

Article

Physiological Plasticity of *Ruditapes decussatus* in Response to Short-Term Changes in Substrate Availability

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Abstract

Substrate availability is a key environmental factor for burrowing bivalves, yet its physiological importance in adult clams remains poorly understood. This study evaluated the effects of short-term substrate manipulation on growth performance, intermediary metabolism, and fatty acid (FA) composition of adult *Ruditapes decussatus*. Clams were reared for 15 months either with or without sediment and subsequently exposed for 40 days to the opposite substrate condition. Growth performance, metabolic parameters (glucose, glycogen, lactate, triglycerides, and cholesterol), and tissue FA composition were determined. No mortality was recorded, and substrate manipulation had no relevant effects on growth performance. In contrast, substrate removal induced significant decreases in glucose, triglyceride, and cholesterol concentrations, together with increased lactate levels, while glycogen remained unchanged. Conversely, substrate addition did not modify metabolic parameters but promoted selective tissue FA remodeling, characterized by reduced saturated FAs and increased unsaturated, particularly long-chain polyunsaturated FAs. These findings demonstrate that adult *R. decussatus* exhibits considerable physiological plasticity, with metabolic and membrane lipid responses differing according to the type of substrate manipulation and the previous acclimation history. The results also highlight the value of integrating metabolic and lipid biomarkers with conventional growth indicators to improve the assessment of the physiological status of cultured clams.

Keywords: substrate; *Ruditapes decussatus*; growth; metabolism; fatty acids

Key Contribution: Temporary changes in substrate availability do not impair the growth of adult *Ruditapes decussatus* but induce rapid metabolic adjustments and selective tissue FA remodeling, revealing a high degree of physiological plasticity.



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1. Introduction

The expansion of aquaculture towards a broader range of cultured species is increasingly regarded as an effective strategy to improve both the sustainability and economic resilience of the sector. Among these emerging resources, bivalve molluscs play a dual role by providing valuable ecosystem services in coastal environments and representing an important source of high-quality protein for human consumption [1]. The grooved carpet clam (*Ruditapes decussatus*) has attracted attention because of its considerable ecological, commercial, and gastronomic importance [1,2]. This native species inhabits coastal and estuarine environments across Europe, where it supports commercially important fisheries and aquaculture, particularly in the Iberian Peninsula [1,3]. Consequently, optimizing culture

conditions has become essential to improve both production efficiency and physiological performance in this species, as the wild populations have declined markedly [4–6].

For infaunal bivalves, the substrate is one of the most influential abiotic factors affecting culture performance. In addition to providing physical support and protection, it plays a fundamental role in regulating several physiological and metabolic processes [7,8]. Consequently, numerous studies have investigated the influence of different substrate types on bivalve aquaculture, with particular attention to growth performance and survival during the early stages of development [9–11]. Despite its recognized environmental and biological benefits, substrate use in aquaculture may affect water quality and increase the risk of pathogenic microbial proliferation [12,13], so its use in aquaculture is under review. Furthermore, under commercial culture conditions, *R. decussatus* may be exposed to temporary changes in substrate availability caused by natural disturbances, such as storms, tidal currents, and wave action, as well as routine farming practices involving substrate maintenance, cleaning, and harvesting operations [14–16].

Regarding *R. decussatus*, substrate is a fundamental abiotic factor that influences a wide range of behavioural, physiological, and biochemical processes, including burrowing activity, predator protection, suspension feeding, and the ingestion and assimilation of nutrients [7,17–19]. As these processes are closely related to energy acquisition and allocation, changes in substrate availability may affect nutrient metabolism and maintenance of homeostasis by altering digestive efficiency and nutrient assimilation. Fatty acids (FAs) are essential dietary components in bivalves that support growth, reproduction, and a wide range of physiological functions [8,20]. Among them, the long-chain polyunsaturated fatty acids (LC-PUFAs) arachidonic acid (ARA; 20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3), and docosahexaenoic acid (DHA; 22:6n-3) are particularly important because they not only enhance the nutritional value of seafood products for human consumption [21] but also maintain membrane integrity and cellular function under changing environmental conditions [22–24]. The ability of bivalves to acclimate to environmental fluctuations largely depends on the composition of membrane lipids, especially phospholipids and cholesterol [25,26]. Furthermore, as a sessile species, *R. decussatus* relies on the storage of FAs as triglycerides to meet its energy demands and maintain physiological homeostasis during periods of environmental stress [8,20,25,27]. Consequently, alterations in substrate availability may influence lipid metabolism and FA composition, potentially affecting the physiological performance and nutritional value of this species.

