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Phenomics enables measurement of complex responses of developing animals to global environmental drivers

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Abstract

Phenomics offers technological advances for high-dimensional phenotyping, facilitating rapid, high-throughput assessment of physiological performance and has proven invaluable in global research challenges including drug discovery and food security. However, this rapidly growing discipline has remained largely inaccessible to the increasingly urgent challenge of assessing organismal functional sensitivity to global change drivers. Here, we investigate the response of an ecologically important marine invertebrate to multiple environmental drivers using Energy Proxy Traits (EPTs), a new approach for measuring complex phenotypes captured on video as a spectrum of energy levels across different temporal frequencies in fluctuating pixel values. We imaged three developmental stages of the common prawn *Palaemon serratus* at different salinities and temperatures, and measured EPTs and heart rate, a major proxy of physiological performance in ectotherms present across stages. Significant interactions were detected between temperature, developmental stage and salinity in frequency-specific energy levels. Despite cardiac activity being a significant contributor to the EPT spectra, treatment interactions were different from those observed on EPTs, highlighting additional phenotypic drivers of EPTs. Elevated temperature resulted in a shift of the EPT spectra towards higher frequency signals, indicating a reallocation of resources within the phenome. Using a non-linear dimensionality reduction, we interrogated the responses of EPT spectra in high-dimensional space. We discovered complex developmental-stage specific sensitivities, highlighting both the complexity of phenotypic responses, and the limits of using univariate approaches with pre-selected traits to assess responses to multiple global environmental drivers. EPTs are a high-dimensional, transferrable method of phenotyping, and are therefore highly relevant to addressing the current limitations of traditional methods of phenotyping applied to assessing biological sensitivity to drivers of global change. We predict that EPTs will become an important tool for indiscriminate phenotyping, transferrable between species, developmental stages and experimental designs.

Introduction

Measuring the effects of multiple environmental drivers on biodiversity is imperative during this time of rapid climate change (Boyd et al 2016, Giménez et al. 2021). Approaches to tackle such a complex task are emerging but the task itself remains formidable, particularly when considering the multi-faceted responses of organisms, let alone ecosystems (Queirós et al 2015, Orr et al. 2020, Tekin et al. 2020). Responses can be trait specific and effects of drivers can be highly variable between species and life-stages (Pandori et al 2018). Thus the choice of biological trait significantly shapes the conclusions of a study and raises questions about its utility as a reliable, universal measure of biological sensitivity. In recent years, -omic approaches have increased the potential to elucidate integrated and multivariate biological responses, with a key advantage of reducing the element of luck associated with study design and trait choice (Houle 2007). Yet, -omic approaches are almost entirely focussed at the molecular level, where changes do not always translate into functional phenotypic-level responses, and can therefore be limited in their ecological relevance. The integration of more holistic, phenotypic level approaches, is therefore urgently required.

The phenotype, an organisms observable characteristics, is the highest and most complex level of biological organisation at the individual level, encompassing an astonishingly broad range of biological traits (Houle 2010). Therefore, it is unsurprising that measuring the phenotype has been less amenable to automated and high-throughput global-omic approaches, including transferability between species, life-history stage and biological application. Key challenges in developing transferrable approaches to measuring the phenotype, collectively termed the phenotyping bottleneck, include the availability of hardware (Kültz 2013); and the management, interpretation and integration of high-dimensional phenotypic datasets (Tardieu et al 2017). The crop sciences are perhaps furthest in realising and addressing the challenges of high-throughput phenotyping and on

capitalising from these approaches to significantly advance the understanding of phenotypic responses to global change drivers in plants (Ninomya 2022, Yang et al 2020).

The phenotyping bottleneck restricts progress in the increasingly urgent research challenge of understanding biological sensitivity to multiple environmental drivers at different life-stages. While life stage-specific sensitives are acknowledged, measuring the phenotype in organisms at their most dynamic, and often most sensitive, stage of life presents an obvious challenge and so unsurprisingly early life stages are under-represented in assessments of sensitivity to global change drivers (Pandori et al 2019). Yet the sensitivities of early life stages are an important contributor to effective species sensitivity assessments, informing environmental management, ecological monitoring and the interpretation of biological responses in an increasingly unpredictable world. Furthermore, the phenotyping bottleneck risks causing an overreliance on lethal endpoints, which provide comparability between species but overlook sublethal impacts (Preslawski et al 2005). These sublethal impacts can be both drivers and early warnings of significant, and highly unpredictable, biological responses (Calosi et al 2013, Collins et al 2019).

