

# Ingestion and respiration rates of a common coastal mysid respond differently to diurnal temperature fluctuation

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## Abstract

Animals face strong environmental variability even on short time scales particularly in shallow coastal habitats, forcing them to permanently adjust their metabolism. Respiration rates of aquatic ectotherms are directly influenced by water temperature, whereas ingestion rates might additionally be influenced by behavior. We aim to understand how respiration and ingestion rates of an aquatic invertebrate respond to changing temperature during a diurnal thermal fluctuation cycle and how both processes are related. We studied the benthopelagic mysid *Neomysis integer* as an important food web component of coastal ecosystems. Mysids were collected at the southern Baltic Sea coast and exposed in the laboratory to either constant temperature of 15°C or daily temperature fluctuation of  $15 \pm 5^\circ\text{C}$ . Short-term (1–2 h) respiration and ingestion rates were measured at four equidistant time points within 24 h and did not differ among time points at constant temperature, but differed among time points in the fluctuating treatment. Respiration was highest at the thermal maximum and lowest at the thermal minimum. Ingestion rates showed the opposite pattern under fluctuation, likely due to differences in underlying thermal performance curves. When temperature transited the average, the direction of temperature change influenced the animals' response in respiration and ingestion rates differently. Our results suggest that respiration is not only instantaneously affected by temperature, but also influenced by the previously experienced direction of thermal change. Our experiment, using an important non-model organism, delivered new insights on the relationship between the crucial organismal processes ingestion and respiration under thermal variability.

Shallow coastal environments are often characterized by strongly variable conditions of abiotic parameters, such as temperature (Franz et al. 2019). Many coastal organisms face

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Additional Supporting Information may be found in the online version of this article.

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challenges of fluctuating conditions by developing broad tolerance windows and high plasticity to stressors. For ectotherms, temperature is the most important driver of changes in metabolic rates, which will adjust continuously to varying temperature. Increasing temperatures that stay within thermal tolerance limits of ectotherms usually lead to accelerated physiological processes (Newell and Branch 1980) that are associated with increasing energy demands. Respiration is a process very closely linked to metabolism. In animal cells, oxygen is needed to generate the energy-rich molecule ATP, fueling metabolic processes. Metabolic demands thus directly affect respiration (Weis 2014). Animals need to acquire sufficient amounts of food by ingestion to satisfy energy demands for ATP production.

Dependent on the time scale, so-called, time-dependent effects' have the potential to shape ectotherms' responses to fluctuating conditions (Kingsolver et al. 2015; Koussoroplis et al. 2017). Important time-dependent effects are stress, compensation, the use of reserves, and acclimation (Sterner and Schwalbach 2001; Schulte et al. 2011; Niehaus et al. 2012). While metabolic responses to thermal stress might be still beneficial at short time scales, the same temperature stress may negatively affect performance at longer time scales (Niehaus et al. 2012). Depending on an organism's rate of change under

temperature fluctuation relative to its acclimation speed, acclimation can both amplify or buffer fluctuation effects on different responses of the organism (Kingsolver et al. 2015; Koussoroplis et al. 2017).

Mean organism performance in fluctuating conditions can in some cases be adequately described based on performance measured in constant conditions with the help of nonlinear averaging, a mathematical principle also referred to as Jensen's inequality (Jensen 1906; Ruel and Matthew 1999). For concave parts of thermal performance curves (TPCs), Jensen's inequality predicts that physiological mean performance under fluctuations is lower compared to performance achieved at constant mean temperature. Nonlinear averaging can be an important tool for predictions on time scales spanning at least one full fluctuation cycle. When time-dependent effects did not play a large role, it has been successfully applied to predict the growth of algae populations and the refuge effect of mussels under thermal fluctuations (Bernhardt et al. 2018; Vajedsamiei et al. 2021). However, it is still unclear how organisms' current performance responds at *different* time points within fluctuation cycles. It is also unclear which short-term effects, such as stress and compensatory mechanisms, play a role in shaping metabolic processes or performance during temperature fluctuations and whether they reflect the long-term mean response of an organism. To better understand an organism's integrated response during full fluctuation cycles, it is important to study the organism's response at different time points during environmental fluctuations.

The mysid *Neomysis integer* Leach, 1814, lives in hyperbenthic waters of estuaries around Europe and is also commonly found in shallow coastal waters of the Baltic Sea (Margoński and Maciejewska 1999). In its habitats, both diurnal and seasonal changes in temperature occur (Seifert 1938; Schiewer 2008). Due to its contribution to nutrient exchange and biomass transfer between benthic and pelagic environments, it has an important role in coastal food webs (Roast et al. 1998), providing protein-rich food (> 70% of dry mass, Raymond et al. 1968) to higher trophic levels. *N. integer* is also an important omnivorous consumer, mainly feeding on phytoplankton and zooplankton, detritus, and benthic material (Lehtiniemi and Nordström 2008). It can be assumed that *N. integer* can switch between two feeding modes, an active hunting mode to attack single prey items and a passive filtration mode for smaller food particles, like the similar mysid *Praunus flexuosus* (Viitasalo and Rautio 1998).

