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Journal of Sea Research 198 (2024) 102467

Contents lists available at ScienceDirect

Journal of Sea Research

journal homepage: www.elsevier.com/locate/seares

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ARTICLE INFO

Keywords:
Starvation
Variegated scallop
Calorimetry
Stable isotopes
Mimachlamys varia
Survival rate

ABSTRACT

In order to better understand *Mimachlamys varia* (Linneaus, 1758) response to nutritional stress, a controlled-condition experiment was conducted. Two scallop batches (*i.e.* juveniles and adults) were completely food-deprived for 3 months. Changes in mass and energy content of tissues (adductor muscle, digestive gland, rest of the soft tissues), as well as stable carbon and nitrogen isotope ratios of the adductor muscle were monitored weekly. Both batches exhibited a 3-phase response to starvation. For adults, phase 1, was characterized by a fast loss in mass, an δ^{15}N-enrichment, a stable calorific power, and a low mortality, corresponding to a transitional stage associated with protein-storage consumption. Phase 2 (day 28–42) exhibited a stabilization of mass and δ^{15}N values, coinciding with a digestive gland calorific power drop and an acceleration in mortality. This corresponds to a "protein sparing" stage where highly energetic fuel such as lipids, which are stored especially in the digestive gland in pectinids, are consumed in priority. Juveniles exhibited a distinct response characterized by a significant mass loss and an increase in calorific power during the first phase (day 0–28). This body weight decrease may involve the remobilization of low-caloric biochemical compounds (*e.g.* proteins) using the structure as internal fuel, thus limiting somatic maintenance costs. During the second month, body mass and calorific power stabilized, indicating a "protein sparing" stage. In a third phase for both age classes, mass decreased again together with a sharp increase in mortality: essential structure was ultimately remobilized. The survival rate of juveniles was higher than that of adults during the first two months of the experiment (97% and 64%, respectively). Although the time required for starvation to deplete of half the cohort is higher for juveniles than for mature individuals, increase in mortality rate after reaching the "Point of No Return", (*e.g.* the beginning of the last phase) was higher in juveniles than in adults. These results highlight the potential impacts of starvation at the population level, beyond the direct impact on individual survival. For instance, individuals may not effectively contribute to reproduction during the spawning period if exposed to a prolonged winter starvation episode. In particular, since highly energetic compounds stored in the digestive gland are often used as fuel to initiate gametogenesis in pectinids. Similarly, decrease in somatic weight in juveniles may delay their sexual maturity and hence their ability to contribute to population reproductive potential.

1. Introduction

Marine species face a number of threats such as climate change and impacts of anthropic activities (*e.g.* fishing, harbour activities and accommodation, pollution (Ipcc, 2023). Various stressors, including predation, disease, climatic variations (Yu et al., 2009; Masanja et al., 2023), acidification (Kroeker et al., 2013), oxygen and food limitation (de Zwaan and Eertman, 1996; Aguirre-Velarde et al., 2017; Cueto-Vega et al., 2022), can directly influence the physiology and survival of marine animals. While food limitation, or in its extreme way, starvation, may not be the primary threat to the survival of a population, it can be a factor limiting population sizes (Malthus, 1798; McCue, 2010; Hatch, 2012; Regular et al., 2022), especially for small populations (Browne et al., 2011). Food limitation is known to play a key role in regulating

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https://doi.org/10.1016/j.seares.2023.102467
Received 24 October 2023; Received in revised form 14 December 2023; Accepted 27 December 2023
Available online 2 January 2024

the populations of many species (Amar et al., 2003; Abbott and Dwyer, 2007). Several studies have explored the impact on biologic traits and metabolism of various types of nutritional stresses such as low diet quality, reduced food intake, fasting or starvation (McCue, 2010; Hatch, 2012; Lignot and LeMaho, 2012; Zeng et al., 2012, 2014). While the effects of starvation directly translate in terms of individual death or degradation of body condition, they can also affect how individuals allocate resources to sustain ecological processes such as foraging, predator avoidance, growth, development, mating and reproduction, defence system, which may therefore indirectly affect individual fitness (Hatch, 2012). Individual ability to overcome nutritional stress may have consequences at the population level.

Along European coastlines, oysters, mussels, clams, and pectinids are among the most commonly exploited bivalve species. For example, scallop fishing is a developing industry, which global production has drastically increased in the second half of the 20th century to about 1.7 million t in 1996 (Bourne, 2000). Among commercial scallops species, the variegated scallop, *Mimachlamys varia* (Linneaus, 1758), holds potential for shellfish production diversification, for instance in Italia (Prato et al., 2020), Croatia (Rathman et al., 2017), or Spain (Iglesias et al., 2012), and has supported important fisheries along the French Atlantic coast, especially in the Bay of Brest (Brittany) (Duncan et al., 2016). *M. varia* is a small East-Atlantic pectinid species that ranges from Norway to Senegal in West-Africa. This species is found from the low intertidal zones, to depths ranging up to 80m (Duncan et al., 2016). It mainly lives byssaly-attached to hard substrates like shells, stones, boulders or rock faces. However, it can also be observed in free-living form in association with epibiotic sponges (Forester, 1979). The typical adult size ranges from 35 to 45 mm in shell height. Unfortunately, since the mid-20th century, its population has been widely declining. In order to restore a sustainable exploitation, better understanding its response to environmental stress, such as food limitation, is necessary.

Individual nutritional status, or body condition, can be defined at each instant as the balance between energy intake and energy consumption. If food intake does not fulfil an organism energetic requirements, consumption of internal resources increases, resulting in fluctuations in organ-specific weight, energy content (McCue, 2010; Lignot and LeMaho, 2012) and biochemical composition across the whole organism. Numerous phyla have varying compositions of reserve and structural tissues. For certain invertebrate species (*e.g.* crustacean; Watts et al., 2014), pectinids (Le Pennec et al., 2001; Telahigue et al., 2013), the digestive gland (or hepatopancreas) is an important organ for lipid-reserve storage, which high energy content is essential for functions such as reproduction. Monitoring the mass, biochemical composition, and energy content of an organism during periods of nutritional stress can provide insights into its response mechanisms and, in an extreme case, its ability to survive.

