Immune defence under extreme ambient temperature

Otto Seppälä^{1,2,*} and Jukka Jokela^{1,2}

¹EAWAG, Swiss Federal Institute of Aquatic Science and Technology, 8600 Dübendorf, Switzerland

²ETH-Zürich, Institute of Integrative Biology, 8092 Zürich, Switzerland

*Author for correspondence (otto.seppaelae@eawag.ch).

Owing to global climate change, the extreme weather conditions are predicted to become more frequent, which is suggested to have an even greater impact on ecological interactions than the gradual increase in average temperatures. Here, we examined whether exposure to high ambient temperature affects immune function of the great pond snail (*Lymnaea stagnalis*). We quantified the levels of several immune traits from snails maintained in a non-stressful temperature (15°C) and in an extreme temperature (30°C) that occurs in small ponds during hot summers. We found that snails exposed to high temperature had weaker immune defence, which potentially predisposes them to infections. However, while phenoloxidase and antibacterial activity of snail haemolymph were reduced at high temperature, haemocyte concentration was not affected. This suggests that the effect of high temperature on snail susceptibility to infections may vary across different pathogens because different components of invertebrate immune defence have different roles in resistance.

Keywords: immune function; immunocompetence; pathogen resistance; *Lymnaea stagnalis*

1. INTRODUCTION

Environmental conditions, such as ambient temperature, often modify host–pathogen interactions [1,2]. Most environments show some natural variation in temperature, but are also affected by the anthropogenic climate change [3]. Owing to climate change, the average air temperature is increasing [4], but also extreme weather conditions such as summer heat waves are expected to become more frequent [5,6]. The increasing frequency of extreme environmental conditions has been suggested to have an even greater impact on ecological interactions than the gradual increase in average temperatures [7]. Extreme weather conditions may also play a major role in determining the outcomes of host–pathogen interactions. For example, during the 2003 European heat wave, pathogen-induced mortality was exceptionally high in three-spined sticklebacks *Gasterosteus aculeatus* [8].

However, the discussion of the effects of climate change on host–pathogen interactions has largely focused on the possible range expansion of disease-causing agents owing to a gradual increase in average temperatures [9,10]. Only very few studies have

examined the effects of temperature variation on host–pathogen interactions in current host and pathogen populations (but see [11–13]). Furthermore, earlier studies have focused on specific interactions between certain host and pathogen species. Such studies are necessarily specific to the study systems being used, and can be confounded by non-immunological factors affecting susceptibility [14]. Therefore, direct investigations examining alterations in host immune function are needed to understand the potential effect of temperature variation on pathogen resistance [15]. In this study, we experimentally examined the effect of extreme ambient temperature on immune function of the great pond snail (*Lymnaea stagnalis*). By quantifying the levels of several immune parameters, we found that snails exposed to high water temperature had a weaker immune defence. Thus, the resistance of snails against pathogens can be expected to be reduced when water temperature is high, which may predispose natural snail populations to disease outbreaks during extreme summer temperatures.

2. MATERIAL AND METHODS

(a) Experimental animals

Lymnaea stagnalis is a holarctic freshwater snail inhabiting shallow littoral zones of stagnant waters such as lakes and ponds. In these habitats, the snails are infected by a community of pathogens. For example, snails are hosts for several trematode parasites [16], many of which are highly virulent by castrating the snails and increasing their mortality. Furthermore, natural habitats of snails (especially small ponds) are susceptible to high temperatures during extreme weather events such as heat waves.

Snails for this study came from a laboratory stock population (F₂ generation) originating from a pond in Zürich, Switzerland (47°22' N, 8°34' E). Before the experiment, we maintained the snails individually in plastic cups filled with 0.2 l of aged tap water at 15°C in two incubators (ICP 700, Memmert GmbH & Co. KG, Germany) for two weeks. We fed the snails with fresh lettuce ad libitum, and transferred them daily into new cups with fresh water.

(b) Experimental design

We maintained the snails (initial size 19–30 mm) as described above and randomly assigned them into two different temperatures (15 and 30°C) and water quality (clean and micro-organism-enriched) treatments (40 individuals per treatment combination). We transferred the snails in 'clean water treatment' daily into new cups with fresh water. We maintained the snails in 'micro-organism-enriched water treatment' in the same cups during the whole experiment, and changed the water within these cups every second day. The aim of water quality treatment was to manipulate snails' immune activity. This method does not predispose snails to any specific pathogens but to a community of opportunistic micro-organisms that grow in water [17]. These micro-organisms invade the snails as a part of normal exchange between snail haemolymph and the surrounding water, which challenges snail immune function [18]. The initial size of the snails in different treatments did not differ (analyses of variance (ANOVA), temperature: $F_{1,146} = 0.004$, $p = 0.952$; water quality: $F_{1,146} = 2.107$, $p = 0.149$).

In this experiment, we used a non-stressful temperature (15°C) and a stressful temperature (30°C) [19] that can occur during summer extremes. We used 30°C as the high-temperature treatment because this temperature periodically occurs in small ponds in Europe during summer extremes, but it is close to the potential temperature maximum snails are exposed to in these habitats (A. Laurila 2010, unpublished data; U. Tobler 2010, unpublished data). It is important to note that this temperature treatment would not be realistic for snails originating from other habitats such as lakes in which the temperature does not get that high.

After one week maintenance in the different treatments, we measured the concentration of haemocytes, phenoloxidase (PO) activity and antibacterial activity of haemolymph from each snail (see a detailed description of the methods in [20]). Ten snails died during the experiment (zero to five individuals per treatment combination), and we excluded them from the data.