We have recently developed energy proxy traits (EPTs), a novel approach to measuring complex phenotypes of developing animals in a high-throughput manner using fluctuations in the brightness of pixel values in video (Tills et al 2018, 2021). To calculate EPTs, the timeseries of mean pixel brightness in different regions of a developing organism are decomposed to a spectrum of energy values associated with different temporal frequencies (Tills et al 2018). These energy values constitute EPTs – a spectral measure of energy in the visible phenotype of an organism. EPTs are an integrated measure of the phenotype encompassing broad responses, containing signals from specific aspects of the phenotype, such as cardiac function, muscular contraction and organismal rotation, and they have proven capable of delimiting lethal end times (Tills et al 2018). Additionally, EPT spectra have been found to have high-treatment specificity and total energy from the spectra during

embryonic development has been identified as being predictive of growth rate, a developmental outcome indicative of rates of energy turnover (Tills et al 2021). EPTs are transferrable between species and developmental stages (Tills et al 2018, 2021), thereby offering a route towards scalable and transferrable phenotyping. However, EPTs are yet to be used to assess phenomic responses to combined environmental drivers and therefore their utility in complex multi-driver studies remains untested.

The aim of this study was to assess phenomic responses of marine invertebrates to combined environmental drivers using EPTs, and to compare such responses with those identified using a single trait approach. To achieve this aim we measured EPTs alongside manual measurements of heart rate from video of the common prawn *Palaemon serratus* at different temperatures (15, 20 °C) and salinities (15, 25, 35, 40) during three distinct, but sequential, embryonic developmental stages (5, 6, 7, Mueller et al 2004). We began with treatment comparisons of the overall energy from the EPT spectra, as a proxy for energy turnover (Tills et al 2021), and compared this with a single trait approach focusing on heart rate. Cardiac activity was selected as it is a dominant contributor to EPTs (Tills et al 2018), it is one of the few traits comparable across developmental stages, and it is widely used as a proxy of physiological performance in ectotherms. To test for integrative, high-dimensional treatment specificity, we transformed EPT spectra to lower dimensional-space using a non-linear dimensionality reduction approach. *Palaemon serratus* brood their embryos and are found berried year around in both sub- and inter-tidal marine environments. Therefore, embryos *in situ* can experience a broad range of temperatures and salinities during a 5-6 week embryonic period (Wear 1974). To assess biological responses associated with a prolonged exposure, we concluded by assessing differences between treatments in the level of energy within particular frequencies during a chronic 12 h exposure, to identify temporal signatures of treatment-response associated with different components of the phenome.

Materials and Methods

Culture and experimental conditions

Berried *Palaemon serratus* were collected (May 2019) using a single beam trawl at Jennycliff Bay in Plymouth Sound (50°20.59'N, 04°07.27'W) and maintained individually in aquaria filled with constantly aerated untreated sea water (S = 35) before transfer to a controlled temperature facility (15°C, 35 S) for a three-day acclimation period. Prawns were fed marine pellet (New Era Aquaculture™, Doncaster, UK) *ad libitium* with the addition of locally sourced seaweeds (*Fucus serratus, Ulva lactuca, Chondrus crispus, Mastocarpus stellatus, Cermamium spp., Cladophora spp.)*. The sea water collected from Plymouth Sound was changed every 48 h, just after feeding.

At the beginning of the experiment embryos were carefully removed from three females, each containing a brood at a different developmental stage (5 - early, 6 - mid, 7 - late, Mueller et al 2004), using a spatula. Embryos were transferred to Petri dishes (diam. = 60 mm) containing sea waters of different salinity (nominally 15, 25, 35, 40, reached at a rate of 10 h^{-1}) and temperature (15, 20 °C). A full factorial experimental design was used (8 embryos * 3 developmental stages * 4 salinities * 2 salinities = 192 embryos). Treatments were constructed by either diluting untreated sea water with deionised water, or by concentrating it by addition of InstantOcean, having pre-warmed the mixtures to either 15 or 20 °C, ie there was no pre-acclimation to 20°C). Embryos were subsequently transferred to individual wells (each, vol. = 350 μL) of two 96 well microtitre plates (Thermo Scientific™, Nunc™, MicroWell™) containing the same treatment solution embryos were previously acclimated to. Embryos were randomly allocated to each treatment.

Once in its well each embryo was orientated in a sagittal/lateral position relative to the bottom of the plate using a fine paintbrush. Microtitre plates were subsequently transferred

to incubation chambers prewarmed to either 15 or 20 °C (Bold Line Cryo, OkoLab, Naples, Italy), mounted in a high-throughput phenotyping system (OpenVIM, Tills et al 2018). Prior to commencement of recording, the positions of individual embryos within each well were recorded using MicroManager (Edelstein et al 2014) to ensure they were within the field of view for each recording schedule. Embryos were imaged individually (20 s duration, 30 Hz frame rate, 750x750 resolution) using a CCD camera (Pike 421B, Stradtroda, Germany) and zooming lens (VHZ20R, Keyence, Milton Keynes), with darkfield lighting achieved using an LED ring-light (LDR2-42-SW2, CCS, London, UK) and this was repeated hourly for a period of 24 h resulting in a series of 25 sequences of 600 frames, for each of the 192 embryos. Camera and XY stage (Scan, Marzhauser, Wetzlar, Germany) were controlled and synchronised using a Multi Dimensional Acquisition in MicroManager, looped hourly.