Accordingly, to improve our knowledge of the physiological consequences of changes in substrate availability, this study investigated the effects of short-term substrate removal and addition on the growth performance and metabolic status of *R. decussatus* previously reared for 15 months under culture conditions with or without substrate. In addition to growth assessment, key metabolic indicators such as glucose, glycogen, lactate, triglycerides, and cholesterol were quantified. Furthermore, the FA profile was analysed to determine whether changes in substrate availability induce lipid remodeling, providing a comprehensive evaluation of the physiological responses of *R. decussatus* to changes in its benthic environment.

2. Materials and Methods

2.1. Clams and Experimental Design

R. decussatus specimens were produced by thermal shock-induced spawning at the experimental hatchery of IFAPA-Agua del Pino (Cartaya, Huelva, Spain). After settlement, clams were cultured for 15 months under two experimental conditions differing only in the presence or absence of substrate. Accordingly, individuals were maintained in randomly arranged baskets placed within a rectangular tank measuring 15 × 2.5 × 0.8 m, with

a total volume of approximately 30 m³ (30,000 L), with each basket containing either a sediment layer or no substrate. Throughout this pre-experimental period, all environmental variables and husbandry practices were kept identical between conditions. Seawater was continuously supplied by pumping raw seawater into the tanks, and clams were fed on the naturally available phytoplankton present in the incoming seawater.

After the 15-month culture pre-experimental period, a total of 640 clams, i.e., 320 clams from each rearing condition (substrate and no substrate), were individually measured and weighed at the beginning of the experimental period (T₀), and then randomly assigned to 16 perforated rectangular baskets (44 × 33 × 10 cm; 2 mm mesh), with 40 individuals per basket. Four replicate baskets from each initial condition were transferred to the opposite substrate condition for 40 days. Thus, clams previously maintained with substrate were transferred to baskets without substrate. In contrast, those reared without substrate were moved to baskets containing a 10 cm sediment layer composed of 95% silica sand and 5% aragonite (CaCO₃), with a particle size of 2–4 mm. In parallel, four additional replicate baskets from each rearing condition were maintained under their original culture conditions and served as the corresponding control groups (CTRL).

The experiment was conducted in a continuous flow-through system supplied with natural seawater at a flow rate of ~150 mL min⁻¹ (~9 L h⁻¹). Throughout the trial period, all experimental units were maintained under identical environmental conditions, including a temperature of 19 ± 0.5 °C, salinity of 36.5 ± 0.5 g L⁻¹, and a 12 h L:12 h D photoperiod. Water quality was routinely monitored by measuring dissolved oxygen, pH, ammonia, nitrite, and nitrate concentrations to ensure stable rearing conditions. As described by Torres-Rodríguez et al. (2026) [8], clams were fed daily with a mixed microalgal diet at a final concentration of approximately 0.5 × 10⁶ cells mL⁻¹, consisting primarily of *Skeletonema costatum* (70–90%), *Chaetoceros gracilis* (10–20%), and *Isochrysis galbana* (approximately 5%).

2.2. Sampling and Growth Performance

Growth performance was evaluated by measuring total body weight (g) and shell length (mm) at the beginning of the experimental period (T₀) and after 40 days in all clams (n = 160 per group). At the end of the experiment, 48 clams per experimental group (12 individuals per basket) were randomly selected, sacrificed, and dissected to determine shell and soft tissue weights. Shells were cleaned, dried, and weighed, whereas the wet weight of the soft tissues was recorded immediately after dissection. The soft tissues were subsequently dried in a forced-air oven at 80 °C for 24 h to determine tissue dry weight.

The somatic indices (%) were calculated according to the following equations [28–30]:

$$\text{Meat Yield} = \text{Wet meat weight (g)} / \text{Total weight (g)} \times 100$$

$$\text{Shell Component Index} = \text{Shell weight (g)} / (\text{Shell} + \text{Wet meat weight (g)}) \times 100$$

$$\text{Condition Index} = \text{Meat dry weight (g)} / \text{Shell dry weight (g)} \times 100$$

Finally, whole soft tissue samples from each experimental group were collected, immediately frozen, and stored at −80 °C until biochemical analyses.

