On their way to the north: larval performance of Hemigrapsus sanguineus invasive on the European coast—a comparison with the native European population of Carcinus maenas

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SUPPLEMENTARY MATERIAL

MATERIAL AND METHODS

1. Animal husbandry, larval rearing and experimental design

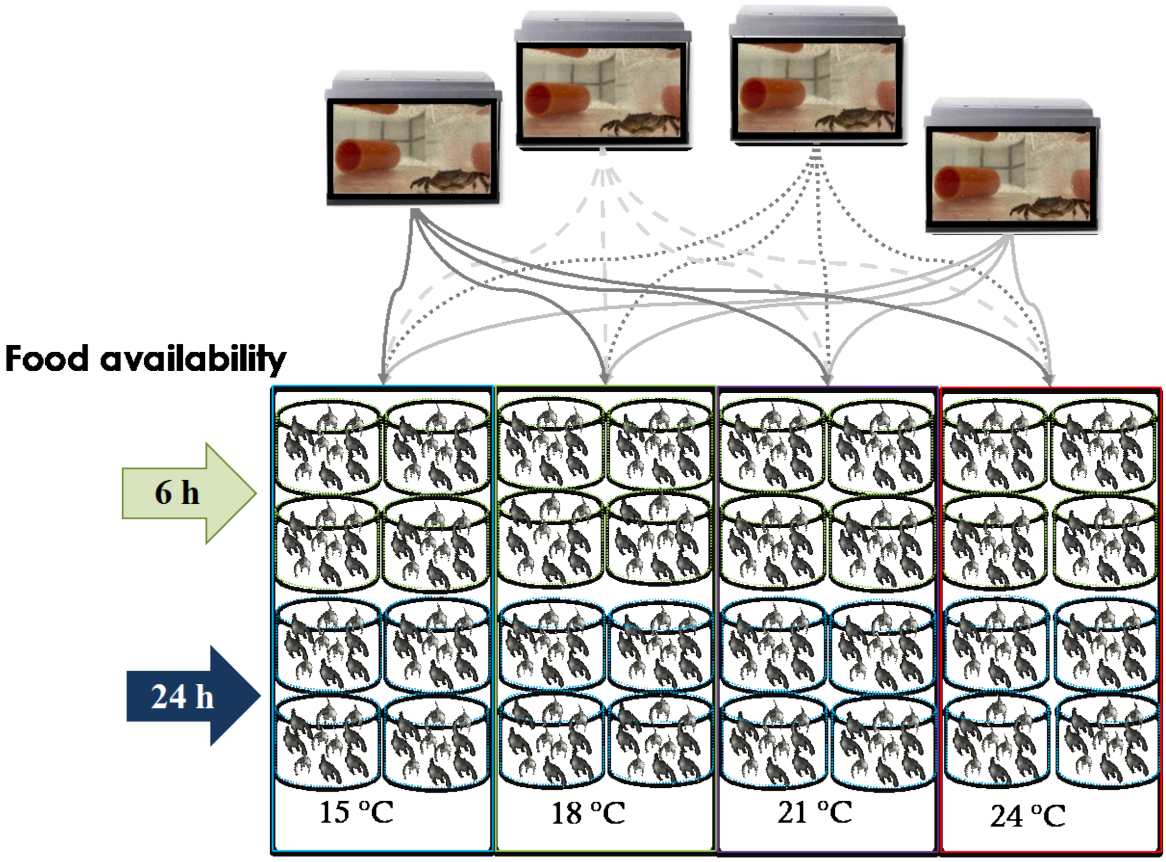

Figure S1. Experimental design to study the effect of limited access to food on larval performance of *Hemigrapsus sanguineus* under increased temperature. We carried out four experiments (one for each of the four females) in four replicates each. Following Torres and Giménez (2020).

Table S1. *Hemigrapsus sanguineus.*Number of megalopae sampled in each rearing vessel for each the temperature and food availability treatment. Abbreviations: ♀: female of origin; RV: rearing vessel F6: limited access to food; F24: permanent access to food

	15 °C								**18 °C**								**21 °C**								**24 °C**							
	F6				**F24**				**F6**				**F24**				**F6**				**F24**				**F6**				**F24**			
RV	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
♀1	3	1	2	0	9	7	6	3	6	7	6	9	6	7	6	9	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
♀2	6	3	6	8	9	6	17	10	6	8	6	6	6	9	6	7	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
♀3	0	2	1	3	2	0	3	0	0	2	1	2	5	6	6	6	0	2	5	5	5	8	5	0	3	2	0	0	6	6	6	5
♀4	0	0	0	0	2	3	0	1	0	0	0	2	6	6	6	6	2	0	3	0	6	6	6	6	0	0	2	0	6	6	4	6

2. Data analysis: details on model simulation

In order to compare the larval performance of *H. sanguineus* with that of *C. maenas*, we calculated the ratios of survival (S_R) and growth rates (G_R) between species. Ratios were thus as follows: $S_R = S_H / S_C$ and $G_R = G_H / G_C$, where S_H and S_C are the survival to the megalopa of *H. sanguineus* and *C. maenas*, respectively, and G_H and G_C the respective instantaneous growth rates (from hatching to megalopa). In order to incorporate the intraspecific variation in the survival and growth responses we simulated 1000 values of the survival and growth rates for each species, and combinations of temperature and access to food. Simulations were carried out in R, after fitting models using the package *nlme* and the functions *simulate* and *sample* (R Core Team 2013). In these simulations, we also considered correlations between survival and growth; hence, each simulated value of growth had a corresponding simulated value of survival.

