Thermal effect on energetic physiology in the ark shell Scapharca subcrenata

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Research

Keywords: Scapharca subcrenata, Physiological ecology, Physiological energetics, Scope for growth, Thermal effect, Korean coast

Posted Date: July 28th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-45639/v1

Thermal effect on energetic physiology in the ark shell *Scapharca subcrenata*

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Abstract

Background: Clams inhabiting temperate coastal zones are affected not only by seasonal thermal variation, but also by changes in the prevailing thermal regime of their habitats. Understanding the physiological processes required for adjusting the energy balance of the ark shell *Scapharca subcrenata* to varying thermal conditions is pivotal for predicting its growth and further phenology, and ultimately promoting successful aquaculture activity. Thermal effects on the physiological processes and the combined energetic physiology at the organism level of *S. subcrenata* were assessed over a temperature range corresponding to field conditions (3–28 °C).

Results: Physiological rates of *S. subcrenata* were well correlated with its dry tissue weight, formalizing allometric relationships. Extremely low weight exponent values for filtration rate and metabolic rate were detected at lower (3–8 °C) compared to higher (8–28 °C) temperatures. In addition to marked reductions at 3 °C, weight exponents were identical and intercept estimates increased progressively with rising temperature over the temperature range (8–28 °C). Identical weight exponents and increasing intercept estimates for both feces production and excretion rates across the experimental temperatures indicated that energy losses by egestion and excretion increased gradually with rising temperature. Scope for growth and net growth efficiency showed relatively constant and positive values at 8–23 °C, suggesting an optimal temperature range for production, but dropped drastically to negative values at 3 and 28 °C, indicating thermal (both cold and heat) stress. The Q_{10} values revealed that the metabolic and filtration rates are more sensitive at 23–28 and 3–8 °C, respectively.

Conclusions: Allometric size-scaling of physiological rates in *S. subcrenata* highlights species-specific responses to changes in temperature. The observed weight exponents and intercept estimates for filtration and metabolic rates reveal the variation of the thermal effects according to size as well as an incapability of acclimation to varying temperatures. Reversed thermal sensitivities in both components confirm that energy acquisition by feeding does not offset the metabolic energy cost

outside the optimal thermal range. Our empirical analysis allowed further understanding of the seasonal energy dynamism and biological cycle of *S. subcrenata* in temperate habitats subject to highly variable thermal regimes.

Keywords: *Scapharca subcrenata*, Physiological ecology, Physiological energetics, Scope for growth, Thermal effect, Korean coast

Background

Marine ectotherms have a capacity for physiological adjustment in response to environmental stresses [1]. These organisms show physiological plasticity to meet the energy requirement by reconstructing their physiological mechanisms during acclimation or acclimatization to environmental changes [2]. To maintain fitness across changing environmental conditions, these organisms regulate the rates of individual physiological components via adaptive strategies [3]. Energy balance in an organism is determined by the difference between energy acquisition (food consumption) and expenditure (egestion, excretion, and metabolism), eventually affecting its growth, reproduction, and survival. A persistent negative energy balance results in weight loss, reproduction inhibition, and even death [4]. Therefore, organisms attempt to optimize their energy balance and the overall adaptive responses can be assessed by determining the energy available for production, i.e., scope for growth (SFG) [3,5]. The mechanism of physiological adaptation can be explicitly explained by integrating the rates of all of the processes that regulate the energy balance in an organism. The regulation capacity for individual physiological processes finally determines the consequence of the organism's adaptive responses to environmental changes [6,7].

The suspension-feeding ark shell *Scapharca subcrenata* (Lischke, 1869) inhabits the soft muddy bottom of the shallow (5–10 m depth) subtidal zone along the northwestern Pacific coasts, including those of Korea, Japan, and China [8,9]. In Korea, this ark shell is a commercially important clam species. As natural seed collection was developed in the 1970s, aquaculture of the ark shell was

pioneered in Yeoja Bay on the southern coast of Korea. The annual production of the cultured clams in the bay has comprised over 80% of the national production since the mid-1990s [10]. The total annual production in Korea was estimated to be 5,000–10,000 tonnes in the 1990s and 2000s, but gradually declined to approximately 3,000 tonnes in the 2010s ([11,12]. Recently, clam production has fluctuated yearly (e.g., 2,369 tonnes in 2017 and 4,439 tonnes in 2018) [10] because of a local shortage of healthy seeds and/or mass mortality of mature ark shells [13].

The ark shell shows a clear biological cycle regarding growth and reproduction, in which spawning takes place mainly during the late spring and summer [14–16]. According to its phenology, the seeds are collected from shallow (<5 m depth) subtidal spawning grounds from June to August. The spats are transplanted to shallow bottom sediment and cultured for 12–18 months until they reach a marketable size [17]. During the culturing period, the ark shells are exposed to a wide range of seasonal temperatures. In addition, due to the accelerating ocean warming, the effect of the increasing water temperature on biological activities is especially significant in coastal zones [18]. Because of their sedentary nature, clams inhabiting temperate coastal zones are affected not only by seasonal thermal variation, but also by changes in the prevailing thermal regime of their habitats [19,20]. As they are exposed to a varying thermal regime in Yeoja Bay, the clams need physiological adjustments that allow them possible to meet their energetic demands. As a consequence, understanding the physiological processes required for adjusting the energy balance of the ark shell to varying thermal conditions would help predict its growth and further phenology, and ultimately promote successful aquaculture activity.

The thermal sensitivity of biological reactions, as defined by the temperature coefficient (Q_{10}), is a traditional approach to measure the temperature dependence of physiological processes [21,22]. The Q_{10} provides significant information regarding the attributes of an individual physiological component in animals [23,24]. Identifying the individual-based reactions induced by environmental changes further requires interpretation of energy dynamics based on measurements of overall physiological processes [25]. Despite a general consensus about the temperature dependence of metabolic rates in marine ectotherms, the species-specific responses deviate from a typical exponential curve, thus mismatching the expected metabolic trend [26]. Some marine ectotherms exhibit increases in metabolic rates at

97 elevated temperatures [26,27], whereas others maintain their metabolic rates in these conditions [28].
98 The increase in the feeding rates to compensate for the metabolic cost allows the animals to maintain a
99 positive energy balance across environmental conditions [29–31]. A lack of such compensation
100 processes may result in negative SFG under unfavorable conditions [32]. The interspecific difference
101 in the capacity for thermal acclimation may add ambiguity to the estimation of thermal tolerance [2,33].
102 Therefore, exploring organismal responses based on measurements of the physiological regulation
103 processes across the exposed thermal regime is fundamental to understand the mechanisms underlying
104 the formation of extreme events, with a particular emphasis on their functional traits.

105 In *S. subcrenata*, a few previous studies assessed the physiological effects of heat stress at
106 relatively high temperatures (17–32 °C) [34,35]. These authors measured the oxygen consumption and
107 ammonia excretion rates, and antioxidant enzyme activities in response to an acute temperature change.
108 Despite some advantages to identify the relationship between fitness and SFG, seasonal measurements
109 of physiological energetics only provided the limited information on probable sources of temporal
110 variation [36]. The present study aimed to examine the physiological adjustment processes of *S.*
111 *subcrenata* to optimize energy balance under a wide thermal range. For this purpose, we measured the
112 physiological processes (e.g., food consumption, egestion, excretion, and metabolism) that determine
113 the energy balance of *S. subcrenata* in response to different thermal conditions (3, 8, 13, 18, 23, and 28
114 °C), a temperature range that is typical of habitats of the native population in the Korean peninsula. We
115 also estimated SFG to evaluate the capacity of net production and optimal temperature ranges of the
116 species. To the best of our knowledge, the present study was the first attempt to investigate the energetic
117 physiology of *S. subcrenata* in response to changes in ambient thermal conditions. Our results will
118 provide clues to explain the manner in which the recent warming of this sea area (thereby changing the
119 thermal regime) affects the seasonal energy dynamism and further biological cycles of this clam species.
120

121 # Methods

122 ## Clam collection, acclimation, and experimental design

123 The ark shell *S. subcrenata* was collected at one of the designated harvesting sites (34°79′ N, 127°52′
124 E) in Yeoja Bay using a clam-harvesting dredge. For biometric analysis, the specimens (n = 100–120)
125 were collected monthly using a clam-harvesting dredge. The specimens of ark shells used for
126 physiological measurements were collected in each season (August and November 2017; February and
127 May 2018) to reduce the physiological disturbance caused by the great differences between the *in situ*
128 acclimatized and experimental conditions. Groups of clams of varying sizes were randomly collected
129 (Table 1) and immediately transported to a 200 l aquarium in the laboratory. The water tank was
130 equipped with a continuous seawater flow system and aeration. On each sampling occasion, 40
131 individuals were then acclimated to the experimental temperature (3, 8, 13, 18, 23, and 28 °C,
132 respectively). This temperature range covered the seasonal variation in the coastal water of the culturing
133 area. The water temperature in the container was controlled by gradually increasing or decreasing it by
134 1 °C a day, until the experimental temperature was reached. After reaching the desired temperature, the
135 specimens were kept under the same conditions for 14 days, before measuring physiological activities.
136 During the acclimation period, the clams were fed daily with a mixed diet (2×10^6 cells l^{-1}, Shellfish
137 Diet 1800®; Reed Mariculture, Campbell, CA, USA) composed of four marine microalgae (40%
138 *Isochrysis* sp., 15% *Pavlova* sp., 20% *Thalassiosira weissflogii*, and 25% *Tetraselmis* sp.);
139 subsequently, the feeding was halted for 48 h before the initiation of measurements to minimize the
140 relevant physiological activities. The specimens were transferred to experimental chambers and
141 reacclimated to chamber conditions for 6 h before the beginning of the experiments.