2.3. Metabolic Parameters

For the biochemical analyses, whole soft tissue from twelve individual clams (3 clams per basket), selected randomly per experimental group (n = 12), was processed according to the protocol previously described by Torres-Rodríguez et al. [8]. To minimize the potential influence of residual microalgal cells on the biochemical and FA analyses, the clams were maintained without food for 12 h before sampling. In addition, pseudofaeces were removed before the animals were processed. These procedures were implemented to reduce the

presence of undigested or recently ingested microalgal material in the digestive tract and, consequently, minimize its potential contribution to the FA profiles obtained from the whole-body homogenates.

Briefly, each wet tissue sample was finely minced and homogenized in ice-cold 0.6 N perchloric acid at a ratio of 1:3.5 (*w/v*). The homogenates were then neutralized with an equal volume of 1 M KHCO₃ and centrifuged at 3500 × *g* for 30 min at 4 °C. Following centrifugation, the supernatants were transferred to 1.5 mL centrifuge tubes for subsequent biochemical analyses. Aliquots for triglyceride and cholesterol determinations were collected before centrifugation. All extracts were stored at −80 °C until further analysis.

Biochemical determinations were performed in duplicate using a Varioskan LUX 3020 microplate reader (Thermo Scientific, Alcobendas, Madrid, Spain) operated with Thermo Scientific SkanIt™ software (version 6.0). Glucose, lactate, cholesterol, and triglyceride concentrations were quantified using commercial enzymatic assay kits (SpinReact SA, St. Esteve d'en Bas, Girona, Spain) adapted for 96-well microplates. Glycogen content was determined according to the method described by Keppler and Decker [31] and Torres-Rodríguez et al. [24].

2.4. Total Lipids and Fatty Acid Analysis

Lipid extraction and FA analyses were conducted following the methodology described by Fernández-Cabanás and Cruzado [32]. Whole soft tissue samples from six randomly selected clams from each experimental group were freeze-dried for 48 h, and approximately 200 mg of the resulting dry tissue was used for lipid extraction. Samples were homogenized in an ice-cold chloroform:methanol solution (2:1, *v/v*) using a MiniG Tissue Homogenizer (SPEX CertiPrep, Metuchen, NJ, USA). Lipid and aqueous phases were separated by adding 0.88% (*w/v*) KCl. After phase separation, the aqueous phase was discarded, and the organic phase containing the lipids was collected and evaporated to dryness under a stream of oxygen-free nitrogen. Total lipid content was subsequently determined gravimetrically after overnight drying in a vacuum desiccator.

Fatty acid methyl esters (FAMES) were obtained from the extracted lipids by acid-catalysed transesterification at 50 °C for 16 h according to the procedure described by Christie [33]. The resulting FAMES were analysed using a Shimadzu GC-2010 gas chromatograph (Teknokroma, Sant Cugat del Vallès, Spain) fitted with a flame ionization detector (280 °C) and a fused-silica capillary column (SUPRAWAX-280, 15 m × 0.1 mm i.d.; Teknokroma, Sant Cugat del Vallès, Spain). Hydrogen was used as the carrier gas. The oven programme consisted of an initial temperature of 150 °C maintained for 1 min, followed by an increase to 250 °C at 8 °C min^{−1} and a final holding time of 3 min. Individual FAs were identified by comparison with a certified reference mixture (Supelco 37 Component FAME Mix, CRM47885) and Fish oil from menhaden (Supelco Menhaden Oil, PHR2983), and their relative abundance was expressed as the percentage of total FAs.