Mysid respiration has been described to be dry mass-dependent (Vilas et al. 2006) and to increase with increasing temperatures in ranges from 0 to 23°C (Weisse and Rudstam 1989; Roast et al. 1999; Szalontai et al. 2003). In mysids, ingestion might be driven by the need to cover metabolic energy demands and additionally by behavioral aspects. Therefore, it can be expected that ingestion shows a less instantaneous response to changing temperature than respiration. Feeding

rates of *N. integer* from the United Kingdom and Germany were found to increase with increasing temperatures up to 15°C (Roast et al. 2000; Hennigs et al. 2022). It is still largely unexplored whether and how respiration and ingestion of ectotherms are related in variable environments and whether these responses follow thermal signals instantaneously.

Recently, it was stressed that thermal fluctuations likely entail phases of augmented stress but also phases of stress relief, from which invertebrates could benefit (Wahl et al. 2015; Morón Lugo et al. 2020). Compensation or shifting activity to less stressful periods of fluctuations could be essential for organisms, allowing them to survive extreme mean thermal conditions that they could not withstand under constant conditions. In fluctuating conditions, which change periodically and thus mimic for instance diurnal temperature changes, animals might use thermal cues that allow them to change their responses (Pincebourde et al. 2008; Hayford et al. 2021). Thus, it might be possible that organisms can perceive and internalize the history of diurnal temperature variation to some extent after experiencing it for several days. In this context the question arises if our research organism is able to anticipate temperature change and may adjust its ingestion to thermal fluctuations accordingly.

To gain a better understanding of how an invertebrate responds in some of its key biological processes to thermal fluctuations, we investigated how ingestion rates as a combination of metabolic and behavioral responses and respiration rates as an immediate metabolic response are related under fluctuating conditions. To do this, we used the non-model organism *N. integer*, collected from a brackish estuary of the southern Baltic Sea. Under laboratory conditions, mysids were exposed to constant (15°C) or diurnally fluctuating ( $15 \pm 5^\circ\text{C}$ ) temperatures. Ingestion was measured throughout a whole 24-h diurnal temperature fluctuation cycle. Following the principle of Jensen's inequality for nonlinear temperature–response relationships, we expected lower ingestion rates under fluctuating compared to constant temperature conditions. In addition, current ingestion and respiration rates were measured for 1–2 h at specific time points during the thermal cycle, that is, when the fluctuating temperature reached both its minimum and maximum and twice at mean temperature, once during the rising and once during the falling temperature phase of the fluctuation cycle. Respiration rates were expected to show an instantaneous response of the mysids to their environment directly associated with their metabolic needs, that is, increasing respiration rates with increasing temperature and vice versa. At non-stressful thermal conditions, ingestion rates were assumed to be positively related to respiration rates, that is, increasing ingestion rate with increasing respiration and vice versa. At higher, more stressful temperatures, we expected the positive relationship between ingestion and respiration rates to disappear, because ingestion rates follow an optimum curve along a temperature gradient with maximal rates at 10–15°C (Hennigs et al. 2022).

## Methods

### Mysid sampling and laboratory conditions

Mysids, mainly *N. integer* and few individuals of *Praunus* sp., were collected on 22 February 2022 at a shallow bay of the Greifswalder Bodden in the southern Baltic Sea (sampling coordinates: 54.097495, 13.455123). At collection from depths < 0.5 m, the water temperature was 4.1°C and the salinity was 8.1 psu. Mysids were transferred to the laboratory into aquaria filled with a mixture of water from their origin (1 liter) and artificial seawater (4 liters; ASW, using Pro-Reef Sea Salt, Tropic Marin). They were fed ad libitum with < 48-h-old *Artemia salina* nauplii and fish pellets (PastillMix, MultiFit) throughout the acclimation period. To acclimate the mysids to our experimental conditions, the temperature in the climate chambers was initially set to 10°C, and the light regime to a 16 : 8-h day–night cycle. After 3 d, the temperature was increased to 15°C and the light regime was changed to continuous low-light conditions, matching subsequent experimental conditions. We chose continuous light conditions to reduce the likelihood of observing signals of possible diurnal feeding patterns. Salinity was set to 10 psu, slightly higher than the ambient value, for comparative purposes with previous experimental studies on *N. integer* (e.g., Roast et al. 1999, 2000; Hennigs et al. 2022).