142 All experiments were carried out in seasonal field conditions in terms of suspended particulate
143 matter (SPM) concentrations and temperature to exclude a confounding effect of the changes in diet
144 concentration and acclimation to experimental temperatures. The feeding and metabolic rates of the
145 clams were determined using 350 ml flow-through closed cylindrical chambers. Each nine experimental
146 chamber was occupied by a single ark shell and one chamber was left empty as a control. The chambers
147 were immersed in a water bath to keep the water temperature constant for each experimental condition.
148 Filtered seawater was continuously flowed into the individual chambers after aeration by a peristaltic
149 pump equipped with a 10-channel pump head (BT 100-1 L; Longer Precision Pump Co. Ltd, Baoding,

China). The concentration and composition of SPM in the experimental waters supplied for feeding were adjusted to minimize variation in the experimental conditions (Table 1). The oxygen concentration in the individual chamber was kept at over 80% saturation [37] and the percentage of particles cleared from the inflow of the chamber was maintained at around 20% [32] by adjusting the flow rates in each chamber to approximately 20 ml min^{-1}. Physiological measurements were carried out over 24 h.

Table 1 Shell length (SL) and dry tissue weight (DW) of experimental individuals and experimental conditions

T (°C)	SL (mm)	DW (g)	SPM (mg l^{-1})	POM (mg l^{-1})	PP (μg l^{-1})	PCH (μg l^{-1})	PL (μg l^{-1})	Energy (J l^{-1})
3	25.9−38.0	0.32−1.59	5.0−7.6	1.8−2.2	276.4−317.2	352.4−491.3	176.7−212.3	24.3−30.1
8	26.8−41.4	0.24−0.46	5.0−6.6	3.0−3.4	393.7−407.5	461.2−488.7	212.1−259.9	31.4−33.6
13	22.5−39.0	0.26−1.38	19.8−20.0	3.2−3.6	423.7−476.0	442.1−515.9	197.4−208.1	31.1−35.4
18	21.8−37.6	0.19−1.24	11.2−11.8	4.0−4.4	387.1−405.7	522.1−548.0	202.8−216.4	34.1−34.5
23	23.8−36.6	0.16−1.11	6.8−7.2	2.8−3.2	313.0−478.5	400.1−479.9	188.8−208.9	26.6−34.1
28	27.9−36.2	0.14−0.63	16.4−19.0	4.2−4.4	472.5−516.8	454.1−534.1	194.4−228.7	32.7−37.3

Composition of suspended particulate matter (SPM) supplied to ark shell: POM, particulate organic matter; PP, particulate proteins; PCHO, particulate carbohydrates; PL, particulate lipids; Energy, energy value of SPM converted by energy equivalents of proteins, carbohydrates, and lipids

Physiological measurements

The filtration rate of the clam was measured by quantifying the particles removed by individuals, which were estimated from the difference between the outflow of the control and each of the experimental chambers. The outflow values of the chambers were collected several times, and 500 ml of seawater was filtered onto the precombusted Whatman GF/F filter (diameter, 47 mm; mesh, 0.7 μm). The filters were lyophilized and the energy values of the particles were calculated using the energy equivalents (J d^{-1}) of their biochemical components (proteins, lipids, and carbohydrates). Protein content was hydrolyzed with a 0.5 N sodium hydroxide and then determined using a colorimetric method [38]. Carbohydrates were extracted in 15% trichloroacetic acid and determined according to the phenol–sulfuric acid method [39]. Lipids were extracted in a mixture of chloroform and methanol [40] and measured using a colorimetric method [41]. The energy conversion factors for 1 mg of proteins,

carbohydrates, and lipids were 24.0, 17.5, and 39.5 J, respectively [42]. Very few pseudofecal materials were observed during the experiments; thus, they were excluded from this analysis because of the difficulty in collecting them. Finally, the filtration rate was considered as the ingestion rate.

Fecal materials produced by clams were collected several times (every 3–4 h) using a micropipette during the 24 h experiment, and transferred into 15 ml precombusted glass tubes. After centrifugation, the fecal pellets were rinsed with distilled water, freeze dried, and weighed. The rate of feces production was estimated from the biochemical composition of the fecal materials and their energy equivalents (J d^{-1}), according to the procedures described above.

The ammonia concentration was measured in the outflow from the chamber for each experiment. Water samples collected several times during the experiments were spectrophotometrically analyzed using the phenol–hypochlorite reaction [43]. The excretion rate was calculated from the differences in ammonia concentration between the control and experimental chambers, and was converted into the energy equivalent (J d^{-1}) using a conversion factor (24.83 J for 1 mg of NH_4-N) [44].

The oxygen concentration inside the measurement chambers was recorded for 24 h according to continuous monitoring protocols [45] and using oximetric probes (Oxy-10 micro; PreSens-Precision Sensing GmbH, Regensburg, Germany). The metabolic rate was determined based on the oxygen consumptions calculated from the differences in oxygen concentration between the control and experimental chambers. The energy conversion factor of oxygen consumption was 14.0 J for 1 mg of O_2 [42].

The thermal sensitivity of physiological rates for individual physiological components was calculated for the 3–8, 8–13, 13–18, 18–23, and 23–28 °C ranges using the following formula: $Q_{10} = (R_2/R_1)^{10/(T_2-T_1)}$, where R represents the rate of the parameter at temperature (T) 1 and 2.

SFG was calculated based on the energy balance equation in an individual organism [3,46] using the energy equivalents of physiological parameters: $SFG = A - (U + R)$, where A is the absorbed energy calculated from the difference between the consumed energy (C) and feces (and pseudofecal) production (F), U is the energy of excretion, and R is the energy equivalent of metabolic losses. The assimilation efficiency (AE) was then assessed using the following formula [47]: $AE = (R + SFG)/C$.

Net growth efficiency (K_2) was calculated using the following formula [48]: $K_2 = SFG/A$.

Biometric measurement

After physiological measurements, the animals were carefully dissected into shells and soft tissues. Shells were dried at 60 °C for 72 h in a drying oven. The shell length of individual specimens was measured using Vernier calipers, and shells were weighed to determine the dry shell weight (SW). Soft tissues were lyophilized for 72 h and weighed, to determine the dry tissue weight (DW).

Size standardization and statistics of physiological measures

The physiological data and DW of the ark shells were logarithmically transformed (base 10), and least-squared regression analyses between all variances were performed for each experimental condition according to the following allometric equation: $Y = aW^b$, where Y is the physiological rate, W is the DW, and a and b are the fitted constants and represent intercepts and slopes, respectively. Allometric equations were compared using an analysis of covariance (ANCOVA). In case of absence of significant differences among the fitted slopes ($P > 0.05$), the intercepts were reestimated using a common slope ($\bar{b}$). A Bonferroni *post hoc* multiple-comparison test was then used to compare the intercepts of the various sets of regression equations. Otherwise, the physiological variables were calculated to the value of a standard-weight animal, which is a grand mean of the covariate (0.6 g DW in this study) of all the clams in the experiments, calculated according to the following equation [49]: $Y_s = (W_s/W_e)^b \times Y_e$, where Y_s is the physiological rate of a standard animal, W_s is its DW, W_e is the DW of the experimental clam, Y_e is the measured physiological rate of the experimental clam, and b is the corresponding allometric coefficient (which corresponded to $\bar{b}$ in the present study). The statistical analyses were performed using SPSS software (v. 22.0; IBM Corp., Armonk, NY, USA).

