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Review

Life extension after heat shock exposure: Assessing meta-analytic evidence for hormesis

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ABSTRACT

Hormesis is the response of organisms to a mild stressor resulting in improved health and longevity. Mild heat shocks have been thought to induce hormetic response because they promote increased activity of heat shock proteins (HSPs), which may extend lifespan. Using data from 27 studies on 12 animal species, we performed a comparative meta-analysis to quantify the effect of heat shock exposure on longevity. Contrary to our expectations, heat shock did not measurably increase longevity in the overall meta-analysis, although we observed much heterogeneity among studies. Thus, we explored the relative contributions of different experimental variables (i.e. moderators). Higher temperatures, longer durations of heat shock exposure, increased shock repeat and less time between repeat shocks, all decreased the likelihood of a life-extending effect, as would be expected when a hormetic response crosses the threshold to being a damaging exposure. We conclude that there is limited evidence that mild heat stress is a universal way of promoting longevity at the whole-organism level. Life extension via heat-induced hormesis is likely to be constrained to a narrow parameter window of experimental conditions.

Contents

1. Introduction

Hormesis is defined as an adaptive response to stressors, whereby low doses stimulate and high doses inhibit an observed trait, such as health, growth or longevity (Calabrese and Baldwin, 2002). Provided that the study design has the capacity to detect a U-shaped dose response, hormetic responses are fairly common in a range of biological models (microbial, plant and animal) and also for exposures to various toxins (Calabrese and Blain, 2011). Hormesis can also occur in response to non-toxicological stressors, such as hypergravity and thermal stress (Le Bourg, 2011). Hormetic responses to heat shock are particularly interesting because we know a potential underlying molecular mechanism, induction of heat shock proteins (HSPs).

Heat shock proteins are molecular chaperones, refolding damaged proteins and inhibiting both stress-mediated and programmed cell death (Verbeke, 2001). The HSPs induction response declines with age in neuronal tissue, skeletal and cardiac muscle and the liver (Calderwood et al., 2009). Accumulated protein

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damage is one of the prime candidates to explain ageing (Rattan, 2008a; Liu and Xu, 2011). Thus, extending the activity of the HSPs response should, in turn, increase an animal's longevity. Increased survival from over-expression of HSPs (Tatar et al., 1997) and a positive correlation between HSPs expression level and maximum longevity (Salway et al., 2011) support this expectation. Stimulation of HSPs synthesis was suggested as a viable anti-ageing strategy (Calderwood et al., 2009; Le Bourg and Rattan, 2008; Rattan and Demirovic, 2010). Therefore, it is not surprising that heat-induced hormesis receives increasing attention in the gerontological literature (Calabrese et al., 2012; Le Bourg, 2011; Rattan, 2008b).

There is strong evidence indicating that HSPs can protect various cell types from protein damage and slow down programmed cell death pathways, postponing cell ageing and death (Calderwood et al., 2009). However, at the whole-organism level, the picture is far from clear (Rattan, 2008b; Sørensen et al., 2007). There are a few major reasons why this is so. Firstly, studies are performed on a range of different animal models (usually invertebrates), which can differ largely in their physiology and life history. Secondly, variable experimental setups, involving heat shocks of different magnitude, duration, number of repeats and spacing, as well as other experimental variables, make direct comparisons among studies practically non-interpretable. Therefore, drawing general patterns and conclusions from the relevant literature has been difficult. To arrive at such general conclusions, one requires quantitative synthesis of the available literature, i.e. meta-analysis. However, combining quantitative data from different species and experiments is methodologically challenging, especially if we have to express difference between survival curves of treatment and control group as a single effect size estimate, which is comparable across studies and species.

Recently, new opportunities for synthesis have been opened by advances in meta-analytic methods, which enable us to deal with the aforementioned issues (Hadfield and Nakagawa, 2010; Hector et al., 2012; Nakagawa and Santos, 2012; Nakagawa et al., 2012). Therefore, the time is ripe for meta-analysis of hormetic effects on longevity. Such a meta-analysis will lead to evidence-based conclusions on this important topic, and it can quantify relative contributions of biological and experimental variables to the observed effects. Furthermore, meta-analysis allows us to identify 'the third factors', which are neither biological nor experimental. Scientists tend to highlight several flagship studies showing clear responses (positive or negative) and studies published in 'high-impact' journals (Murtaugh, 2002; Nieminen et al., 2007). Also, results of experiments with no statistically significant effects are less likely to be published (known as publication bias; Rothstein et al., 2006). These third factors, or biases, might well result in a distorted picture of our understanding of the topic of interest. Indeed, such biases can be systematically examined in meta-analysis, identifying potential factors distorting our perception of the status quo.

The main aim of the current meta-analysis is three-fold: (1) to quantify the overall effect of heat shocks on longevity across animal models, (2) to measure the sensitivity of the hormetic response to a range of experimental parameters and (3) to examine bias in the literature of heat-induced hormesis, which may affect our understanding of this topic of increasing importance.