Previous to the simulation (Fig S2a), we fitted a model for growth of each species (G_i), where female of origin (F_i) was a repeated measured factor, with and additive effect of survival (S_i) and interacting effects of temperature (T) and access to food (F), using the formula:

$$G_i \sim F \times T + S_i, correlation = CorcompSymm(form = \sim 1|F) \quad \text{(S1)}$$

The growth model used for the simulation was applied to values averaged by female of origin because data on *C. maenas* were not sufficient to perform a simulation considering variability within each hatch (as done for *H. sanguineus*). In addition, data shows that the largest source of variability in survival (both species) and growth (*H. sanguineus*) corresponds to that among larvae hatching from different females. In the growth model, female of origin is still incorporated as a within subject factor to account for potential female-related sources of variation in growth not explained by variations in survival. Model selection (methods described in the main body of the article) retained the model described in equation S1.

Table S2. *H. sanguineus* and *C. maenas*. Results of model selection explaining the role of survival (S) as predictor of growth in addition to the interaction between temperature (T) and access to food (F) for *C. maenas* and *H. sanguineus*. Best models are marked in bold.

Terms	*C. maenas*	*H. sanguineus*
	AIC	AIC
FxT +S	**-188**	**-218**
FxT	-183	-178
S	-174	-203

The simulation was carried out in two steps. In step 1 (Fig S2b, grey boxes), survival proportion was simulated from the statistical models fitted to the responses of both species [for *H. sanguineus*: model in Table S2; *C. maenas*: model in Torres & Giménez (2020)]. The simulated values were then averaged by female of origin and then used to simulate growth rates (blue boxes). Notice here that the simulation needs to be carried out in sequence in order to account for the correlation between growth and survival. In this step, and for each species separately, the output were 1000 pairs of values of survival-growth per female of origin, temperature and access to food (i.e. 32,000 values = 1000 x 4 females x 2 food treatments x 4 temperatures per species). Iterations giving survival = 0 or negative were removed (usually 1 or 2 iterations) and the remaining results stored.

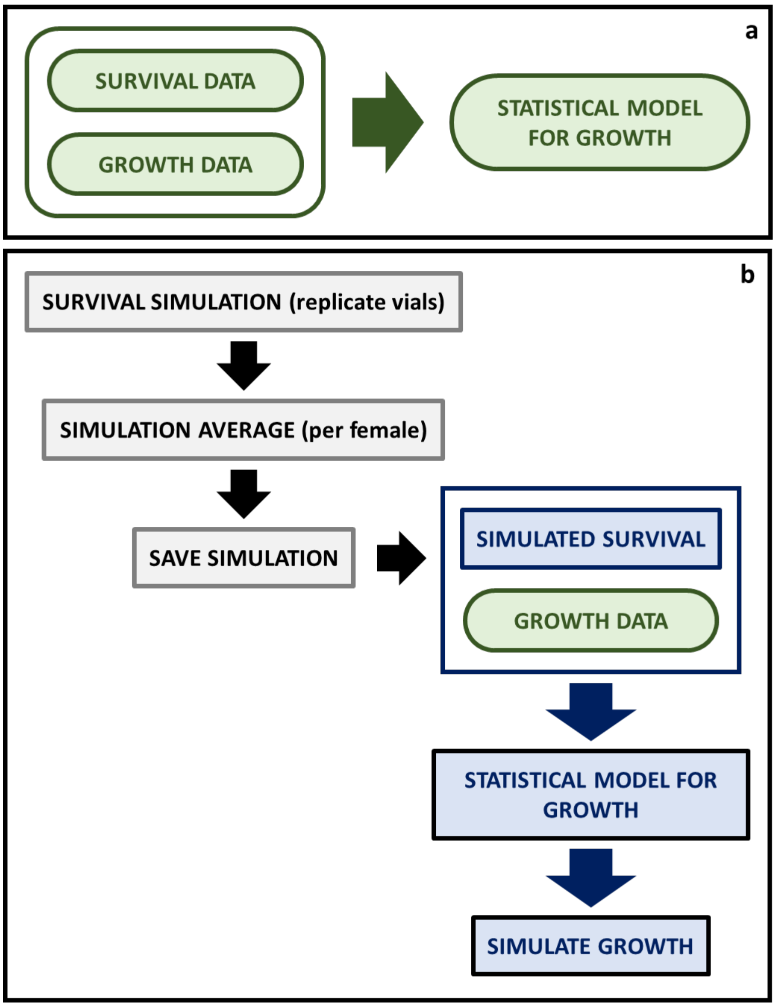

Figure S2. *H. sanguineus* and *C. maenas*. Simulation of survival and growth for *H.sanguineus* and *C. maenas*. (a) Previous to the simulation, a model is fitted to growth rates with survival as predictor (equation S1 for details). (b) Survival data are simulated and values are averaged by female of origin; those values are used as predictors in the statistical model predicting growth rate data. Each simulated growth value is paired with the corresponding simulated survival value and stored for use in the next step (see text below and Figure S3). The sequence shown in (b) is carried out 1000 times, for each species, female of origin, and combination of temperature and access to food.

In the second step of the simulation (Fig S3) 4000 pairs of survival-growth values of each species were combined at random (using the function *sample*, with replacement) to calculate the survival and growth ratios (S_H/S_C and G_H/G_C). Values were then averaged by groups of four females of origin, keeping the fact that both studies were based on larvae hatched from four different females; averages values are plotted in Figure 5.