Results

Filtration rates

226 The regression analyses between the physiological rates and DW of *S. subcrenata* from Yeoja Bay
227 indicated significant relationships at all six experimental temperatures for each variable ($P < 0.05$; Table
228 2, Fig. 1). Significant positive relationships were detected between the filtration (i.e., ingestion) rate
229 and DW values at all experimental temperatures (Table 2, Fig. 1a). ANCOVA revealed a significant
230 heterogeneity in the estimates of the slope of the regressions ($F_{5,42} = 2.723$, $P = 0.032$), which precluded
231 further comparison of differences among intercepts. Therefore, the regressions were divided into two
232 groups based on temperature dependency; within the lower (3–8 °C) and higher (8–23 °C) temperature
233 groups, no significant differences were detected between the estimates of slope ($F_{1,14} = 0.535$, $P = 0.477$;
234 $F_{4,35} = 0.783$, $P = 0.554$, respectively). A common slope ($\bar{b}$) for each group (0.183 ± 0.027 and 0.336 ±
235 0.019, respectively) was calculated, showing the presence of a steeper slope at higher temperatures
236 ($F_{1,59} = 19.007$, $P < 0.001$). ANOVA of the filtration rates standardized to a grand mean DW (0.6 g)
237 indicated a significant difference at all experimental temperatures ($F_{5,48} = 253.55$, $P < 0.001$; Fig. 2a).
238 A subsequent Tukey *post-hoc* test revealed a significant effect of temperature on filtration, denoting a
239 gradual increase from 3 °C to 28 °C ($P < 0.05$).
240
241 **Oxygen consumption**
242 The regressions between oxygen consumption and DW values for all the experimental temperatures
243 were significantly positive (Table 2, Fig. 1b). ANCOVA showed a significant difference among the six
244 slopes of the oxygen consumption–DW regressions ($F_{5,42} = 2.553$, $P = 0.042$), which enables further
245 comparison of the differences among elevations. There were no significant differences among the
246 estimates of the slope of the regressions within the groups incubated at lower (3–8 °C) and higher (8–
247 28 °C) temperature ranges ($F_{1,14} = 1.196$, $P = 0.293$; $F_{4,35} = 0.664$, $P = 0.621$, respectively). ANCOVA
248 revealed a significant difference in the slopes between the two groups ($F_{1,59} = 5.928$, $P = 0.018$), with
249 higher-temperature groups exhibiting a steeper common slope ($\bar{b}$) of 0.370 ± 0.025, vs. 0.182 ± 0.031
250 at lower temperatures. ANOVA of oxygen consumption rates, which were adjusted to a grand mean
251 DW (0.6 g), indicated a significant difference among means at the experimental temperatures ($F_{5,48} =$
252 173.05, $P < 0.001$; Fig. 2b). A subsequent Tukey *post-hoc* test denoted a gradual increase in oxygen

consumption at all experimental temperatures, with the exception of the constant values observed between 13 and 18 °C, suggesting a significant temperature effect on oxygen consumption ($P < 0.05$).

Table 2 Regression coefficients of allometric relationship between physiological rate and dry tissue weight

Parameter			a	b	r	$\bar{a}$
Ingestion energy (*I*)						
	3 °C		210.8	0.174	0.873*	210.7
	8 °C		412.3	0.227	0.885*	349.2
	13 °C		512.6	0.315	0.944**	516.6
	18 °C		615.7	0.329	0.962**	618.9
	23 °C		799.7	0.359	0.966**	787.5
	28 °C		948.4	0.398	0.904**	891.5
	Fs	df	Significance		$\bar{b}$ ± 95% CI	
All	2.723	5,42	$P = 0.032$		-	
3–8 °C	0.535	1,14	NS ($P = 0.477$)		0.183 ± 0.027	
8–28 °C	0.783	4,35	NS ($P = 0.554$)		0.336 ± 0.019	
Respiration energy (*R*)						
	3 °C		240.4	0.167	0.876*	239.0
	8 °C		347.0	0.257	0.800**	284.9
	13 °C		412.3	0.332	0.951**	418.3
	18 °C		459.5	0.371	0.922**	459.0
	23 °C		627.6	0.428	0.922**	603.7
	28 °C		898.9	0.415	0.922**	859.7
	Fs	df	Significance		$\bar{b}$ ± 95% CI	
All	2.553	5,42	$P = 0.042$		-	
3–8 °C	1.196	1,14	NS ($P = 0.293$)		0.182 ± 0.031	
8–28 °C	0.664	4,35	NS ($P = 0.621$)		0.370 ± 0.025	
Feces energy (*F*)						
	3 °C		9.1	0.121	0.855*	9.9[a]
	8 °C		26.1	0.185	0.895*	31.4[b]
	13 °C		48.6	0.415	0.759	47.4[c]
	18 °C		91.4	0.389	0.902**	88.7[d]
	23 °C		90.9	0.336	0.903**	91.7[d]
	28 °C		97.9	0.406	0.780	92.6[d]
	Fs	df	Significance		$\bar{b}$ ± 95% CI	
All	1.289	5,42	NS ($P = 0.287$)		0.350 ± 0.040	
Excretion energy (*U*)						
	3 °C		1.4	0.192	0.885*	1.5[a]
	8 °C		1.8	0.202	0.921**	2.0[b]
	13 °C		3.3	0.323	0.763	3.3[c]
	18 °C		3.3	0.278	0.718	3.3[c]
	23 °C		3.9	0.328	0.769	3.8[c]
	28 °C		6.6	0.350	0.891*	6.2[d]
	Fs	df	Significance		$\bar{b}$ ± 95% CI	
All	0.366	5,42	NS ($P = 0.869$)		0.294 ± 0.036	

Regression coefficients of allometric equation $Y=aW^b$ between physiological rate (Y, J d^{-1}) and dry tissue weight (W, g) of the ark shell *Scapharca subcrenata* at different temperatures. Y represents ingestion, respiration, feces production and ammonium excretion. a and b indicate estimates of regression intercept and slope (exponent), respectively. Nine individuals were used for each experimental condition. All regressions are significant at $P < 0.05$, $^{*}P < 0.01$, and $^{**}P < 0.001$. Results of analysis of covariance (ANCOVA) to test significance of differences in slope are summarized at the bottom of each physiological parameter. $\bar{a}$, recalculated using common slopes ($\bar{b}$) obtained from ANCOVA. CI, confidence interval.

Feces production

Significantly positive relationships were observed between the energy loss caused by feces production and DW values at all the experimental temperatures (Table 2, Fig. 1c). ANCOVA performed on the feces production and DW values revealed no significant differences ($F_{5,42}$ = 1.289, P = 0.287) and suggested a common slope ($\bar{b}$) of 0.350. Moreover, significant differences were detected between the rate of feces production–DW regression elevations revealed by ANCOVA ($F_{5,47}$ = 267.780, P < 0.001). A Bonferroni test further indicated a significant effect of temperature on feces production, denoting a gradual increase at 3–13 °C, followed by constant values at 18–28 °C (P < 0.05).

Ammonium excretion

The energy loss by ammonium excretion also displayed significant positive relationships with increasing DW at all six temperatures (Table 2, Fig. 1d). No significant differences among the slopes of the six regressions were found by ANCOVA of excretion ($F_{5,42}$ = 0.366, P = 0.869); thus, a common slope ($\bar{b}$) of 0.294 was obtained. ANCOVA performed on the elevations of the regressions of ammonium excretion and DW revealed significant differences in the rate of excretion among the experimental temperatures ($F_{5,47}$ = 108.470, P < 0.001). A Bonferroni test denoted a significant difference in ammonium excretion between 3–8 °C, and a gradual increase through 13–23 °C to 28 °C (P < 0.05).

Assimilation efficiency (AE) and O:N ratio

The adjusted values for AE to a grand mean DW (0.6 g) ranged from 85% at 18 °C to 95% at 3 °C, displaying a decreasing trend in this parameter with increasing experimental temperature (Fig. 3). The atomic ratio of oxygen consumed to nitrogen excreted (O:N ratio) of the standard animal remained at high levels (>120) for all experimental temperatures (Fig. 4).

Temperature coefficient (Q_{10})

Q_{10} values indicated a pronounced thermal sensitivity of the physiological rates (>1) with increasing temperature over the entire temperature range (Table 3). The Q_{10} for filtration rates displayed the most drastic change at the lowest temperatures (3–8 °C) and the most reduced thermal sensitivity at the highest temperature range (23–28 °C). The Q_{10} for metabolic rates showed a high thermal sensitivity at higher temperatures (18–28 °C) compared with lower temperatures, with the exception of the lowest temperature range (3–8 °C). The Q_{10} for feces production rates showed a similar trend to that of filtration rates, with higher values recorded at lower (3–18 °C) vs. higher (18–28 °C) temperatures. Ammonia excretion exhibited high Q_{10} values at 8–13 °C and at the highest temperature range (23–28 °C).