Phenotyping

Energy proxy traits are a measure of the distribution of energy across different temporal frequencies within timeseries of mean pixel intensity fluctuations in regions of a video (Tills et al 2021). Calculation of EPTs involved first calculating the mean pixel values for each of 64 regions of the video associated with each time point in an 8x8 grid. This process was repeated for each sequence of video recorded at a particular time point, for each embryo. Spectral decomposition (Welch 1967) was subsequently applied to each signal to quantify energy in the frequency range 0.01 to 6 Hz for each video timepoint (12 timepoints per embryo, upper limit of the range determined as approx. ¼ of the frame rate). A mean of the resulting set of 64 energy spectra from each video/timepoint was calculated and this was used in all downstream experimental analyses. The EPT spectra for individual embryos across timepoints were stored in an H5 data format for downstream analysis (192 embryos, 12 timepoints). A pipeline for calculating EPTs runnable online, *via* Google Colab is accessible at: https://doi.org/10.5281/zenodo.4680830.

Cardiac activity was visible at each developmental stage and was measured manually as rate of heart beat from the first video time point, for each embryo using ImageJ (Schindelin et al 2015). Beats were recorded by manual counting for the duration of the 20 s video and this measure was subsequently converted to beats per minute (BPM).

Statistical analysis

Total energy was used as an aggregate measure of energy turnover in the embryo by summing all values of EPT for each individual over the course of the experiment, integrating phenotypic responses at all frequencies, including signals such as heart rate, muscular contractions, etc. Frequency specific energy values were also calculated by binning particular frequency bands to elucidate more specific phenotypic responses. EPTs were also used in their most granular form – retaining all 300 frequency bands – for a dimensionality reduction using Uniform Manifold Approximation and Projection (UMAP, McInnes et al 2018).

Effects of temperature, salinity, developmental stage, and their interactions, on both heart rate and global levels of EPT, calculated as the $\log_{10}$ of the sum of energy in the EPT spectra at the beginning of the study, were tested for using analysis of variance ANOVA. Treatment differences in the levels of EPT spectra within different frequency bins during the 12h experimental period were tested for using a repeated measures ANOVA.

UMAP was used to reduce the high-dimensional EPT spectra to three dimensions in order to interrogate broad-scale treatment-level responses. This approach produced three coordinates for each embryo on the basis of their EPT spectra. Treatment differences in the coordinates for each individual were tested for using a multivariate analysis of variance (MANOVA).

Results

The novel application of EPTs for capturing whole-organism phenome-level responses to multi-stressor environments has highlighted some key patterns. Distinctions between EPTs and more traditional phenotype measurements (in this case heart rate) are evident. Differences in energy within particular frequency bins within the EPT spectra indicate changes in the phenome during development and in response to environmental conditions, yet total energy turnover - represented by the sum of EPT measurements within the EPT spectra - did not vary between treatments.

Heart rate and global EPT spectra energy

Heart rate increased during the course of the experiment in all treatment combinations, with significant differences also apparent between temperatures ($F_{1,105}$ = 99.44, P ≤ 0.001), and developmental stages ($F_{2,105}$ = 315.4, P ≤ 0.001), but also their interaction ($F_{2,4603}$ = 12.31, P ≤ 0.001), at the outset of the experiment (Figure 1). There was, however, no effect of temperature ($F_{1,155}$ = 1.59, P = 0.209), salinity ($F_{3,155}$ = 2.61, P = 0.053), or developmental stage ($F_{2,155}$ = 0.623, P = 0.538) on the total levels of energy in the EPT spectra. The absence of differences in total energy between developmental stages was particularly interesting, since heart rate did increase with development, and cardiac function is the most visible physiological feature in the video at each of the three developmental stages studied.

Global combinatorial effects of EPTs

To assess global treatment differences in EPTs a non-linear dimensionality reduction (UMap McInnes et al 2020) was applied to 300 frequencies of EPT (from 0 to 6 Hz with 0.04 Hz resolution) averaged across the 12 h experimental period for each embryo. Clustering of embryos by both temperature and developmental stage was apparent in the visualisation of all three UMap dimensions (Figure 2). This clustering is supported by a MANOVA indicating significant differences between temperature (Pillai's Trace = 0.744, $F_{3,159}$ = 153.94, P ≤

0.001), developmental stage (Pillai's Trace = 0.835, $F_{2,161}$ = 38.2, P ≤ 0.001) and their interaction in embryo coordinates along the three UMap dimensions (Pillai's Trace = 0.635, $F_{6,161}$ = 24.81, P ≤ 0.001). Temperature-driven responses of all developmental stages were evident along both the 1st and 2nd UMap dimensions (Y and X in Figure 2), but unlike the mid- and late- stages, the early stage embryos also exhibited notable responses along the third dimension (see colour mapping in Figure 2). There was, however, no significant effect of salinity on the coordinates of embryos in the UMap dimensionality reduction (Pillai's Trace = 0.785, $F_{9,161}$ = 1.44, P = 0.167).