(c) Statistical analyses

To examine the effects of experimental treatments on snail immune defence, we first analysed the variation in snail immune function using a multivariate analysis of variance (MANOVA). In this analysis, we used haemocyte concentration, PO activity and antibacterial activity of snail haemolymph as response variables. We log-transformed all variables to fulfil the assumptions of MANOVA. In the analysis, we used a model with temperature (15 and 30°C) and water quality (clean and micro-organism-enriched) as fixed factors. We included the interaction between the factors into the model. After this, we analysed the variation in each response variable separately using ANOVA and similar models as described above to examine whether the observed effects were similar across different immune parameters.

3. RESULTS

In general, high (30°C) water temperature reduced snail immune defence when compared with snails maintained at 15°C (MANOVA: Pillai's trace = 0.599, $F_{3,143} = 71.079$, $p < 0.001$; figure 1). Water quality did not have a main effect on immune function (MANOVA: Pillai's trace = 0.037, $F_{3,143} = 1.852$, $p = 0.140$), but its impact depended on water temperature revealed by a significant interaction between the factors (MANOVA: Pillai's trace = 0.064, $F_{3,143} = 3.252$, $p = 0.024$). However, the above effects varied between the measured immune parameters (table 1 and figure 1). Neither temperature nor water quality affected haemocyte concentration of snail haemolymph, but PO activity and antibacterial activity were reduced in snails maintained at high temperature (table 1 and figure 1). Furthermore, micro-organism-enriched water induced higher PO activity at 15°C, but not at 30°C (table 1 and figure 1).

4. DISCUSSION

In this study, we examined how immune parameters of a freshwater snail *L. stagnalis* are affected by ambient temperature. By exposing snails to 15°C (non-stressful temperature) and 30°C (extreme temperature that occurs during summer extremes), we found that high temperature reduced snail immune defence. This suggests that extreme weather events can reduce pathogen resistance of snails, which may predispose natural snail populations to disease outbreaks, for example, during heat waves.

The effect of temperature on snail immune function, however, varied among studied immune traits. Haemocyte concentration of snail haemolymph was not affected by temperature, but the levels of PO activity and antibacterial activity were reduced at high temperature. Furthermore, high temperature reduced the ability of snails to induce higher PO activity when exposed to micro-organism-enriched water. The last result may, however, be confounded by potential qualitative and quantitative differences in micro-organism communities among temperature treatments if, for example, their species compositions depend on temperature. The reason for the differences between immune traits is unknown, but it is possible that haemocyte concentration responds differently to environmental factors when compared with other immune parameters (see also [20]) because haemocytes are involved, not only in immune defence, but also in many other functions

Figure 1. (a) Haemocyte concentration (cells μl^{-1} ; mean $\pm$ 95% CI), (b) phenoloxidase (PO) activity (milliunits; mean $\pm$ 95% CI) and (c) antibacterial activity (milliunits; mean $\pm$ 95% CI) of haemolymph in *L. stagnalis* snails maintained in different water temperatures (15 and 30°C) and water quality (clean (open circles) and micro-organism-enriched (filled circles)) for 7 days.

of the haemolymph [21]. Nevertheless, these findings suggest that the increase in snail susceptibility to infections owing to high ambient temperature may vary across different pathogens. This is because

Table 1. ANOVAs for the haemocyte concentration, PO activity and antibacterial activity of snail haemolymph by water temperature (15 and 30°C) and water quality (clean and micro-organism-enriched).

	source	d.f.	MS	F	p
haemocyte concentration	temperature (<i>T</i>)	1	0.195	0.642	0.424
	water quality (<i>W</i>)	1	0.403	1.328	0.251
	<i>T</i> × <i>W</i>	1	0.287	0.947	0.332
	error	145	0.303		
PO activity	temperature (<i>T</i>)	1	10.591	63.662	<0.001
	water quality (<i>W</i>)	1	0.216	1.299	0.256
	<i>T</i> × <i>W</i>	1	1.238	7.444	0.007
	error	145	0.166		
antibacterial activity	temperature (<i>T</i>)	1	4.815	65.331	<0.001
	water quality (<i>W</i>)	1	0.219	2.966	0.087
	<i>T</i> × <i>W</i>	1	0.052	0.706	0.402
	error	145	0.074		

different components of the invertebrate immune system have different roles in defence; haemocytes are important for phagocytosis and encapsulation [22], PO is a component of oxidative defences [23] and humoral antimicrobial proteins are used against microbial infections [24].

It is important to note, however, that as we did not expose snails to any pathogens, the possible effects of water temperature on host–pathogen interactions cannot be addressed from pathogen perspective in the present study. While studies using experimental infections would also be interesting, we concentrated on host responses to temperature variation by directly measuring several immune parameters. We chose to do this because experimental infections are necessarily specific to the particular pathogens being used, and they can be confounded by non-immunological factors affecting susceptibility [14]. Furthermore, understanding the effects of temperature variation on pathogen epidemiology would require experiments at all stages of pathogen life cycle [11].

To conclude, we found that snail immune defence is context dependent so that exposure to high ambient temperature reduces snail immune function. This suggests that the increasing frequency of extreme weather events owing to climate change can pose a threat for natural host populations by predisposing them to disease outbreaks (see also [15]). However, further research is needed to understand how changes in immune defence are related to other life-history traits, and if organisms are able to adapt to changing environmental conditions (i.e. if they show genetic variation in tolerance for high temperatures). Such studies are important as they can reveal whether observed alterations are owing to physiological stress or phenotypic adaptation to changed conditions (e.g. terminal investment), and estimate the risk of climate change for natural populations in the long run.

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