Table 3 Q_{10} values for physiological rates of the ark shell *Scapharca subcrenata* at different temperatures

Temperature	Ingestion	Oxygen consumption	Feces production	Excretion
3–8 °C	3.44	1.74	10.18	1.78
8–13 °C	1.49	1.43	2.27	2.73
13–18 °C	1.43	1.21	3.51	1.02
18–23 °C	1.62	1.73	1.07	1.32
23–28 °C	1.29	2.02	1.02	2.63
All (3–28 °C)	1.73	1.60	2.45	1.77

SFG and K_2

The SFG recalculated to different sizes of ark shells (0.2, 0.6, and 1.0 g DW) exhibited a clear effect of temperature (Fig. 5a). The SFG was negative at the lowest (3 °C) and highest (28 °C) experimental temperatures, whereas positive SFG values were recorded at 8–23 °C in all size groups, peaking at 23 °C. K_2 displayed a similar pattern to the SFG variation in all size groups (Fig. 5b). The K_2 values were negative at 3 and 28 °C, but were positive at 8–23 °C.

Discussion

The seasonal cycle of soft tissues in the ark shell *S. subcrenata* is characterized by a fast storage of

energy reserves and concomitant growth during the spring, and a rapid exhaustion of reserves and weight loss [16]. Because the accumulation of energy reserves is synchronized with gametogenic development, the physiological optimization of the energy balance to meet energy requirements for reproductive activity during spring–summer and for maintenance during autumn–winter is of particular importance to the life-history strategy and population dynamics of a species [50–52]. In this context, an advancement of the gametogenic development was recently observed in the ark shell on the southern coast of Korea [16]. The authors speculated that physiological adjustments to the elevated temperature in winter–early spring observed in recent decades would advance its seasonal biological cycle. In case of *S. subcrenata*, empirical information on the physiological processes that determine the energy budget to sustain growth and maintenance is lacking. Therefore, the interpretation of its seasonal energy dynamism and, thereby, biological cycle in a warming world should be based on the physiological responses of other bivalve species to changes in temperature [3,53,54].

In the present study, the physiological processes that regulate the energy budget of an individual were measured through the responses of energy acquisition (i.e., filtration rate) and expenditure (i.e., metabolic rate, feces production rate, and excretion rate) to the exposure to seasonal variation over a temperature range corresponding to field conditions (3–28 °C). The ark shell displayed differentiated patterns in the responses of individual physiological components to thermal conditions, highlighting its physiological plasticity. Further allometric scaling ($Y = aDW^b$) of physiological rates (Y) to DW generated positive values of the exponents (b), indicating the increase of the rates with increasing size at all experimental temperatures. While the effect of temperature was not paralleled in all physiological traits, the thermally regulated physiological responses were integrated into the energy budget in terms of SFG. As mentioned earlier, no comparable data are available for the allometric weight exponents of physiological rates in *S. subcrenata*. Such allometric relationships provide a straightforward way to identify species-specific physiological characteristics [53,55], compute SFG, and further parameterize bioenergetics growth models [52,56].

The filtration rate of *S. subcrenata*, which constitutes the main component of energy acquisition, exhibited a disparity in allometric weight exponents (b values) over the experimental temperature range

342 (Table 2), suggesting the presence of varying degrees of thermal effects between large and small
343 individuals. The exponent values for filtration of suspension-feeding bivalves vary, with a relatively
344 broad range of 0.310–0.820 (overall mean, 0.616 ± 0.127) [3]. Regarding interspecific variation, a
345 similar variation in filtration rate (as also represented by the clearance rate, [53,55] has been observed
346 even within species. The weight exponent (0.336) obtained for *S. subcrenata* at higher temperatures (8–
347 28 °C) corresponds to the lower limit of those of other suspension feeders, whereas the value (0.183)
348 obtained at lower temperatures (3–8 °C) falls outside the range. Interspecific variations in weight
349 exponents for filtration are attributable to the experimental conditions, i.e., studies performed using
350 different diets and temperatures. It has long been documented that the filtration rates of suspension-
351 feeding bivalves are highly susceptible to changes in diet quality and quantity [3,55]. The measurement
352 of filtration rates in the presence of cultivated microalgal diets yields consistently overestimated rates
353 compared with those using natural seston [357,58]. The low exponent values found for *S. subcrenata*
354 suggest that a relative suppression of feeding activity in larger individuals plays a part in lowering the
355 exponent value in the experimental conditions of natural seston diets [59]. Those authors found that the
356 filtration rates were constant in small mussels (*Perna canaliculus*), whereas they varied according to
357 food concentration or quality in large mussels. Conversely, infaunal bivalves, such as *S. subcrenata*,
358 may reduce their filtration rates in the flow-through experiment outside their normal sediment
359 substratum [57,60]. Therefore, a possible reduction in the filtration rates measured in the present study
360 caused by suboptimal conditions cannot be excluded completely.

361 The intercepts (*a* values) in allometric equations revealed a significant intraspecific variation in
362 the filtration rate of *S. subcrenata* according to changes in temperature. The intercept values may vary
363 both among species and depending on experimental conditions [3,62]. The effect of temperature on
364 feeding activity in temperate regions categorizes bivalves into two groups [53,55,63]. Those authors
365 reported that while some bivalves possess temperature-independent or fairly constant filtration rates
366 over the broad temperature range of 10–26 °C, the majority exhibit filtration rates that increase linearly
367 with temperature [64]. In either case, filtration declines markedly and even ceases at low temperatures
368 (5°C or below). Consistent with that observed for other bivalves, filtration in *S. subcrenata* was

markedly reduced at 3 °C, with no complete cessation, as indicated by a dramatically increased Q_{10} recorded at 3–8 °C (3.44 vs. 1.73 at 3–28 °C). The extremely low allometric weight exponent value found at low temperatures (3–8 °C) indicates a more pronounced suppression of feeding activity in larger individuals in cold conditions. Given that our experiments were conducted in a narrow range of food concentrations, a progressive increase in *a* values with rising temperature over the temperature range (8–28 °C), within which a common weight exponent was warranted, reveals a clear thermal effect on the filtration rate of the ark shell. The increase in the filtration rate of *S. subcrenata* with rising temperature is compatible with previous findings [8]. The lack of temperature acclimation of the filtration rate in bivalves has been considered an evolutionary adaptation that enables them to offset metabolic energy loss and consequently maintain a potential for growth [63,65]. Because such an effect of temperature on the filtration rates of *S. subcrenata* may be masked by food concentration, more realistic bioenergetics should be accomplished based on physiological responses to the combination of temperature and food availability [31].

Metabolic rate (measured here by oxygen consumption) is a key component that represents the energy expenditure and thereby determines growth rates of an individual. Allometric weight exponents for metabolic energy expenditure in *S. subcrenata* varied from 0.182 at 3–8 °C to 0.370 at 8–28 °C, in a manner similar to that observed for the filtration rate across the experimental temperatures. The exponent values in different suspension-feeding bivalves varied between 0.438 and 1.090, with a mean of 0.727 ± 0.130 [3]. The values obtained at high temperatures (8–28 °C) in the present study even fell to the lower limits of the range. Our respiratory measurements represented routine metabolic rates associated with physiological costs, including feeding, digestion, excretion, and biosynthesis. Given that the exponent values for metabolic rate in ectothermic animals can vary among species according to activity level [66], the observed scaling exponents may reflect diversified responses, such as clam size or the limited food supply provided in the present study compared with the above-mentioned measurements. The extremely low weight exponent value for metabolic rate observed at low temperatures (3–8 °C) is comparable to the consistently low weight exponent value for filtration rate, possibly because of declined feeding in larger individuals in similar cold conditions. This result may

396 support a modification of the size-dependent thermal effects on metabolic rate in the low temperature
397 range.

398 In addition to the abrupt fall in the weight exponent at 3–8 °C, the comparisons between the
399 regression intercepts for respiration rate over the temperature range (8–28 °C), within which a common
400 weight exponent was calculated, highlighted the presence of a significant thermal effect on metabolic
401 rate. Metabolic energy expenditure can be adjusted to thermal acclimation in some bivalves, such as
402 *Mytilus edulis* [67] or *Crepidula fornicata* [28] or can be temperature dependent in others, such as
403 *Ostrea edulis* [68] or *Ruditapes philippinarum* [31]. As mentioned previously, our physiological
404 measurements were performed seasonally in a narrow range of diet concentrations to extract thermal
405 effects on physiological rates (Table 1). Consistent with the latter bivalve group, the broad range in
406 metabolic cost of *S. subcrenata* and its increase with increasing temperature denote an incapability of
407 acclimation to seasonal temperatures [34,35]. Considering this temperature-dependent filtration rate,
408 this result further indicates the increased metabolic costs of feeding during the warm summer season.
409 The increased Q_{10} value obtained for metabolic rate, as opposed to filtration rate, between 23 and 28 °C
410 supports the relatively enhanced thermal effect on metabolic rate observed at 28 °C.