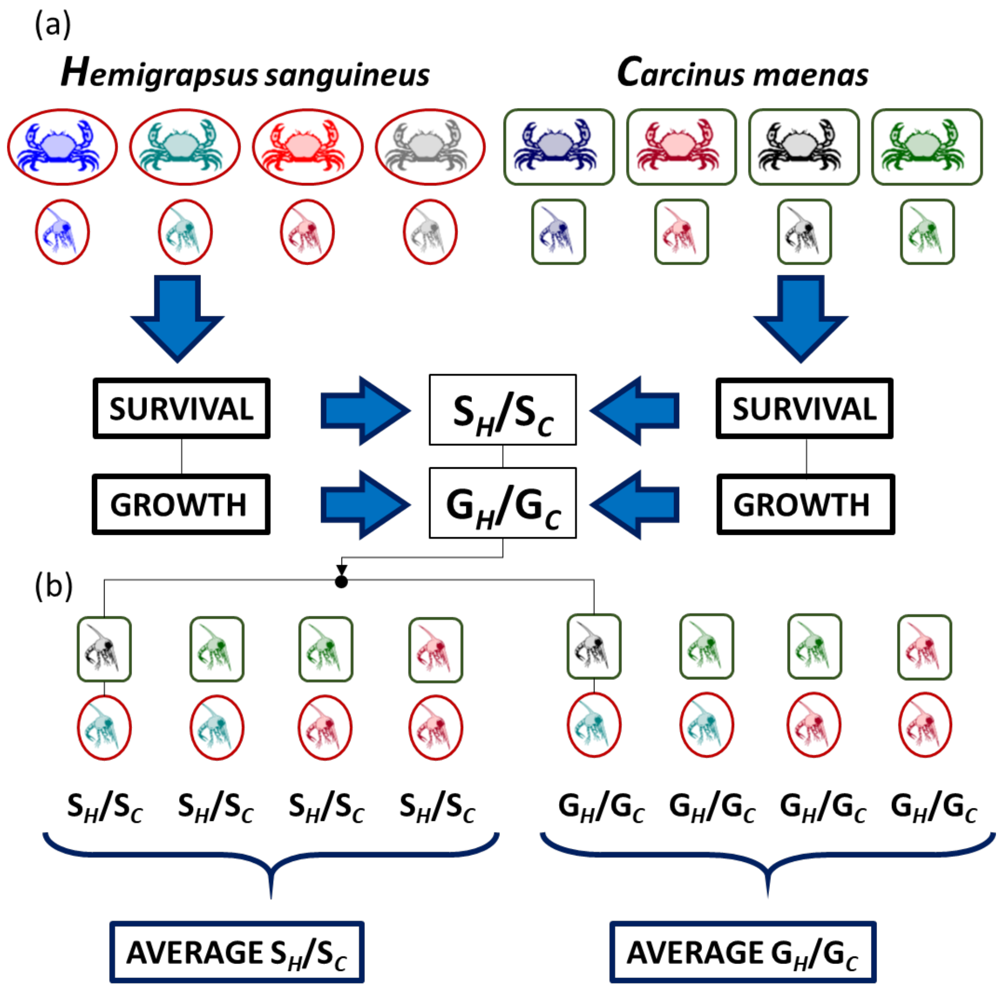

Figure S3. Random sampling and averaging of ratios. (a) Simulated data corresponding to larval survival and growth of a given female per species is randomly sampled. Species are coded with ellipses (*H. sanguineus*) and rectangles (*C. maenas*) around the drawings; females and the respective larvae are coded in different colours. In (a) an iteration of the simulation is represented: average values of survival and growth from female 2 of *H. sanguineus* are paired (see connecting line) with those of female 3 of *C. maenas* and the ratios are calculated; ratios are then used in the subsequent averaging procedure. (b) Four ratios are averaged (without replacement): in this averaging procedure, there is the chance that the mean ratios are over-represented by larvae from a particular female. In the diagram, an averaging procedure is shown where ratios are over-represented by larvae from females 2 and 3 of *H. sanguineus* (colour coded) and by larvae from female 4 of *C. maenas*. Drawings are based on modified images taken from Wiki under Creative Commons (CC) licence.

There are two additional points to consider in the simulation. For figure 5, we did not plot the 4000 values because the simulation was aimed at comparing larval performance at the level of populations; comparisons at the level of families do not make sense because the larval seasons of *H. sanguineus* and *C. maenas* do not fully overlap. Plots using the 4000 values show similar average responses as in Fig 5, but much wider confidence ellipses. Plots based on averages by a larger number of females (e.g. averaging by 10 instead of 4 values) result in narrower confidence ellipses. However, we do not think it is appropriate to average over more than number of females used in the experiment because we would be underestimating the intraspecific variation in the responses. For instance, in an experiment based on e.g. larvae originated from 10 females there is a higher chance that rare larval phenotypes are sampled (and extreme values of survival and growth are obtained) than in an experiment based on larvae from 4 females.

The second point concerns the consequences of the averaging data procedure on how the correlation between survival and growth affects the output of the simulation. The correlation is manifested in the inclination of the major axes of the ellipses with respect to the vertical and horizontal axes. Three additional preliminary simulations showed that: (1) If the correlation is not considered, the average responses (centroid of ellipses) give similar results as in the used simulation. However, the two major axes of the ellipses run (respectively) in the vertical and horizonal direction (Fig S4a). The effect of the correlation on the inclination of the axes is removed by: (2) Calculating the ratios *after* survival and growth are averaged for all the females used in the experiment (Fig S4b). (3) Calculating the averages over the four females of each species, instead of sampling four females at random with replacement (Fig S4c).