Direct measurements of an organism's calorific content can be obtained through calorimetry. Although the results from this method may slightly vary from indirect measurements through the analysis of tissue biochemical composition, they remain reliable and have been used in numerous studies focusing on bivalves (Beukema and de Bruin, 1979; Young et al., 2017; Sacristán et al., 2020), including *M. varia* (Shafee, 1981). The impact of nutritional stress on metabolism and hence biochemical tissues composition variation has been largely investigated through nitrogen and carbon stable isotope signatures of animal tissues (McCue and Pollock, 2008; Hatch, 2012; Doi et al., 2017). δ^{15}N-enrichment observed in animal tissues is a good indicator of nutritional stress (Hatch, 2012), which is well-documented for numerous organisms (Doi et al., 2017) under starvation conditions.

Environmental pressures may have simultaneous and cumulative impacts (Gunderson et al., 2016; Aguirre-Velarde et al., 2017), making difficult the investigation of the effects of starvation on animals in the field (McCue, 2010; Hatch, 2012; Lignot and LeMaho, 2012). Thus, a prolonged starvation-controlled experiment was conducted to investigate the nutritional stress response of *M. varia*. In order to address consequences at the population level, we considered two different life stages (juveniles and adults). For that, we monitored the mortality together with changes in individual body weight, energy content, carbon and nitrogen stable isotopes composition throughout the experiment. This will provide knowledge into the individual mitigation mechanisms employed to survive during extended periods of nutritional stress and to assess the ability of *M. varia* population to persist.

2. Material and methods

2.1. Biological material

Two batches of 500 variegated scallops produced by Le Tinduff hatchery and reared in separated cages in the Bay of Brest (48°21.4′N, 4°33.23′W) were transferred to Le Tinduff hatchery experimental facilities on November 2020. One batch was composed of 6-months old individuals (13.30 ± 1.96mm shell height) and the second one composed of 1.5-year old individuals (35.10 ± 3.23mm shell height). All individuals were previously cleaned from their epibionts. For the first batch (younger and smaller individuals), scallops are supposed to be juveniles, according to literature (Dalmon, 1935; Shafee and Lucas, 1980), and laboratory observations (*i.e.* non mature gonads). The older batch was considered an adult batch.

2.2. Experimental setup

The experiment started on November 23th 2020 (day of transfer of scallops in the laboratory) and was conducted over 12 weeks until reaching consistent mortalities in both baches. All scallops were placed in a single 800-L tank, maintained suspended in sub-surface in mesh trays. The tank was continuously supplied with well aerated 0.5-µm filtered seawater with a renewal rate of the tank volume per day. Seawater was thermoregulated at 14°C close to field temperature at the date of transfer. Temperature within the tank was monitored every 15 min using two temperature data loggers (Electricblue® EnvLogger T2.4 27 mm). Salinity was controlled by the hatchery: it remained within a range of 30.5 to 32‰ throughout the experiment, except for a very short period (January 2 to 4, 2021) when a minimum salinity of 28.5‰ was observed. No additional food was supplied.

During the 8 first weeks of the experiment, 30 living individuals from each batch were sampled weekly, and fortnightly for the 4 last weeks. Individuals were either stored at −80°C before further analysis, either treated fresh (Table 1). Mortality was assessed daily for both batches. Dead scallops were counted and stored at −20°C.

Table 1
Sampling schedule: sampling dates and duration of the experiment since the beginning of starvation (t_0). At each sampling date, 30 individuals from each batch were collected. When individuals could not be processed fresh the sampling day, they were deep-frozen at −80°C and further processed after thawing at 6 °C.

Sample	Date	Days since t_0	Preservation
P0 (t_0)	2020-11-23	0	Deep-Frozen (−80°C)
P01	2020-11-30	7	Deep-Frozen (−80°C)
P02	2020-12-07	14	Deep-Frozen (−80°C)
P03	2020-12-14	21	Deep-Frozen (−80°C)
P04	2020-12-21	28	Deep-Frozen (−80°C)
P05	2020-12-28	35	Deep-Frozen (−80°C)
P06	2021-01-04	42	Deep-Frozen (−80°C)
P07	2021-01-11	49	Treated fresh
P08	2021-01-18	56	Treated fresh
P09	2021-02-01	70	Treated fresh
P10 (t_f)	2021-02-15	84	Treated fresh

2.3. Analyses

2.3.1. Biometric measurements

Individuals dorso-ventral height was measured using a Vernier-calliper (larger shell height in dorso-ventral axis, H_{sample}, nearest 0.1mm). For the large individuals, soft tissues were removed from the shells and separated (adductor muscle, *AM*; digestive gland, *DG*; and remaining soft tissues, *RST*). Then, tissues were dried (48h at 60°C), and weighed independently to the nearest 1 mg (dry weight, *DW*). The experiment was conducted during the gonadal resting period of the species (October to February, Lubet, 1959; Shafee, 1981). Gonads remained empty throughout the experiment, thus gonads were pooled with the RST. The total soft tissues (*TST*) dry weight, was calculated as the sum of these three compartments. Because the DG was not clear of food content at the beginning of the experiment (t_0), it was not analysed for the large individual batch. Thus *DG* and *TST* ash free dry weights *(AFDW)* are not given for t_0. For the small individuals batch, due to their small size, total soft tissues were collected together, dried and weighted. Ash content was estimated using the remaining pools of tissues used for calorimetric analysis (560°C, 5h). The proportion of ash in each tissue/sample alowed to calculate the individual *AFDW* of the corresponding tissue/sample. The initial body condition index (*CI*) was calculated as $CI = 1000*TST_{AFDW}/(H^3_{sample})$ according to Nagasawa and Nagata (1992).

In order to compare values obtained for individuals of different size, weights were standardized for height differences between individuals using Bayne et al. (1987) formula, based on the assumption that weight scales with cubed length:

$$W_{Std} = W_{Sample} \times \left(\frac{H_{Std}}{H_{Sample}}\right)^3$$

where,

W_{Std} is the individual tissue standardized weight (*DW* or *AFDW*), in mg,

W_{Sample} the weighed individual tissue weight (*DW* or *AFDW*), in mg,

H_{Std} the average shell height of all individuals from each batch, in mm (35 mm and 13 mm for great and small batch respectively),

H_{Sample} the shell height of the individual considered of measured shell weight W_{Sample}, in mm.

This allowed to standardize DW and the AFDW for each tissue and whole body (without shell) for standard shell height of 35mm and 13mm for the large and the small batch respectively. These standardized values were used for the data analyses.