2. Methods

2.1. Literature search

We followed a systematic review approach, detailed in the PRISMA statement (Moher et al., 2009), as far as it could be applied to a review of multiple-species based studies. We performed the initial literature search using *SCOPUS* database with a query "TITLE-ABS-KEY (horme* AND thermal OR temperat* OR heat*) AND SUBJAREA (mult OR agri OR bioc OR immu OR neur OR phar)", which resulted in a list of 185 publications. From this list we identified potentially relevant literature reviews and experimental studies, which were then used to perform further backward and forward citation searches. We also ran similar queries on *ISI Web of Knowledge* and *Google* to locate any missed publications and 'grey literature' (e.g. unpublished works and dissertations). We used different combinations of the terms "heat", "thermal", "temperature" with "horme*", "shock", "stress", "harden*", "acclimat*", "adaptation", "*treatment" and with the terms "longevity", "life extension" and "life span". We contacted experts in the field asking for help finding relevant studies and for unpublished data. Collected experimental studies and reviews were used for further extensive backward and forward searches. We decided to include studies from grey literature and non-English language studies.

2.2. Study selection and eligibility criteria

Two authors (KLH, ML) independently searched and reviewed potential studies according to a set of eligibility criteria and a final study set for data extraction was identified by consensus. Studies were initially included in the analysis if they passed the following criteria: (1) the study must present experimental data on the whole-organism level (simulated datasets or experiments on cell lines were excluded), (2) the animals in the study must not be genetically modified or selected for traits that could affect heat treatment outcomes (e.g. short or long-lived mutants or heat-resistant selection lines), (3) the study must have a control and heat shock treatment group, on the same diet and standard living conditions, (4) survival of the treated and control individuals was assessed at multiple time points after exposure (represented as survival curves or raw data allowing for construction of survival curves), (5) animals were treated at adult or sub-adult stage (not as eggs), (6) the total duration of heat shock was less than the remaining expected lifespan (i.e. not a continuous exposure to heat stress, but we allowed for multiple shocks of the same intensity at regular intervals (temperature fluctuations) above the standard thermal conditions), and (7) the animals must not be treated with additional challenges such as toxins, parasitism or predation. The final assessment of the studies was based on the full-text publication and additional information obtained from authors, where necessary. During the data extraction stage we also had to exclude any work that failed to provide essential variables for analysis (e.g. number of individuals). The list of excluded studies, with reasons for exclusion, was compiled (see Supplementary data A; Table A.1). Wherever possible, we attempted to request additional data and study details from the study authors.

2.3. Data extraction and coding

Two researchers (KLH, ML) independently extracted the data and resolved disagreements by discussion. We used the *GetData* program (GetData Graph Digitizer, S. Federov, Moscow, Russia) and GraphClick (Arizona Software, Switzerland) to extract information from the published survival curves following the method described in Nakagawa et al. (2012). We measured the percentage of treatment animals that were still alive when 90%, 80%, 70%,. . .20% and 10% of the control animals were alive, for a total of 9 points of comparison (Fig. A.1). We were able to convert the multiple survival comparisons between heat shock and control groups into a single effect size estimate (the logarithm of hazard ratio, ln(HR)) with its associated error (variance(ln(HR))) (Parmar et al., 1998; Williamson et al., 2002; for details see Supplementary data A). A negative ln(HR) value indicated that heat shock treatment

Table 1
List of studies included in our meta-analyses: species name, number of control-treatment comparisons (*N*) extracted from each of the studies, study publication reference and source of data used (figures and tables in the original publications, raw data from the authors).