After 2 d at 15°C constant temperature, mysids were transferred to climate cabinets with two different treatment conditions, to which they had been exposed for additional 3 d prior to the measurements. In the climate cabinets, mysids were distributed to five aquaria per treatment, each filled with 4 liters ASW and fed ad libitum with freshly hatched *A. salina* nauplii and fish pellets until they were used for the ingestion or respiration measurements. Feeding was aimed at avoiding overestimation of ingestion rate measurements using starved animals and to ensure equal food supply for mysids used in respiration rate measurements independent of the measurement time point. In this study, treatment conditions consisted of two regimes, (1) a constant temperature of 15°C, and (2) a fluctuating temperature regime with 15°C as mean  $\pm$  5°C within a 24-h period (Fig. 1). The mean of 15°C was chosen for comparative reasons with previous studies (e.g., Roast et al. 1999; Hennigs et al. 2022). The amplitude of  $\pm$ 5°C, even though somewhat higher than fluctuations observed near the collection site ( $\pm$  3°C; Supporting Information Fig. S1), is a reasonable fluctuation amplitude observed in shallow waters (e.g.,  $\pm$  4°C observed by Franz et al. (2019) in 1.2 m depth at Lübecker Bight in the southwestern Baltic Sea). With our experiment, we aimed at studying fundamental responses of coastal marine invertebrates to thermal fluctuations. Choosing a somewhat higher fluctuation amplitude than the one observed at the collection site, we also aimed at examining potential impacts of extreme thermal events on mysids that may be expected in near future due to climate change.

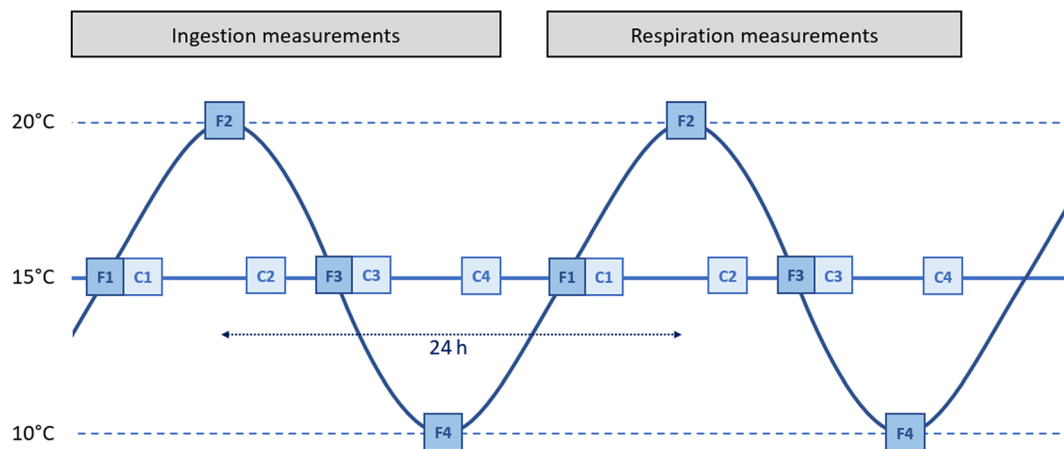
### Ingestion and respiration measurements

Ingestion and respiration of individual mysids were measured on 2 consecutive days after the 3 d on the respective

treatment conditions. On Day 1, ingestion was measured, and on Day 2, respiration was measured. Different individuals were used for each measurement (i.e., no repeated measurements on individuals). At each time point, 10 mysids per thermal condition were randomly caught from the 5 aquaria and used as independent replicates. For logistical reasons, and to avoid damage and additional stress to animals, species identity was not determined before but after the measurements on the preserved individuals. Short-term measurements (1–2 h) were conducted at four time points during the fluctuation cycle: (1) at the mean temperature during rising temperature, (2) at the maximum temperature, (3) at the mean temperature during falling temperature, and (4) at the minimum temperature. The measurements at constant temperature followed immediately after the completion of measurements at fluctuating conditions (Fig. 1).

For the 24-h ingestion measurements, 10 mysids per treatment were placed individually in plastic beakers containing 800 mL of temperature-acclimated ASW and 480 freshly hatched *A. salina* nauplii. These trials covered the entire fluctuation cycle. After 24 h in the treatments, replicates were treated as described below for the short-term measurements. For the 1-h ingestion measurements, mysids were placed individually in cups containing 50 mL of temperature-acclimated ASW and 30 freshly hatched *A. salina* nauplii (following Hennigs et al. 2022). After 1 h, mysids were transferred to ASW without *A. salina* nauplii at 15°C and kept for at least 24 h without food to empty their digestive tract for unbiased dry mass determination. Then, mysids were preserved in ethanol for species, sex, length, and dry mass determination. *A. salina* from the experiment that had remained in the cup were counted and ingestion rates were calculated. From the overall 60 individual mysids, only few were lost due to zero ingestion ( $n = 4$ ), individuals identified as *Praunus* sp. ( $n = 2$ ), and a missing dry mass measurement ( $n = 1$ ). This resulted in replicate numbers of  $n = 10$  per treatment, except of the time points C1 with  $n = 7$ , F3 with  $n = 8$ , and F4 with  $n = 9$ .