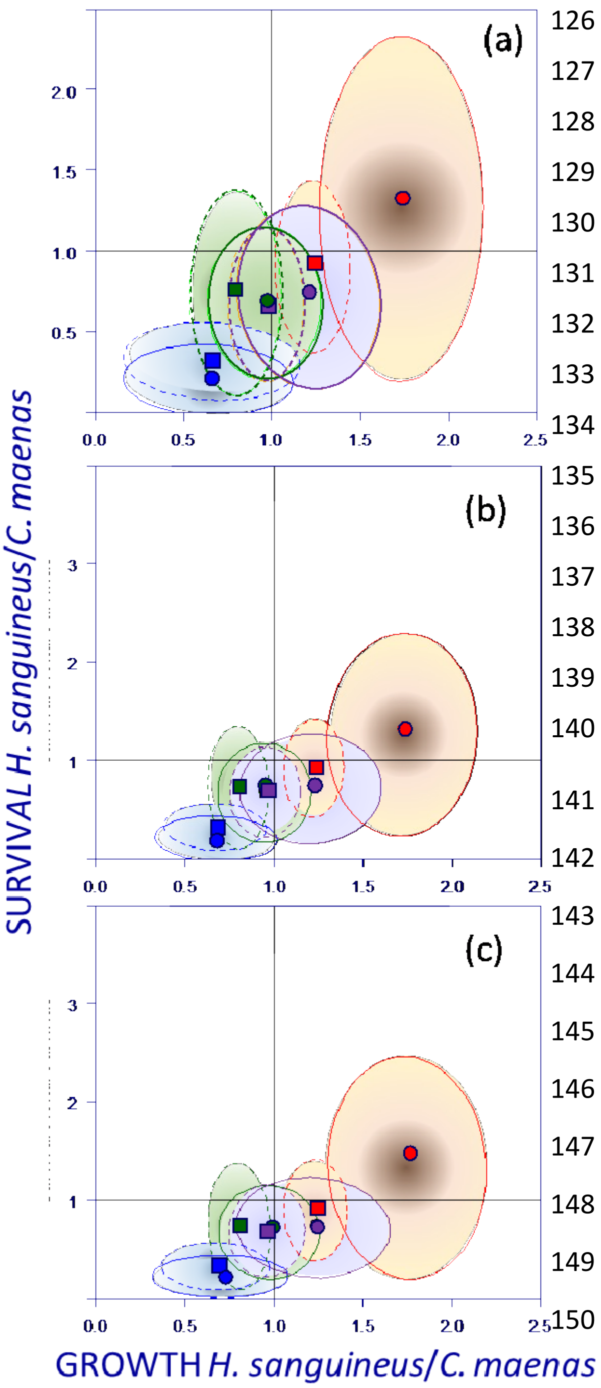

Figure S4. *H. sanguineus* and *C. maenas*. Output of three alternative simulations. (a) Without correlations between growth and survival; simulated survival and growth are averaged over the four females of each species and then ratios are calculated. (b) With correlations between survival and growth; averaging occurs as in (a). (c) With correlations between survival and growth; ratios calculated and then four ratios averaged so that the four females of each species are included in each average. The simulation presented in the main text is as (c) but with ratios sampled at random, so that particular larvae from particular females can be over-represented in the average value.

RESULTS

Table S3. *Hemigrapsus sanguineus.* Model selection for survival to consecutive stages, data transformed logistically (on the left) and logarithmically (on the right). Model selection was performed using the adjusted Akaike information criteria (AICc). Abbreviations: ♀: female of origin; F: food condition; T: temperature, ZII: zoea II, ZIII: zoea III, ZIV: zoea IV, ZV: zoea V, and M: megalopa, respectively). Highlighted in bold: the best overall model (containing both the best random and fixed term).

Model selection	Scale									
Random (REML)	Logistic					Logarithmic				
	ZII	ZIII	ZIV	V	M	ZII	ZIII	ZIV	V	M
♀:F:T	**-234**	**-156**	**-136**	**-123**	**-121**	**-172**	-58	**3**	**77**	330
♀:T	-227	-151	-107	-90	-84	-167	**-62**	14	93	**317**
♀:F	-224	-139	-91	-83	-116	-161	-50	41	129	343
♀	-218	-142	-90	-73	-86	-158	-55	37	128	337
Fixed (ML)										
F:T	-273	**-192**	-167	-153	-151	**-206**	**-95**	**-20**	61	310
F+T	-272	-191	**-168**	-159	**-155**	-203	-86	-19	54	**305**
T	**-273**	-190	-160	-154	-143	-205	-87	-15	56	308
F	-266	-180	-159	**-164**	-147	-198	63	-9	**48**	369
Null	-268	-180	-153	-159	-138	-200	-64	-5	50	372

Table S4. *Hemigrapsus sanguineus*. Model selection for survival considering the models per females of origin (♀1: female 1, ♀2: female 2, ♀3: female 3, ♀4: female 4). Model selection was performed using the adjusted Akaike information criteria (AICc). Abbreviations: F: food condition; T: temperature. Highlighted in bold: the best model.

Fixed (ML)	AICc			
	♀1	♀2	♀3	♀4
F:T	**62.8**	**33.4**	95.2	86.7
F+T	69.6	48.5	**91.5**	**80.34**
T	73.7	48.2	115	114
F	88.5	73.3	94	94.1
Null	89.3	71.8	112	115

