1 High thermal plasticity and vulnerability in extreme environments at the
2 warm distributional edge: the case of a tidepool shrimp

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1 **1. Abstract**

2 Climate change threatens the resilience of species, especially at their warm
3 distributional edge in extreme environments. However, not much is known about the
4 thermal vulnerability of marine intertidal species at this edge. We investigated the
5 thermal vulnerability of the tidepool shrimp, *Palaemon elegans* in the fast-warming
6 southeastern Mediterranean, its warm distributional edge. Tidepool organisms
7 experience strong and fast thermal fluctuations. This might make them more resilient
8 to change, but also bring them closer to their thermal limits during extreme conditions.
9 To test the shrimp's resilience, we tested three hypotheses: (1) *P. elegans* in the
10 southeast Mediterranean has higher critical thermal maximum (CTMax) than in cooler
11 regions, (2) the shrimp possess seasonal acclimatization, but (3) long exposure to
12 extreme summer temperatures might erode its thermal performance making it
13 vulnerable to future climate change. We characterized the shrimp's thermal
14 environment and population dynamics, determined CTMax and tested diverse
15 physiological performance attributes (respiration, digestion, activity, growth) under a
16 wide range of temperatures during winter and summer. *P. elegans* has a wide optimum
17 performance range between 20-30 °C during summer and its CTMax is 38.1 °C, higher
18 than its Atlantic counterparts. However, its warming tolerance is only 0.3 °C, indicating
19 low capacity for dealing with further warming in pools compared to northeast Atlantic
20 populations that have wider tolerance. Prolonged exposure to current mean summer
21 values in open water (~ 32 °C) would also significantly reduce its performance and
22 increase mortality. This suggests that its population viability may be reduced under
23 continuous regional warming and intensification of extreme events.

24

25 **Keywords:** Climate change, ocean warming, Thermal tolerance, Acclimation,
26 Phenotypic plasticity, Rocky shores.

1 **2. Introduction**

2 There is a growing body of evidence concerning the increasing impacts of
3 anthropogenic climate change (CC) on marine ecosystems and their biodiversity, which
4 is predicted to continue and increase in the near future (Gattuso et al., 2015). Ocean
5 warming poses one of the biggest threats to sea life, with evidence that the ocean surface
6 has already warmed by a global average of 0.87°C compared to the pre-industrial
7 period, and is expected to increase further by 1-3°C, depending on the emission scenario
8 (IPCC, 2019). Temperature affects all biological and chemical processes and
9 ectotherms are directly affected by temperatures since they lack the ability to control
10 their own body temperature. Effects of temperature change at the organismal level, i.e.,
11 physiology and behavior (which affect growth, reproduction and levels of activity),
12 ultimately lead to impacts on species global distribution, seasonal timing of biological
13 events (phenology), and food web structure (Pörtner and Farrell, 2008; Verberk et al.,
14 2016). At the physical-chemical-physiological interface, increasing temperature leads
15 to increases in the kinetic energy of molecules, and therefore increasing metabolic rates
16 such as enzymatic reaction as well as mitochondrial activity that in turn intensifies the
17 respiration rate of an organism (Levinton, 2001; Schulte et al., 2011).

18 Temperature increase under global warming is heterogeneous in space and time and
19 some regions warm faster than others (Macias et al., 2013). Because in many locations
20 or marine habitats organisms already live close to their thermal limits (Harley et al.,
21 2006; Somero, 2002; Vinagre et al., 2018) even slight increases in temperature can
22 negatively affect their performance, and ultimately their survival (Pörtner and Farrell,
23 2008; Rivetti et al., 2014). The warm or trailing edge of species distributions is where
24 species are expected to be frequently thermally challenged, and where the viability of
25 the population should start to erode first under global warming (Bates et al., 2014;

1 Donelson et al., 2019; Somero, 2010). At the warm edge, even thermal refugia can be
2 lost in extreme environments such as the intertidal zone (Lima et al., 2016).

3 The southeastern Mediterranean Sea, represents the warm edge of most Atlanto-
4 Mediterranean species and can be used as a testbed of marine species vulnerability at
5 this edge. The Mediterranean Sea, as a whole, experiences one of the strongest ocean
6 warming rates because of its small size and the fact that it is almost enclosed, in addition
7 to its geographic location and the effect of a recent change in the Atlantic Multidecadal
8 Oscillation (Macias et al., 2013; Marbà et al., 2015). Therefore, the Mediterranean is
9 expected to be more strongly influenced by global change than the open ocean, and
10 indeed there is accumulating evidence about the footprint of climate change on
11 Mediterranean Sea biota, mostly for its well-studied western basin (Marbà et al., 2015;
12 Rivetti et al., 2014). For example, the two major mass mortality events that followed
13 heat waves in the northwest basin in the past two decades (Rivetti et al., 2014). The
14 East Mediterranean Sea (EMS) is warming faster than the western basin, especially in
15 winter (Pastor et al., 2019), and offshore *in-situ* measurements over the past three
16 decades revealed warming at an average rate of 0.12°C per year or almost three degrees
17 in the past three decades (Ozer et al., 2016), an order of magnitude larger than the global
18 average. Nonetheless, the near absence of long-term biological time-series makes it
19 difficult to identify the ecological footprint of climate change in this basin. Recently,
20 the collapse of many native reef invertebrate species has been reported on the
21 southeastern coast of the Levant basin (Israel), and it was suggested that rapid ocean
22 warming in this region may be partially responsible for that (Rilov, 2016). At least for
23 sea urchins, which have become extremely rare on this coast in the past few decades,
24 there is evidence that they die when water temperature rises above 30.5°C, which occurs
25 every summer on this coast in the past decade (Rilov, 2016; Yeruham et al., 2015).

1 One of the ecosystems where climate warming is expected to have a strong influence is
2 the rocky intertidal zone. This zone exists at the margin between the terrestrial and
3 marine realms, so organisms in it are subjected to frequent changes in both water and
4 air temperatures. Within this ecosystem, tidepools may be especially vulnerable to
5 change in temperatures (Harley et al., 2006; Helmuth et al., 2006). Tidepools are highly
6 structured environments that provide food and shelter for diverse biological
7 communities (Vinagre et al., 2015). Each tidepool has its own unique environmental
8 characteristics, since they can be found in a variety of shore levels, sizes and volumes,
9 as well as having different levels of connection to the open sea (Metaxas & Scheibling
10 1993, Vinagre et al., 2015). Because of their small water volume, tidepools have low
11 thermal inertia, unlike other aquatic environments, and can thus warm or cool very fast,
12 reaching extreme values in a matter of hours (Vinagre et al., 2018). This requires
13 organisms inhabiting them to be able to deal with a large range of abiotic conditions,
14 but also possibly make them more prone to extreme change in their fitness (Madeira et
15 al., 2012b). A limited global analysis across realms suggested that animals from more
16 stable environments have greater capacity for acclimation compared to unstable ones,
17 but pointed out that generality of such patterns is limited because much of the world is
18 undersampled (Seebacher et al., 2015).