For 2-h respiration measurements, 4-mL glass vials with optical sensor spots mounted at their bottom (©PreSens, Germany) were used as respiration chambers and filled with pre-acclimated ASW. Mysids were placed individually into the vials. Two vials without animals served as controls per measurement. The vials were sealed with lids and we visually checked that there were no air bubbles when incubation started. Phase values were measured every minute by SensorDish® Reader units (©PreSens, Germany) and the ©PreSens software SDR v4.4.0. Depending on the reached oxygen decrease, measurements were stopped after at least 1.5 h, and the mysids were preserved in ethanol for later species, sex, and dry mass determination. The calculation of oxygen consumption was based on a time frame of measurements in which all replicates of a treatment showed a linear oxygen decrease for at least 30 min. At least the first 15 min of the measurements were discarded to exclude side effects, such as



**Fig. 1.** Ingestion and respiration of *Neomysis integer* were measured under constant (C) and fluctuating (F) temperature at four equidistant time points (1–4). Measurements were conducted on 2 consecutive days, first ingestion, then respiration. For each measurement, 10 independent replicates (individual mysids) were used. Ingestion measurements had a duration of 1 h, respiration measurements of 1.5–2 h.

stress responses of the individuals due to transfers into vials. Oxygen concentration in the vials was calculated from measured phase values and a previously established calibration at constant temperature. Since the PreSens software (SDR v4.4.0) only allows to enter one measurement temperature, the realized temperature in the treatments was measured every 5 min with a temperature logger (HOBO® Pendant® MX Temp/Light MX2202) placed in a jar of similar volume to the respiration vials. The realized temperature was used for the adjustment of the calibration. We calculated individual oxygen consumption per minute from the linear slope of oxygen concentration change over time subtracted by the mean oxygen concentration change of the control vials. From the overall 40 individuals, very few replicates were lost due to errors during measurements ( $n = 3$ ) and individuals identified as *Praunus* sp. ( $n = 2$ ). This resulted in replicate numbers of  $n = 10$  per treatment, except of the treatments C1, C2, C4, F1, and F3 with  $n = 9$ .

The species, sex (based on sexual morphological differences), and length (from the base of eyestalks to the end of the last abdominal segment, excluding uropods and telson) of preserved mysids were determined using a stereo-microscope. Dry mass of mysids was measured after drying for 3 d at 60°C using an electronic microbalance ( $\pm 1 \mu\text{g}$ , Sartorius ME5, Germany). The mysids varied between 5.5 and 14.3 mm in length over all measurements. Juveniles had lowest dry masses down to 0.5 mg, and females were the heaviest individuals with up to 6.4 mg.

### Data analyses

Respiration and ingestion rates of the individuals were related to their dry mass. These mass-specific respiration and ingestion rates were analyzed by a linear model using dry mass, time point and the thermal environment (constant temperature vs. fluctuating temperature) as independent variables.

Dry mass was included as continuous explanatory variable to account for the negative allometric relationship seen as decreasing mass-specific rates with increasing dry mass (Supporting Information Figs. S2, S3). The continuous variable dry mass was used in interactions with the factorial explanatory variables time point and thermal environment to account for potential differences in slopes of the relationship between dry mass and mass-specific rates (Supporting Information Figs. S2, S3). Analyzing our data using models without interactions involving dry mass did not change the general outcomes (cf. Table 1; Supporting Information Table S2). Pair-wise comparisons of the mass-specific rates among the four time points within each thermal environment were done by estimated marginal means using the “emmeans” package (Lenth 2022), which adjusts for the dry mass dependency of the rates. To test for differences in the 24-h ingestion measurements between the constant and fluctuating temperature treatments, mass-specific ingestion rates were analyzed using a linear model with dry mass as continuous and thermal environment as factorial explanatory variables (Supporting Information Table S1).

To test how respiration rates and ingestion rates are related to each other, linear regressions between respiration rates and dry mass from animals of the respiration rate measurements (Supporting Information Fig. S4) were used to predict respiration rates for the individual animals of the ingestion rate measurements (“predicted respiration rates”). Calculating predicted respiration rates for the analysis of the relationship between respiration and ingestion rates was necessary as the two different rates were measured on different individuals, that is, independent replicates. Since the “time point” did not affect mass-specific respiration rates in the constant environment (comparison of the estimated marginal means, Fig. 2), one general linear regression between respiration and animal dry mass was calculated for the measurements from constant

**Table 1.** Results of general linear models, testing for the effects of dry mass, time point, thermal environment (constant vs. fluctuating temperature) and their interactions on mass-specific respiration and mass-specific ingestion rates of *Neomysis integer*. Significant effects ( $p < 0.05$ ) in bold.