Species	*N*	References	Data source for effect size calculations
Drosophila nasuta	4	Ranjini and Ramachandra (2011)	Fig. 2A
Drosophila buzzatii	2	Gómez et al. (2009)	Fig. 1A
Drosophila subobscura	3	Maynard Smith (1958)	Fig. 1
Drosophila melanogaster	2	Hercus et al. (2003)	Fig. 2A
Drosophila melanogaster	2	Kuether and Arking (1999)	Fig. 1A
Drosophila melanogaster	6	Le Bourg et al. (2001)	Fig. 1
Drosophila melanogaster	84	Sarup and Loeschcke (2011)	Supplementary Material 1, raw data
Drosophila melanogaster	2	Sørensen et al. (2007)	Fig. 1
Drosophila melanogaster	2	Minois and Vaynberg (2002)	Raw data
Drosophila melanogaster	2	Le Bourg et al. (2009)	Raw data
Drosophila melanogaster	4	Le Bourg et al. (2004)	Raw data
Bicyclus anynana	2	Janowitz and Fischer (2011)	Fig. 2
Helicoverpa armigera	34	Mironidis and Savopoulou-Soultani (2010)	Fig. 4
Trichogramma brassicae	12	Hoffmann and Hewa-Kapuge (2000)	Fig. 5, raw data
Aphidius avenae	2	Roux et al. (2010)	Fig. 4, raw data
Ophraella communa	5	Zhou et al. (2011)	Fig. 1
Panstrongylus megistus	6	Garcia et al. (2003)	Fig. 4.2
Caenorhabditis elegans	8	Galbadage and Hartman (2008)	Fig. 1A, raw data
Caenorhabditis elegans	6	Lithgow et al. (1995)	Fig. 3D
Caenorhabditis elegans	24	Michalski et al. (2001)	Fig. 1 and Table 1
Caenorhabditis elegans	11	Olsen et al. (2006)	Fig. 1A
Caenorhabditis elegans	1	Wiegant et al. (2009)	Fig. 9
Caenorhabditis elegans	10	Wu et al. (2009)	Fig. 1A
Caenorhabditis elegans	3	Yokoyama et al. (2002)	Fig. 3
Saccharomyces cerevisiae	1	Shama et al. (1998a)	Fig. 7a
Saccharomyces cerevisiae	1	Shama et al. (1998b)	Fig. 1
Saccharomyces cerevisiae	2	Swieciło et al. (2000)	Fig. 4

extended the lifespan of the treatment group relatively to the control group; note that we considered 95% highest posterior densities (HPD; also known as credible interval), which did not span across zero as statistically significant in this study. On Cohen's scale, the absolute ln(HR) effect size values of approximately 0.2, 0.6 and 1.1 correspond to "small", "moderate" and "large" effects, respectively (Nakagawa et al., 2012). Estimating the effect size from direct comparison between two survival curves within a species allowed us to compare multiple species with varying life spans.

We also collected the following information for each data point for use as moderators to explain potential heterogeneity and bias in the data: (1) publication information on the paper (publication year, journal name, Thomson ISI 2011 journal impact factor), (2) taxonomic information (strain, species, genus, family, class), (3) sample size of the control and treatment groups, (4) sex of the animals (female, male, mixed-sex, hermaphrodite), (5) control temperature at which the animals were maintained, (6) heat shock treatment details (temperature, duration, number of repeats, recovery time between heat shocks, where applicable, total and relative exposure time, age when the first heat shock started). In many cases, multiple treatment groups (heat shocked at different temperatures or durations) were compared against a single control group, which was also coded in the dataset. We recorded the time at which 10% of the control animals were still alive as a proxy for maximum longevity of a given laboratory population at given experimental conditions.

2.4. Statistical analysis

All statistical analyses were performed in *R* v.2.11 (R Development Core Team, 2011). We modelled the data using Bayesian Markov Chain Monte Carlo generalised linear mixed-effects models implemented in the *MCMCglmm* package (Hadfield, 2010). Model 1 (Table A.2) had only the intercept as its fixed factor with species identity, experiment identity and study identity as random factors (i.e. normal meta-analysis); these random factors accounted for correlated structures arising from shared identities (see also Supplementary data A for the explanation of a correlated structure used due to shared measurement error covariance, which we could not fully account for in our analyses). Different taxonomic levels were largely overlapping in our dataset (e.g. one strain and one species per genus). We selected species as the most informative taxonomic variable, after testing the other taxonomic levels, because it best described the key sources of variation. Model 2 built upon Model 1 by accounting for phylogeny (i.e. similarity between species due to shared evolutionary history). Model 3 is comparable to Model 1, but with species identity as fixed effect. It was used to extract the differences in the effects among species. Model 4 is our full model (i.e. meta-regression); it is equivalent to Model 1 with a full set of moderators (predictors: sex of the animals (hermaphrodite, mixed-sex, female, male), age, heat shock temperature, heat shock duration, heat shock repeats number, heat shock spacing time, control temperature; control longevity; study publication year and impact factor) we expected to influence observed effects. Because the relationship between dose and hormetic response is expected to be U-shaped (Calabrese, 2008), we also included potential quadratic effects for the heat shock temperature and duration, as the main determinants of the heat shock dose (101 out of 241 data points were for experiments with single heat shock). All moderators had been appropriately transformed and scaled prior to inclusion in the models. When interpreting meta-regression results, regression coefficients of scaled continuous moderators can be interpreted as the amount of change in ln(HR) when the moderator value changes by one standard deviation, with all other moderators fixed at their average value (Gelman, 2008). Model 5 is the same as Model 4, except that the sex variable was removed for easier interpretation of the results. All models are summarised in Table A.2.

Heterogeneity was quantified using a modified I^2 statistic for each random effect (see Supplementary data A for the details). We conducted sensitivity analyses by removing the three most influential studies, which contributed a total of 142 data points to the dataset, and re-running our analyses on the remaining 99 effect sizes.