19 On the rapidly warming Israeli coast, which is the southeastern edge of distribution of
20 most Mediterranean and Atlanto-Mediterranean species, and where the water is
21 naturally hottest in the basin, tidepool ecology has not been studied before, but it is
22 assumed that the pools can get extremely hot when disconnected from the sea. This
23 study focuses on the small rockpool shrimp *Palaemon elegans* (Rathke 1837). *P.*
24 *elegans* is native to the Mediterranean Sea, the Black Sea, and the East Atlantic coast,
25 ranging from Norway in the north to Namibia in the south. It inhabits coastal tidepools,

1 shallow rocky areas, submerged vegetated areas, lagoons, estuaries and man-made
2 structures (Bilgin et al., 2009; Glamuzina et al., 2014). Its reproductive season is May
3 to September (in the Black Sea), and when conditions are favorable, the female can
4 reproduce twice a year (Zaharia et al., 2005). The shrimp is euryhaline, with a high
5 salinity tolerance of 1-40 PSU (Glamuzina et al., 2014). It is omnivorous, feeding
6 predominantly on algae, small crustaceans and foraminifera (Dalla Via, 1985;
7 Glamuzina et al., 2014). In Israel, it almost exclusively lives in tidepools on the rocky
8 shore (Tsumamal, 1963). *P. elegans* distribution expanded extensively in the past
9 century, as it had invaded the Caspian Sea (1930), Aral Sea (1960), Persian Gulf (1972),
10 Baltic Sea (2000) and the shores of Massachusetts, North America (2010) (Fofonoff et
11 al., 2003). As a result, the species gained widespread attention in the fields of ecology,
12 biology, and in relation to commercially-related impacts on food security and its use as
13 bait in fisheries (Al-Khafaji et al., 2016; Janas and Mańkucka, 2010; Janas et al., 2013).
14 Other studies from the Black Sea, Caspian Sea and the north Mediterranean Sea
15 investigated its growth and reproductive performance, larval development, fecundity,
16 individual and group behavior, population structure and habitat conditions (Bilgin et
17 al., 2009; Dalla Via, 1985; Duran et al., 2006; Glamuzina et al., 2014; Janas and
18 Mańkucka, 2010; Tsumamal, 1963). A few studies showed that *P. elegans* has a strong
19 resistance and a high tolerance to a wide range of conditions. It achieves this by
20 exhibiting a high production of heat shock proteins (HSP70) and by expanding its
21 habitat range through occupying vacant ecological niches (Madeira et al., 2012a;
22 Szaniawska and Łapińska, 2006; Tsumamal, 1963). Other studies examined the
23 vulnerability of this species (among other species) to global warming in the Northeast
24 Atlantic and in the west Mediterranean Sea, by investigating the CTMax and its
25 acclimation capacity through different acclimation states at different life stages

(Madeira et al., 2015; Madeira et al., 2012b; Magozzi and Calosi, 2015; Ravaux et al., 2016; Vinagre et al., 2013; Vinagre et al., 2016) (Table 1).

Here, we investigate the effects of temperature on *P. elegans* at its distributional trailing (warm) edge, the EMS, where it possibly experiences the most extreme conditions in both open water and tidepools. A species' relative sensitivity to warming depends on its thermal tolerance and also on its thermal acclimation potential, defined as the plasticity of its behavioral, physiological or morphological characteristics in response to environmental temperature (Angilletta Jr, 2006). Hence, the present study specifically aims to investigate the vulnerability of the EMS population of *P. elegans* to the predicted rise in temperature. For that, we measured temperature and population dynamics in different pool types occupied by the shrimp, and tested its CTMax and thermal performance of various physiological and behavioral parameters under a wide range of temperatures and at different seasons (for possible seasonal adaptations). Information regarding the shrimp's optimal thermal performance range and its upper thermal limits (CTMax) will help to evaluate the present and future effects of CC on the species' fitness and ultimately its distributional range. The thermal performance curve (TPC) describes the effects of temperature on the rate of biological processes, and demonstrates the thermal range of performance, the optimal temperature (T_{opt}) and the points at which performance is zero (T_{crit}). CTMax explores the upper thermal tolerance of a species and is a good index and standard for evaluating the thermal requirements and physiology of an organism (Lutterschmidt and Hutchison, 1997). We specifically tested three working hypotheses: (1) CTMax of the southeast Mediterranean shrimp is higher than that of the East Atlantic one because of the extreme environmental condition it experiences in the EMS (Vinagre et al., 2016); (2) T_{opt} will be higher in the summer than in the winter as a result of seasonal acclimatization, which

1 makes the shrimp more tolerant to higher temperatures in the warm months (Chapperon
2 *et al.*, 2016), and (3) longer exposure to high temperatures will erode the performance
3 of the shrimp, reducing its optimum values.

4

5 **3.Materials and methods**

6 *3.1 Study site*

7 We studied the shrimp population at the beach of the Shikmona marine reserve, Haifa,
8 Israel (Fig. 1, 32° 49' 32.27"N, 34° 57' 18.21"E). This site has a gently-sloping
9 limestone rocky shore at the back of a vermetid reef platform, with multiple tidepools
10 (rockpools) of varying sizes and connectivity to the open waters. The environmental
11 conditions and population dynamics of *Palaemon elegans* were studied in five tidepools
12 of different sizes, and varying degrees of connection to the open sea (Fig. 1).

13 *3.2 Environmental conditions*

14 In order to determine the dynamics and annual range of temperatures the study species
15 is naturally exposed to in tidepools in the study region, water temperatures were
16 recorded hourly between November 2016 and January 2018, using HOBO pendant
17 temperature Data Logger (model UA-002-64, Onset Computer Corporation, USA)
18 deployed at the bottom of the five pools and attached to the rock with stainless-steel
19 bolts similar to Kelly et al (2012). Measurements of nearshore open-water temperatures
20 were obtained from a logger deployed at a depth of 0.5 m at the seaward edge of the
21 rocky shore (serving IOLR's National Monitoring Program). Measurements of air
22 temperatures were obtained from a weather station located on the roof of IOLR, about
23 200 m from the study site.

