 1    | 2860 | 214  | <0.001| 14    | 63.6| <0.001|
| W              | 1    | 215  | 16   | <0.001| 0.3   | 1.3 | 0.25  |
| P              | 2    | 2799 | 105  | <0.001| 0.3   | 0.8 | 0.47  |
| S              | 1    | 4040 | 303  | <0.001| 163   | 745 | <0.001|
| G × W          | 1    | 227  | 17   | <0.001| 0.2   | 0.8 | 0.36  |
| G × P          | 2    | 404  | 15   | <0.001| 0.14  | 0.32| 0.73  |
| W × P          | 2    | 1334 | 50   | <0.001| 0.31  | 0.7 | 0.5   |
| G × S          | 1    | 71   | 5    | 0.022 | 2.1   | 3.3 | 0.002 |
| W × S          | 1    | 57   | 4    | 0.030 | 0.02  | 0.3 | 0.94  |
| P × S          | 2    | 69   | 3    | 0.078 | 0.26  | 3.3 | 0.55  |
| G × W × P      | 2    | 349  | 13   | <0.001| 0.19  | 0.08| 0.65  |
| G × W × S      | 1    | 29   | 2    | 0.140 | 0.01  | 0.005| 0.93  |
| G × P × S      | 2    | 6    | 0.06 | 0.810 | 0.87  | 0.06| 0.14  |
| W × P × S      | 2    | 92   | 5.90 | 0.030 | 0.63  | 1.5 | 0.2   |
| G × W × P × S  | 2    | 69   | 2.57 | 0.080 | 0.28  | 0.6 | 0.5   |
| Residuals      | 239  | 3186 | 52   |

Table 2. Results of ANOVA showing the effects of warming (W, ambient, warmed (ambient +1.9 °C)), Precipitation (P, increased (30% increased), average (average of past 53 years), and decreased (30% decreased)), the presence of galls (G, intact, removed), and sex (S) as well as their interactions on timing of adult fly emergence and adult fresh mass.
emergence was significant (Table 2), as indicated by the earlier emergence of adults (by 13.2 days on average) in the gall-removed than gall-intact treatment (Fig. 2).

In addition, warming and precipitation had a non-significant effect on adult fresh mass, whereas females had higher fresh mass than males, regardless of whether the galls were intact or removed (Fig. 3). Gall removal significantly increased adult fresh mass, but the increase was stronger in males than in females, as indicated by the significant interaction between gall and sex (Table 2).

Discussion

Our study shows that warming significantly affected the mortality rate and the timing of adult emergence, but these warming effects depended on the amount of precipitation. Moreover, the results also indicate that the direction and magnitude of the warming effect on mortality rate and timing of emergence depended on whether the galls were intact or removed, and that the precipitation effect was significant on both the mortality and the timing when the galls were intact but non-significant when the galls were removed. These findings not only indicate that the effect of altered environmental conditions on species performance was significantly mediated by galls, but also suggest that the galling-making habit may improve *U. stylata’s* adaptability to fluctuating spring climate at the Tibetan Plateau.

The finding of warming increasing the survival and advancing the timing of emergence confirms our expectations since the physiological process rates of ectoderm insect activities are largely dependent upon external environmental temperature, which is often positively associated with enzyme activities. The warming effect on species performance found here is consistent with results of many previous studies addressing insect survival and phenology. However, the positive effect of warming occurs theoretically only when other factors that are responsible for physiological processes are not limited. For example, warming should increase the energy demand and nutrient uptake of animals, and hence, if nutrient level is low enough, this may slow down animal metabolic rates. In this study, the larval maggots almost reached their maximum body size at the end of the growing season when the plants die off, and larval maggots do not consume plant tissues in the next spring.
(X. Xi and S. Sun; personal observation). This suggests that the larval response to warming likely was not limited by nutrients. Other potential factors that can confound the warming effect include photoperiod and water availability. Several studies showed an interaction effect between temperature and photoperiod on animal phenology, in which insects were inactive despite a temperature increase in the season of hibernation. The hibernating maggots of our study species are covered by galls, which usually are 5 mm to 10 mm thick and may totally prevent light penetration.

We have shown that precipitation significantly interacted with warming to affect the performance of the studied species. Water is an essential element of all animal body components and metabolic activity, and precipitation is a major signal for many insect species living in dry areas. For example, in tropical savanna and desert systems, many beetle species become active only after a large precipitation event, and then they complete growth, development, and reproduction in a short rainy season. Importantly, the precipitation effect was stronger in warmed than in the ambient temperature treatment for the maggots with intact galls. This indicates that the larval maggots are more likely to survive and emerge earlier under warmer and more humid conditions. Such species responses to warming and precipitation could help to adapt to environmental change in the spring of the Tibetan Plateau.