Temporal spectral responses

Differences in the EPT response of embryos between 15 and 20 ºC were detected for all developmental stages, across salinity treatments, but responses were specific to different treatments and frequency ranges of EPT (Figure 3, Table 1). Temperature had a significant main effect on levels of energy in the frequencies 1-2, 4-5 and 5-6 Hz, whereas salinity interacting with developmental stage had a significant impact on energy at 5-6 Hz. There was a significant main effect of time for 0-1, 1-2, 4-5 and 5-6 Hz, but the interaction of developmental stage and salinity occurred in 2-3 Hz and 5-6 Hz, with the addition of temperature to this interaction term at 4-5 Hz.

Trends were observed in the treatment responses of frequency specific EPT energy levels in both temporal changes in frequency specific energy, and in treatment-differences to the distribution of energy across the frequency spectra (Figure 3). A general trend towards increased dominance of higher frequencies in 20 ºC was observed across the three developmental stages in the frequency specific energy spectra associated with the 12 h experimental period (Figure 3). However, EPT spectra were also markedly different between developmental stages. Developmental stage-specific treatment-responses were apparent in

the interaction terms reported in 2-3, 4-5 and 5-6 Hz frequency bands. Coherent bands of frequencies with elevated energy were evident in examination of the timeseries (Figure 3) of each treatment group,and appeared strongest in the mid- and late- developmental stages. These bands appeared to correspond to the frequencies associated with heart function. Spectral transformations, such as those used in the measurement of EPTs, quantify the harmonics of a signal, meaning a heart rate of 2 Hz, will also be detectable in the energy values associated with frequencies of 4 Hz. In comparison of developmental stage and salinity treatment groups between temperatures (Figure 3) the frequency of the bands of elevated energy in the EPT timeseries was evidently raised in concordance with the temperature effect evident in the manual measures of heart rate (Figure 1). Indications of greater variability in heart rate at 20 compared to 15 °C were evident, corresponding to elevated energy across a broader range of frequencies. Considerable variability in inter-beat timings of heart function has previously been described in embryonic stages of an amphipod (Tills et al 2018) and this would be reflected as energy within a breadth of EPT frequencies – associated with the frequencies of beat-to-beat timings.

Late-stage embryos in S = 15 at 20 ºC exhibited noticeably supressed levels of energy associated with phenotypic responses other than heart function, compared to the other salinity treatments at 20 ºC (Figure 3). The absence of energy within broader frequencies is indicative of a lack of broader muscular driven phenotypic responses. Within the 0-1 Hz frequency bin the only significant treatment effect was time. In late-stage embryos, some increase in energy within these frequencies was evident and is likely indicative of a move towards muscular flexing associated with breaking the chorion beginning the process of hatching, however no embryos hatched within the 12 h experimental period. In both the early- and mid-stages there appeared to be an effect of time on time specific overall energy, with increases evident at the early-stage and a general trend towards a decrease in the mid-stage embryos (Figure 3).

Discussion

Phenomics has proven an invaluable approach to measuring complex phenotypic responses in fields including crop science, livestock science and medicine with previously unattainable scale and resolution in the measurements of life at its highest level of organisation. Yet such approaches remain largely inaccessible in assessing broader biological responses to the drivers of global environmental change – significantly hindering this increasingly important research endeavour. Here, we use energy proxy traits (EPTs), a powerful new approach to measuring complex phenotypes (Tills et al 2018, 2021). Significant interactions were detected between temperature, developmental stage and salinity in frequency-specific levels of energy, but these were not driven solely by cardiac activity, suggesting that there are additional phenotypic drivers of EPTs. We identified complex developmental-stage specific sensitivities, highlighting the complexity of phenotypic responses to multiple global change environmental drivers. We demonstrate the efficacy of ETPs in detecting complex and multi-faceted responses to combined environmental drivers. We conclude that a key advantage of phenomics is overcoming the limitation of selecting, *a priori*, a limited number of individual traits and to move towards quantifying multifaceted responses more closely representative of the high-dimensional nature of whole organismal biology (Houle 2007).