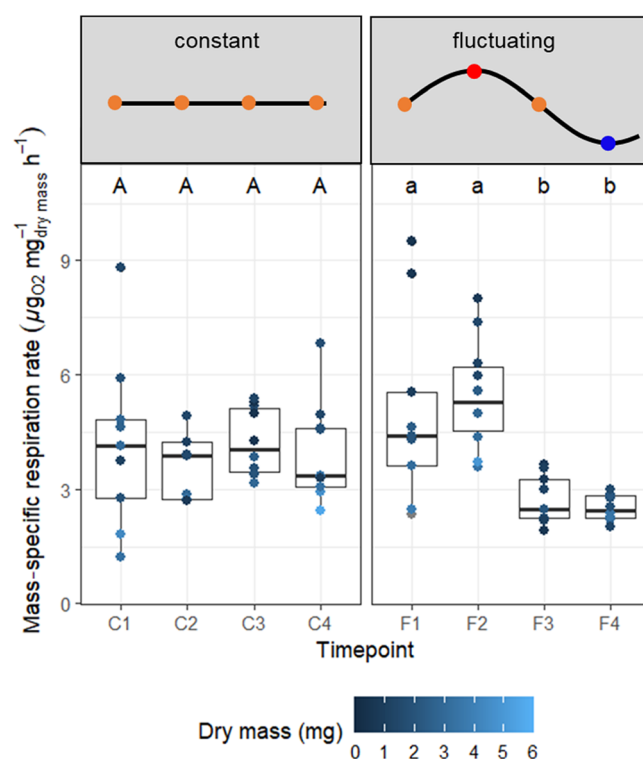
| Response                  | Variables       | F-value <sub>df, resid</sub> | p-value           |
|---------------------------|-----------------|------------------------------|-------------------|
| Mass-specific respiration | Dry mass (DM)   | $F_{1,59} = 28.57$           | <b>&lt; 0.001</b> |
|                           | Time point (T)  | $F_{3,59} = 13.55$           | <b>&lt; 0.001</b> |
|                           | Environment (E) | $F_{1,59} = 0.81$            | 0.37              |
|                           | DM × T          | $F_{3,59} = 4.35$            | <b>0.008</b>      |
|                           | DM × E          | $F_{1,59} = 1.90$            | 0.17              |
|                           | T × E           | $F_{3,59} = 13.92$           | <b>&lt; 0.001</b> |
|                           | DM × T × E      | $F_{3,59} = 1.46$            | 0.23              |
| Mass-specific ingestion   | Dry mass (DM)   | $F_{1,58} = 34.07$           | <b>&lt; 0.001</b> |
|                           | Time point (T)  | $F_{3,58} = 3.22$            | <b>0.029</b>      |
|                           | Environment (E) | $F_{1,58} = 2.29$            | 0.14              |
|                           | DM × T          | $F_{3,58} = 1.24$            | 0.30              |
|                           | DM × E          | $F_{1,58} = 0.22$            | 0.64              |
|                           | T × E           | $F_{3,58} = 3.97$            | <b>0.012</b>      |
|                           | DM × T × E      | $F_{3,58} = 0.18$            | 0.91              |

temperature conditions at all time points. For fluctuating temperature measurements, respiration rates were predicted for time points 1 and 2 by one regression, and for time points 3 and 4 by another regression, according to the differences in estimated marginal means (Fig. 2). To investigate the relationship between ingestion and respiration, a ratio of the measured mass-specific ingestion rate and the predicted mass-specific respiration rate was calculated. The dependence of the ratio on the two factors thermal environment and time point was analyzed by a linear model, and differences among the four time points within each of the thermal environments were investigated using pairwise comparisons of the estimated marginal means.

All statistical analyses were performed in R version 4.1.2 (R Core Team 2021). Assumptions for linear models were visually checked using the “autoplot” function of the “ggfortify” package (Tang et al. 2016) and were considered to have been met.