Previous climatic records show that precipitation in spring (April to June) fluctuates considerably with a maximum of 220 mm and minimum of 76 mm, and with less than 100 mm in eleven out of 53 years (data from Hongyuan County climate station). This indicates that spring drought is not unusual at the study site, which would be detrimental to both plant and animal activities. Indeed, modeling work shows that the spring greening of the plateau plants is not only driven by temperature but also triggered by precipitation. Thus, such plastic responses to precipitation and temperature might help synchronize the timings of fly emergence and its host plant flowering.

The comparison of *U. stylata* performance in the gall-intact and gall-removed treatments shows that the galling-making habit plays a decisive role in the sensitivity of larval maggots to precipitation as well as in modulating the warming effect on the survival and timing of adult emergence. Specifically, the precipitation effects on
both the mortality and the phenotype disappeared when the galls were removed. Moreover, the warming effect on mortality rate varied in direction depending on whether galls were intact or removed. Galls mediated climate effects, and this particular function can be attributed to the properties of the gall tissue. The galls mainly consist of plant fibers, and after plants die off, they are usually well insulated against temperature fluctuations outside, unless they get wet\textsuperscript{39}. Consequently, when the larvae are living within galls, they can detect the humidity of gall tissues: when they are naked they hardly ‘sense’ the precipitation intensity (presumably because of quick water loss and ‘transient feeling’), as reflected by our results. As noted above, having galls and sensing precipitation is beneficial to the species for survival and phenological adaptation, but it can also be costly\textsuperscript{44}. For example, the gall formation needs the costs of the larval physiological processes to manipulate plant nutrient transport and cell division\textsuperscript{32}. In extremely dry years, having galls may cause a higher mortality rate because dry galls could be too compact for adults to emerge. Consistently, our results show that galls significantly decreased the adult fresh mass and induced a high mortality rate in the reduced precipitation treatment. It should be noted that it is not clear why males and females differed in their responses in the timing of emergence and adult fresh mass to the treatment factors. The differences may have unknown consequences for the fitness of the focal species, which needs further exploration.

In summary, we revealed that warming and altered precipitation interactively affected the mortality rate and the timing of adult emergence in spring for *Urophora stylata* in an alpine meadow of the Tibetan Plateau. Moreover, our results also demonstrated the important role of gall-making habit in mediating the response of the studied species to climate change. These results collectively suggest that the effect of elevated temperature on the performance of species may depend on other environmental conditions, such as variations in precipitation, and species traits like the formation of galls. Long-term studies are needed to explore the interactive effects of multiple environmental change agents as well as that of species traits on species population dynamics in a changing world.

**Methods**

**Study site.** This study was conducted in Hongyuan Country (32°48′N, 102°33′E), Sichuan province, China, in the eastern part of the Tibetan Plateau. The altitude is ca. 3500 m above sea level, and the climate is characterized by a short and cool summer and a long and cold winter. Mean annual temperature is 6.9 °C, with the maximum monthly average temperature being 10.9 °C (July) and the minimum monthly average temperature being −10.3 °C (January); mean annual precipitation is 744 mm, which mainly occurs during May to August\textsuperscript{45}. Meteorological data during 1961 and 2013 collected by the local climate station showed that the mean annual temperature has increased at a rate of 0.29 °C per decade during this time period\textsuperscript{46}.

Alpine meadow is the major vegetation type in the study area. Plant coverage mostly exceeds 90% in the meadow. Sedges like Kobresia setchwanensis and Blysmus sinocompressus dominate lowland and high soil moisture communities, while forbs like Potentilla anserina, Saussurea nigrescens, and Anemone trullifolia var. linearis are dominant species in communities with relatively low soil moisture content.

Arthropod species, such as dung decomposers\textsuperscript{47}, pollinators\textsuperscript{48}, and herbivorous insects\textsuperscript{49} are diverse and abundant in the meadow.

**Focal species.** *Urophora stylata* (Diptera: Tephritidae) is a common univoltine fly, which is a pre-dispersal seed predator species of *Carduus nutans* (Asteraceae). The plant species is a biannual forb occurring along the roadside and other disturbed sites in the study area. Height of mature plants is between 0.5 m and 1.5 m, and each plant individual bears 5 to 50 capitula, which flower from late May to late August.