While the responses are complex, major patterns are clear. Elevated temperatures modified the distribution of energy in the EPT spectra towards higher frequencies. There were, however, some interactions between developmental stage and salinity in the level of energy at particular frequencies. Maia et al (2022) identified temperature and pH induced alteration to the biochemical profile of later stage juvenile prawn embryos in a congeneric species *P. elegans.* Investigating links between EPT spectral responses and the extent to which these either reflect biochemical profiles, or may result from differences in the energetic costs of particular spectral phenotypes could be particularly rewarding in understanding causation at the level of biological outcomes. Specificity in the response at different stages of development of particular aspects of the phenotype are well reported (Burggren & Reyna

2011, Burggren & Mueller 2015, Przeslawski et al 2015, Tills et al 2010), and this developmental-stage specificity contributes significantly to the challenge of assessing biological sensitivity within a multistressor experimental approach. However, using EPTs, we consider this sensitivity as alterations in a spectrum of energy across different frequency bands, with a distinct advantage of being applicable across different species and stages of development (Tills et al 2018, 2021). The broad and high-dimensional developmental-stage specific treatment responses we observe here demonstrate that EPTs are a spectral phenotype with high sensitivity for measuring and distinguishing integrated biological responses to combined environmental drivers.

Measuring biological sensitivity to combined environmental drivers is a major research focus within the life sciences (Przeslawski et al 2015, Boyd et al 2018, Kling et al 2019, Polazzo et al 2021, Sage 2020), now given renewed impetus and relevance by its role in informing predictions of biological responses *in situ* particularly to rapid global change. Here, we show the potential of a new and powerful means of assessing biological sensitivity using EPTs, a spectral phenotyping approach, to test for developmental-stage specific responses of complex phenotypes to combined environmental drivers. In ecology, spectral approaches to the study of complex biotic and abiotic systems have a rich history (Platt and Denman 1975, Jervis et al 1989), and owing to the significant increase in data availability is increasingly being used to investigate complex systems (Cazelles et al 2008). We demonstrate the applicability of EPTs as a transferrable approach to understanding complex responses of dynamic periods of life, a significant current challenge in assessing biological responses to environmental drivers.

There was no effect of temperature, salinity or developmental stage on global energy levels across the EPT spectra. The absence of a treatment level effect on global energy, but effects on energy within specific frequencies and also the combinatorial signal from the EPT spectra in high-dimensional space is intriguing and suggests nuanced and integrated

phenotypic responses. Tests of thermal sensitivity of EPTs in the embryonic development of a brackish water gastropod identified changes in global energy (Tills et al 2021). While species-specific responses to environmental drivers are unsurprising, it is also important to note that here we use three developmental stages from the approximately two-month embryonic development of *P. serratus* (T = 12.5 ºC, Wear, 1974), whereas Tills et al (2021) measured EPTs at hourly intervals throughout the shorter developmental period of 14 days (T = 20 ºC) for *R. balthica*. Of particular interest is that Tills et al (2021) found that overall levels of EPT were a significant predictor of growth rate, with greater levels of overall energy throughout development corresponding to faster growing embryos and it was therefore posited that EPTs may be indicative of the metabolic rate of embryos. *Palaemon serratus* embryos fill the egg capsule from an early stage of development and consequently growth was not measured in this study. However, if EPTs are indeed indicative of metabolic rate then the highly-treatment specific responses within particular frequencies reported here may suggest that the overall energy expended by an embryo does not vary under the tested conditions, but that the prioritisation of biological processes may instead change under these scenarios. Further study is required to understand the significance of EPTs within the context of biochemical energy usage and how alterations to an EPT spectra may correspond to energy allocation.

Heart rate was higher in later developmental stages and at elevated temperature and is one of the conspicuous biological responses visible in the video of *P. serratus* and within the EPT spectra itself (Figure 3). However, as indicated by the differences in treatment response of overall levels of EPT compared to heart rate, it was not the sole contributor to EPT spectra. Energy proxy traits are emerging as a useful approach to isolate and measure specific physiological rates, such as heart rate (Ibbini et al 2022), but also as integrators of higher-dimensional phenotypic responses, such as, in the case of *P. serratus,* appendage beating, gut contractions, whole-body muscular flexing and blood flow. EPTs are a quantification of fluctuations in pixel brightness as a spectrum of energy at different temporal

frequencies, and therefore if individual phenotypic traits are associated with particular frequencies they can be isolated and quantified from the spectrum. However, much of the phenotype, particularly in developing organisms, is not easily quantifiable owing to the transitory and integrated nature of the traits involved. A key strength of EPTs within the context of phenomics is the ability to integrate and quantify these traits, alongside those such as heart rate that are more easily quantifiable and discrete. To investigate high-dimensional treatment responses in the EPT spectra we used Unifold Manifold Approximation and Projection (UMap, McInnes et al 2018) to collapse the high-dimensionality of EPTs to just three explanatory axes. Dimensionality reduction is an increasingly popular tool for interpreting high-dimensional phenotypic data, particularly in the plant sciences which benefit from being further forward in building capacity for high-dimensional phenotyping (Tardieu et al 2017). We discovered developmental-stage specific environmental sensitivities in high-dimensional space highlighting complex biological responses in the EPT spectra of embryos. Berried *Palaemon serratus* have been found year around, inhabiting both sub- and inter-tidal marine environments, thus embryos can experience a broad range of temperatures and salinities *in situ* during their development (Taylor 1988). Univariate phenotypic responses of developing aquatic embryos are known to impact biological outcomes including survival (Bitterli et al 2016),but given the high-dimensional and integrated nature of the phenotype, understanding the biological consequences of responses to global change drivers in high-dimensional space must be a priority.