As mentioned earlier, typical publication bias occurs when papers are "missing" from the literature because journals may be

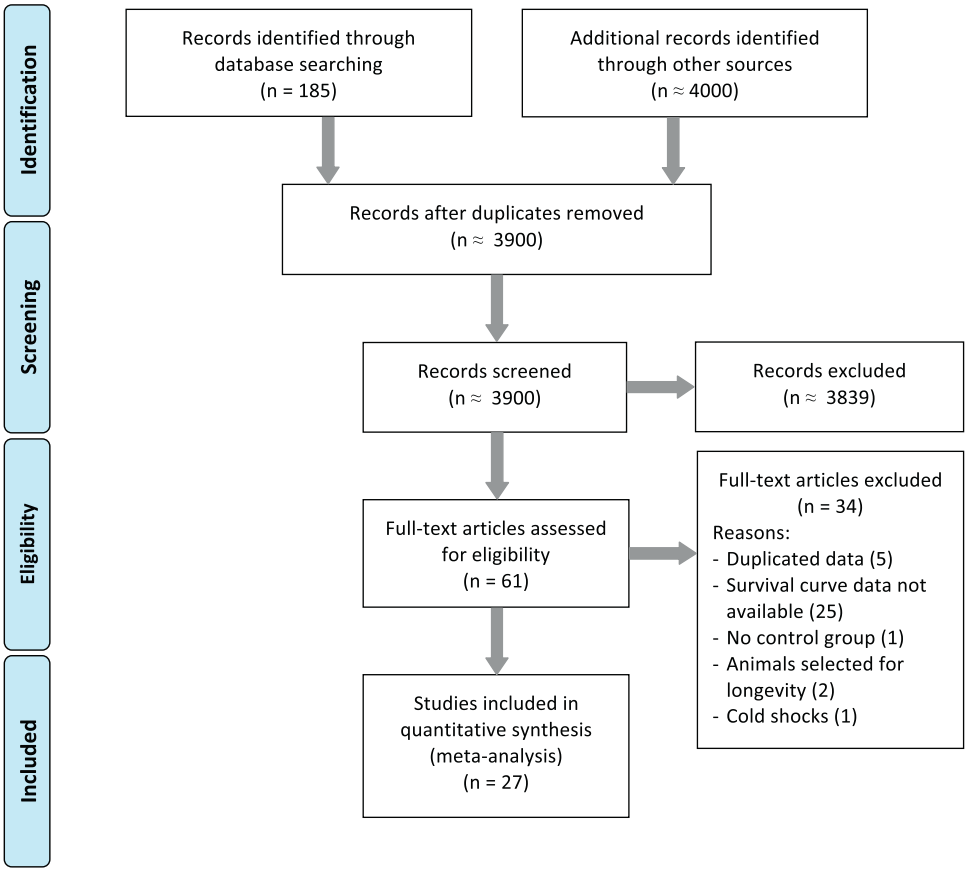

Fig. 1. PRISMA flow chart of search results and study selection process.

less likely to publish studies reporting statistically non-significant results (Rothstein et al., 2006). We assessed publication bias in our dataset by using two approaches: (a) visual inspection of symmetry of funnel plots for the raw data and for the residual effect sizes from the intercept model, and (b) Egger's regression method (Egger et al., 1997) modified to account for data non-independence (details in Supplementary data A). The impact factor of study publication journal was included as one of the moderators in meta-regression, because it was shown that negative results are not only less likely to be published at all, but also when they are published, it tends to be in journals with lower impact factors (Easterbrook et al., 1991; Murtaugh, 2002). Therefore, association between journal impact factor and effect size could indicate potential presence of publication bias. We note that journal impact factor was negatively correlated with publication year in our dataset ($r_s = -0.653$, $t = -4.308$, $df = 25$, $p = 0.0002$). Thus, we conducted additional sensitivity analysis by removing the study which had the outstandingly high impact factor (36.28, Fig. A.2), and which also was published over 30 years earlier than all other studies, and re-running our analyses.

3. Results

The outcomes of our literature search are presented as a PRISMA diagram (Fig. 1). We contacted 33 authors requesting raw data, or additional information, for 25 studies and received 16 replies. Eight authors shared raw data and study details, from which 5 raw data files were included in our analysis (Fig. A.3). In summary, our data set consisted of 241 data points (comparisons of survival between treatment and control group) from 27 studies (Table 1). We included data on twelve species from nine genera of invertebrates: *Drosophila*, *Bicyclus*, *Helicoverpa*, *Trichogramma*, *Aphidus*, *Ophraella*, *Panstrongylus*, *Caenorhabditis*, and *Saccharomyces*.