To estimate the bias due to preservation methods (fresh or deep-freezing), the weight of soft tissues was compared in a subset of variegated scallops from the same sample ($N = 50$), with 30 variegated scallops dissected under fresh conditions and 20 variegated scallops dissected after being deep-frozen for several days. The difference between fresh and deep-frozen samples was found to be significant for adults DG and RST AFDW (Student *t*-test, *p*-value <0.001). Consequently, the bias was corrected for all deep-frozen samples (Table 1). The ratio between the mean AFDW of fresh samples and deep-frozen samples were calculated for each tissue, all weights of deep-frozen samples were corrected using this ratio.

2.3.2. Calorimetric analysis

For each sampling date and batch, each tissue (A*M*, *DG*, *RST* for large batch, *TST* for small) from all individuals was pooled and grinded into a fine homogeneous powder, and then freeze-dried during 24 h. Calorimetric content of pool of tissue was measured using an IKA C-4000 adiabatic bomb calorimeter (IKA-WerkeGmbh & Co. KG) on 100-mg or 80-mg triplicates (weighed to the nearest 0.1 mg). Note that, at the last sampling date, the quantity of material available for small batch (TST) analysis was not sufficient for a calorific content measurement. Repeatability among triplicate was checked, with a tolerance for coefficient of variation of 3%. Calorific power (*E*, KJ g^{-1}) was calculated as:

$$E = \frac{C \times (T_f - T_i) - K_{para}}{M}$$

where,

C is the effective heat capacity (J K^{-1}), 9251 J K^{-1} for this system,

T_i, T_f the system balancing temperature before and after combustion, respectively (K),

K_{para} the parasitic heat (J), estimated to be constant during the analyses, 637 J in this system,

M the sample ash free dry weight (*AFDW*) analysed (g).

The individual calorific content (without shell) was calculated using the *AFDW* and the mean calorific power of each tissue (A*M*, *DG*, *RST*, or *TST*). As the specific AFDW of the DG and the TST was not available for the adults at the initial time (t_0), their energy content could not be calculated.

2.3.3. Stable isotope analyses

Nitrogen and carbon stable isotope signature of animal tissues is a good indicator of nutritional stress (McCue and Pollock, 2008; Hatch, 2012), which is well-documented for numerous organisms (Doi et al., 2017) under starvation conditions. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes (*SI*) of adductor muscle were analysed at each sampling date. For this purpose, 1000µg of dry powder were weighted in 6 × 4mm tin capsules. *SI* analyses were performed on a Thermo Delta V isotope ratio mass spectrometer (IRMS) interfaced to a NC2500 elemental analyser at Cornell Isotope Laboratory (COIL, USA). Each sample was measured in triplicates. Organic carbon was expressed as the percentage of total organic matter, and stable isotope ratios were expressed in the conventional δ notation in units (‰) and based on international standards (Vienna Pee Dee Belemnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$) according to the formula:

$$\delta_{Sample} = \left[\left(R_{Sample}/R_{Standard}\right) - 1\right] X1000$$

where,

R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios for $\delta^{13}C$ or $\delta^{15}N$ respectively.

2.4. Statistical analyses

All statistical analyses were performed using R software (version 4.3.0; R core Team, 2023), with a significance level of $\alpha = 0.05$. Normality was assessed with Shapiro tests. When normality was achieved, the evolution of AFDW and energy content over time were tested using an ANOVA with post-hoc Tukey HSD. When it was not, a Wilcoxon-Mann-Whitney test, followed by a Kruskal-Wallis post-hoc test was performed. Trends and breakpoints in mortality dynamics were explored through segmented linear regressions using the "segmented" package (Muggeo, 2008).

3. Results

3.1. Effect of starvation on weight

For the two batches, weight loss of animals represented >60% of their total soft tissues (TST) during the 84 days of experiment, and was statistically significant (Fig. 1A, B). Unlike large individuals exhibited a regular weight decrease over time (Fig. 1B), small individuals seemed to show a 3-phase dynamics (Fig. 1A*)*. Indeed, the average TST weight did not significantly vary from day 0 to 14, and was followed by a drop occurred to a second step during which the weight kept quite constant from day 28 to 56. Then, the small individuals TST weight markedly decreased between day 56 and day 70, and remained again almost constant until the end of the experiment. The body condition index was not significantly different between the two batches at the beginning of the experiment, at day 7 (*t*-test, *p*-value = 0.286).