## Results

Mass-specific respiration rates were lower in heavier animals and differed between time points (Fig. 2; Supporting Information Fig. S2; Table 1). After accounting for this dry mass dependency by using the estimated marginal means, respiration rates showed different patterns over time depending on the thermal environment (Fig. 2; Table 1, significant  $T \times E$  interaction). Over time respiration rates were similar under constant temperature, but clearly differed in the fluctuating temperature treatment (Fig. 2). At the mean temperature, respiration was higher at rising temperature (F1) than at

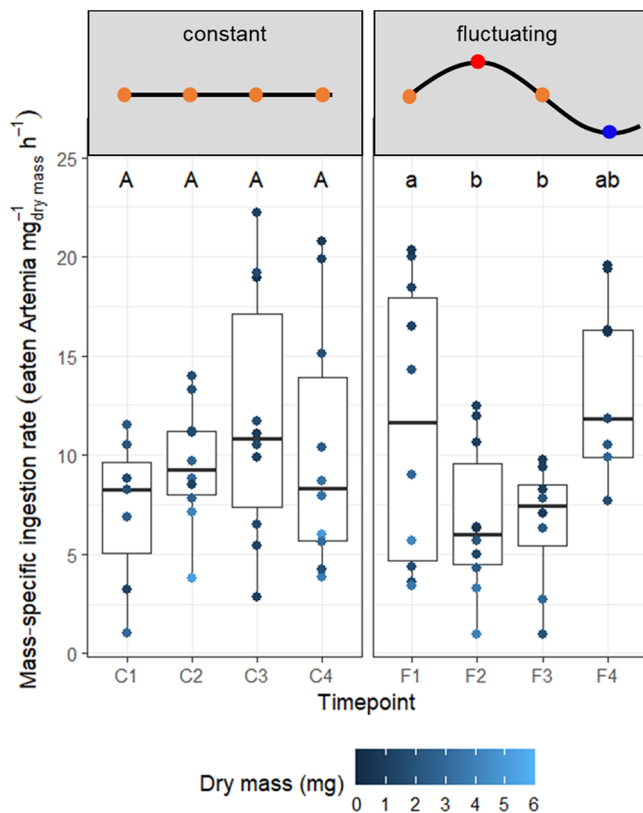


**Fig. 2.** Mass-specific respiration rates of *Neomysis integer* for the different time points under constant and fluctuating thermal environment. Different letters indicate significant differences among time points within each thermal environment after adjusting for the dry mass dependency (by estimated marginal means) using pairwise comparison.

declining temperature (F3; Fig. 2). Respiration also differed between extreme temperatures (high respiration at maximum temperature, 20°C, F2, and low respiration at minimum temperature, 10°C, F4; Fig. 2).

Mass-specific ingestion rates were generally lower in heavier animals (Fig. 3; Supporting Information Fig. S3; Table 1). Using estimated marginal means to adjust for the dry mass dependency, ingestion rates showed also different patterns over time depending on thermal environment (Fig. 3; Table 1, significant  $T \times E$  interaction). They were similar under constant temperature conditions, but differed among time points when temperature fluctuated (Fig. 3). As for respiration, ingestion in the fluctuating treatment differed between the time points at mean temperature (15°C, time points F1 and F3), and was higher at rising (F1) than at declining temperature (F3; Fig. 3). At extreme temperatures, and in contrast to the responses observed for respiration, ingestion showed opposite trends, with lower ingestion at maximum temperature and higher ingestion at minimum temperature.

In the 24-h measurements, no difference was observed between thermal environments (Supporting Information Fig. S5; Table S1), indicating no reduced ingestion in the fluctuating treatment as predicted by Jensen's inequality. This is supported by the missing effect of the thermal environment



**Fig. 3.** Mass-specific ingestion rates of *Neomysis integer* for the different time points of the 1-h measurements under constant and fluctuating thermal environment. Different letters indicate significant differences (see figure legend of Fig. 2).

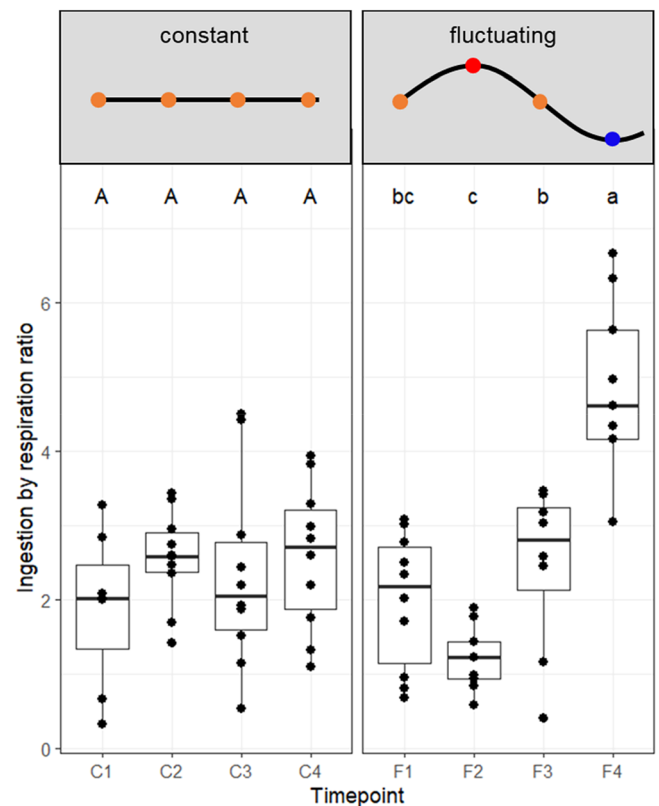
in the 1-h measurements of mass-specific ingestion rates (Table 1). Twenty-four-hour ingestion rate measurements were in the range of variation of the 1-h ingestion rate measurements (Supporting Information Fig. S5).