The results of statistical modelling are summarised in Table A.3. Our intercept-only (null) model, with species, study and experiment as random effects, indicated that there is no significant overall effect of heat shock treatment on longevity, although the HPD interval was fairly wide (Bayesian mixed-effects meta-analysis, BMM: ($\beta_{[\text{meta-analytic mean}]}$) = 0.043, 95% highest posterior density (HPD) = −0.191 to 0.278). When only species effects were evaluated, none of the species was significantly affected by the heat shock treatment, overall (Fig. 2a). Phylogenetic relationships among species explained little variance when added to the null model (Bayesian Phylogenetic Generalised Linear Mixed-effects Model, $I^2_{[\text{phylogeny}]} = 1.5\%$), and therefore, the random effect for phylogeny was omitted from further analyses. This is in congruence with the intercept-only model, where we observed high overall heterogeneity ($I^2 = 95.6\%$), owing to between-paper variation ($I^2_{[\text{paper}]} = 33.3\%$), between-experiment variation ($I^2_{[\text{exp}]} = 18.4\%$) and residual variation ($I^2_{[\text{residual}]} = 43.3\%$), but with between-species variation being low ($I^2_{[\text{species}]} = 0.6\%$) (Table A.2).

In our full model (Model 4, Table A.2), sex, heat shock temperature (linear and quadratic terms), heat shock duration (linear and quadratic terms), number of heat shock repeats, recovery period between repeated heat shocks, control (baseline) temperature, maximum longevity of the control group, paper publication year and journal impact factor were included. When evaluating our models with scaled moderators fitted, it is important to note that the overall intercept reflects the reference values of the moderators included. This means that the overall intercept is based