2.5. Statistical Analysis

Statistical analyses were performed after assessing data normality using the Shapiro–Wilk test, and homogeneity of variances was evaluated using Levene's test. Outlying observations were identified using the ROUT method with a Q value of 1%. Data are presented as mean ± standard error of the mean (SEM). Growth parameters were compared among experimental groups using one-way analysis of variance (ANOVA), with statistical significance set at *p* ≤ 0.05. When significant differences were detected, Tukey's HSD test was used for post hoc multiple comparisons. Differences in metabolic compounds and FA composition between groups differing in substrate availability during the experimental period were evaluated using independent-samples Student's *t*-tests. To account for multiple

comparisons and reduce the risk of false-positive results, p -values were adjusted using the Bonferroni correction, with statistical significance considered at an adjusted $p \leq 0.05$. The effects of pre-experimental condition, experimental condition, and their interaction on metabolic parameters and FA composition were further evaluated using two-way ANOVA. This analysis was used to assess the main effects of each factor and their interaction. All statistical analyses and figure preparation were conducted using GraphPad Prism version 8.0 (GraphPad Software Inc., San Diego, CA, USA).

3. Results

3.1. Growth Performance

No mortality was recorded throughout the experimental period. Growth performance after the 40-day experimental period is shown in Table 1.

Table 1. Growth parameters of *Ruditapes decussatus* reared with or without substrate for 15 months (T_0) and subsequently exposed for 40 days to the opposite substrate conditions (presence or absence of substrate). Data are presented as mean $\pm$ SEM of 160 (*) and 48 clams (**). Different letters (a, b) indicate significant differences (one-way ANOVA and Tukey's test, $p \leq 0.05$) among the initial sampling (T_0), clams maintained under their original substrate conditions (CTRL), substrate-deprived clams (NS), and clams transferred to substrate (S).

	Substrate			No Substrate		
	T_0	CTRL	NS	T_0	CTRL	S
Total Weight (g) *	8.73 $\pm$ 0.40 ^a	10.04 $\pm$ 0.30 ^b	9.93 $\pm$ 0.30 ^b	4.57 $\pm$ 0.07 ^a	5.23 $\pm$ 0.12 ^b	5.22 $\pm$ 0.11 ^b
Meat Wet Weight (g) **	3.06 $\pm$ 0.21 ^a	3.76 $\pm$ 0.11 ^b	3.86 $\pm$ 0.10 ^b	1.70 $\pm$ 0.09	1.80 $\pm$ 0.05	1.82 $\pm$ 0.06
Meat Dry Weight (g) **	0.50 $\pm$ 0.03 ^a	0.62 $\pm$ 0.02 ^b	0.64 $\pm$ 0.01 ^b	0.28 $\pm$ 0.01	0.30 $\pm$ 0.01	0.30 $\pm$ 0.01
Shell Weight (g) **	4.79 $\pm$ 0.29 ^a	5.79 $\pm$ 0.20 ^b	5.51 $\pm$ 0.17 ^{ab}	2.48 $\pm$ 0.08	2.53 $\pm$ 0.08	2.58 $\pm$ 0.13
Shell Length (mm) *	35.06 $\pm$ 0.24	36.02 $\pm$ 0.32	35.51 $\pm$ 0.35	27.30 $\pm$ 0.15 ^a	28.63 $\pm$ 0.22 ^b	28.81 $\pm$ 0.20 ^b
Meat Yield (%) **	37.36 $\pm$ 1.15	37.43 $\pm$ 0.29	38.46 $\pm$ 0.34	33.89 $\pm$ 2.72	29.17 $\pm$ 1.25	30.00 $\pm$ 1.14
Shell Component Index (%) **	55.63 $\pm$ 0.79 ^{ab}	56.32 $\pm$ 0.26 ^b	55.26 $\pm$ 0.30 ^a	60.41 $\pm$ 0.43 ^b	58.04 $\pm$ 0.35 ^a	58.19 $\pm$ 0.28 ^a
Condition Index (Dry Weight) **	9.93 $\pm$ 0.45	9.89 $\pm$ 0.12	10.40 $\pm$ 0.15	10.84 $\pm$ 0.19 ^a	12.03 $\pm$ 0.18 ^b	11.81 $\pm$ 0.15 ^b

In clams initially reared with substrate, significant differences were only detected for the shell component index, which was significantly lower in clams deprived of substrate (NS) than in the control group (CTRL). No significant differences were observed between the CTRL and NS groups for any of the remaining growth parameters. However, total weight, meat wet weight, meat dry weight, and shell weight increased significantly in both the CTRL and NS groups compared to the initial sampling (T_0).