Female flies oviposit in the capitula of host plants before flowering, and larval maggots feed on developing seeds within the capitula until pupating. The larvae usually form visible galls two weeks after oviposition, and then they continue to grow and develop within the galls. In late September before winter, the galls, together with the maggots, drop down to the ground surface, as a result of the die-off of aboveground plant parts. Larval maggots overwinter within the galls and then pupate and emerge out of the galls next spring. However, it is not unusual that the galls are found damaged and the maggots are exposed to external environments due to unknown reasons.

**The experiment.** We conducted a three-factorial experiment involving three factors including artificial warming (ambient vs. warmed), manipulated precipitation (30% increase, average of the past 53 years, 30% decrease), and gall presence (gall intact vs. removed), resulting in 12 treatments in total.

We collected medium sized galls from the fenced meadow in late April 2015 when maggots living in capitula were inactive. There were $4.2 \pm 0.6$ ($N = 20$) maggots in each gall. For the treatments with intact galls, the galls were placed onto 48 plastic pots (Diameter = 29.8 cm, depth = 25 cm), in which soil collected from the same site was filled to depth of 27 cm. Each pot had 6 galls that were evenly distributed, and pots were replicated eight times, resulting in 288 galls in total (48 pots × 6 galls). For the treatments with galls removed, we carefully removed the gall tissues using a knife and collected 90 naked larval maggots, each of which was enclosed by a 2 × 2 cm paper bag to prevent too quick water loss from the larval bodies, as did by Charlet (1989) for larval weevils (*Cylindrocopturus adspersus*) that were moved out of flower stalks. Each of the six gall-removed treatments had 15 replicates (paper bags). All the paper bags of the same treatment were placed onto one pot, such as done for the gall-intact treatment.

Warming was achieved by placing the pots under a 1 m long 1600 W infrared heater that hang 1.2 m above the ground surface. This infrared heater raised the temperature by 1.9 °C on average during the experiment (Supporting materials, Fig. S1A). The average precipitation was calculated from daily precipitation between 1961 and 2013, as recorded by Hongyuan County climatic station. Precipitation was manipulated every third day by increasing (+30%) and decreasing (−30%) precipitation and comparing it with a control treatment with average precipitation of the past 53 years. For each time, the water (Supporting materials, Fig. S1B) was directly sprayed to the galls with at speed of in ca. 200 ml per min.
The experiment was started on 28-Apr-2015 in a fenced plot within a typical alpine meadow. On 31-May-2015 before adult emergence, we enclosed all the galls as well as the paper bags and associated naked maggots using bags made of steel screen with the mesh size of 2 × 2 mm. During the experiment, the paper bags were replaced for the naked maggots at the same frequency as for precipitation manipulation.

Starting on 01-Jun-2015, we checked every day if there was a newly emerged adult fly in the steel bags. Once found, the adult was put into a 2 ml plastic tube, which was kept at 0 °C in a refrigerator after being weighed. We recorded the emergence day as the days after the beginning of the experiment for each gall (larva) and each paper bag. Ten days after the last flies had emerged from the galls, we removed galls or bags and found that all the larvae or pupae were dead in the galls or bags.

Data analysis. The normality of the data on each variable was tested before analysis. Nested Four-way ANOVAs (individual maggots nested in galls) were used to determine the effect of warming, precipitation, gall, and sex on the on the timing of emergence and fresh body mass of the adult flies. Sex was included as factor because males and female flies largely differ in life-history traits (see also ref. 50). Post hoc Tukey HSD tests were employed to determine difference between treatments whenever a significant effect (P < 0.05) was found. General linear model (GLM; with binomial errors) was employed to determine the effects of warming, precipitation, and gall on the mortality rates of the larval maggots. As larval male and females were visually indistinguishable, sex was not included as a treatment factor here. "glft" within “multcomp” package was used to conduct post hoc tests after GLM. All the data analyses were performed by R51.