411 The common weight exponent (0.350 ± 0.040) on egestion (as measured based on feces
412 production) over the temperature range was very close to that of the filtration rate at 8–28 °C. As
413 recalculated using the present allometric equations, fecal energy loss was closely associated with the
414 energy acquired by filtration (r^2 = 0.90), corresponding to 5%–15% of the energy consumed.
415 Furthermore, a constant assimilation efficiency (85%–95%) on diets offered to feeding trials indicates
416 a temperature-independent assimilation activity [36]. In a similar manner, based on a common weight
417 exponent (0.294 ± 0.036) for excretion rates (as measured based on ammonia excretion), the
418 comparisons between *a* values indicate an effect of rising temperature on the excretion energy loss of
419 *S. subcrenata*. However, in terms of the energy balance, the energy expenditure by excretion was
420 negligible (<1%) among the total energy losses in the present study. Despite its minor role in
421 determining the energy budget, its measurement is useful as an indicator of changes in the catabolism
422 of substrates occurring along the seasonal cycle [3]. The consistently high O:N values (range, 120–170)

423 detected over the experimental temperature range indicate that *S. subcrenata* catabolizes carbohydrates
424 and lipids predominantly, relative to proteins, for maintenance (and growth) metabolism. Therefore,
425 our results suggest that the observed size- and temperature-dependent trends (*b* and *a* values,
426 respectively) in egestion and excretion rates may reflect temperature-dependent filtration in different-
427 size individuals.

428 In *S. subcrenata*, the regulation of the SFG and K_2 values was attained by adaptive adjustments of
429 the physiological processes that determine the energy budget over a wide temperature range,
430 summarizing the thermal impact on energy balance. A maximum and nearly constant positive SFG (and
431 K_2) was observed between 8 and 23 °C, respectively, providing optimal thermal conditions for
432 production. Both the SFG and K_2 were independent of temperature over that temperature range. This
433 positive energy balance reflected a synchronous increment in the filtration and respiration rates [29–
434 31]. In contrast, the SFG and K_2 dropped drastically to negative values at 3 and 28 °C, indicating thermal
435 (both cold and heat) stress. The Q_{10} of metabolic rate was maximized by 2.02 at high temperatures of
436 23–28 °C compared with the minimum (1.29) of filtration rate at the same temperatures, suggesting that
437 metabolic rate is more sensitive to temperatures above the optimal value. This implies that the feeding
438 rate did not increase as much because it compensated for the increased metabolic cost at 28 °C [25,32].
439 Conversely, the Q_{10} of filtration rate was maximized by 3.44 at low temperatures of 3–8 °C, indicating
440 a more-sensitive response than that (1.74) of the metabolic rate to temperature variation. A more
441 pronounced feeding suppression restricts the compensation, even for the lowered metabolic cost at 3
442 °C.

443 The ark shells in shallow subtidal soft-bottom habitats of temperate zones experience temperature
444 extremes because of the distinctly seasonal regime. The present study highlighted the effect of
445 temperature on the physiological components that determine the energy balance at the organism level
446 in *S. subcrenata*. Mechanistic adjustment of energy budget based on feeding, assimilation, excretion,
447 and metabolism across thermal conditions in its natural habitats may explain the seasonal cycle of the
448 accumulation and consumption of reserves and, thereby, growth, development, and maintenance
449 [16,17]. Our physiological observations provide further empirical evidence that physiological stresses

and, thereby, an energy imbalance in *S. subcrenata* after spawning during the warm summer season might severely threaten their survival as hypothesized based on its high summer mortality [17]. More importantly, according to the daily sea-surface temperatures in the southern coastal waters of Korea recorded by the National Institute of Fisheries Science (http://www.nifs.go.kr/kodc/coo_list.kodc) over the past 6 decades, the winter minimum temperature fell below 3 °C before 1990, but gradually increased up to 7 °C during the subsequent 30 years (unpublished data). In the context of the energy dynamism of the ark shell, winter warming would provide physiological benefits that support the accumulation of energy reserves during winter–early spring, leading to advanced gametogenesis [16], as indicated by the SFG and K_2 values at 3 and 8 °C, respectively. Our results highlighted the physiological regulation of ark shells by the seasonal fluctuation in temperature. Given that the seasonal growth pattern of bivalves is well linked to seasonal changes in the quantity and composition of food [3,53,55], further information on the combined effects of temperature and food availability on physiological mechanisms is needed to better understand their energy dynamism in relation to climate forcing in their natural habitats [31].

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by the National Institute of Fisheries Science [grant number R2020048].

Authors' contributions

HYK conceptualized research, analyzed data, and wrote original draft. JS performed physiological measurements and curated data. YJL performed physiological measurements and performed biochemical analyses. CK prepared figures for visualization and performed nutrient analyses. WCL was responsible for resources and project administration, and interpreted data. CKK designed research, wrote, edited, and revised the manuscript. All authors read and approved the final manuscript."

Acknowledgements

Not applicable.

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Figure captions

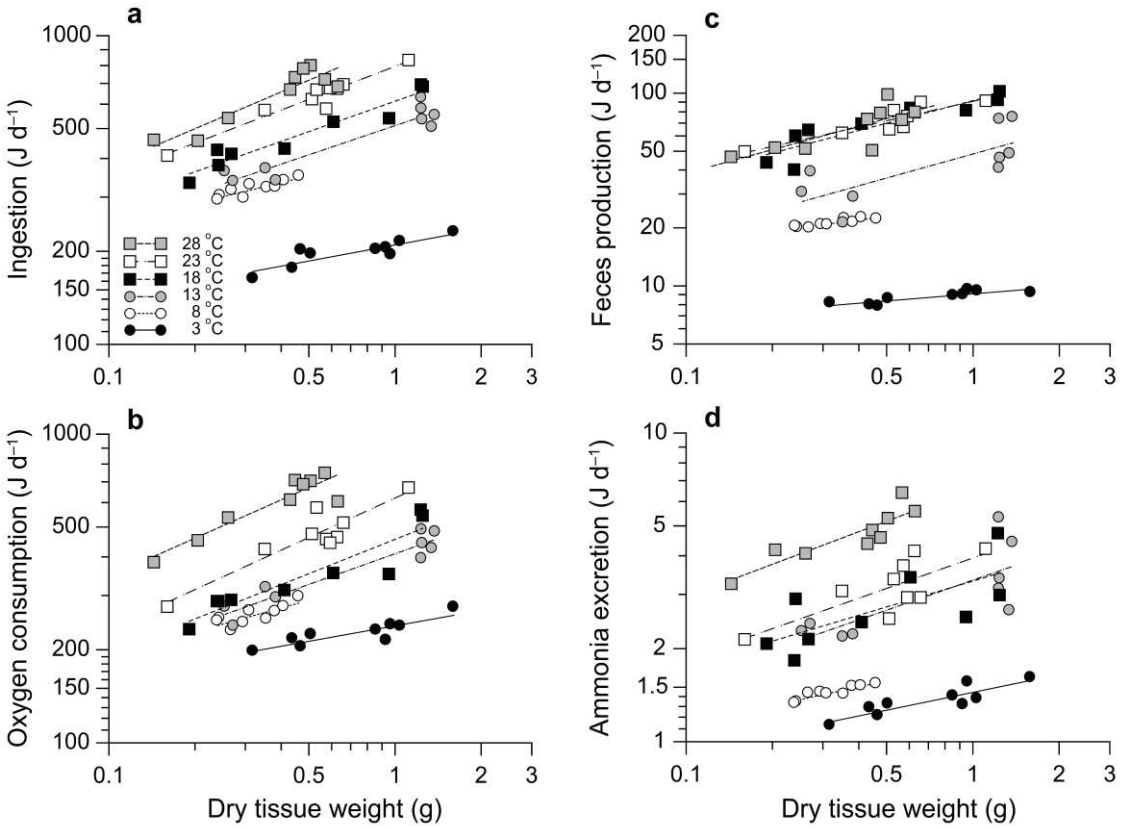
Fig. 1 Physiological rates of the ark shell *Scapharca subcrenata* as a function of dry tissue weight at different temperatures. **a** Ingestion rates, **b** oxygen consumption rates, **c** feces production rates, and **d** ammonia excretion rates