24

1 3.3 *Population dynamics*

2 In order to detect seasonal population dynamics and determine the reproductive and
 3 recruitment season, the population of *P. elegans* was sampled monthly in the five
 4 tidepools between December 2016 and December 2017 using a hand net (12 x 10 cm,
 5 1mm mesh). The net was moved slowly along the sides (walls) and near the bottom of
 6 the pool (areas where the shrimp can normally be found) for three minutes, and all
 7 individuals caught were recorded and placed back to the same pool after handling.
 8 Because this species is nocturnal, the sampling occurred at night when the sea is calm
 9 and during low tide. The recording included measuring the size with a digital caliper
 10 (INSIZE, electronic caliper series 2111) and weight with an analytical scale (Analytical
 11 balances ASB-220-C2 ($\pm 0.2\text{mg}$)).

12 3.4 *Specimen collection and preparation for experiments*

13 Specimens of *P. elegans* were collected from tidepools (different pools than the
 14 monitored ones, but at the same site, from depths of 0.1-0.5m) for the different
 15 experiments, using a hand net in January, May, June and September of 2017. The
 16 average water temperature in the tidepools at the time of collection was 17°C, 22°C,
 17 25°C and 29°C, respectively. Immediately after collection, the shrimp were transferred
 18 to the lab for an initial acclimation period of five days in outdoor aquaria (20x38x21cm,
 19 maximum 25 shrimp in each aquarium) with a continuous seawater flow at ambient
 20 conditions (supply directly from the sea).

21 3.5 *Temperature performance experiments*

22 Different measurements of the shrimp's thermal performance under a range of
 23 temperatures were taken three times: adults were tested in winter and summer and
 24 juveniles during the main reproductive season in spring. After the acclimation period

1 in the aquaria, the shrimp were transferred to individual glass jars set in ten separate
2 20L units of an outdoor (allowing natural photoperiodicity) custom-made thermobath
3 system (see description in Guy-Haim et al., 2016) for an additional five days of
4 acclimation at a constant ambient temperature, corresponding to the average seawater
5 temperature measured at the sampling site at each season. The temperature in each
6 thermobath was controlled independently by NOVUS N1020 temperature controllers
7 ($\pm 0.4^{\circ}\text{C}$). Individuals of *P. elegans* were each placed in separate 0.75L glass jars (n=6)
8 that were submerged in the thermobaths. Every 48 hours, 100% the water was replaced
9 manually and gradually in each container with filtered Mediterranean seawater (2 μm
10 carbon filter) that was pumped from a depth of 1m and warmed or cooled beforehand
11 to the temperature currently present in each thermobath treatment in order to reduce
12 possible stress. Food supply was *ad libitum*. Before placement in the jars, each shrimp
13 was weighed with an analytical scale (Analytical balances ASB-220-C2 ($\pm 0.2\text{mg}$)) after
14 being carefully dried with blotting paper. Salinity was kept at the levels of the nearby
15 coastal water and was on average 39.0 ± 0.1 PSU. Water motion and temperature
16 homogeneity within the containers were ensured by aerators and submersible pumps.
17 After five days of the acclimation period, the temperature in each bath was differentially
18 increased or decreased by $0.5^{\circ}\text{C} \cdot 12\text{h}^{-1}$ until reaching their target temperatures at the
19 same date. This rate of change was not greater than the diurnal temperature range in the
20 collection site at that period (1°C). The winter experiment (temperature range: $8\text{--}34^{\circ}\text{C}$)
21 started on February 4th, 2017; target temperatures were reached on February 24th, 2017
22 (25 days after sample collection); and were maintained until the end of the experiment,
23 on March 17th, 2017. The late spring juvenile growth experiment (temperature range:
24 $12\text{--}35^{\circ}\text{C}$) started on June 2nd, 2017; target temperatures were reached on June 20th, 2017
25 (23 days after sample collection) and were maintained until the end of the experiment,

1 on June 26th, 2017. The late summer experiment (temperature range: 17-35°C) started
 2 on September 25th, 2017; target temperature was reached on October 10th, 2017 (20
 3 days after sample collection) and was maintained until the end of the experiment, on
 4 October 21st, 2017. We tested the performance of four types of animal functions that
 5 could be affected by temperature, and thereby can affect the shrimp's fitness:
 6 respiration, digestion rate, growth (in juveniles) and activity (movement).

7 *3.5.1 Respiration* was measured as the change in dissolved oxygen (DO) during 1-3-
 8 hour incubations. The incubations were performed three times on individual specimens
 9 in gas-tight 0.3L containers to which the shrimp were temporarily relocated. DO
 10 concentration ($\mu\text{mol O}_2 \cdot \text{L}^{-1}$) was measured inside the containers using an oxygen
 11 optode (Oxi3315, WTW Weilheim, Germany). The first incubation was done after a
 12 five-day acclimation period at ambient temperatures, the second, four days after
 13 reaching the target temperatures and the last - a week after the second incubation, in
 14 order to see if longer exposure had shifted the shape of the performance curve as
 15 suggested by our third hypothesis. In the winter experiment, a fourth incubation was
 16 done, ten days after the third incubation. The shrimp were weighed after each
 17 incubation. Net respiration in terms of oxygen consumption ($R_{\text{net}(\text{O}_2)}$) in units of μmol
 18 $\text{O}_2 \cdot \text{liter seawater (L)} \cdot \text{gram shrimp (GS)} \cdot \text{hour (H)}^{-1}$) was calculated as the difference
 19 between initial and final dissolved oxygen concentrations (ΔDO) per liter seawater per
 20 gram of shrimp divided by the duration of the incubation (ΔT) as follows:

$$(1) R_{\text{net}} = \Delta\text{DO} \cdot \text{L} \cdot \text{GS} / \Delta T$$

22 *3.5.2 Gut resident time (GRT)* as a measure of digestion rate was tested three days after
 23 the second incubation. Food in the shrimp's gut is easy to follow because of its highly
 24 transparent body. Before the test, the shrimp were starved for two days and their glass
 25 container was cleaned every day from shrimp feces and small algae that grew on the

1 jar sidewall. After this period, the digestive organs of the shrimp were clearly empty.
2 Then, each individual received five red pellets of fish food (SERA Granured), and all
3 shrimp were continuously observed to detect the first time they defecated. GRT = the
4 interval between feeding and first defecation.

5 *3.5.3 The growth rate* of juveniles (smaller than 7mm) was assessed by measuring their
6 weight and size in the beginning of the experiment and once every two weeks for two
7 months. This was done by weighing the shrimp with an analytical scale (Analytical
8 balances ASB-220-C2 ($\pm 0.2\text{mg}$)), and photographing them with a digital camera (Sony-
9 RX100, 5) to assess size change with the Image-J software.