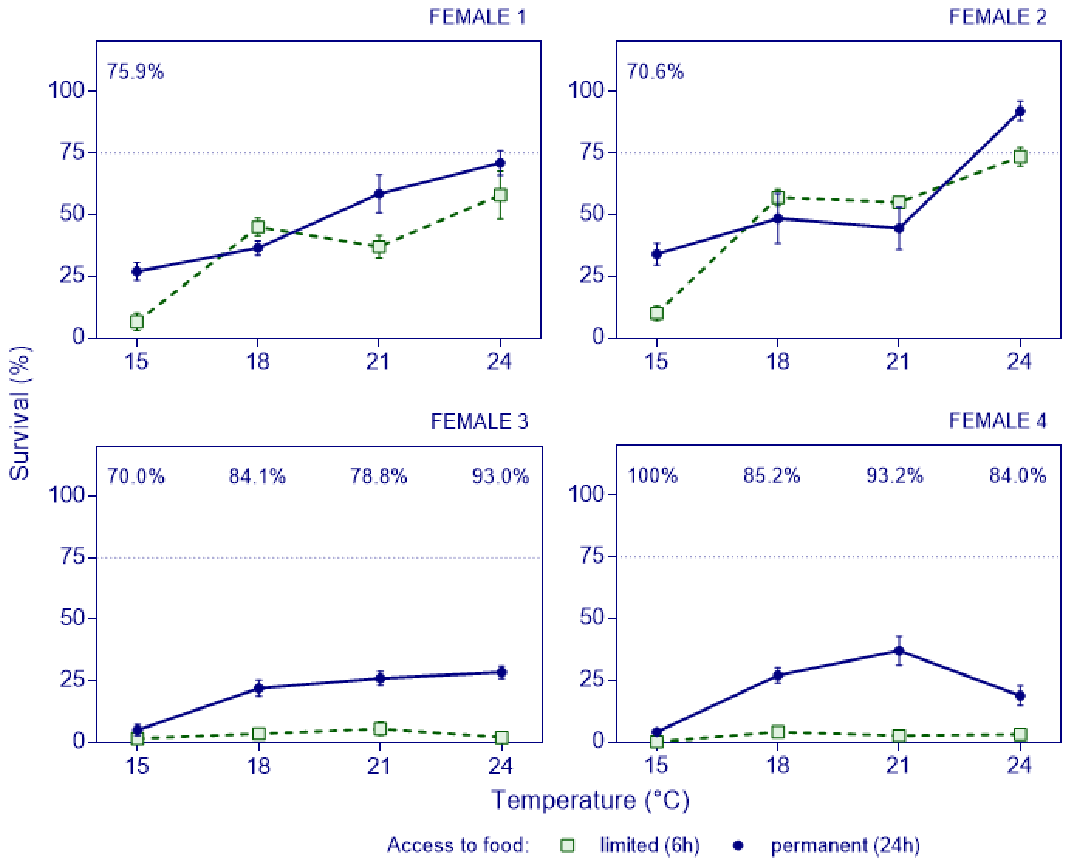

Figure S5. *Hemigrapsus sanguineus*. Average survival from hatching to megalopae as a response to temperature and food availability, discriminated by female of origin. Data shown as average values ± SE. Permanent access to food: blue circles and continuous line; limited access to food: green squares with dashed line. Percentages on top (only when significantly different): percent difference in survival between permanent and limited access to food treatments for each temperature.