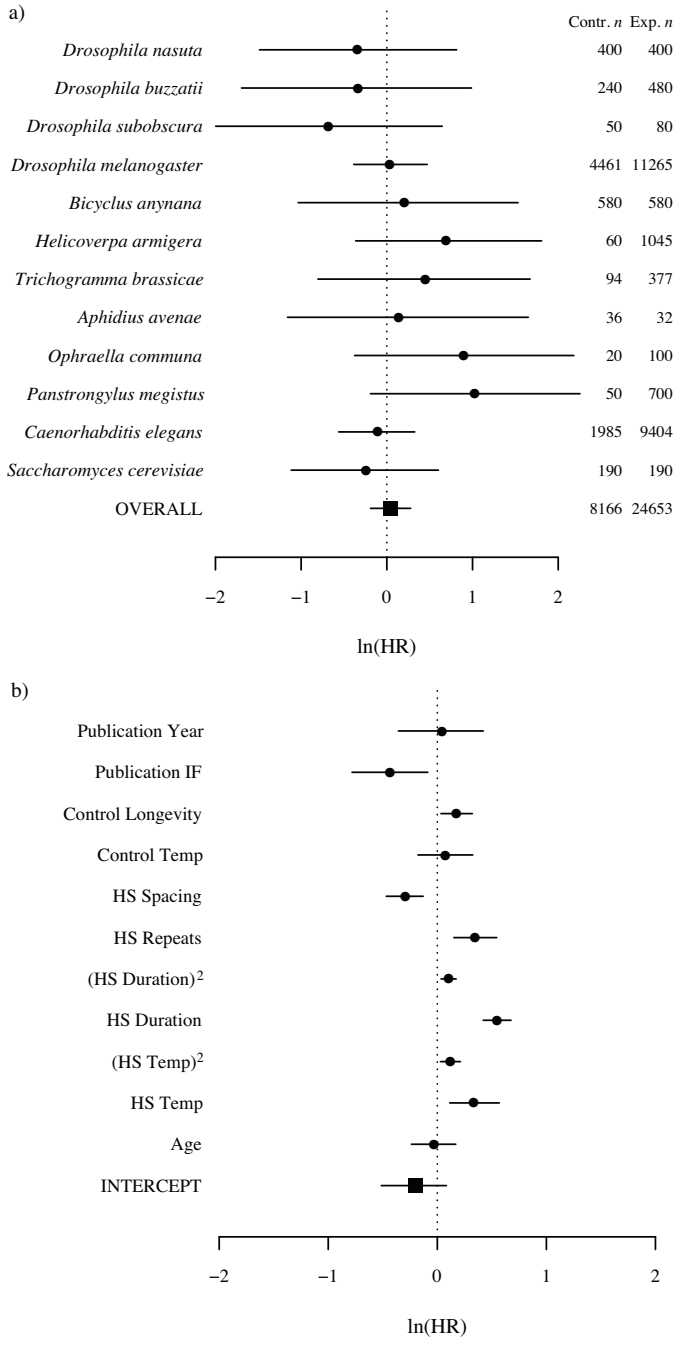

Fig. 2. Forest plots of effect size (logarithm of hazard ratio ln(HR)) estimates for the relationship between heat shock (HS) treatment and survival. A negative ln(HR) value at the intercept indicates that the treatment group outlived the control group for the intercept, but it is not statistically significant effect, because its 95% HPD interval (shown as error bars) includes zero (Nakagawa et al., 2012). Positive ln(HR) values for moderators can be interpreted as decreased likelihood of hormetic effect with increasing values of a moderator. Panel (a) shows the overall meta-analytic mean, as estimated from the intercept model (Model 3), and the individual species effects (as random factors, controlling for experiment and study). Total numbers of individuals contributing to these estimates are provided in the columns to the right. Panel (b) shows the moderators included in the full model (Model 5, Table A.2) with experiment, study and species as random factors, and the overall intercept controlling for the moderators. Included moderators: Publication Year – the year in which the study reporting an effect was published; Control Longevity – maximum longevity of the control group as a proxy of the species and experiment-specific longevity; Control Temp – control temperature used in the experiment; HS Spacing – length of time between the start of subsequent heat shock treatments; HS Repeats – number of the heat shock treatments; HS Duration – duration of a single heat shock treatment; HS Temp – temperature of a heat shock treatment; Age – age of animals at the start of the heat shock treatment; Journal IF – impact factor of a publication journal. All moderators were appropriately transformed and centred, such that the intercept reflects the average value of each continuous variable.

on hermaphrodites (as the binary reference to each of the other sex types: male, female and mixed-sex group, Model 4) and the average values for each of the continuous moderators (Model 4 and Model 5). Overall, as expected from the result from Model 1, heat shock was found to have no significant effect on longevity (BMM: ($\beta_{[\text{meta-analytic mean}]}$) = −0.514, 95% HPD = −1.401 to 0.433, Table A.3). Based on the full model (Model 4, Table A.3), heat shock temperature significantly influenced experimental outcomes, with higher temperatures decreasing the likelihood of any life extension (BMM: ($\beta_{[\text{Shock temperature}]}$) = 0.319, 95% HPD = 0.0750–0.560) and a significant, although small quadratic term, indicative of curvature in the response (BMM: ($\beta_{[(\text{Shock temperature})^2]}$) = 0.129, 95% HPD = 0.038–0.220). Similarly, longer durations of heat shock also decreased the chance of a hormetic response (BMM: ($\beta_{[\text{Shock duration}]}$) = 0.550, 95% HPD = 0.427–0.686), with increasing slope steepness (BMM: ($\beta_{[(\text{Shock duration})^2]}$) = 0.102, 95% HPD = 0.032–0.170). Of the 241 control-treatment group comparisons in our analysis, 102 had only a single exposure to heat shock. More shock repeats decreased the likelihood of life extension (BMM: ($\beta_{[\text{Shock repeat number}]}$) = 0.320, 95% HPD = 0.105–0.536). The recovery period between repeated heat shocks for the other 120 experiments ranged from 1 min to 11 days. Longer periods of recovery between repeated heat shocks were found to increase the likelihood of life extension (BMM: ($\beta_{[\text{Shock spacing time}]}$) = −0.306, 95% HPD = −0.481 to −0.137). There were no significant effects of sex, age at the start of the experiment, control temperature or longevity of the control group (Fig. 2b and Table A.3). However, in the full model (Model 4) control longevity had a tendency to be positively related to the life-extending effects of heat shocks, and this relationship became significant when sex was dropped from the model (Model 5), suggesting that life-extension is more likely in short-lived species and strains. Moreover, we observed that stronger effect sizes were published in journals with higher impact factors (BMM: ($\beta_{[\text{JournalIF}]}$) = −0.494, 95% HPD = −0.857 to −0.130) and we observed no effect of the publication year. The results from Model 4 and Model 5 (without sex) were qualitatively identical, except for the small difference indicated above.

Overall, inclusion of the moderators in our full model explained some heterogeneity in the data (DIC value of 339.64 for the full model vs. 422.61 for the intercept-only model), although the I^2 value decreased very little, from 95.6 to 94.0% (Table A.2). The heterogeneity in the full model was more heavily dependent on variation between species ($I^2_{[\text{species}]}$ = 23.3%) and experiments ($I^2_{[\text{exp}]}$ = 21.6%) then between papers ($I^2_{[\text{paper}]}$ = 9.9%).

Sensitivity analysis revealed that our results are robust to the exclusion of the three studies contributing over the half of the data points and, thus, introducing significant non-independence to our full data set. The results of the subset analyses were qualitatively identical to the results obtained from the full data set, with the same moderators being significant and all effects in the same direction, although there were some changes to the strength of contributions by the linear and quadratic components of the heat shock temperature and duration and the effect of animal age became significant (Tables A.4 and A.5).