Fig. 2 Adjusted values to the grand mean (0.6 g) of dry tissue weight for **a** ingestion rates and **b** oxygen consumption rates of the ark shell *Scapharca subcrenata* at all six experimental temperatures, based on the common slopes ($\bar{b}$) at both temperature ranges (3–8 °C and 8–28 °C).Results of an analysis of variance and subsequent Bonferroni *post hoc* test on the adjusted values were embedded in the figures

Fig. 3 Assimilation efficiency of the ark shell *Scapharca subcrenata* at different temperatures. The efficiency (%) was calculated using the adjusted rate values to a grand mean of dry tissue weight (0.6 g)

Fig. 4 Atomic ratio of oxygen consumed to nitrogen excreted (O:N) of a standard animal (0.6 g, a grand mean of dry tissue weight in the experiments) of the ark shell *Scapharca subcrenata* at different temperatures

Fig. 5 Energetic physiology for different size-class ark shells (0.2 g, 0.6 g, and 1.0 g in dry tissue weight) at different temperatures. **a** Scope for growth (SFG) and **b** net growth efficiency (K_2)



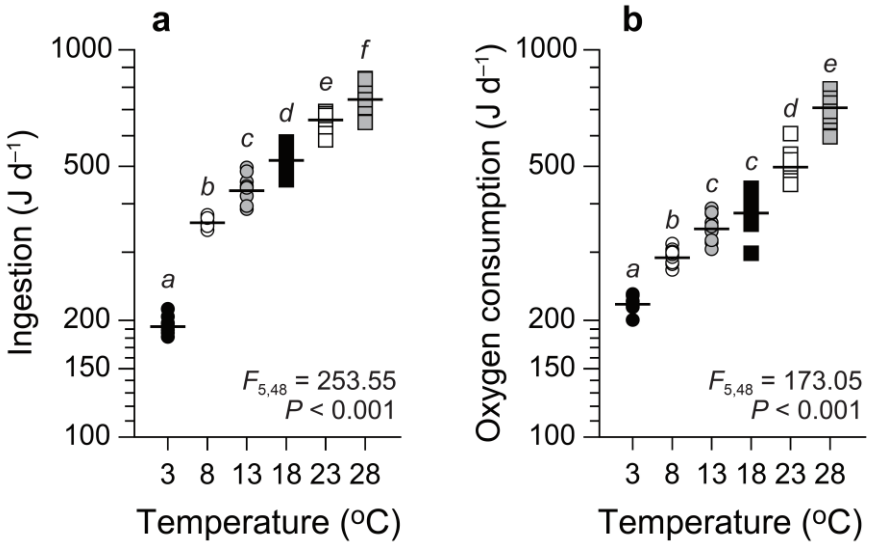
665

666 **Fig. 1** Physiological rates of the ark shell *Scapharca subcrenata* as a function of dry tissue weight at

667 different temperatures. **a** Ingestion rates, **b** oxygen consumption rates, **c** feces production rates, and

668 **d** ammonia excretion rates

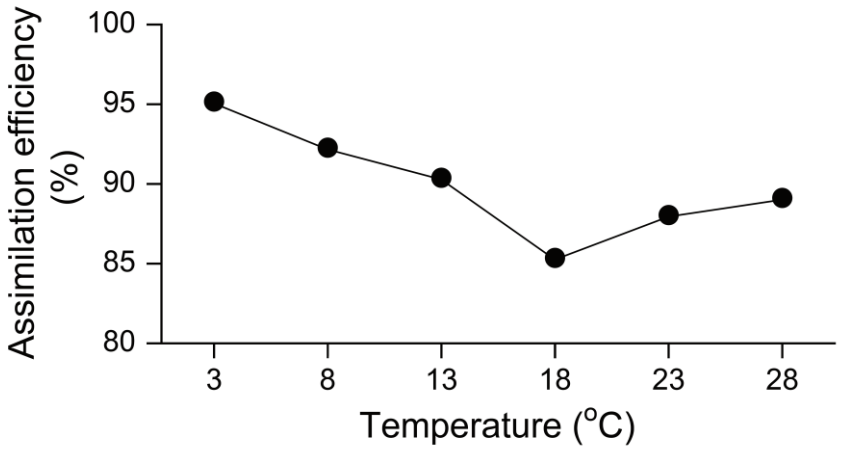
669



670

671 **Fig. 2** Adjusted values to the grand mean (0.6 g) of dry tissue weight for **a** ingestion rates and **b** oxygen
672 consumption rates of the ark shell *Scapharca subcrenata* at all six experimental temperatures, based
673 on the common slopes ($\bar{b}$) at both temperature ranges (3–8 °C and 8–28 °C).Results of an analysis of
674 variance and subsequent Bonferroni *post hoc* test on the adjusted values were embedded in the
675 figures

676



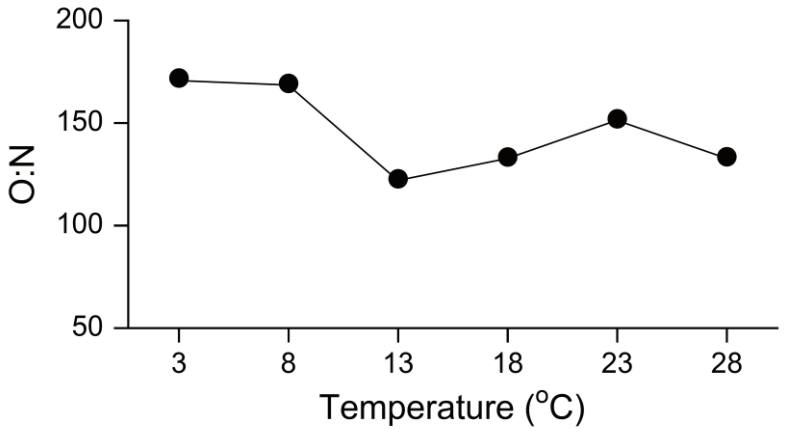
677

678 **Fig. 3** Assimilation efficiency of the ark shell *Scapharca subcrenata* at different temperatures. The

679 efficiency (%) was calculated using the adjusted rate values to a grand mean of dry tissue weight (0.6

680 g)

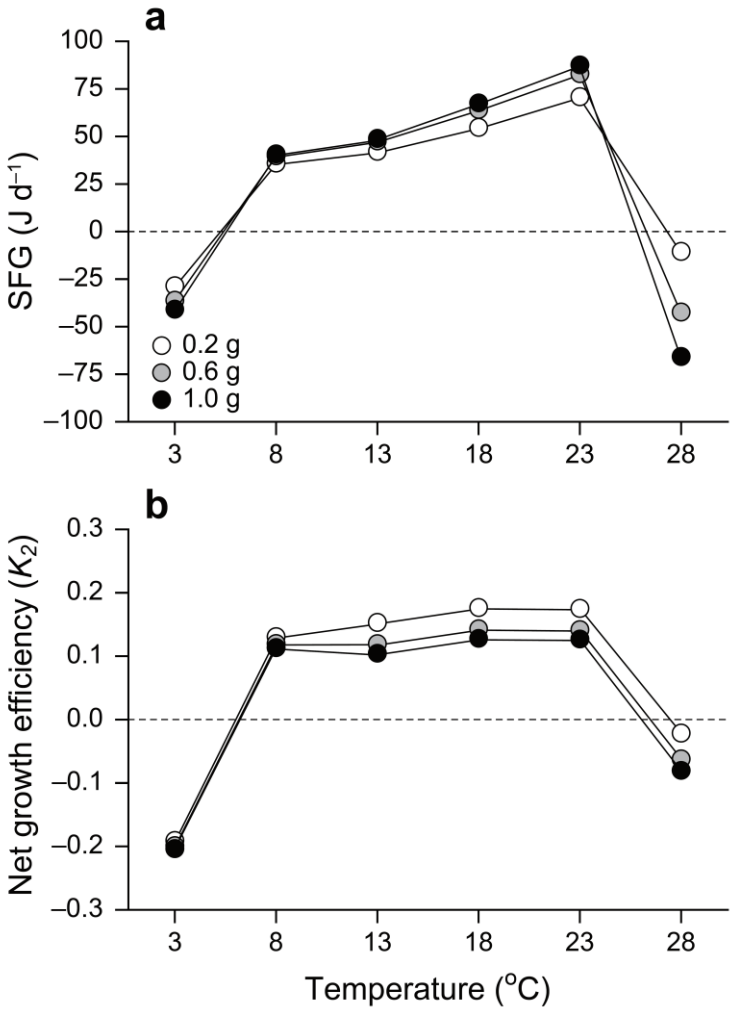
681



682

683 **Fig. 4** Atomic ratio of oxygen consumed to nitrogen excreted (O:N) of a standard animal (0.6 g, a grand
684 mean of dry tissue weight in the experiments) of the ark shell *Scapharca subcrenata* at different
685 temperatures