The ratio of mass-specific ingestion rates to the mass-specific predicted respiration rates in the 1 h measurements was similar under constant temperature conditions but differed under fluctuating temperatures (Fig. 4, linear model, factor time point:  $F_{3,66} = 12.50$ ,  $p < 0.001$ , thermal environment:  $F_{1,66} = 0.90$ ,  $p = 0.35$ ; interaction:  $F_{3,66} = 10.64$ ,  $p < 0.001$ ). When temperature fluctuated, the ratio was similar at the mean temperature (F1 and F3), but differed at extreme temperatures, with lowest ratios at highest temperature (F2) and highest ratios at lowest temperature (F4; Fig. 4).

## Discussion

### Relationship between respiration and ingestion

Mass-specific respiration and ingestion rates of the mysid *N. integer* did not vary over time at constant temperature of 15°C, whereas both rates varied over time when temperature fluctuated (Figs. 2, 3). Under thermal fluctuation, ingestion showed opposite responses to respiration at temperature



**Fig. 4.** Ratio of mass-specific ingestion rates and predicted mass-specific respiration rates (both per mg dry mass and per hour) of *N. integer* for the different time points in the constant and fluctuating thermal environment. Different letters indicate significant differences among time points within each thermal environment using pairwise comparison by estimated marginal means.

extremes. An explanation for these different responses might be that optimal temperatures for the mysids' ingestion could vary from thermal optimums for respiration, as observed for the Baltic blue mussel (Vajedsamiei et al. 2021). The TPC for ingestion of *N. integer* shows an optimum around 10°C for males and around 15°C for females (Hennigs et al. 2022) and could be shifted toward colder temperatures for animals used in this study, which were collected at colder in situ temperatures than in the previous study. The TPC for respiration of Baltic *N. integer* shows increasing respiration for temperatures up to 15°C and higher, or at least equal, respiration rates for temperatures up to 20°C depending on exposure time (Laughlin and Lindén 1983). At mean temperature under thermal fluctuation, mysids in our study showed both higher ingestion and respiration when temperature increased (F1) compared to when temperature decreased (F3; Figs. 2, 3). Similarly, respiration and feeding rate of the Baltic blue mussel also showed different responses at mean temperature depending on whether temperature was increasing or decreasing (Vajedsamiei et al. 2021). The opposite responses of ingestion and respiration we observed at the temperature extremes might be also

caused by effects of time-dependent energy storage (Supporting Information Box 1).

To investigate how ingestion and respiration are related, we used the ratio among both rates at each of the four time points. The pattern of that ratio mirrored the temperature cycle in the fluctuating environment at the horizontal axis (Fig. 4). At the time points where mysids experienced the mean temperature of the fluctuation, respiration and ingestion responded similarly (both higher at F1 and lower at F3), thus resulting in a similar ratio (Fig. 4). This suggests a consistent, in this case positive relationship between the two responses at mean temperature conditions, which presumably were not stressful to the mysids. When considering each rate individually, both responses were influenced not only by the ambient measurement temperature, but also by the previously experienced temperature extreme and the direction of the temperature change. However, linking ingestion and predicted respiration via their ratio revealed a similar ingestion performance relative to their respiratory performance, regardless of whether temperature increased or decreased at the thermal mean. The observed responses at the extremes of the temperature fluctuation indicate that the thermal optimum of the studied *N. integer* population might be close to 10°C, the temperature minimum within the fluctuation, because ingestion performance relative to respiratory requirements was highest at minimum temperature (Fig. 4). An optimum of 11°C has been described for the feeding activity of male mysids of this population earlier (Hennigs et al. 2022). Similarly, in our study, ingestion at 10°C was one of the highest rates measured, while the metabolic response, reflected by predicted respiration rates, remained relatively low. This suggests only little or no thermal stress at the thermal minimum of the fluctuation.

### Respiration

In the fluctuating thermal environment, mass-specific respiration of mysids was highest at maximum and lowest at minimum temperature (Fig. 2). Considering the van 't Hoff or RTG rule, the higher physiological response at warm temperature was expected (Mundim et al. 2020). However, deviations from that assumption were reported for another mysid species, in which lower respiration of *Neomysis americana* was unexpectedly observed at increased temperature, possibly caused by metabolic disruption (Chapina et al. 2020). Our studied mysid population showed different patterns in mass-specific respiration rates around mean temperature by being higher at rising temperature and lower at decreasing temperature (Fig. 2). This contradicts our expectations and may result not only from the different direction of the ongoing temperature change, but also by the previously experienced thermal extreme.