10 *3.5.4 Movement rates (activity)* were measured using a method similar to the one
11 described by Chapman et al (2013) with small modifications. We used a 19x24x17 cm
12 aquarium that was divided into 12 equally-sized square grids (6.3x6 cm). Individual
13 shrimps were placed in the aquarium for three minutes of acclimation, then their
14 movement was recorded with a digital video camera (Sony-RX100, 5) for another three
15 minutes. We considered movement as follows: every time the shrimp moved from one
16 square to the next or moved upward. The activity rate was defined numerically as the
17 number of movements.

18 *3.6 Critical temperature maximum (CTMax) and vulnerability assessment*

19 The thermal tolerance of the shrimp was determined by the dynamic method described
20 by Mora and Ospina (2001). The aim was to determine the CTMax, which is defined as
21 the “arithmetic mean of the collective thermal points at which the end-point is reached”
22 (Mora and Ospina, 2001). The end-point was a loss of equilibrium (LOE) when the
23 shrimp starts to swim upside-down. To determine the CTMax, individual shrimp were
24 placed in an aquarium (19x20x38 cm) with a water depth of 13 cm. Water was warmed

1 at a constant rate of 1°C h^{-1} using an ENDA EPC8420 profile controller with SCHEGO
 2 Heizer 300-watt heater and a temperature sensor. The shrimp were observed
 3 continuously until they reached the end-point. The end-point temperature was recorded
 4 and then the CTMax was calculated as follows:

$$5 \quad (2) \text{CTMax} = \sum (T_{\text{end-point}}) / n$$

6 Where $T_{\text{end-point}}$ is the end-point temperature for individual 1, individual 2, individual n,
 7 divided by n individuals that were tested ($n=X$). The warming tolerance was determined
 8 as the difference between CTMax and the maximum habitat temperature (MHT).

9 3.7 Survival

10 While *P. elegans* were in the thermobath system, their survival was measured from the
 11 point when they got to the target temperature. The daily survival rate of *P. elegans* in
 12 the experiment was calculated as follows:

$$13 \quad (3) \text{DSR (Daily survival rate)} = 1 - \frac{\sum_{i=1}^k (y_i)}{\sum_{i=1}^k (x_i)}$$

14 Where K is the total number of animals in the sample, y_i is 1 if animal i died during the
 15 reporting period and 0 if animal i was still alive at the end of the reporting period, x_i is
 16 the number animal days (number of day the individual was alive) for animal i
 17 (DeMaster and Drevenak, 1988).

18 3.8 Establishing performance curves

19 The performance parameters were plotted against temperature and fitted with models
 20 commonly used to describe performance curves (Angilletta Jr, 2006). The corrected
 21 Akaike Information Criterion (AICc), estimating information loss upon model
 22 selection, was calculated for each model, where the lowest AICc scores indicate the

1 best fitting model. AICc and r^2 indicators were used to assess the goodness-of-fit of
2 thermal performance curves. Data exploration was performed to find $P_{\max}$ (the value of
3 the measured parameter at peak performance) and T_{OPT} (the optimal temperature where
4 performance levels are maximal). The assessment of model fitting was performed using
5 CurveExpert Professional 1.6.10.

6 *3.9 Statistical analysis*

7 One-way analysis of covariance (ANCOVA) was performed to test the effect of
8 treatment-temperature and season-winter/summer and their interaction with respiration,
9 GRT and movement. For the effect of time since reaching the target temperature on
10 respiration rate, one-way repeated measures ANOVA was conducted. For growth rate,
11 differences between treatments were tested using one-way ANOVA. A subsequent
12 Bonferroni post-hoc test was applied to reveal which treatment differs from the others,
13 and how. All assumptions were tested prior to the analysis and a significance level of
14 0.05 was used. In cases where samples were not normally distributed (Shapiro Wilk's
15 test) or not homoscedasticity (Levene's test), data was first transformed using Log(10)
16 transformation, and in cases where prerequisites for parametric tests were not achieved,
17 the non-parametric Kruskal-Wallis test was performed. Statistical analysis did not
18 include dead shrimp. All statistical tests were performed using SPSS statistics version
19 21. Tabular results of the statistical analyses can be found in the Supplementary
20 Material section.

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4. Results

4.1 Tidepools and open sea temperatures

Pool depth varied between 20 and 95 cm and their area ranged from 0.72 to 367.3 m² (Supplementary Table S2). The temperature varied considerably both daily and seasonally between pools (Fig. 2. A-F), with maximum daily fluctuations in the closed pools reaching 10°C. At summer time, in closed disconnected pools (e.g. pool 1) the temperature raised above 35°C, for short periods of 6-7 hours maximum and cooled at night (Fig. 3). Minimum and maximum extreme values of 5.9°C in November and 37.8°C, respectively, in July were measured at pool 1. In the shallow open coastal waters near the reef, the minimum temperature was 14.9°C in December and the maximum was 32.18°C in July (Fig. 2G) and maximum daily fluctuations were 3°C during the sampled year.

4.2 Population dynamics

Individuals of *Palaemon elegans* were observed in the monitored tidepools throughout the year. The highest number of *P. elegans* was caught in tidepool 1 (remote and disconnected) and the lowest in tidepool 2 (big open pool). Ovigerous females were present from March-April and July-October. Juveniles were present between May-June and September-October. The highest number of juveniles were caught in May in tidepool 1 (Supplementary Table S1).

4.3 Thermal performances

4.3.1 Respiration. In the first incubation (at ambient water temperature) of adults in each season, we examined the relationship between the respiration rate (μmol O₂ h⁻¹) and the shrimp biomass (in grams). In both seasons, the expected increase in the respiration rate with body mass (see, Ivleva 1980) was evident (winter: $r^2 = 0.45$,

1 summer: $r^2 = 0.64$, Supplementary Fig. S1). The best fitting models for the performance
2 parameters measured after acclimation to target temperatures are detailed in Table 2.

3 When dead individuals are included in the analysis we see a clear optimum that is
4 shifting with the duration of exposure and with season (Fig. 4). When dead individuals
5 are excluded, a steady increase in respiration rate with temperature is evident, with
6 some leveling or even slight decrease (Fig. 4D-E) at the higher temperatures (insets in
7 Fig. 3). A clear reduction in T_{opt} of several degrees, with the increase in duration of
8 exposure to target temperature, was evident in both winter and summer (Table 2, Fig.
9 4). In winter, P_{max} was also reduced considerably with duration of exposure (Table 2,
10 Fig. 4). Significant differences were found in respiration rate between winter and
11 summer, after four days in the target temperature (incubation 2), with an interaction
12 between season and temperature (ANCOVA, $P < 0.05$, Supplementary Table S3).