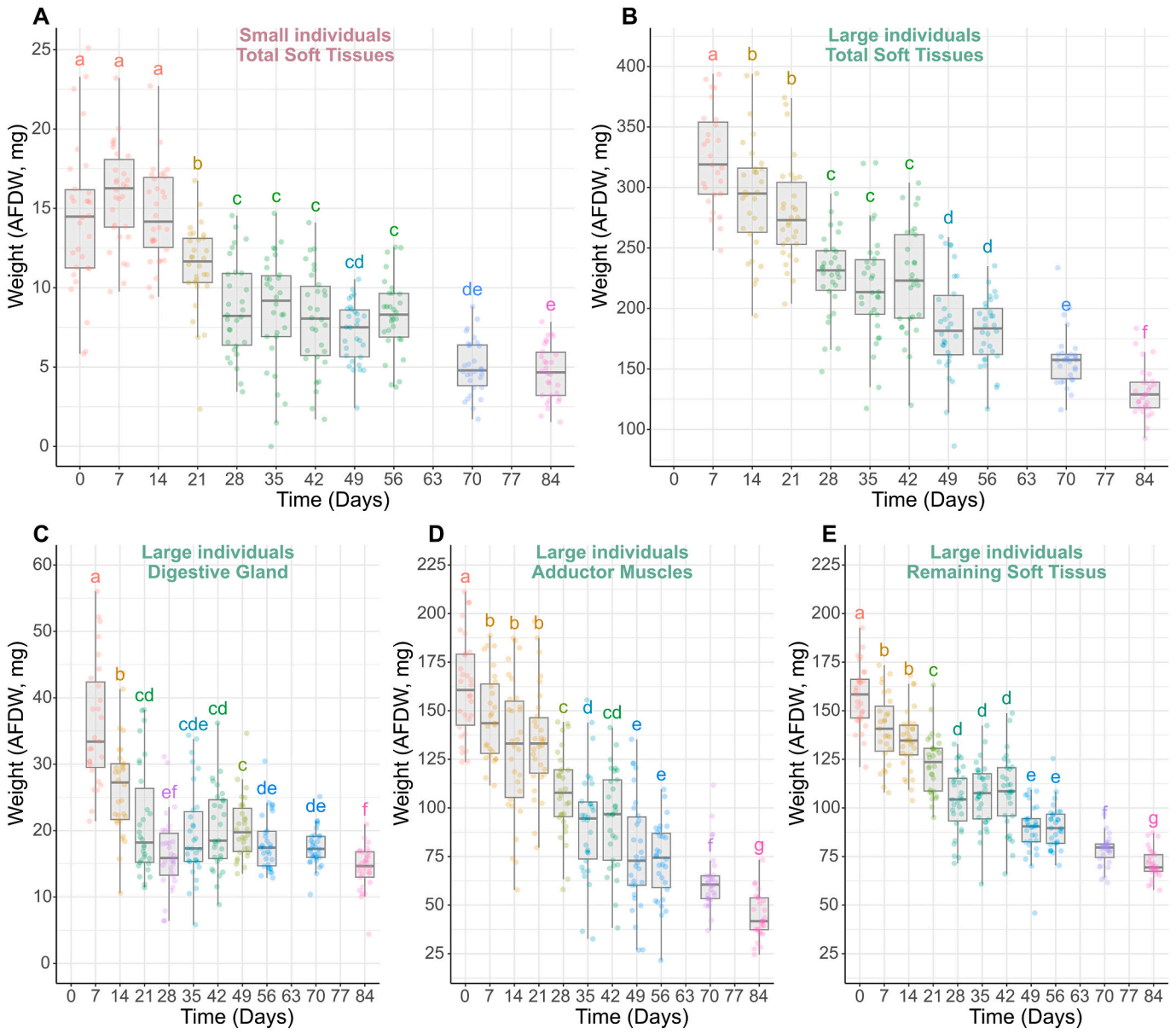

Fig. 1. Ash free dry weight evolution of total soft tissues during the experiment for small (A) and large (B) individuals batches; and tissues separately for large individuals batch: digestive gland (C), adductor muscle (D) and the remaining soft tissues (E) during the 84-day experimental period. At each sampling, $n = 30$. Boxes in the box plots indicate median ± quartiles. Letters and colours indicate statistical differences between sampling assessed by a one-way ANOVA (*p*-value $<2\ 10^{-16}$) and Tuckey test (p-value <0.05) for small batch (A), and by a Kruskal-Wallis (p-value $<2\ 10^{-16}$) and Wilcoxon test (p-value <0.05) for large batch (B, C, D, E). The distribution of samples sharing the same letter is not statistically different.

Results showed that the weight loss dynamics varied between organs (only analysed for the large batch). The weight of digestive gland (DG) steeply decreased from day 0 to day 28 and stabilized until the end of the experiment, with a loss percentage between 44 and 60% since the beginning (Fig. 1C). Conversely, the weight loss of the adductor muscle (AM, Fig. 1D) and the remaining soft tissues (RST, Fig. 1E) was steady over time leading to a loss of 72% and 55% at the end of the experiment, respectively. Note that, for these two tissues (and then the TST), the weight seemed to stabilize between 28 and 42 days before dropping again until the end of experiment (Fig. 1B, D & E). The AM weight represented 52 to 38% of the TST weight. Its contribution to the total soft tissues AFDW follows a significant negative linear relationship with monitoring time ($p < 2\ 10^{-16}$, $R^2 = 0.27$; $y = -0.14\%\ x + 52\%$).

3.2. *Impact of starvation on energy dynamics*

The calorific power of the small individuals (TST), did not show clear pattern, and stayed quite constant during the experiment (linear model, *p*-value > *0.05*, Fig. 2), but seemed to increase in a first period, achieved its maximum in day 28 (22 KJ g^{-1}), and has recovered its initial value on day 56 (20.5KJ g^{-1}). The calorific power of AM and RST of the large individuals remained stable during the whole experiment (linear model, *p-value > 0.05,* Fig. 2). The mean calorific power of these three tissues were very close: 21.3 ± 1.1 KJ g^{-3} for the small individuals TST, and 21.4 ± 0.7KJ g^{-3} and 21.7 ± 0.6KJ g^{-3} for the large individuals AM and RST respectively. Conversely, the digestive gland exhibited a three-phase dynamics with higher values from day 0 to 35 and lower values after day 49 separated by a steep drop. Segmented linear regression highlighted breakpoints at day 33 and day 45. During the first phase, DG's calorific power (24.17 ± 0.28KJ g^{-1}) was significantly higher than

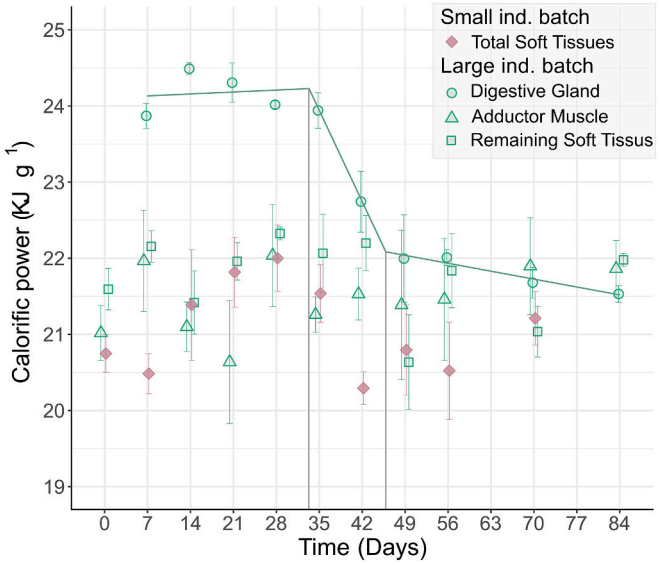

Fig. 2. Evolution of soft tissues calorific power: total soft tissues (small individuals, in pink fool diamonds), and digestive gland, adductor muscle, and remaining soft tissues (large individuals, in green: empty circles, squares and triangles respectively). Bars indicate standard deviation. The green line indicates the segmented linear regression for the digestive gland, grey arrows show the two break-points (at 33.3 and 45.8 days, respectively). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

during the second phase (21.80 ± 0.24KJ g^{-1}, Wilcoxon-test, *p*-value < *0.05*). Before the drop, its calorific power was significantly higher than other tissues (average 21.47 ± 0.45KJ g^{-1}, 21.75 ± 0.53KJ g^{-1}, 21.08 ± 0.60KJ g^{-1} for AM, RST and small batch TST respectively; Kruskal-Wallis test, *p*-value < *0.05* and Wilcoxon post-hoc test, *p*-value < *0.05*), but did not differ significantly from any of the other tissues during the second phase (Wilcoxon post-hoc test, *p*-value > *0.05*).