An imbalance in the rates of data acquisition capable at the phenotypic level compared with lower biological levels risks an inadequacy in the contribution of the phenotype to our understanding of biological sensitivity to environmental drivers of global change. This situation is particularly concerning given that the phenotype is the highest level of organismal organisation, and also the one at the forefront of most conservation efforts. Biologists using non-model organisms – a common situation when assessing responses to

global change drivers – are still hindered by the limited range of methodological approaches available to them. Here, we find that EPTs were effective at each of the three dynamic developmental stages used, enabling complex and multifaceted responses to multiple environmental drivers to be captured. EPTs are a method of extracting data from videos, are highly transferrable, and this study advances previous work (Tills et al 2018, 2021) by showing their applicability to the significant challenge of assessing phenotypic sensitivity to combined global environmental change drivers. Comparable phenomic approaches are increasingly used in the crop sciences for research to support food-security in a changing climate, and arguably the greatest challenge remains the integration, analysis and interpretation of data, as opposed to its acquisition (Tardieu et al 2017). We suggest that for animal phenomics, this challenge may be greater-still, but so too could be the rewards. After all, it is only by developing and applying new methods of phenotyping that we can begin to appreciate the full extent of the phenome and the value of it in understanding responses to changing environments. Advances in the areas of phenomic data acquisition and analysis, and our ability to interpret the output, should be a key priority in advancing our ability to assess biological responses to global environmental change. We predict that EPTs will become an increasingly important method for indiscriminate phenotyping, transferrable between species, developmental stages and experimental designs.