686



687

688 **Fig. 5** Energetic physiology for different size-class ark shells (0.2 g, 0.6 g, and 1.0 g in dry tissue weight)

689 at different temperatures. **a** Scope for growth (SFG) and **b** net growth efficiency (K_2)

Figures

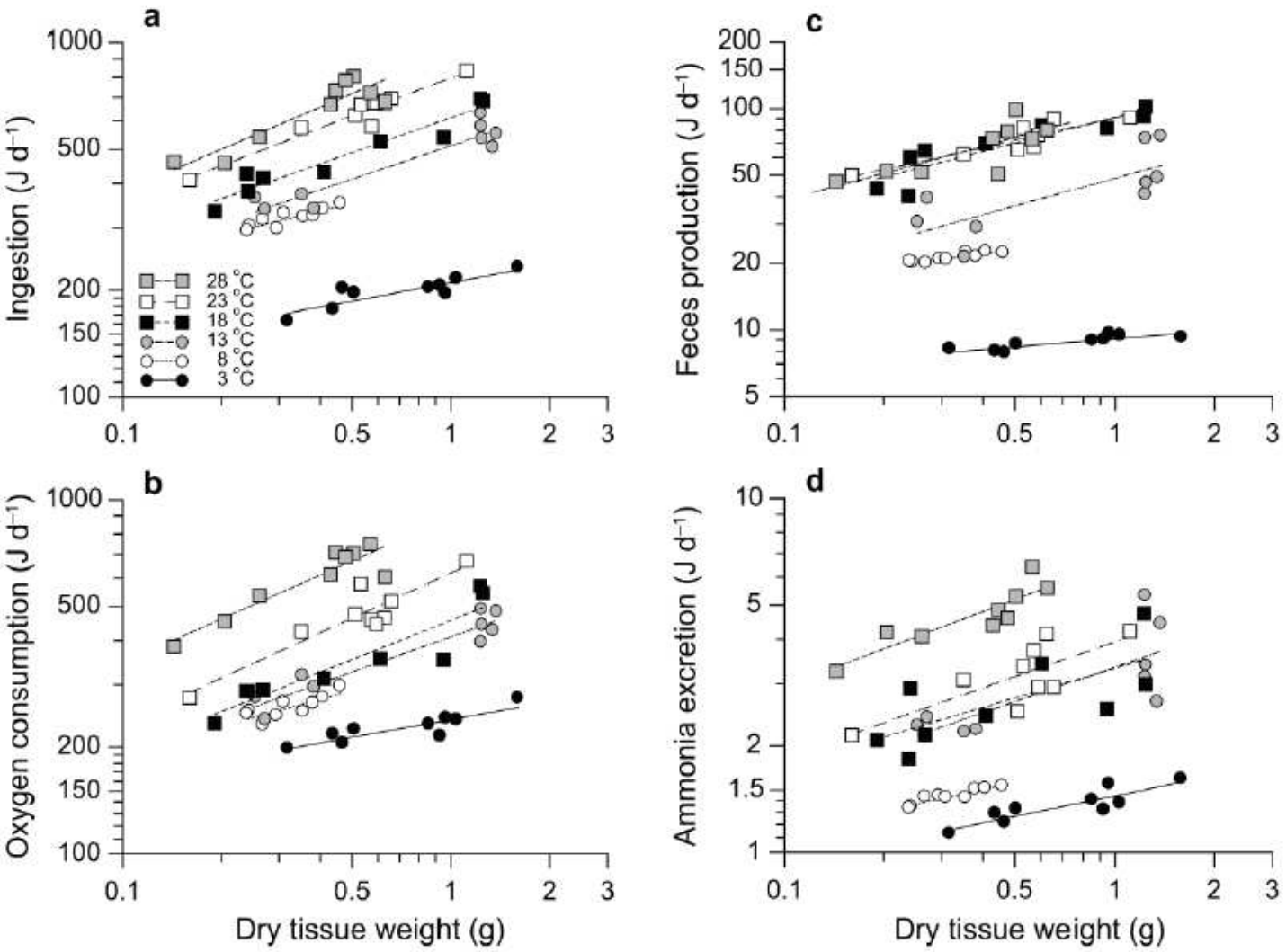


Figure 1

Physiological rates of the ark shell Scapharca subcrenata as a function of dry tissue weight at different temperatures. a Ingestion rates, b oxygen consumption rates, c feces production rates, and d ammonia excretion rates

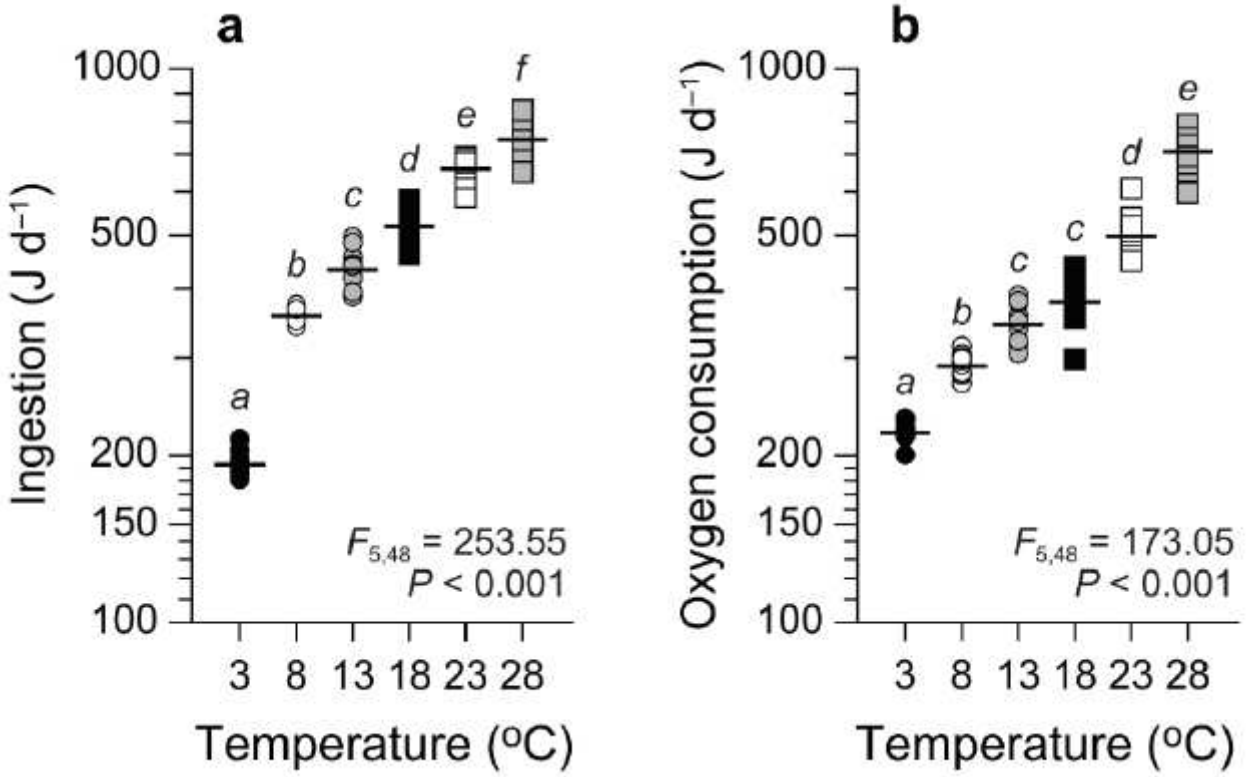


Figure 2

Adjusted values to the grand mean (0.6 g) of dry tissue weight for a ingestion rates and b oxygen consumption rates of the ark shell Scapharca subcrenata at all six experimental temperatures, based on the common slopes (▯▯) at both temperature ranges (3–8 °C and 8–28 °C).Results of an analysis of variance and subsequent Bonferroni post hoc test on the adjusted values were embedded in the figures