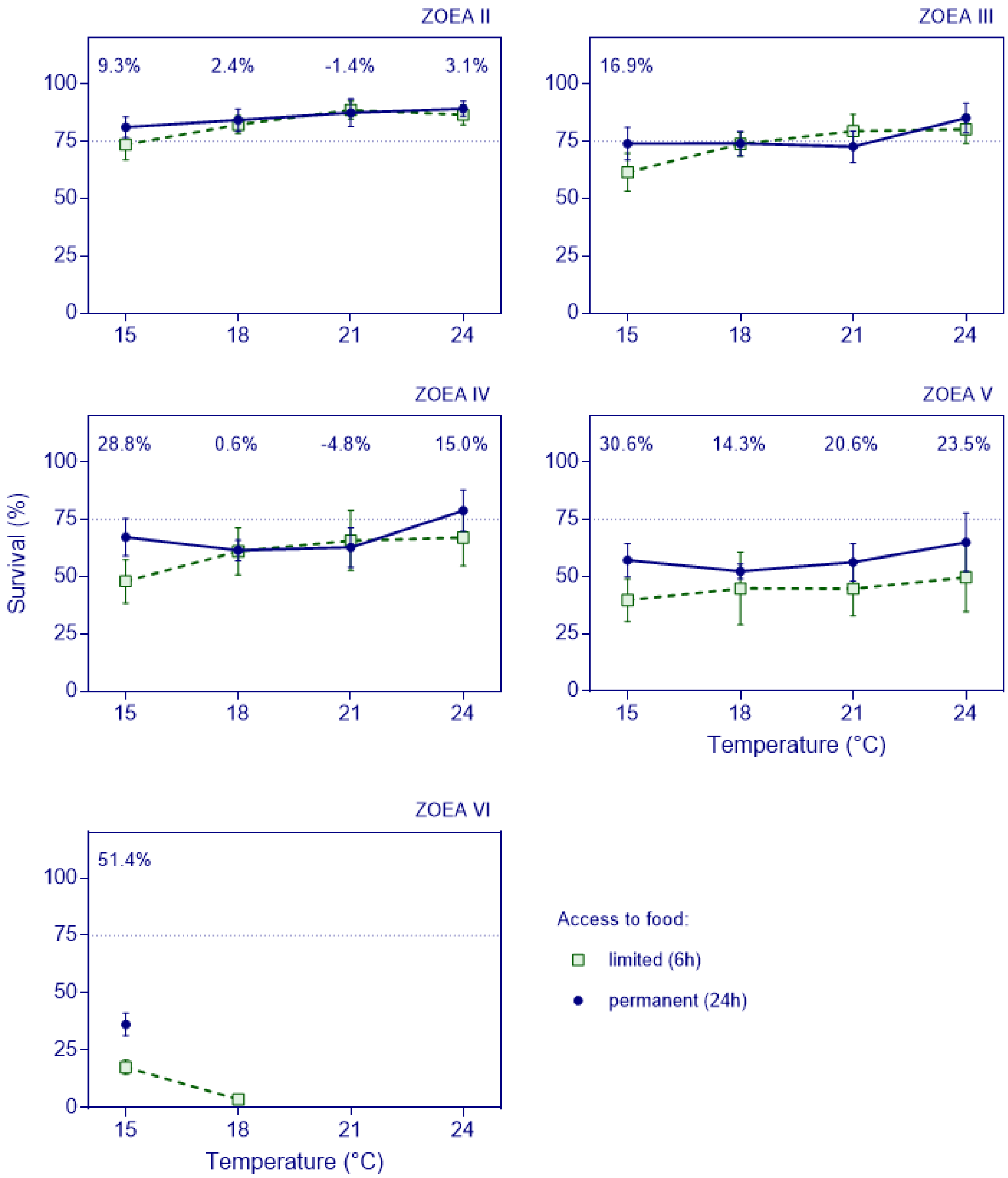

Figure S6 *Hemigrapsus sanguineus*. Average survival from hatching to zoea II, III, IV, V, and VI as a response to temperature and food availability. Symbols as in Fig. S1. Percentages on top (only when significantly different): percent difference in survival between permanent and limited access to food treatments for each temperature.

Table S5. *Hemigrapsus sanguineus*. Model selection for duration of development to consecutive stages, data analysed in the raw scale (on the left) and logarithmically. Model selection was performed using the adjusted Akaike information criteria (AICc). Abbreviations: ♀: female of origin; F: food condition; T: temperature; ZII: zoea II, ZIII: zoea III, ZIV: zoea IV, ZV: zoea V, and M: megalopa. Highlighted in bold: the best overall model (containing both the best random and fixed term).

Model selection	Scale									
Random (REML)	Raw					Logarithmic				
	ZII	ZIII	ZIV	V	M	ZII	ZIII	ZIV	V	M
♀:F:T	**208**	389	464	472	379	**-291**	**-237**	**-222**	-286	-192
♀:T	248	386	480	460	370	-232	-212	-176	-273	-196
♀:F	223	390	**461**	464	375	-264	-207	-209	**-294**	-195
♀	247	**383**	478	**458**	**370**	-232	-214	-178	-275	**-198**
Fixed (ML)										
F:T	203	378	**465**	**458**	**375**	-328	-277	-260	**-341**	**-235**
F+T	199	**375**	470	478	389	-331	-277	**-265**	-306	-213
T	**199**	379	501	526	411	**-331**	**-278**	-261	-255	-182
F	240	751	522	937	616	-287	-225	-216	-249	22
Null	240	749	554	936	616	-288	-228	-209	-201	23