Considering that calorific power is stable over time, except for the digestive gland, which only accounts for 7.2 to 11.1% of the total weight, the energetic content dynamics (Table 2) are similar to the tissues' weight dynamics (Fig. 1). The small individuals have lost close to 40% of their initial energy content in day 28, after the quick loss in their weight. Large individuals lost >20% of TST energy content in day 28, and 40% after 49-day. According to the standardized shell height and the mean energy content per volume at the beginning of the experiment was 0.16 ± 0.02J mm^{-3} for the large individuals batch, and 0.14 ± 0.04J mm^{-3} for the large individuals batch.

3.3. Survival dynamics

Survival exhibited different trends between the two batches (Fig. 3). Mortality was observed in large individuals from the beginning of the experiment, while it was initially very low for small individuals. In both batches, mortality accelerated in a second phase. Segmented linear regressions reveal mortality acceleration breakpoints at days 36 and 68 for large and small individuals, respectively (Table 3). Slope of survival was higher in the second phase for small individuals than for the large one. The second-phase regressions allowed to estimate time for 50% mortality: 77 days for large individuals, and 88 days for small individuals respectively.

3.4. Evolution of nitrogen and carbon stable isotope ratios

During the experiment, the $\delta^{15}N$ signature of adductor muscle gradually increased by <1‰ ($\Delta^{15}N = 0.67‰$) and ranged from 8.9‰ to 9.7‰ (Fig. 4A). In the middle of the experiment, between days 28 and 56, the $\delta^{15}N$ values seemed to stabilize, before increasing again. The pattern of AM $\delta^{13}C$ variation is unclear over time (Fig. 4B) and slightly

Table 2
Soft tissue energetic content (mean ± sd) during the 84-day experimental period of small and large individuals batches ($n = 30$). The percentage correspond to energy content loss proportion in each compartment separately since the beginning of the experiment, at day 7. Letters indicate statistical differences between sampling for each compartment separately, assessed by a one-way ANOVA (*p*-value <$2\ 10^{-16}$) and Tuckey post-hoc test (*p*-value <0.05) for small batch, and by a Kruskal-Wallis (*p*-value <$2\ 10^{-16}$) and Wilcoxon post-hoc test (*p*-value <0.05) for large batch. The distribution of samples sharing the same letter is not statistically different.

Duration time (Day)	Energy content (KJ, mean ± sd)						
	Large ind.					Small ind.	
	Digestive gland[a]	Adductor muscle	Remaining soft tissues	Total soft tissues[a]		Total soft tissues	
				Energy content	Percentage loss	Energy content	Percentage loss
0	–	3.40 ± 0.54 *a*	3.46 ± 0.48 *a*	–	–	0.52 ± 0.10 *a*	–
7	0.86 ± 0.23 *a*	3.23 ± 0.49 *a*	3.12 ± 0.40 *b*	7.21 ± 0.91 *a*	–	0.48 ± 0.06 *a*	–
14	0.65 ± 0.18 b	2.76 ± 0.74 *b*	2.89 ± 0.34 *c*	6.30 ± 1.03 *b*	2.85%	0.49 ± 0.06 *a*	3.13%
21	0.52 ± 0.20 *c*	2.78 ± 0.56 *b*	2.70 ± 0.34 *d*	5.99 ± 0.89 *b*	8.45%	0.37 ± 0.06 *b*	21.88%
28	0.40 ± 0.14 *d*	2.38 ± 0.48 *c*	2.32 ± 0.37 *e*	5.09 ± 0.75 *c*	25.00%	0.32 ± 0.07 *c*	40.63%
35	0.47 ± 0.17 *efg*	1.95 ± 0.59 *de*	2.35 ± 0.37 *e*	4.77 ± 1.00 *c*	19.98%	0.32 ± 0.08 *c*	43.75%
42	0.46 ± 0.14 *def*	2.04 ± 0.55 *d*	2.40 ± 0.43 *e*	4.90 ± 0.98 *c*	23.63%	0.29 ± 0.06 *cd*	50.00%
49	0.45 ± 0.10 *def*	1.65 ± 0.61 *ef*	1.84 ± 0.27 *f*	3.93 ± 0.89 *d*	37.44%	0.22 ± 0.04 *ce*	53.13%
56	0.40 ± 0.09 *de*	1.56 ± 0.42 *f*	1.97 ± 0.25 *f*	3.93 ± 0.59 *d*	41.89%	0.26 ± 0.05 *c*	46.88%
70	0.38 ± 0.06 *fg*	1.37 ± 0.34 *g*	1.64 ± 0.16 *g*	3.39 ± 0.50 *e*	42.58%	0.19 ± 0.04 *e*	65.63%
84	0.31 ± 0.07 *g*	0.99 ± 0.29 *h*	1.57 ± 0.16 *g*	2.87 ± 0.45 *f*	54.11%	–	–

[a] Because of the initial digestive gland calorific content is unknown, the total soft tissues (large individuals) is calculated without digestive gland, and the percentage of energy content loss since the beginning is calculated from day 7.