When the oldest study with highest impact factor was excluded from the full data set and the analyses were repeated, the pattern of stronger effect sizes being published in journals with higher impact factors disappeared (Tables A.6 and A.7). Visual inspection of the funnel plots (Fig. 3b) did not show perfect symmetry, however the intercept of Egger's regression performed on the residuals and measurement errors was not significantly different

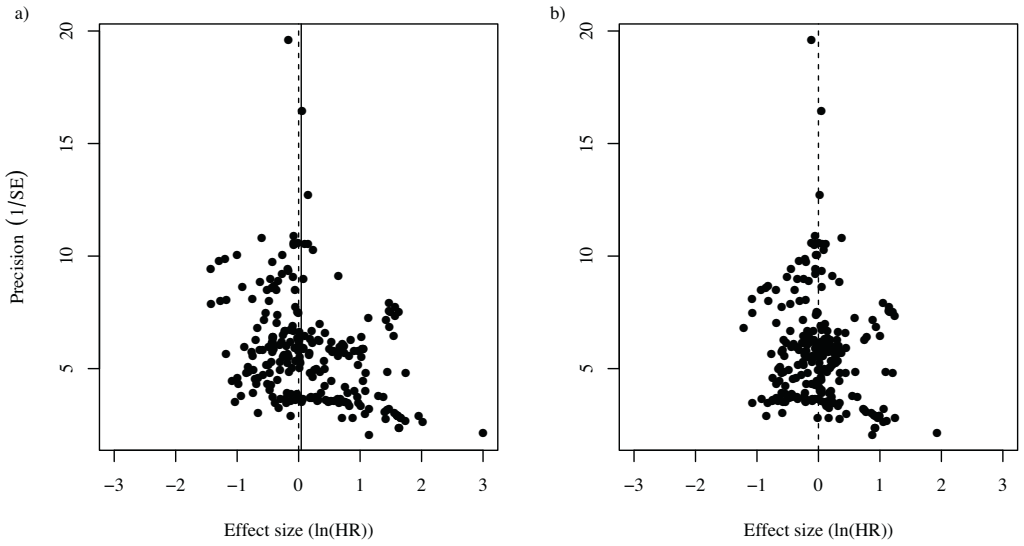

Fig. 3. Funnel plots used to estimate publication bias in the data set. The solid line indicates the overall meta-analytic mean from the intercept model and the dashed line indicates zero, i.e. no effect size. Panel (a) shows the raw effect size estimates from each control versus heat shock treatment group comparison plotted against their precision (1/SE). Panel (b) shows the residual effect sizes from the intercept model.

from zero, indicating little evidence of publication bias (BMM: $(\beta_{[\text{intercept}]}) = 0.669$, 95% HPD = −0.200 to 1.702).

4. Discussion

We aimed to answer three main questions in the presented meta-analysis. We revealed that: (1) the overall effect of heat shocks on longevity in different animal species is close to zero, although very high heterogeneity was observed, (2) heat shock temperature, duration, number of repeats and their spacing can affect the magnitude of hormetic response, and (3) there is no strong evidence for publication bias in the data included in our study. Below, we discuss our main findings in turn.

Firstly, we were unable to detect a general hormetic effect on longevity from heat shock exposure. This is not surprising given that heat shock effects are "often modest and the window for a positive effect to be observed is narrow" (Le Bourg, 2011). Thus, our results are likely to reflect the technical difficulty in detecting hormetic responses (Calabrese, 2008). Also, it is possible that, when a hormetic life-extending effect is induced, its magnitude is probably smaller than that usually observed for biomarkers and short-term life-history traits (most maximum responses in the experimental group being 30–60% greater than in the control group; Calabrese and Baldwin, 2002). However, we cannot relate the magnitude of hormetic effects from our data directly to the single-point endpoint measurements, like those just mentioned, because we were comparing the shapes of the survival curves between two groups of animals. In our data set, the largest recorded difference between survival curves was ln(HR) = −1.43, which on Cohen's scale corresponds to "large" effect (Nakagawa et al., 2012). This, together with the overall effect being close to zero, suggests that while large positive hormetic effects on longevity are possible, they may be more of an exception than the rule, at least within the limits of the data available for our analyses.

The ability to display hormetic response was thought to be independent of the biological model, with possible differences in susceptibility to a given shock factor existing among species (Calabrese, 2008). In our study, the 12 invertebrate species included responded to heat shock in a similar way, indicating that biological variables other than species identity influence whether heat shocks elongate or shorten lifespan. Therefore, we expected that the significant heterogeneity in observed effect sizes could be explained by some of the moderators, i.e. experimental variables.

The meta-regression approach allowed us to go beyond summarising the overall effect by modelling the variables on which the effect potentially depends (our second aim). This means we looked at a range of experimental variables, such as: shock temperature, duration, number of repeats and their spacing, control temperature and longevity, animals' sex and age at the start of experiments. As expected, most of these moderators affected how heat shock treatments shaped longevity of experimental animals. Moreover, the effects of significant moderators were all in the direction expected of hormesis. Heat shocks at temperatures too high, or for durations too long, may push the physiological response beyond the point of beneficial stimulation to an increased likelihood of cellular damage, resulting in shortened lifespans (Rattan, 2008b). In addition, longer time periods between repeated heat shocks were more likely to result in life extension, suggesting that allowing the organism ample time to recover between shocks may be a key issue if repeated stressors are employed (Calabrese and Baldwin, 2001). However, unlike results reported in Olsen et al. (2006), we also observed that animals stressed multiple times were less likely to live longer. This contradiction can be possibly explained by our inclusion of the experiments where large numbers of heat shocks were applied over long periods of time, rather than using a small number of shock repeats, as in Olsen et al. (2006).