13 Pairwise-comparisons for the winter incubation 2 (Kruskal-Wallis, Supplementary
14 Table S4) show significant differences ($P < 0.05$) between 8°C and 25°C, 28°C, 30°C
15 and 32°C. Pairwise-comparisons for summer incubation 2 (Bonferroni post-hoc test,
16 Supplementary Table S5) show significant differences ($P < 0.05$) between 17°C and
17 28°C, 30°C, 31°C, 32°C, 33°C, 34°C, and between 21°C and 31°C, 32°C and 33°C.

18 The same result (interaction effect of season and temperature) was found after eleven
19 days in the target temperature (ANCOVA, $P < 0.001$, Supplementary Table S6).

20 Pairwise-comparisons for winter incubation 3 (Bonferroni post-hoc test, Supplementary
21 Table S7) show significant differences ($P < 0.05$) between 25°C, 8°C and 12°C.

22 Pairwise-comparisons of temperature for summer incubation 3 (Bonferroni post-hoc
23 test) did not show significant differences between temperatures.

24 The effect of duration of exposure in the winter experiment (Repeated measured, $P <$
25 0.001) was significant (Bonferroni post-hoc test, $P < 0.05$) (Supplementary Table S8)

1 between 12°C and 21°C, 25°C and 28°C, as well as between 17°C and 25°C. The effect
2 in the summer had significant differences ($P < 0.05$) between 17°C and 31°C and 32°C
3 (Supplementary Table S9).

4 *4.3.2 Gut residence time (GRT)*

5 In both seasons, there was a strong negative correlation ($r^2=0.644$) between temperature
6 and GRT (Fig. 5), but at the highest temperature treatments (winter: 34°C, summer:
7 35°C) shrimp showed low interest in food or did not eat at all (thus data are not shown
8 for these temperatures). GRT in the summer was much lower in all tested temperatures
9 compared to winter (Fig. 4a). There was a significant difference between the interaction
10 of season and temperature (ANCOVA, $P < 0.001$, Supplementary Table S10). Pairwise-
11 comparisons of temperatures were done separately for winter and summer and showed
12 significant differences (Kruskal-Wallis, $P < 0.05$, Supplementary Tables S11, S12): in
13 winter between 28°C and 8°C and 12°C, in summer: between 25°C and 33°C.

14 *4.3.3 Shrimp activity*

15 Activity was highly variable among individuals at all temperatures, resulting in a low
16 power of analysis (Fig. 6). Nonetheless, the interaction between season and temperature
17 was significant (ANCOVA, $P < 0.05$, Supplementary Table S13), but pairwise-
18 comparisons, done separately for winter and summer, found significant differences only
19 in the summer, between 17°C and 33°C (Bonferroni post-hoc test $P < 0.05$,
20 Supplementary Table S14).

21 *4.3.4 Growth rate*

22 Growth rates were also highly variable among individuals at all temperatures, resulting
23 again in low power of analysis (Fig. 7A). The optimum juvenile growth rate was
24 measured between 21-27°C with maximum growth at 24°C (Fig. 7A). The ANOVA

1 test revealed a significant difference between groups ($P < 0.05$) but the Bonferroni post-
 2 hoc test did not find significant differences between temperatures (Supplementary
 3 Table S15).

4 4.3.5 Critical Temperature Maximum and warming tolerance

5 The CTMax was lower in winter (36.46°C) compared to summer (38.1°C, t-test Mann-
 6 Whitney Test, $U = .000$, $Z = -3.341$, $P < 0.001$, Supplementary Table S16). When
 7 considering MHT of 37.8°C (measured in pool 1 in July 2017), the warming tolerance
 8 for tidepools is only 0.3°C, but in the open coastal waters, it is much higher: 5.9°C.

9 4.4 Survivorship

10 In all three experiments, DSR differed significantly among temperatures (Kaplan-
 11 Meier, $P < 0.001$, Supplementary Tables S17-S19). *P. elegans* did not survive in the
 12 highest target temperatures. In the winter experiment, all shrimp died at 34°C within 4
 13 days, and at 33°C within 3 days (Fig. 8A). At 32°C, 80% of the shrimp died by the end
 14 of the experiment. All other treatments showed survival $> 50\%$. In the summer
 15 experiment (Fig. 8B) all the shrimp died at 35°C within 6 days and at 34°C within 9
 16 days. At 32°C, 50% of the shrimp were dead at the end of the experiment. All other
 17 treatments showed survival $> 50\%$. In the juvenile growth spring experiment, DSR was
 18 much lower since the experiment lasted two months (Fig. 7B). All juveniles died within
 19 6 days at 35°C, within 11 days at 33.5°C and within 27 days at 32°C. At 29.5°C, 80%
 20 of the shrimp died by the end of the experiment. At 12°C, 60% of the shrimp died by
 21 the end of the experiment and at 30.5°C and 28°C, 50% survived until the end of the
 22 experiment. All other treatments showed survival rates $> 50\%$.

23

24

5. Discussion

Using multiple lines of evidence our results show that *Palaemon elegans* lives close to its upper thermal limits in the most thermally-stressful tidepools (small and often disconnected from the open sea), but it is still within a wide safe zone in more open habitats. Nonetheless, long exposures to temperatures slightly higher than current summer values may affect its population viability over time. We also found that the shrimp's CTMax in the southeast Mediterranean, the warmest part of its distribution range, is higher compared to that of populations from the colder Atlantic shores, and that summer populations have higher thermal tolerance than winter ones, confirming our two working hypotheses related to local adaptation and seasonal acclimatization.

5.1 Tidepool conditions and population dynamics

As expected, our tidepool temperature time series revealed a wide daily variation of 10°C and seasonal variation of more than 32°C in the smaller, more isolated pools. Water temperature in these pools can drop to 6°C (after a few days of cold eastern winds) or climb up to 37.8°C and stay at temperatures greater than 35°C for more than six hours in summer. A few meters away, the open coastal waters have much lower temperature variability, showing a daily variation of 2-3°C, and seasonal variations of 17°C. In July and August, the open water temperature stayed above 30°C for more than two months, indicating the potential stress of residing in a shallow water system. Nonetheless, *P. elegans* populations were present in the tidepools throughout the year at variable densities and were more abundant at the higher part of the intertidal zone where the tidepools are less connected to the open sea and have the highest temperature fluctuations. The highest density of shrimp occurred during the recruitment season in

1 the spring in the most isolated tidepools, similar to the findings of Vinagre et al (2015)
2 who suggested that these tidepools may function as safe nursery grounds.