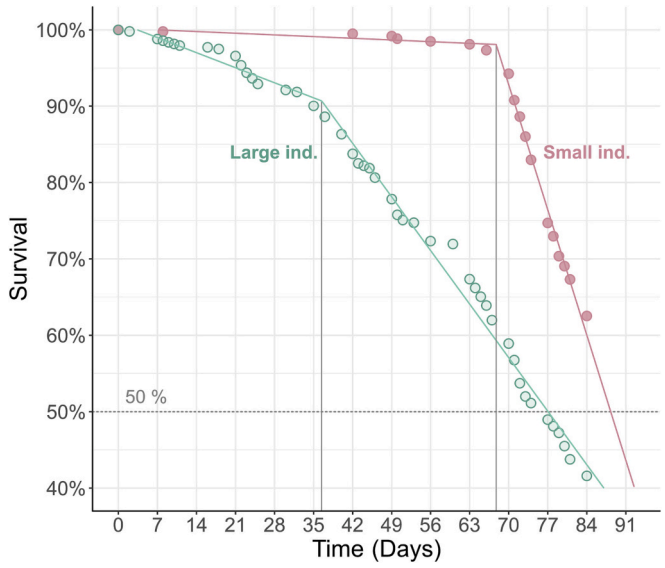

Fig. 3. Survival curves of small and large individuals batches of *Mimachlamys varia* (full pink and empty green points respectively) during the starvation monitoring. The lines indicate for each batch the segmented linear regression, with one breakpoint (at 67.8% for small individuals and 36.4% for large, the grey arrows). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Estimated breakpoints using a segmented linear regression for the large and small individuals batches ($R^2 > 0.99$ for both batches). The time required for half of a population to succumb to starvation LT_{50} (Lindstedt and Boyce, 1985) was estimated using the 2nd phase linear regression as the day when the survival percentage falls below the 50% threshold. The stars indicate the significant level: ** *p*-value <0.01, *** *p*-value <0.001.

Batch	Breakpoint (day)	Linear model slope (day^{-1})		LT_{50} (day)
		1st phase	2nd phase	
Large ind.	36.4	−0.3% *** $R^2 = 0.93$	−1.0% *** $R^2 = 0.98$	77
Small ind.	67.8	−0.03% ** $R^2 = 0.75$	−2.3% *** $R^2 = 0.99$	88

varied from −18.2‰ to −17.7‰. The ratio seemed to increase during the 49 first days of the experiment ($\Delta^{13}C = 0.38‰$), and then it decreased to return to values close to its initial value at the end of the monitoring period (respectively −18.15 ± 0.23‰ and − 18.06 ± 0.15‰).

4. Discussion

4.1. Physiological process

Our study highlighted a three-phase response to starvation in adults of *Mimachlamys varia*, each characterized by specific features. The first phase (day 0–28) was characterized by a rapid mass loss, a $\delta^{15}N$-enrichment, a stable calorific power for all tissues particularly for the digestive gland (DG), and a low mortality. During the second phase (day 28–42), mass and $\delta^{15}N$ values remained stable, coinciding with a DG calorific power drop and an acceleration in mortality. The third phase (from day 42) showed a stable calorific power for all tissues, with DG values close to those of other tissues, a mass decrease, a resumed $\delta^{15}N$-enrichment, and a persistently high mortality. This three-phase starvation dynamic has been reported in a wide range of animals (*e.g.* molluscs, snakes, mammals), and likely depends on metabolic requirements and mobilization mechanisms during starvation (McCue, 2010). Regarding metabolic requirements, energy first comes from various reserves including reserves stored for specific processes (*e.g.* reproduction, or spring growth resumption; Le Pennec et al., 2001) mostly involving carbohydrates, then lipids and reserve proteins, and ultimately by structure remobilization through protein catabolism (McCue, 2010; Lignot and LeMaho, 2012). This three-phase dynamic is also visible regarding protein metabolism (Olive et al., 2003; McCue, 2010; Lignot and LeMaho, 2012). During the first phase, metabolism is in transition between fed and starving states (Lignot and LeMaho, 2012) and basal protein catabolism persists, leading to protein limitation. Progressively, remaining proteins are saved and/or recycled, leading to a reduction in nitrogen excretion. The third phase is characterized by structural catabolism (Lignot and LeMaho, 2012).

In this study, the transition between phase 1 and 2 occurred between days 21 and 28, where the average weight loss equalled 16% and 31%, respectively. In comparison, Shafee (1981) observed a winter weight loss in the wild of the same order of magnitude (20%) mostly due to glycogen mobilization. Although biochemical analyses have not been performed in this study, it is likely that the main physiological fuel used during phase 1 is the glycogen, as documented in a number of taxa (Pinheiro, 1996), including bivalves (Bayne and Scullard, 1977; Patterson et al., 1999; Cordeiro et al., 2016). Nitrogen isotopic composition can provide information on the proteic metabolism involved during starvation. For instance, a preferential excretion of ^{14}N during the catabolic breakdown of body tissues (protein) would lead to a $\delta^{15}N$-enrichment in tissues (Hobson et al., 1993; Gannes et al., 1997). In our study, such an enrichment in adductor muscles (AM) was observed during phase 1, indicating the persistence of a protein catabolism. However, the $\delta^{15}N$-enrichment appears weak (+0.3‰) in comparison to a recent meta-analysis conducted across 130 species in various phyla (Doi et al., 2017) with reported values ranging between −0.82 and 4.30‰. The low AM isotopic turnover rate of bivalves (Lorrain et al., 2002; Hill and McQuaid, 2009; Ezgeta-Balić et al., 2014) may explain this low value.

During phase 2, the metabolism enters a “protein sparing” stage, during which the stored reserves, particularly the lipids, are consumed (Lignot and LeMaho, 2012). This may lead to a low nitrogen excretion, and subsequently a $\delta^{15}N$ stabilization, a pattern consistent with our findings. Such a stabilization of $\delta^{15}N$ preceded by a sharp increase under starvation has already been observed in the polychaete *Nereis virens* (Olive et al., 2003). This transition can be explained by a reduction in protein oxidation (allowing their conservation) followed by an increased consumption of lipids (Cherel et al., 1988; Castellini and Rea, 1992). In pectinids as in other bivalves, the DG is known to act as an energetic reserve organ, mainly storing lipids (Comely, 1974; Taylor and Venn, 1979; Barber and Blake, 1981; Robinson et al., 1981; Napolitano and Ackman, 1992; Pazos et al., 2003). This reserve may be mobilized for fuel maintenance and/or when facing acute energy demand, such as during gametogenesis, when AM reserves are depleted (Shafee, 1981; Le Pennec et al., 2001; Pazos et al., 2003). During phase 1 we observed a significantly higher calorific power in this tissue which may be explained by the storage of very energetic compounds, presumably lipids. The strong decline in DG calorific power observed after day 35 owing to a mobilization of lipids during phase 2 is consistent with this hypothesis.