All mysids were fed ad libitum until they were used for measurements. Consequently, respiration in this experiment included the need to cover energy expenditure for food processing, the specific dynamic action (SDA; Secor 2009).

SDA for *N. integer* is not reported in the literature, but was estimated to be 18% of the assimilated food for another mysid of the Baltic Sea, *Mysis mixta* (Rudstam 1989) based on literature data of other plankton crustaceans (Kjørboe et al. 1985; Lampert 1986). However, SDA might differ among mysid species and ingested prey categories. Thus, the SDA for *N. integer* fed *A. salina* nauplii should be measured first, in case of an intended quantitative use of our reported values.

### Ingestion

We observed decreasing mass-specific ingestion rates of *N. integer* with increasing dry mass (Supporting Information Fig. S3), consistent with findings of a previous study on other mysid species (Jerling and Wooldridge 1995) and an expected negative allometric relationship between size and ingestion. As expected, the mysids' mass-specific ingestion did not differ between the measured time points under constant temperature conditions, whereas thermal fluctuation led to different responses (Fig. 3).

Mass-specific ingestion rates measured over the full fluctuation cycle did not differ between constant and fluctuating thermal environments (Supporting Information Fig. S5), suggesting no decreasing effect of fluctuating temperature on ingestion as expected based on the principle of Jensen's inequality. This is in contrast to our hypothesis and to observations of a previous study, where thermal fluctuation caused a decrease in *N. integer*'s ingestion on a short-term scale (Hennigs et al. 2022). Our results suggest that throughout a whole diurnal fluctuating cycle, mysids might be able to compensate for the potential stress of periodic temperature changes. We know of only one study that also measured time point-dependent feeding of brackish invertebrates throughout a sinusoidal thermal fluctuation, in which it was reported that feeding of the sea star *Asterias rubens* was higher at minimum temperature than at the other time points (Morón Lugo et al. 2020). This feeding pattern is similar to the results of our study and suggests a TPC of the sea star with an optimum at low temperature. Consequently, decreased mean responses under fluctuating conditions would have been expected at warmer temperatures, which would be located in the concave downwards part of the TPC. However, thermal fluctuation did not affect the mean energy uptake of the sea star over the course of the entire experiment in comparison to the constant treatment (Morón Lugo et al. 2020). Thus, as in our study, the predictions of Jensen's inequality of a decreased mean response in fluctuating conditions were not observed. Both studies underline the importance of time-dependent effects, such as compensation, in understanding the consequences of fluctuating environments on organism performance (Koussoroplis et al. 2017).

### Conclusions and perspectives

Our study demonstrated that ingestion and respiration showed opposite responses at the temperature extremes

during a diurnal thermal fluctuation cycle. However, at mean temperatures of the fluctuation, the ratio of ingestion to respiration was similar, although single responses differed depending on the positive or negative direction of temperature change. We conclude that the response profile of ingestion rates is not directly related to immediate respiration rates. The observed opposite response patterns of ingestion and respiration might be caused by different thermal optima of each rate. Thus, it would be interesting for future studies to determine TPCs of mysids for different responses in combination with investigating their reactions to temperature variation. We found that mysids were able to compensate for periodic deficits in energy uptake through ingestion over the applied diurnal fluctuation cycle, because they did not show reduced ingestion under fluctuating compared to constant temperature during the 24-h measurements (as would be expected according to Jensen's inequality). However, given the relatively short exposure duration applied in our study, further investigations are needed to verify if mysids can compensate over longer time periods. It would be also interesting in future experiments to compare responses of mysids to thermal fluctuations they currently experience in situ with conditions they may experience in future due to climate change (such as stronger fluctuation amplitudes or higher mean temperatures caused by heat waves).

The physiological and ecological response of aquatic invertebrates to environmental variation is influenced by multiple stressors, their interactions, changing mean levels, and differences in frequency, amplitude, and phase of fluctuations (Gunderson et al. 2016; Koussoroplis et al. 2019; Gerhard 2022). Thus, studying how organisms respond to changes in fluctuations of multiple environmental drivers in combination would be the next step to gain more insights into challenges that mysids or other invertebrates will experience in their natural environment. With our experiment, we investigated two main short-term responses, respiration and ingestion, of an important coastal invertebrate and generated knowledge about their relationship in a changing thermal environment. Such information may be useful for future response estimations on larger scales.

#### Data Availability Statement

The data that support the findings of this study are openly available in "figshare" at doi:10.6084/m9.figshare.23695257.

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#### Conflict of Interest

None declared.

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