3 *5.2 Respiration response to warming*

4 The respiration rate of the shrimp increases with temperature until it levels off (or even
5 slightly declines under long exposure in the summer), and when dead individuals are
6 included in the analysis, clear optimum and lethal temperatures are evident (Fig. 4). As
7 we hypothesized, respiration rates increase (and P_{max}) from winter to summer, and T_{opt}
8 also increases between seasons (28.2°C to 32°C), and so does CTMax (34.5°C to
9 35.5°C), suggesting seasonal acclimatization for the higher summer temperatures.
10 Seasonal acclimatization of oxygen consumption rates has been previously documented
11 in intertidal crabs (Newell, 1973). Hopkin et al (Hopkin et al., 2006) tested eight crabs
12 species and in most, summer-captured animals had significantly higher CTMax values
13 than winter captured animals. Evidence of seasonal acclimatization in cardiac responses
14 to heat stress and thermal tolerance are commonly found in the literature. For instance,
15 the temperature for peak heart rate in the gastropod, *Lymnaea stagnalis* (Harrison,
16 1977) was found to be lower in winter (30.5°C) than in summer (37.5°C). A recent
17 study found a clear seasonal acclimatization of the cardiac function of the limpet *Patella*
18 *vulgata*, that allows individuals to be more tolerant to higher temperatures in the warm
19 months (Chappon et al., 2016). A fast seasonal physiological acclimatization is a
20 valuable feature that may lead to adaptive shifts in thermal breadth, thermal optima and
21 higher fitness (Somero, 2002). Acclimatization is a known mechanism that enables the
22 organism to cope with the changing environmental temperature that occurs seasonally.
23 Acclimatization (in the wild) or acclimation (in the laboratory) can change the
24 organism's response to environmental conditions (Fangue et al., 2009; Lachenicht et
25 al., 2010; Ravaux et al., 2016; Somero, 2002; Tomanek, 2010). For *P. elegans*, this

1 acclimatization is critical, as summer shallow waters have already warmed
 2 considerably, by 2-3 degrees, in the past three decades (Ozer et al., 2016; Rilov, 2016).
 3 The duration (and frequency) of exposure to extreme conditions is also a critical
 4 parameter that can determine a species' survival or fitness. In both winter and summer,
 5 *P. elegans* showed a reduction in the TPC parameters including $P_{\max}$ and T_{opt} , with
 6 increased exposure to high temperatures, and thus support our third working hypothesis.
 7 *P. elegans* use energy to produce stress proteins for repairing temperature-induced
 8 damage (Madeira et al., 2015; Madeira et al., 2012a; Williams et al., 2016). Therefore,
 9 the long exposure to constant high temperatures probably has high demands on the
 10 shrimp's metabolic functions and the accumulated stress weakens it, reducing its
 11 performance and shifting its thermal breadth to the left, as evident in our study. This is
 12 also expressed in the increased mortality at the highest temperatures after extended
 13 exposure.

14 The summer T_{opt} represents a threshold for respiration at 32°C. Beyond T_{opt} , the rate
 15 drops sharply to zero. At 35°C mortality was 100% in six days and at 34°C, all shrimp
 16 died before the third incubation (less than ten days). In the wild, in tidepools, *P. elegans*
 17 experience much higher temperatures, above 35°C, but only for short periods of 6-7
 18 hours maximum (with cooling at night, Fig. 2). Animals living in the intertidal zone
 19 develop different mechanisms to cope with short-term extreme temperatures. Some
 20 animals modify their exposure to extremes behaviorally, through the selection of
 21 thermally favorable microclimates, by hiding in crevices and escaping to shady areas
 22 (behavioral thermoregulation). Others have physiological adaptations where they divert
 23 their energy flow towards the production of a cellular stress response (CSR) and shift
 24 to anaerobic metabolism that is less efficient in generating energy (Han et al., 2013;
 25 Somero, 2002). These mechanisms require a change in the energy budget, which comes

1 at the expense of growth, reproduction and other vital functions (Williams et al., 2016).
 2 With an expected 2 degrees increase in temperature in the future, and the subsequent
 3 increase in the duration and frequency of hot days which are expected to rise, we predict
 4 that in isolated tidepools CTMax will be frequently surpassed, leading to increased *P.*
 5 *elegans* mortality.

6 In the open coastal waters, at summer time, *P. elegans* currently experience
 7 temperatures that are constantly above 30°C for more than two months (average August
 8 temperatures around 31.5°C). Based on our results, an increase of two degrees in sea
 9 surface temperature (SST) in the open sea will lead to a severe reduction in individual
 10 performance and fitness and possibly in the population's viability in the region. A total
 11 collapse of a regional populations due to warming (and possibly competition for food
 12 with an invasive species) has indeed already occurred in southeast Levant urchins
 13 (Yeruham et al., 2015; Yeruham et al., 2019). It is possible that semi-isolated tidepools
 14 might still offer temporal thermal refuge at night that can sustain the resilience of
 15 individuals living there and thus the population in the region. In future experiments, a
 16 simulation of real tidepool fluctuating conditions will shed more light on the actual
 17 robustness of the shrimp. Nonetheless, we assume that in tidepools disconnected from
 18 the open sea for long periods, multiple exposures to extreme peak temperatures in the
 19 heat of the day can gradually erode resilience – another aspect that deserves testing.
 20 The highest mortality that we witnessed in the field actually followed a sharp drop in
 21 temperature (down to 6°C); thus, cold stress in insulated pools during cold nights might
 22 also be a factor.

23 *5.3 Gut resident time and activity respond to warming*

24 Gut resident time reduced with temperature and was higher in winter than summer for
 25 the same temperatures, suggesting again a seasonal acclimatization in metabolic

1 functions (faster digestion). Another crustacean study showed that at higher
2 temperatures, there is a disassociation between feeding and gut evacuation rates and
3 suggested that mechanisms other than digestion rate might limit feeding rate (Chippis,
4 1998). In our study, we did not test the feeding rates of *P. elegans*, but we did observe
5 a decrease in appetite at high temperatures (personal observations), even though we
6 found high rates of digestion at such temperatures in both seasons. These results suggest
7 that an increased metabolic rate at higher water temperatures plus low feeding rates and
8 high gut evacuation rates at the highest livable temperatures, likely reduce the overall
9 scope for growth under excessive warming.