Once lipids reserve are fully depleted (after day 42), the scallops metabolism must rely on the energy stored within its structure (taken as somatic compounds excluding reserves, according to Kooijman (2010) to cover somatic maintenance. In this third phase, a second decline in mass occurs concomitantly with an $\delta^{15}N$-enrichment of the AM, suggesting a resumption of the protein catabolic activity (Goodman et al., 1980; Le Maho et al., 1981). Beyond this point, individual’s death can occur rapidly due to rapid protein loss, corresponding to a “point of no return“ (*PNR*, Da Costa et al., 2012). Such a threshold has been widely reported, in a number of taxa and across all life-stages and inevitably leads to individual’s death even if food resumes. During our experiment

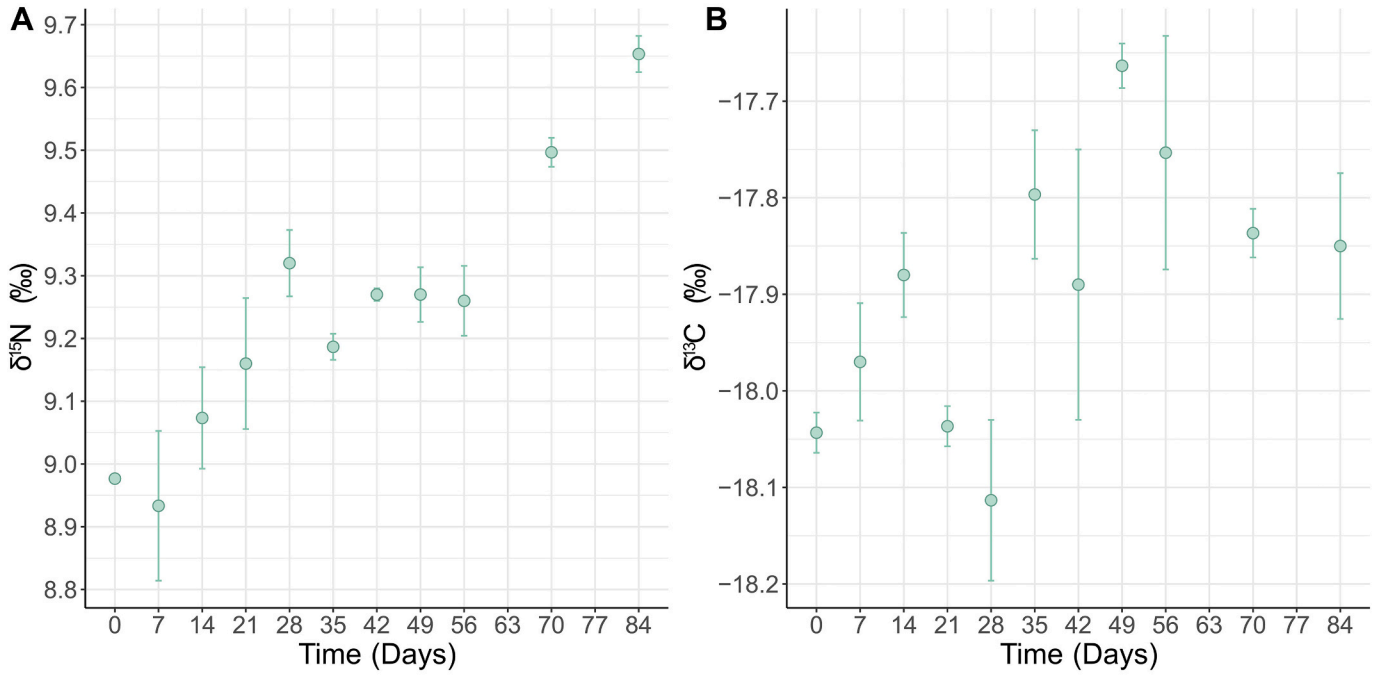

Fig. 4. Mean $\delta^{15}N$ (A) and $\delta^{13}C$ (B) (± sd) of *Mimachlamys varia* adductor muscle tissues from the large individuals batch during the experiment. At each sampling, measurements were taken in triplicates on pooled ground muscles from the 30 sampled individuals.

(*i.e.* dissections), the degradation of the gills and buccal pieces (labial palps, lips) macrostructure were observed. We hypothesized that such macroscopic degradation, probably resulting from somatic tissue degradation, can lead to the incapacity to ensure nutritional functions, and may be one of the factors leading to this critical point.

Trend in $\delta^{13}C$ was unclear and did not followed this three-phase dynamic. In the polychaete *Nereis virens*, Olive et al. (2003) also found a complex pattern in $\delta^{13}C$, presumably because $\delta^{13}C$ variation depends on the dynamic of several compounds (lipids, proteins; see *e.g.* Doucett et al., 1999; Hatch, 2012; Doi et al., 2017), suggesting that $\delta^{13}C$ is an unreliable indicator of starvation.

4.2. Size and life stage

Due to the limited material collected for our analyses, less information is available for juveniles (small individuals' batch) than for adults, limiting the interpretation of the processes underlying the three-phase dynamic. For both, juveniles and adults, the variation in energy content primarily depends on the variation regarding the dry mass of the different tissues and their relative proportions, which is in accordance with Shafee (1981) observation in the Bay of Brest. Nevertheless, weight loss patterns during the experiment diverged between juveniles and adults. Juveniles exhibited an important mass loss until day 28 (50% from the beginning of the experiment), coinciding with a moderate but significant increase in calorific power. We hypothesize that this structural weight decrease involves the remobilization of low-calorific biochemical compounds (*e.g.* proteins) using the structure as internal fuel, while conserving high-calorific components such as lipids. Catabolized proteins are recycled to ensure remaining proteins turn as suggested by Hatch (2012). After day 28, weight loss stabilizes (mass loss ~50% from the beginning of the experiment) and the calorific power increase stops. Total calorific content then remains constant for about 40 days until the mortality break-point is reached (65.6% of the initial content). This phase may be interpreted as the second starvation phase, and can be regarded as the "protein sparing" stage identified for adults by Lignot and LeMaho (2012). In addition, juveniles displayed higher survival rates than adults during the first two months of the experiment, with 97% and 64% survival rates respectively after 70 days. Interestingly, although LT_{50} is higher for juveniles, the increase in mortality rate after the "Point of No Return" is reached at higher energy loss for juveniles than for adults. Defining the PNR as the end of the lipids-digestive gland remobilization phase for adults and as the mortality acceleration rate for juveniles, the PNR is reached at 44% mass and 43% energy loss for adults, while for juveniles, the PNR is reached at 75% mass and 65% energy content loss.