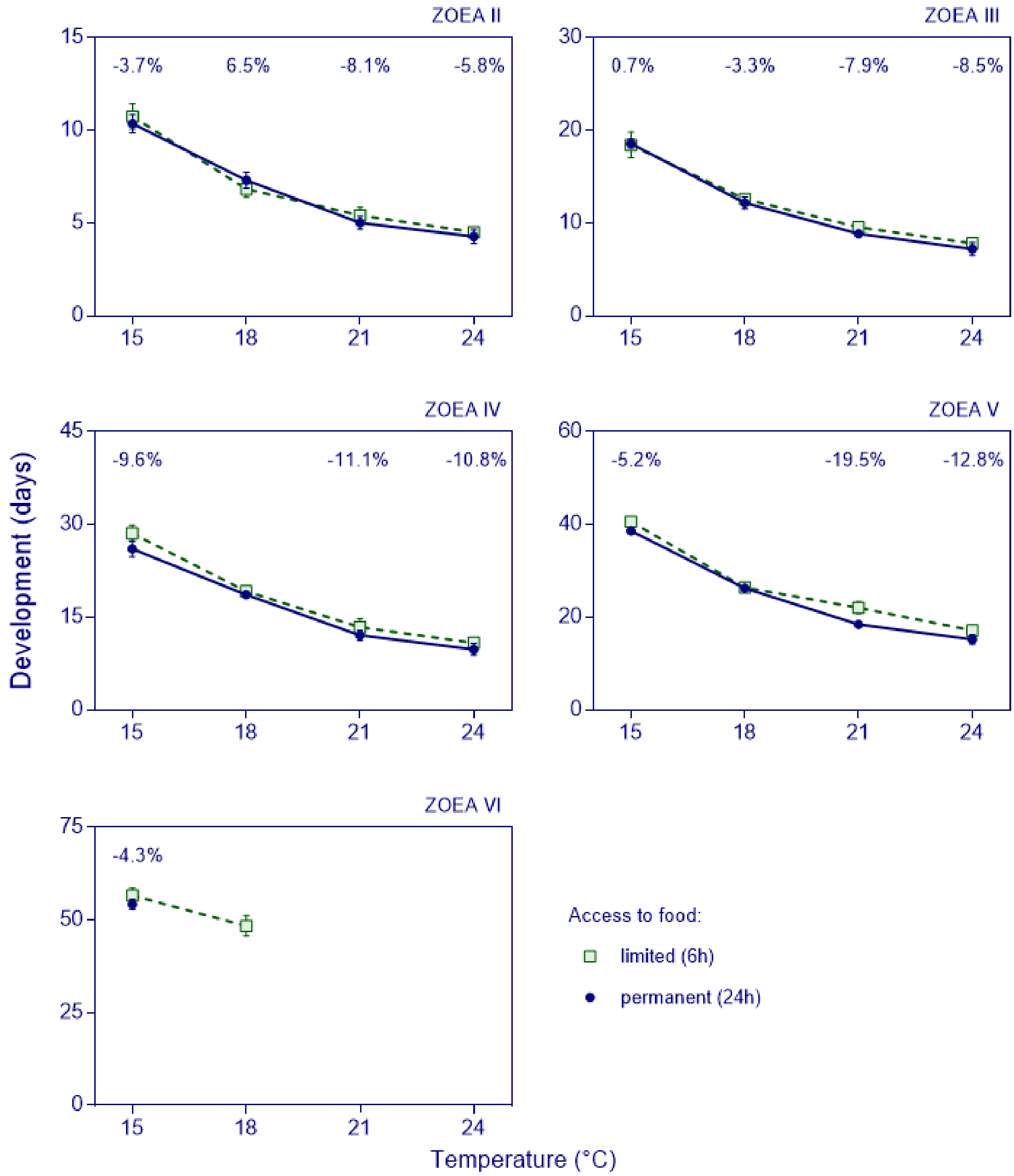

Figure S7. *Hemigrapsus sanguineus*. Average duration of development from hatching to zoea II, III, IV, V and VI as a response to temperature and food availability. Symbols as in Fig. S1. Percentages on top (only when significantly different): percent difference in development time between permanent and limited access to food treatments for each temperature.

Table S6. *Hemigrapsus sanguineus.* Megalopa: model selection for dry mass (DW), carbon (C) and nitrogen (N) content per individual; instantaneous growth in terms of dry mass (IgDW), carbon (IgC) and nitrogen (IgN) and C/N ratio. Model selection was performed using the adjusted Akaike information criteria (AICc). Abbreviations: ♀: female of origin; F: food condition; T: temperature. Highlighted in bold: the best overall model (containing both the best random and fixed term).

Note: Model without including data for larvae exposed to 15 °C

Random (REML)	AICc						
Term	DW	C	N	IgDW	IgC	IgN	C/N
♀:F:T	750	579	363	**-512**	**-514**	**-511**	**-100**
♀:T	751	574	355	-474	-487	-479	-106
♀:F	**743**	**571**	355	-505	-509	-507	-92
♀	749	571	**352**	-476	-489	-481	-92
Fixed (ML)							
Full factorial	**778**	**592**	**357**	**-567**	**-571**	**-567**	**-135**
F+T	782	599	360	-555	-558	-556	-137
T	825	644	415	-553	-556	-552	-139
F	780	598	370	-523	-527	-525	-111
Null	838	652	419	-522	-525	-522	-113

Table S7. *Hemigrapsus sanguineus.* Megalopa: model selection for dry mass (DW), carbon (C) and nitrogen (N) content per individual; instantaneous growth in terms of dry mass (IgDW), carbon (IgC) and nitrogen (IgN) and C/N ratio. Model selection was performed using the adjusted Akaike information criteria (AICc). Abbreviations: ♀: female of origin; F: food condition; T: temperature. Highlighted in bold: the best overall model (containing both the best random and fixed term).

Note: Model without including data for larvae from female 4

Random (REML)	AICc						
Term	DW	C	N	IgDW	IgC	IgN	C/N
♀:F:T	754	586	380	-494	**-**492	-491	**-**71
♀:T	741	**571**	**364**	-457	-474	-460	-88
♀:F	743	575	368	**-493**	**-495**	**-491**	-83
♀	**739**	569	362	-459	-476	-463	**-90**
Fixed (ML)							
Full factorial	786	599	370	**-570**	**-569**	**-566**	**-130**
F+T	**783**	**597**	**367**	-562	-561	-559	-134
T	829	652	429	-558	-557	-555	-120
F	820	625	379	-522	-520	-520	-102
Null	851	666	432	-518	-514	-515	-94