10 The activity test suggests again that higher metabolic rates, in summer compared to
11 winter, are manifested with increased movement. There was a rise in the performance
12 with temperature, but individual variability was very high, resulting in non-significant
13 relationships. The higher metabolic rates in summer may express seasonal
14 acclimatization to high temperatures.

15 *5.4 Critical temperature maximum*

16 The CTMax is considered to be the most reliable parameter in macrophysiological
17 comparative studies in ectotherms (Lutterschmidt and Hutchison, 1997). It is used for
18 assessing the upper thermal limits and the thermal tolerance of a species in relation to
19 the maximum habitat temperature that a species experience. The CTMax, the thermal
20 tolerance and the acclimation capacity for *P. elegans* were previously tested on the other
21 side of the species distribution range, in the West Mediterranean and East Atlantic-
22 which are colder regions compared to the EMS (Table 1). Madeira et al. (2012b)
23 measured the CTMax of coastal and estuarine organisms including *P. elegans* on the
24 coast of Portugal, where the mean water temperature was 24°C and MHT was 35°C,
25 and established a CTMax of 34°C. The authors concluded that "the most vulnerable

1 organisms to sea warming were those that occur in the thermally unstable environments
2 because, despite their high CTMax values, they live closer to their thermal limits and
3 have limited acclimation plasticity". These results were based on MHT measured in air
4 and therefore their conclusion should be considered with caution, as it was not based
5 on the actual MHT that *P. elegans* experience (in tidepool waters, which might be lower
6 than that of the air). Vinagre et al. (2016) measured the CTMax and acclimation
7 capacity of tropical and temperate coastal organisms, among them *P. elegans* from west
8 Portugal. They found a CTMax of 33.4°C after 7 day exposure at ambient temperature
9 (20°C). When testing the acclimation capacity of its thermal limits after acute and
10 chronic exposure, they found that *P. elegans* has the highest acclimation capacity under
11 long-term low stress (30 days +3°C, CTMax = 36.2°C) or short-term high stress (10
12 days +6°C, CTMax = 36.6°C) conditions compared to all other test species. Because in
13 their study the MHT measured in a tidepool on a hot summer day was 30°C, they
14 concluded that *P. elegans* is still far from its upper thermal limits in its natural
15 environment.

16 In the western Mediterranean, Ravaux et al. (2016) examined the CTMax of *P. elegans*
17 during ontogeny, and found that adults have the ability to adjust their CTMax through
18 long-term acclimation (4 to 8 months at 10°C or 20°C). Although they used a slightly
19 different procedure (heating rate of 1°C per minute) they found that the CTMax for
20 zoea larva is significantly lower than that of adults, both reared at 20°C. The CTMax
21 for adults acclimated to 10°C and 20°C was 27±0.8°C and 33.2±1.5°C, respectively.
22 The higher value of CTMax for adult *P. elegans* is very similar to the CTMax of *P.*
23 *elegans* measured in the study by Vinagre et al. (2016). Together, these findings
24 indicate that the CTMax is strongly influenced by habitat temperatures experienced by
25 the species.

1 Evidently, the CTMax values measured in our study for *P. elegans* at its trailing, warm
 2 edge (winter, 36.4°C; summer, 38.1°C) was considerably higher than those measured
 3 in populations from the leading edge of its distribution, as described above, even after
 4 acute exposure to extreme heat shown by Vinagre et al. (2016). The higher summer
 5 CTMax value (compared to winter) yet again is probably the result of acclimatization
 6 to the very high temperatures that this species experiences during this season. This
 7 result supports our hypothesis that the CTMax of *P. elegans* from this warmer region
 8 will be higher compared to that of western populations. It also supports the assumption
 9 that the upper thermal limit is related to the local thermal habitat. The mechanism
 10 behind it may be adaptation (perhaps through selection) to the higher temperatures in
 11 the region. Local adaptation was shown to be widespread in marine invertebrates
 12 including ones with planktonic larvae (Sanford and Kelly, 2011), such as *Palaemon*.
 13 However, isolated populations with high local adaptation might have very limited
 14 capacity to adapt to further warming, as was shown at the leading edge of a copepod
 15 that lives in high intertidal tidepools (Kelly et al., 2012).

16 *Juvenile growth*

17 The optimum temperature for juvenile growth in the EMS was found to be 24°C (similar
 18 to late spring SST in coastal waters), and growth rates were highest between 21-27°C.
 19 In the field, juveniles were abundant in May and June, and the average temperature in
 20 pool 1, that was populated by the highest abundance of juveniles, during that period
 21 was 25.7°C and 29.3°C respectively, indicating that May has the average optimal
 22 temperature for growth. During that period, the maximum temperature recorded was
 23 33.2°C (May) and 36.3°C (June); however, these extreme temperatures were short in
 24 duration and thus did not result in mass juvenile mortality. The survival data (Fig. 7B)
 25 showed that juvenile shrimp are more vulnerable to high and low temperatures

1 compared to adults. Post-larva of *P. elegans* from the northwest Mediterranean were
2 found to have lower CTMax than adults, based on their capacity for heat shock response
3 (see, Ravaux et al., 2016). In our study, all juveniles died above 33°C, and mortality
4 was greater than 50% at temperatures of 12, 28, 29.5 and 30°C.

5 In summary, *P. elegans* appears to have a high capacity for acclimatization (high
6 plasticity) both biogeographically (although selection for more heat resistant genotypes
7 might be involved in the process), and seasonally. *P. elegans* in the EMS has higher
8 CTMax than its more northwestern counterparts, but in small, isolated tidepools it
9 experiences short exposures to extreme temperatures that are much closer to the CTMax
10 than those in the western populations. In light of the projected continuation of global
11 warming of both air and water (IPCC, 2014), conditions in temporarily isolated
12 tidepools are expected to become more extreme for *P. elegans* and especially for
13 juveniles, which may negatively affect the population along the coast. Furthermore, the
14 recently described prolonged desiccation events (PDEs) on southeastern Mediterranean
15 rocky shores, generated by dry easterly winds that can expose the vermetid reefs to air
16 continuously for days during spring (Zamir et al., 2018), can leave many tidepools
17 isolated from the open sea for very long periods, creating extreme conditions in them
18 that are harmful for juvenile shrimp. The frequency of PDE generating synoptic systems
19 has more than doubled in the past four decades during winter and spring (Alpert et al.,
20 2004; Zamir et al., 2018), and this trend is expected to continue in the near future
21 (Hochman et al., 2018a; Hochman et al., 2018b), adding additional stress to this already
22 challenging habitat. The combination of all these stressors has the potential to reduce
23 the fitness and viability of the population on a regional scale with potentially little
24 chance of adaptation as warm-tolerant phenotypes in the trailing edge could reduce

1 genetic diversity and constrain the potential for adaptation for other stressors (Donelson
2 et al., 2019).