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FIGURE LEGENDS

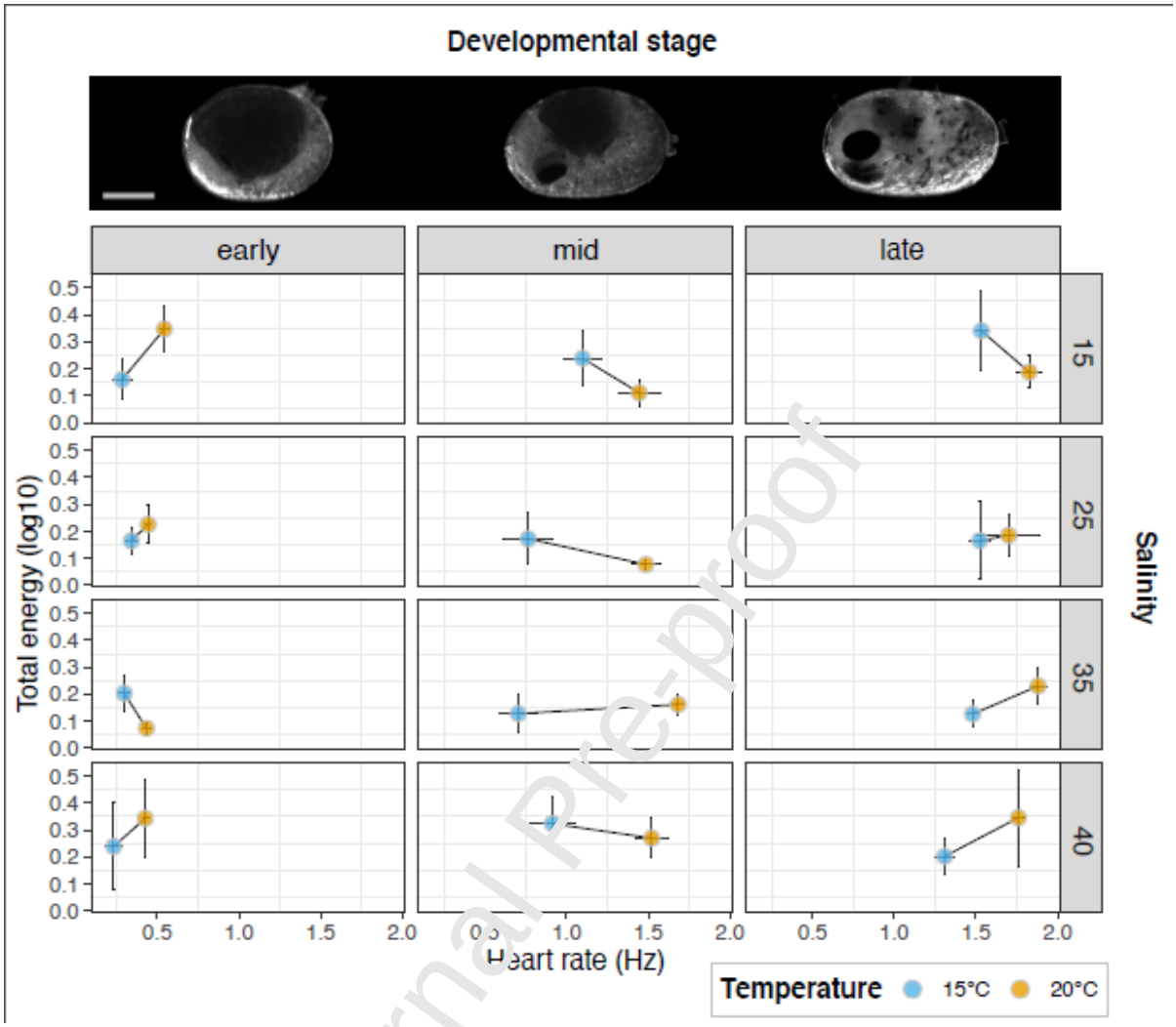


Figure 1. Responses of heart rate and total energy (mean ± SE) in prawn embryos of different developmental stages exposed to a range of salinities, and temperatures. Scale bar for top banner of embryo micrograph images = 250 µm.

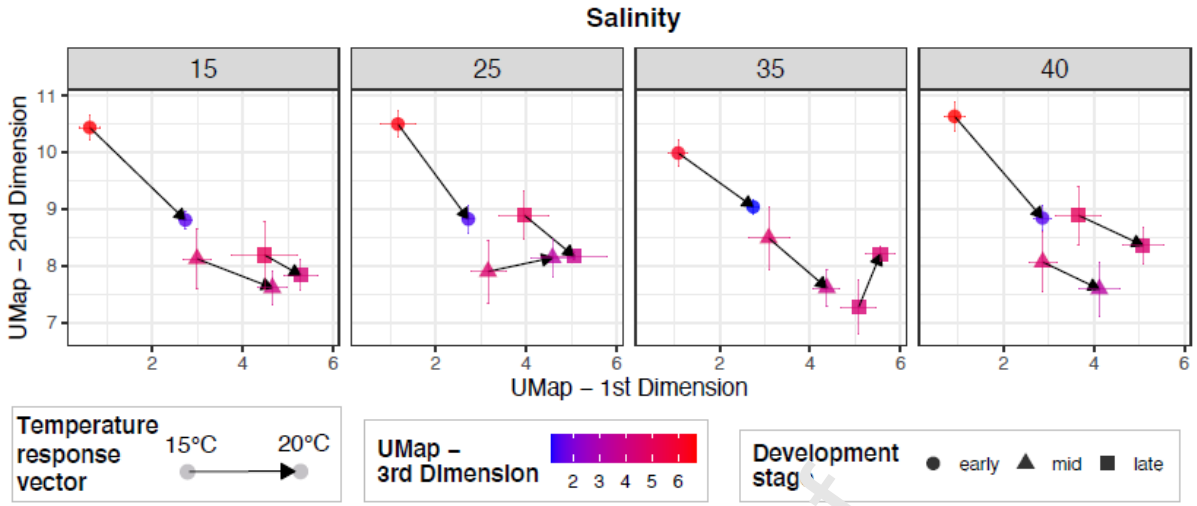


Figure 2. Mean treatment projection of embryos on the basis of EPT spectra in low dimensional space (UMap), grouped by developmental stage, temperature and salinity experimental treatments along three axes from a UMap non-linear dimensionality reduction of responses to EPT frequencies.