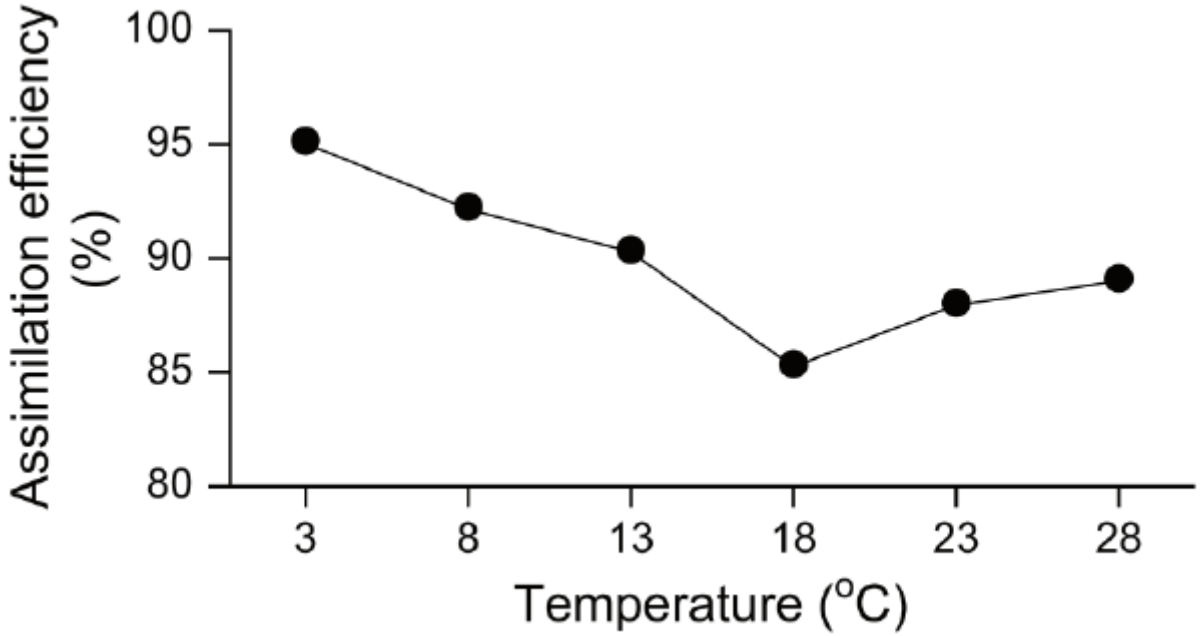


Figure 3

Assimilation efficiency of the ark shell Scapharca subcrenata at different temperatures. The efficiency (%) was calculated using the adjusted rate values to a grand mean of dry tissue weight (0.6 g)

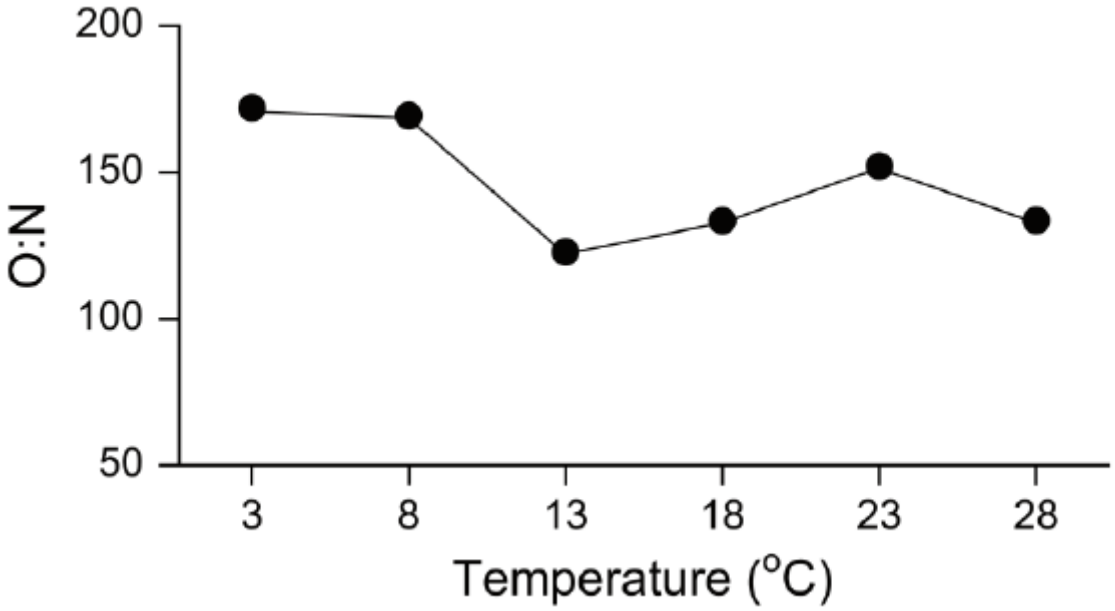


Figure 4

Atomic ratio of oxygen consumed to nitrogen excreted (O:N) of a standard animal (0.6 g, a grand mean of dry tissue weight in the experiments) of the ark shell Scapharca subcrenata at different temperatures

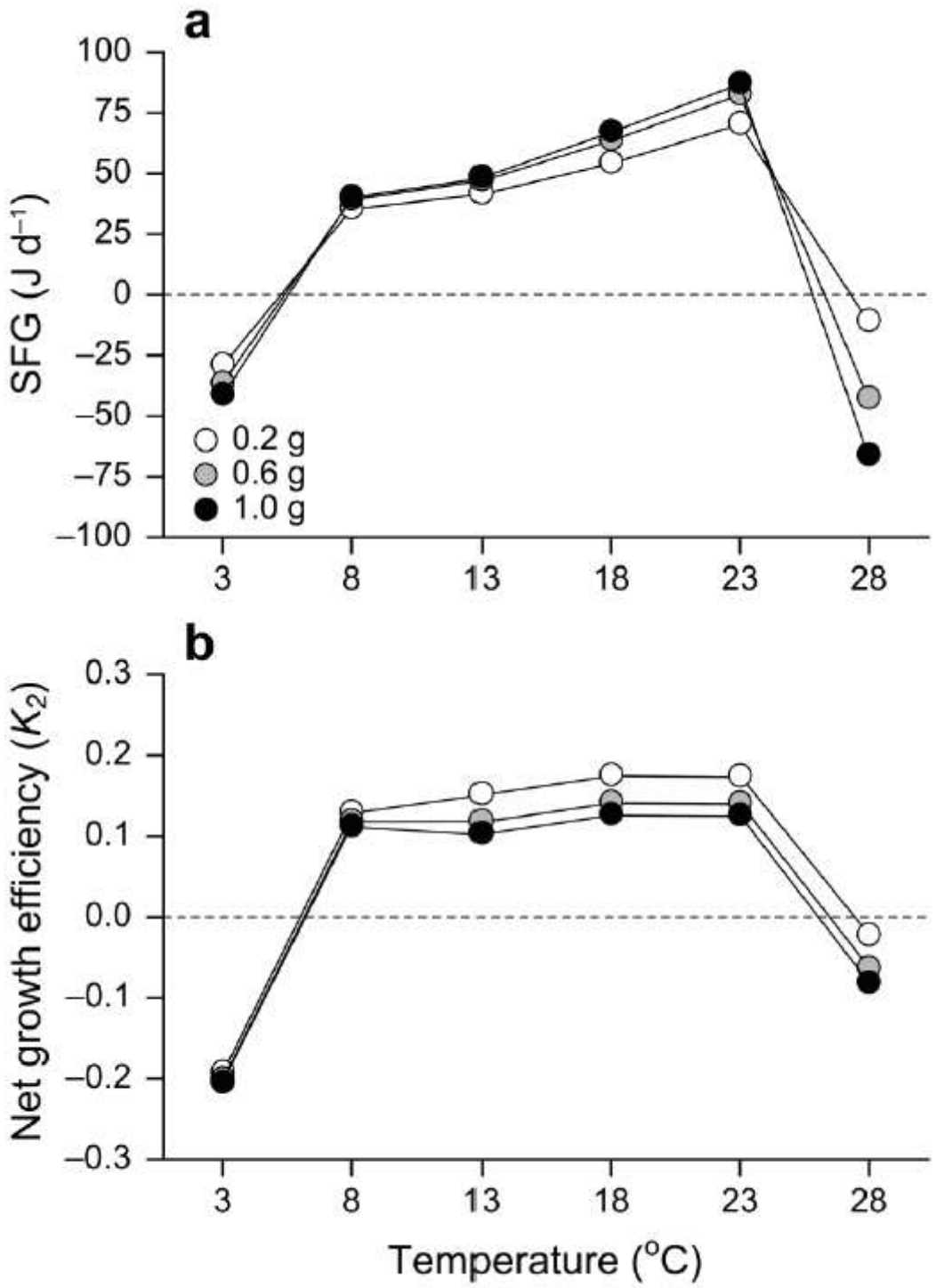


Figure 5

Energetic physiology for different size-class ark shells (0.2 g, 0.6 g, and 1.0 g in dry tissue weight) at different temperatures. a Scope for growth (SFG) and b net growth efficiency (K2)