The authors of the aforementioned paper also showed that hormetic response in younger animals is stronger than in older animals. We did not detect such age effect when analysing the full data set. However, when the three studies contributing many non-independent data points were excluded, we were able to find an age effect in the expected direction. This whole-organism-level result is in line with the experimental data showing diminished expression levels of heat shock proteins in aged cells and organisms after stress treatments (Alsbury et al., 2004; Sørensen and Loeschcke, 2002). Reduced ability to respond to stress at older age may therefore be an obstacle for the use of hormetic effects for life extension in aged individuals.

Additional moderators used in our analyses included quantification of the baseline/control conditions, which often differed between species and studies. The high variability in the control group is an important factor in estimating the effectiveness of an hormetic treatment (Calabrese et al., 2013). Even animals from the

same species and the same laboratory are likely to show some variation in the shape of their survival curves (Lints et al., 1989). Also, although in most cases the control temperature used in the experiments was the same as the standard rearing temperature for a given species, in some studies it was not the case. Thus, we wanted to be certain that the control group and the control conditions to which the treatment group was compared did not unduly affect our results. Our analyses showed that these two variables did not affect the overall outcomes of the meta-analysis, but it is possible that short-lived strains and species are more likely to benefit more from the hormetic treatment.

We did not observe marked difference between males and females, although several published studies reported sex-specific hormetic effects (e.g. Gómez et al., 2009; Le Bourg et al., 2002; Sarup and Loeschcke, 2011; Sørensen et al., 2007). This result is also surprising in the wider context, as longevity in males and females was shown to respond differently to other manipulations, such as dietary restriction (Nakagawa et al., 2012), regulation of steroid hormones (Tricoire et al., 2009) and inbreeding (Bilde et al., 2009). At the cellular level, different patterns of HSPs expressions are known to be induced in males and females after heat shock treatment (Dahlgaard et al., 1998; Mikulski et al., 2011). Overall, although HSPs are involved in hormetic responses, the relationship between heat shocks and their effect on longevity is complex and most likely depends on the cumulative stress level and the physiological state of cells and organisms.

Finally, publication bias can potentially affect the conclusions of meta-analysis if selective publication of results occurs. We found no evidence for such bias in our dataset, which strengthens our conclusions. Also, a special type of dissemination bias, with strong results supporting heat-induced hormesis being published in journals with higher impact factors (so called place-of-publication bias, Barto and Rillig, 2012) was absent after the oldest paper with highest impact factor was removed from the analyses. Therefore, it seems that the evidence available for testing our research questions is well balanced.

Obviously, one of the limitations of any meta-analysis lies in the set of studies that were included, even if no publication bias was detected. We had to exclude from our analyses many experiments in which survival after heat shock treatment was measured only at one or two time points after exposure (usually 24 h or 48 h), as well as any study for which only summary statistics were available (mean longevity, maximum lifespan, etc.). This is because we were not able to convert these results into a single comparable measure allowing us to combine effects of heat shocks on longevity from multiple studies. We also acknowledge that there exists a significant body of literature available on the hormetic effects of heat treatments on survival of cells (Rattan and Demirovic, 2010), but which was outside of the scope of our analyses.

The main open question in hormesis research is the potential use of mild stress to increase life expectancy in humans. The answer to this question has to be based mainly on data from animal models, since there are no experimental data on humans. Our main conclusion, based on meta-analysis of animal models, is that in the case of heat treatments, the resulting life-extending hormesis is not a consistent phenomenon. This conclusion does not rule out that an optimal heat treatment could be identified for each species, population and individual (Sarup and Loeschcke, 2011), although possibly small lifespan extension can be achieved in a limited window of experimental parameters. Heat shock temperature and duration, as well as spacing of multiple heat shocks are the most important variables shaping hormetic response to heat shocks. Future research could aim to systematically explore whole parameter space of these variables to find optimal parameters for maximum life-extending effect. More promisingly, the research effort should be directed into quantifying hormetic effects on certain aspects of health, such as strengthening responses to additional stressors, improving early survival ability, and extending overall healthspan. We recommend using meta-analytical approaches to summarise accumulating evidence on health-related outcomes of mild stress.

Conflict of interest

The authors have no conflict of interest to declare. None of the studies included in our analyses declared conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.arr.2013.03.005.

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