References

1. Walther, G.-R. et al. Ecological responses to recent climate change. Nature 416, 389–395 (2002).
2. Parmesan, C. Ecological and Evolutionary Responses to Recent Climate Change. Annu. Rev. Ecol. Evol. S. 37, 637–669 (2006).
3. Visser, M. E. & Both, C. Shifts in phenology due to global climate change: the need for a yardstick. Proc. Roy. Soc. Lond. B. Bio. 272, 2561–2569 (2005).
4. Visser, M. E. Keeping up with a warming world; assessing the rate of adaptation to climate change. Proc. Roy. Soc. Lond. B. Bio. 275, 649–659 (2008).
5. Stireman, J. O. III et al. Climatic unpredictability and parasitism of caterpillars: implications of global warming. Proc. Natl. Acad. Sci. USA 102, 17384–17387 (2005).
6. Abarca, M. & Lill, J. T. Warming affects hatching time and early season survival of eastern tent caterpillars. Oecologia. 179, 901–912 (2015).
7. Doiron, M., Gauthier, G. & Lèvesque, E. Trophic mismatch and its effects on the growth of young in an Arctic herbivore. Glob. Change Biol. 21, 4364–4376 (2015).
8. Sentis, A., Hemptinne, J.-L. & Brodeur, J. Effects of simulated heat waves on an experimental plant–herbivore–predator food chain. Glob. Change Biol. 19, 833–842 (2013).
9. Ouyang, F. et al. Early eclosion of overwintering cotton bollworm moths from warming temperatures accentuates yield loss in wheat. Agr. Ecosyst. Environ. 217, 89–98 (2016).
10. Badeck, F. W. et al. Responses of spring phenology to climate change. New Phytol. 162, 295–309 (2004).
11. Lemoine, N. P. & Buxkepile, D. E. Temperature-induced mismatches between consumption and metabolism reduce consumer fitness. Ecology 93, 2483–2489 (2012).
12. Bale, J. S. et al. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. Glob. Change Biol. 8, 1–16 (2002).
13. Visser, M. E. & Holleman, L. J. M. Warmer springs disrupt the synchrony of oak and winter moth phenology. Proc. Roy. Soc. Lond. B. Bio. 268, 289–294 (2001).
14. van Asch, M. & Visser, M. E. Phenology of forest caterpillars and their host trees: the importance of synchrony. Annu. Rev. Entomol. 52, 37–55 (2007).
15. Jepson, J. U. et al. Rapid northwards expansion of a forest insect pest attributed to spring phenology matching with sub-Arctic birch. Glob. Change Biol. 17, 2071–2083 (2011).
16. Logan, J. A., Regnaître, J. & Powell, J. A. Assessing the impacts of global warming on forest pest dynamics. Front. Ecol. Environ. 1, 130–137 (2003).
17. Kiritani, K. Predicting impacts of global warming on population dynamics and distribution of arthropods in Japan. Popul. Ecol. 48, 5–12 (2005).
18. Kurz, W. A. et al. Mountain pine beetle and forest carbon feedback to climate change. Nature 452, 987–990 (2008).
19. Robinet, C. & Roques, A. Direct impacts of recent climate warming on insect populations. Integ. Zool. 5, 132–142 (2010).
20. Battisti, A. et al. Expansion of geographic range in the pine processionary moth caused by increased winter temperatures. Ecol. Appl. 15, 2084–2096, doi: 10.1899/04-1903 (2005).
21. Bale, J. S., Harrington, R. & Clough, M. S. Low temperature mortality of the peach–potato aphid Myzus persicae. Ecol. Entomol. 13, 121–129 (1988).
22. Crotzier, L. Warmer winter drive butterfly range expansion by increasing survivorship. Ecology 85, 231–241 (2004).
23. Primack, R. B. et al. Spatial and interspecific variability in phenological responses to warming temperatures. Biol. Conserv. 142, 2569–2577 (2009).
24. Ellwood, E. R. et al. Disentangling the paradox of insect phenology: are temporal trends reflecting the response to warming? Oecologia 168 (2011).
25. Hodgson, J. A. et al. Predicting insect phenology across space and time. Glob. Change Biol. 17, 1289–1300 (2011).
26. Bale, J. S. & Hayward, S. A. Insect overwintering in a changing climate. J Exp. Biol. 213, 980–994 (2010).
27. Trenberth, K. E. Changes in precipitation with climate change. Clim. Res. 47, 123 (2011).
28. Donat, M. G., Lowry, A. L., Alexander, L. V., Ogorman, P. A. & Maher, N. More extreme precipitation in the world’s dry and wet regions. Nature Clim. Change advance online publication, doi: 10.1038/nclimate2941 (2016).
29. Eskafi, F. M. & Fernandez, A. Larval–Pupal Mortality of Mediterranean Fruit Fly (Diptera: Tephritidae) from Interaction of Soil, Moisture, and Temperature. Environ. Entomol. 1670 (1990).
30. D’Amico, V. & Elkinton, J. S. Rainfall effects on transmission of gypsy moth (Lepidoptera: Lymantriidae) nuclear polyhedrosis virus. Environ. Entomol. 24, 1144–1149 (1995).