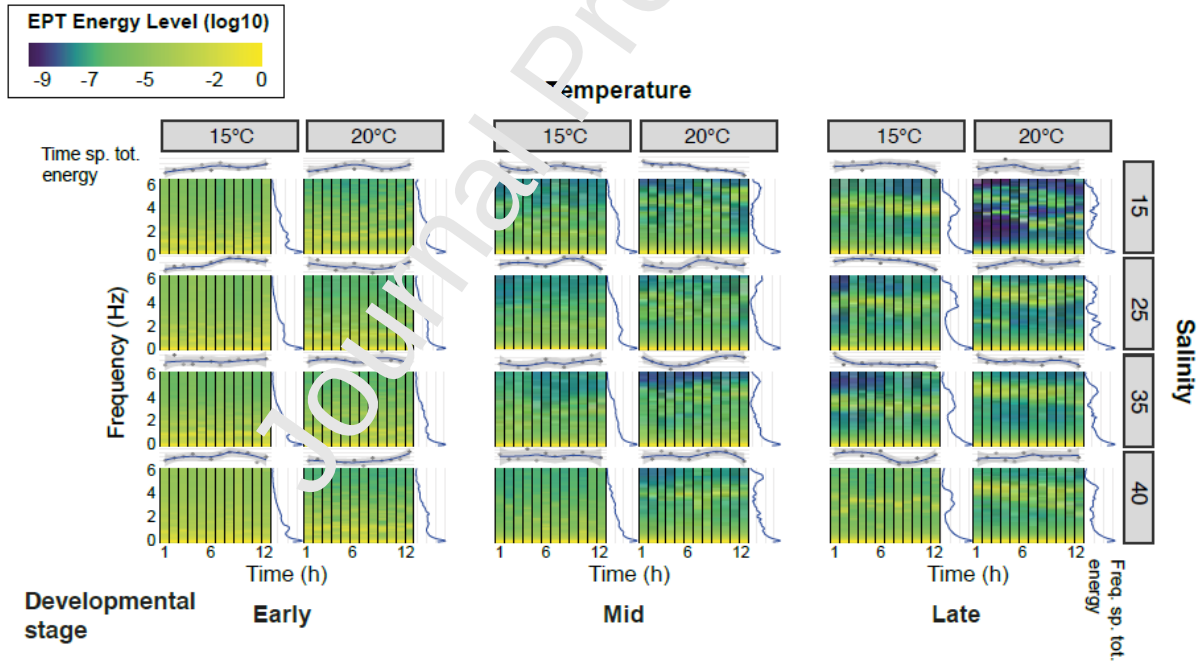


Figure 3. Heatmaps display the magnitudes (logged mean) of energy proxy trait at different temporal frequencies during the 12 h experimental period. Changes in overall levels of EPT during the 12 h period of the experiment are shown in the line plots above each heatmap. The mean energy spectra throughout the 12 h experimental period is shown in the line plot adjacent to the right of each heatmap, indicating relative change within each treatment. See

Table 1 for associated significant statistical tests and Supplementary 1 for the complete statistical results.

Table 1. Summary of significant results of a repeated measures ANOVA for EPTs binned at 1 Hz intervals. Levels of significance are indicated as P ≤ 0.05 (*), ≤ 0.01 (**), and ≤ 0.001 (***). Full results included in Supplementary 1.

	Between-subjects						Within-subjects					
Frequency Bin (Hz):	0-1	1-2	2-3	3-4	4-5	5-6	0-1	1-2	2-3	3-4	4-5	5-6
Temperature (Temp.)		*			***	***						
Time							**	**			***	
Dev Stage * Salinity						*						
DevStage * Time											*	
DevStage * Salinity * Time									***			***
Temp. * DevStage * Salinity * Time											*	

Author Contributions Statement

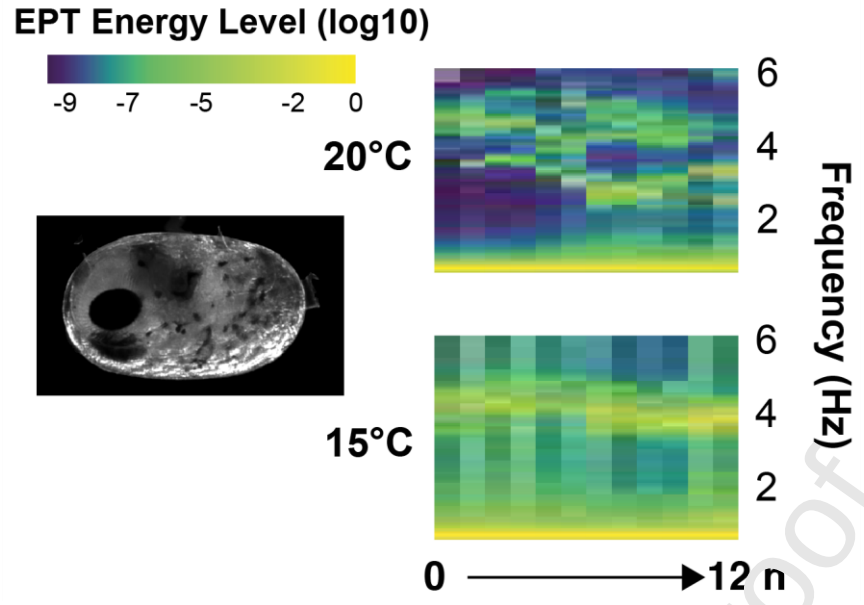
Oliver Tills: Conceptualisation, Funding acquisition, Software, Methodology, Formal analysis, Writing. **Luke Holmes**: Formal analysis, Writing, **Elliot Quinn**: Investigation, Writing, **Anthony Quinn:** Investigation, Writing, **Manuela Truebano**: Visualisation, Writing, **John Spicer**: Conceptualisation, Methodology, Writing

Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical abstract



Highlights

- Analysis of fluctuating pixel brightness quantifies complex biological responses
- Responses of the common prawn to global change drivers are high-dimensional.
- Innovation is urgently needed to more-fully capture whole-organismal responses
- High-dimensional phenomic approaches can be transferrable and scalable.
- Such transferrable approaches are urgently needed to assess biological impacts.