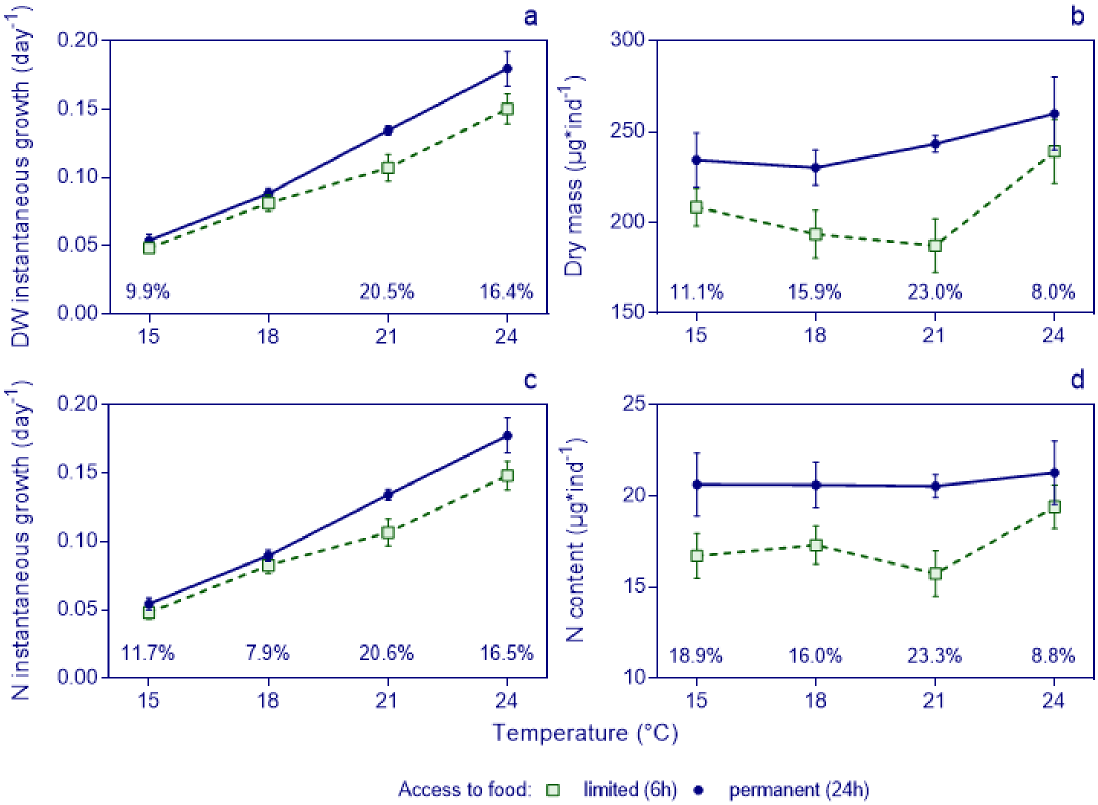

Figure S8. *Hemigrapsus sanguineus*. (a) Average dry mass growth rates. (b) Average dry mass. (c) Average Nitrogen growth rates. (d) Average Nitrogen content. Data corresponds to the responses, from hatching to megalopa, to temperature and access to food. Data shown as average values ± SE. Symbols as in figure S1. Percentages on top or below (only when significantly different): percent difference in development time between permanent and limited access to food treatments for each temperature.

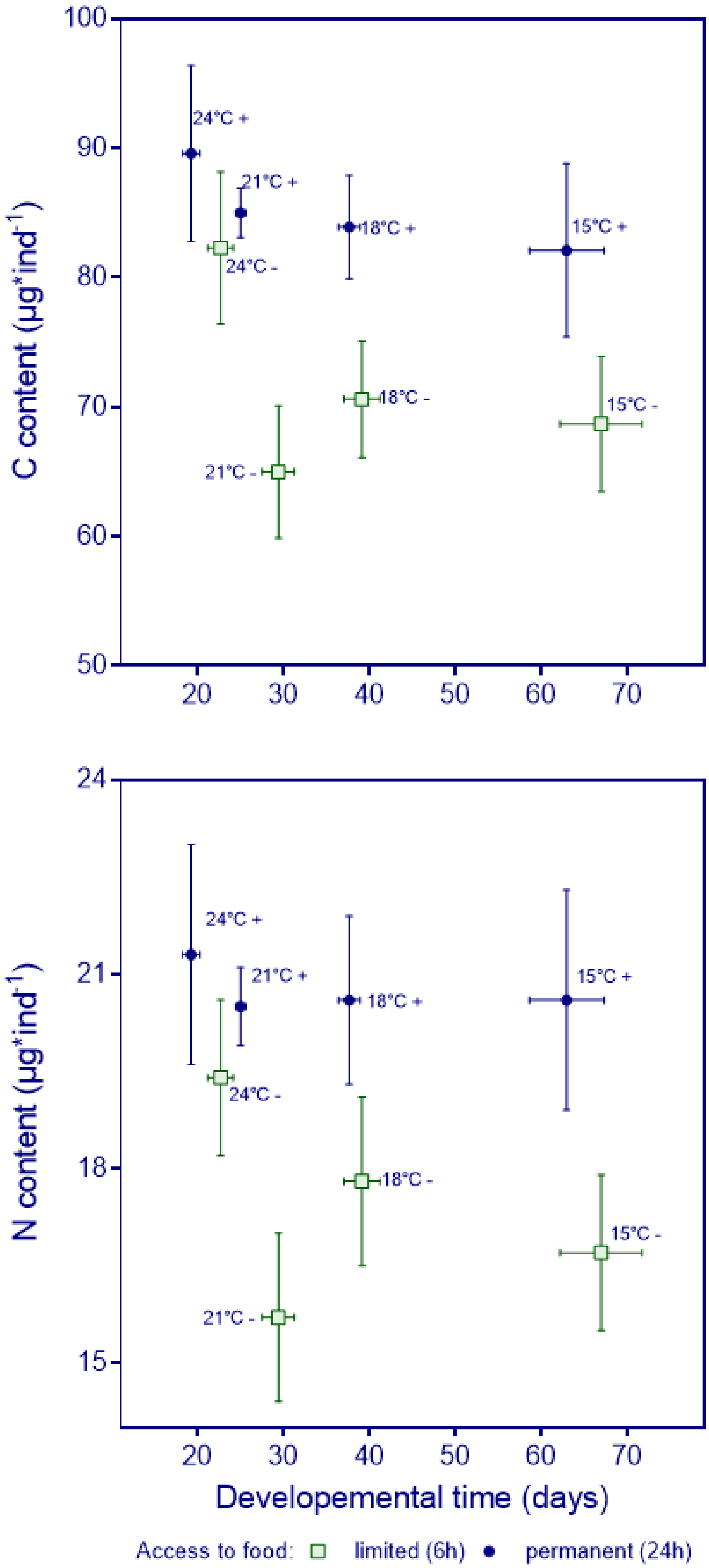

Figure S9. *Hemigrapsus sanguineus*. Integrated responses of carbon content (upper panel) and nitrogen content (lower panel) and duration of development under the different treatments of temperature and food availability. Data shown as average values ± SE for both variables. Symbols: permanent access to food is represented with blue circles (indicated as `+´) and limited access to food with green squares (indicated as `-`), temperature is indicated in the graph next to the symbols.

REFERENCES

R Core Team. (2013). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-proje ct.org/

Torres G, Giménez L (2020) Temperature modulates compensatory responses to food limitation at metamorphosis in a marine invertebrate. Funct Ecol 34: 1564-1576. https://doi.org/10.1111/1365-2435.13607