31. Hajek, A. E., Tobin, P. C. & Haynes, K. J. Replacement of a dominant viral pathogen by a fungal pathogen does not alter the collapse of a regional forest insect outbreak. Oecologia 177, 785–797 (2015).
32. Abrahamson, W. G. & Weis, A. E. Evolutionary ecology across three trophic levels: goldeneers, gallmakers, and natural enemies Vol. 29 (Princeton University Press, 1997).
33. Irwin, J. T. & Lee, R. E. Mild winter temperatures reduce survival and potential fecundity of the goldenrod gall fly, Eurosta solidaginis (Diptera: Tephritidae). *J. Ecol. Appl.* 46, 655–661 (2000).
34. Liu, X. & Chen, B. Climatic warming in the Tibetan Plateau during recent decades. *Int. J. Climatol.* 20, 1729–1742 (2000).
35. Qiu, J. China: the third pole. *Nature News* 454, 393–396 (2008).
36. Shen, M., Piao, S., Cong, N., Zhang, G. & Jassens, I. A. Precipitation impacts on vegetation spring phenology on the Tibetan Plateau. *Glob. Change Biol.* 21, 3647–3656 (2015).
37. Piao, S., Fang, J. & He, J. Variations in Vegetation Net Primary Production in the Qinghai-Xizang Plateau, China, from 1982 to 1999. *Climatic Change* 74, 253–267 (2006).
38. Sinclair, B. J., Vernon, P., Jako Klokh, C. & Chown, S. L. Insects at low temperatures: an ecological perspective. *Trends Ecol. Evol.* 18, 257–262 (2003).
39. Angilleta Jr, M. J., Niewiarowski, P. H. & Navas, C. A. The evolution of thermal physiology in ectotherms. *J. Therm. Biol.* 27, 249–268 (2002).
40. Wright, S. J. Seasonal drought and the phenology of understory shrubs in a tropical moist forest. *Ecology* 1643–1657 (1991).
41. Parker, A. R. & Lawrence, C. R. Water capture by a desert beetle. *Nature* 414, 33–34 (2001).
42. Li, R. et al. Leaf unfolding of Tibetan alpine meadows captures the arrival of monsoon rainfall. *Sci. Rep.* 6, 20985 (2016).
43. Stone, G. N. & Schönrogge, K. The adaptive significance of insect gall morphology. *Trends Ecol. Evol.* 18, 512–522 (2003).
44. Shorthouse, J. D., Wool, D. & Raman, A. Gall-inducing insects – Nature’s most sophisticated herbivores. *Basic Appl. Ecol.* 6, 407–411 (2005).
45. Zhao, J., Yang, Y., Xi, X., Zhang, C. & Sun, S. Artificial Warming Facilitates Growth but Not Survival of Plateau Frog (*Rana kukunorisis*) Tadpoles in Presence of Gape-Limited Predatory Beetles. *PLoS One* 9, e98252 (2014).
46. Mu, J. et al. Artificial asymmetric warming reduces nectar yield in a Tibetan alpine species of Asteraceae. *Ann. Bot.* (2015).
47. Wu, X., Griffin, J. N., Xi, X. & Sun, S. The sign of cascading predator effects varies with prey traits in a detrital system. *J. Anim. Ecol.* 84, 1610–1617 (2015).
48. Mu, J. et al. Domesticated honey bees evolutionarily reduce flower nectar volume in a Tibetan lotus. *Ecology* 95, 3161–3172 (2014).
49. Xi, X., Griffin, J. N. & Sun, S. Grasshoppers amensalistically suppress caterpillar performance and enhance plant biomass in an alpine meadow. *Oikos* 122, 1049–1057 (2013).
50. Xi, X., Wu, X., Nylin, S. & Sun, S. Body size response to warming: time of the season matters in a tephrabrid fly. *Oikos* 125, 386–394 (2016).
51. R Development Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/. (Accessed 1th June, 2014).

Acknowledgements
We thank Yangheshan Yang, Hanxiang Chen and Rui Cao for field assistances and Qinghai-Tibetan Research Base of Southwest Normality University for providing research convenience. This study was supported by National Program on Key Basic Research Project (973 Program, 2013CB956300), National Natural Science Foundation of China (31470482, 31325004 and 31500395). All experiments were performed in compliance with the laws of the People’s Republic of China.

Author Contributions
X.X. and S.S. conceived and designed the experiments, X.X. and D.L. performed the experiments, X.X. and D.L. analyzed the data, X.X., D.L., Y.P., N.E. and S.S. wrote the manuscript.

Additional Information
Supplementary information accompanies this paper at http://www.nature.com/srep
Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Xi, X. et al. Experimental warming and precipitation interactively modulate the mortality rate and timing of spring emergence of a gallmaking Tephritid fly. *Sci. Rep.* 6, 32284; doi: 10.1038/srep32284 (2016).

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/

© The Author(s) 2016