Facing prolonged starvation is appears that juveniles and adults exhibit different strategies. Adults may have various mobilizable energy pools, particularly in the DG (Le Pennec et al., 2001) while juveniles potentially exhibit a higher ability to reduce their structural weight, making it possible to reduce their maintenance costs and enhance their survival ability. In addition, at the beginning of our experiment, juveniles had a body condition index similar to that of adults likely because juveniles may not have accumulated energy for reproduction, implying a different energy partitioning between size batches. Given that the ability of an extended phase II depends on the status of non-proteic content (glycogen and/or lipid reserves; McCue, 2010), the absence of a high-energetic pool in juveniles – such as lipids which are usually stored for reproduction – may explain the high increase in mortality observed right after the *PNR* was reached.

4.3. Population impact

The ability of a species to handle prolonged starvation has been shown to play a key role for population dynamics (McCue, 2010), and can be captured through estimates of *PNR* and LT_{50} (Chase and McMahon, 1995; McCue et al., 2015). However, caution is advised when using these proxies because their estimates strongly varies with temperature in ectotherms owing to increased metabolism activity. Indeed, Chase and McMahon (1995) showed that these thresholds are reached faster at higher temperatures due to faster metabolism.

In our study, temperature was maintained at 14 °C, corresponding to the average sea temperature in the Bay of Brest at the beginning of the experiment. However, winter average temperature (*i.e.* the period during which starvation takes place in the wild) in the bay have been

observed to vary between 8 and 11 °C (calculated between 2006 and 2022; data extracted the 08–22-23 from the database SOMLIT: Service d'Observation en Milieu Littoral; www.somlit.fr). Temperature and food limitation in our experiment may have been more severe than winter field conditions in the Bay of Brest. We observed a 20% mass loss between days 21 and 28, with *PNR* being reached 5 days later, after a 1-month starvation. In the while Shafee (1981) observed a 20% mass and total energy content loss during the winter period (*i.e.* ~2.5 months when sea temperature averages 8–9°C, Shafee, 1980) suggesting that in natural conditions, the physiological limit of the scallop to handle starvation is close to being reached. A projected global increase in sea surface temperature (Ipcc, 2023), may lead to earlier *PNR* and LT_{50}, indicating that starvation can be a concern for natural populations in the near future. For instance, despite a higher resistance to starvation, the higher mortality rate of juveniles after *PNR* is reached suggests that changes in environmental conditions could trigger the loss of an entire cohort, with a severe impact on population dynamics.

Beyond survival, starvation may indirectly affect populations by impacting on the reproduction success. Previous studies have shown that bivalves, including *M. varia* (Iglesias et al., 2012), use DG reserves acquired during the previous season of abundant food (*i.e.* summer or autumn) for gametogenesis (Barber and Blake, 1981; Le Pennec et al., 2001; Pazos et al., 2003; Watts et al., 2014). *M. varia* exhibits two spawning peaks, in early spring and in autumn (Lubet, 1959; Shafee and Lucas, 1980; Iglesias et al., 2012), where the intensity of the second peak is conditioned on the energy accumulated during spring and summer (Shafee, 1981). We observed a reduction of the energy content of DG during the phase 2 of starvation suggesting a mobilization of reserves that may be used to fuel gametogenesis. Such a reduction can affect the intensity, phenology and number of spawning events in the wild with consequences for populations way before the *PNR* is reached.

Reaching sexual maturity is crucial for population maintenance. In *M. varia*, sexual maturity occurs in the spring following their birth (*i.e.* ~ 1 year old) and depends, as well as first gametogenesis, on the energy stored during the first growing season. Even if we found that juveniles were more resistant to prolonged starvation than adults, the observed drastic decrease in their body mass, could limit their ability to reach sexual maturity in case of severe food limitation during the first winter. Under this scenario, and considering the synchronicity of juvenile response to nutritional stress, an entire cohort may not participate in the population's spawning effort. Coupled with the fact that some adults may also have been affected, nutritional stress could have a large impact on population dynamics.

5. Conclusion

Juveniles and adults showed similar patterns but distinct strategies facing nutritional stress. Adults start by consuming the internal pool of energy reserves allocated to basal metabolism, and ultimately rely on reserves stored for specific processes (*i.e.* early gametogenesis, growth resumption, or predator escape) in case of severe and prolonged starvation. In contrast, juveniles quickly lose mass, reducing their somatic volume before stabilizing with low maintenance requirements. The time required for half of the population to succumb to starvation occurs more rapidly in adults than in juveniles, but death is less synchronous among adults. While these two strategies can make it possible for the population to persist in the short-term, effects on the reproductive success (through gametogenesis and sexual maturation) can have long-lasting consequences on the maintenance of the population. Finally, although our study was conducted under controlled conditions, given current climate disruptions, our results indicate that an intensified winter food stress could represent a significant threat for *M. varia* populations within the Bay of Brest. To more precisely assess the impact of starvation on populations, future research could focus on the biochemical composition of the various compartments and organs throughout the seasonal cycle, while also studying the processes affecting *M. varia* sexual maturity.

CRediT authorship contribution statement

Laure Régnier-Brisson: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Aline Blanchet-Aurigny:** Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Writing – review & editing, Methodology. **Philippe Cugier:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing. **Florian Breton:** Conceptualization, Methodology, Resources. **Jean-Dominique Gaffet:** Methodology, Resources. **Fred Jean:** Conceptualization, Supervision. **Jonathan Flye-Sainte-Marie:** Conceptualization, Investigation, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

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

Aline Blanchet-Aurigny reports financial support was provided by France Filière Pêche. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgment

The authors would like to thank Allan Stervinou for his support during the experiment. The LBH laboratory (Ifremer) is also appreciated for granting access to the calorimeter. Special thanks go to Sophie Le Mestre for sharing her technical knowledge and practical expertise, and to Erwan Le Gall for his assistance with the calorimeter setup. Thanks to Laura Fradet for her contribution during the laboratory work. Finally, the authors would like to thank Mathieu Chevalier, Amélia Curd and Martin Marzloff for proof-reading the manuscript, and having improved the english quality. This work is part of a PhD thesis, and received financial support from the research project "MaSCoET" (Maintien du Stock de Coquillages en lien avec la problématique des Efflorescences Toxiques) financed by France Filière Pêche, Brest Métropole, Région Bretagne and the Ifremer Scientific Direction.

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