3 The potential for plasticity in thermal tolerance, and/or adaptation, which may mitigate
4 the effects of global warming is largely unknown due to the lack of knowledge about
5 global and taxonomic patterns of variation in tolerance plasticity (Gunderson and
6 Stillman, 2015). Donelson et al (2019) further stress the importance of such knowledge
7 to improve species distribution models under climate change and for marine
8 conservation. Gunderson and Stillman's (2015) global analysis of terrestrial, freshwater
9 and marine ectotherms, suggested that “overheating risk will be minimally reduced by
10 acclimation in even the most plastic groups”. The findings of our study strengthen the
11 case for the clear context-dependency of the vulnerability of ectothermic species that
12 live in extremely stressful and fluctuating environments to climate change. It
13 demonstrates the potential biogeographical thermal physiological flexibility and
14 adaptability (whether genotypic or phenotypic) of such species, but also their high and
15 increasing vulnerability at the trailing edge of their population under climate change as
16 suggested by Somero (2010).

17

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1 Tables

2 **Table 1.** CTMax values of *Palaemon elegans* tested in studies conducted in the north
3 east Atlantic and west Mediterranean Sea and determined under different temperature
4 exposure regimes. Acl = Acclimation tretment, D = days.

Authors	Location of collection	Time of collection (mm-yy)	Water temp at collection time	Maximum habitat temp	Heating rate	<i>P. elegans</i> CTMax
Diana et al	Portugal	Jul-10	24°C	35°C (based on max air temp)	1°C\H	34°C
Magozzi & Calosi	England	Jun-12	12.4°C	Not reported	0.75°C\ M	Acl 10°C: 30°C Acl 15°C: 33°C Acl 20°C: 34°C Acl 25°C: 35°C
Catarina et al	Brazil and Portugal	Sep-14	20°C	30°C (June 2014)	1°C\H	7D: 33.4°C 30D: 36.2°C 10D: 36.6°C
Juliette et al	France	Oct-11	17°C	Not reported	1°C\M	Acl 10°C: 27.5°C Acl 20°C: 33.2°C

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1 **Table 2.** Best-fitting models for the tested TPCs in different seasons and for the
2 performance of respiration at different exposure durations after reaching target
3 temperatures. The goodness-of-fit indicators are Akaike Information Criterion (AICc)
4 and r^2 .
5

Performance variable	N	Best-fit model	AICc/ r^2	T _{OPT} (°C)	P _{max}	CT _{Max}
Respiration, 4 days, winter	58	Rational	131.6/0.6	28.2	11.3	33.5
Respiration, 11 days, winter	58	Rational	122.7/0.56	25.5	9.5	33.5
Respiration, 20 days, winter	53	Rational	51.86/0.45	25.5	4.8	34.5
Respiration, 4 days, summer	49	Rational	166.4/0.35	32	14.4	35.5
Respiration, 11 days, summer	49	Rational	152.66/0.54	26.3	14.7	34.8
Activity winter	42	Rational	261.4/0.08	29	33	34.5
Activity summer	42	Heat capacity	260.09/0.1	26-30	40	N/A
Growth	64	Rational	1141.3/0.3	24	0.0003	N/A

1 **Figure legends**

2 **Figure 1. Study site.** Location of the study site on the Israeli coast and a drone photo
3 showing the location of the five monitored tidal pools on the rocky shore at
4 Shikmona.

5 **Figure 2. Tidepool monthly temperature variability.** (A)-(E) Mean monthly water
6 temperature in the five pools during the study period. (F) Average temperature of five
7 pools in the study site. (G) Coastal temperature in the open water at 0.5 m depth
8 during the study period. (H) Air temperature during the study period. Red, gray and
9 blue lines represent the maximum, average and minimum values that measured,
10 respectively (gaps in data are periods when the logger failed and replaced later). In
11 three pools, loggers were lost or damaged resulting in gaps in data.

12 **Figure 3. Hourly pool temperature variability.** Four days of temperature
13 fluctuations measured in July 2017 illustrating the present and predicted future
14 summer conditions in the most extreme (isolated tidal pool 1, solid red line) and
15 benign (open sea, solid blue line) habitats. The red and blue dashed lines represent
16 increase of +2°C in water temperature. The upper black dashed line represents the
17 measured critical temperature maximum and the lower line represents the measured
18 threshold point for *P. elegans* after four days of exposure to constant temperature
19 (32°C).

20 **Figure 4. The effect of temperature on respiration.** Temperature effects on the
21 dissolved oxygen (DO) consumption in *P. elegans* measured at 10 temperature levels
22 in the winter between 8-34°C and in the summer between 17-35°C (n=6 per temperature
23 level). Measurements were conducted after reaching the target temperature. In the
24 winter: after 4 days (A), after 11 days (B) and after 20 days (C). In the summer: after 4
25 days (D) and after 11 days (E). A 95% confidence interval bend is marked by shaded

1 areas. Insets show the data and curves when all dead individual are removed. The model
2 best fitting to the data were rational (**A-D**) and Heat capacity (**E**).

3 **Figure 5. The effect of temperature on the gut residence time** in *P. elegans* measured
4 in the winter and summer at 10 temperature levels between 8-34°C and 17-35°C,
5 respectively (n=6 per temperature level). Resident time is shown as means $\pm$ SE.

6 **Figure 6. The effect of temperature on activity** of *P. elegans* measured in the winter
7 and summer at 10 temperature levels between 8-34°C and 17-35°C, respectively (n=6
8 per temperature level). Activity measurements that were conducted six days after
9 reaching the target temperature. Each dot represents a measurement. A 95% confidence
10 interval band is marked by shaded areas. The fitted models were Rational in the winter
11 and Heat capacity in the summer.

12 **Figure 7. The effect of temperature on the growth rate (A) and on the daily**
13 **survival rate (DSR) (B) in juvenile *P. elegans*** measured in the spring at 11
14 temperature levels between 12-35°C (n=6 per temperature level). The red arrow
15 represents the time of reaching the target temperatures. The best fitted model in A was
16 the Rational model.

17 **Figure 8. *P. elegans* daily survival rate (DSR) under 10 tested temperatures in the**
18 **winter (A) and summer (B) experiments** (n=6 per temperature level). The red arrows
19 represents the time it took to reach the target temperatures. Each color represents a
20 different treatment.

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