In clams initially reared without substrate, no significant differences were detected between the CTRL and substrate-transferred (S) groups for any of the growth parameters evaluated after the 40-day experimental period. Likewise, meat wet weight, meat dry weight, shell weight, and meat yield did not differ significantly among the T_0 , CTRL, and S groups. The shell component index was the only parameter with higher values in the T_0 group than in the CTRL and S groups. In contrast, total weight, shell length, and condition index increased significantly in both the CTRL and S groups compared to T_0 .

3.2. Metabolic Parameters

Metabolic parameters after the 40-day experimental period are presented in Table 2, and the results of the two-way ANOVA are summarized in Table 3. In clams initially reared with substrate, substrate deprivation (NS) significantly reduced cholesterol, triglyceride, and glucose concentrations compared to the control group (CTRL). In contrast, lactate

concentration was significantly higher in the NS group than in the CTRL group. Glycogen concentration was not significantly affected by substrate removal.

In clams initially reared without substrate, transfer to substrate (S) did not significantly affect any of the metabolic parameters evaluated. Cholesterol, triglyceride, glucose, glycogen, and lactate concentrations remained comparable between the CTRL and S groups.

Table 2. Metabolic parameters of *Ruditapes decussatus* reared with or without substrate for 15 months and subsequently exposed for 40 days to the opposite substrate conditions (presence or absence of substrate). Data are presented as mean $\pm$ SEM (n = 12). Asterisks (*) indicate significant differences (*t*-Student, $p \leq 0.05$) among clams maintained under their original substrate conditions (CTRL) and substrate-deprived clams (NS) or clams transferred to substrate (S). Values are expressed as mmol per gram of the wet weight of tissues (mmol g⁻¹ w.w.).

	Substrate		No Substrate	
	CTRL	NS	CTRL	S
Cholesterol	4.30 $\pm$ 0.22	3.31 $\pm$ 0.19 *	2.15 $\pm$ 0.13	2.27 $\pm$ 0.25
Triglycerides	4.40 $\pm$ 0.40	2.74 $\pm$ 0.23 *	1.94 $\pm$ 0.29	1.61 $\pm$ 0.20
Glucose	2.68 $\pm$ 0.12	2.15 $\pm$ 0.15 *	11.76 $\pm$ 0.62	9.78 $\pm$ 1.15
Glycogen	26.98 $\pm$ 0.25	26.38 $\pm$ 0.28	17.43 $\pm$ 1.11	16.17 $\pm$ 1.25
Lactate	0.73 $\pm$ 0.05	1.12 $\pm$ 0.07 *	1.24 $\pm$ 0.19	1.12 $\pm$ 0.16

Table 3. Full Type III two-way ANOVA summary for metabolic parameters measured in *R. decussatus*. The table shows the effects of pre-experimental rearing conditions, experimental rearing conditions, and their interaction on cholesterol, triglycerides, glucose, glycogen, and lactate. Columns report the F-statistic, *p*-value, and 95% confidence interval (95% CI). S: Substrate; NS: No substrate.

Variable	Effect	F	<i>p</i>	95% CI
Cholesterol	Pre-experimental (S-NS)	45.55	<0.001	1.123 to 2.066
	Experimental (S-NS)	3.364	0.0711	−0.038 to 0.905
	Pre-experimental $\times$ Experimental	5.525	0.0217	0.167 to 2.053
Triglycerides	Pre-experimental (S-NS)	25.75	<0.001	1.091 to 2.505
	Experimental (S-NS)	7.895	0.0065	0.288 to 1.703
	Pre-experimental $\times$ Experimental	3.484	0.0663	−0.092 to 2.737
Glucose	Pre-experimental (S-NS)	347.3	<0.001	−9.268 to −7.437
	Experimental (S-NS)	12.05	0.0017	0.513 to 1.990
	Pre-experimental $\times$ Experimental	4.026	0.0545	−2.925 to 0.030
Glycogen	Pre-experimental (S-NS)	181.3	<0.001	8.359 to 11.40
	Experimental (S-NS)	2.803	0.1097	−0.230 to 2.099
	Pre-experimental $\times$ Experimental	0.3346	0.5694	−2.975 to 1.683
Lactate	Pre-experimental (S-NS)	5.709	0.0197	−0.472 to −0.042
	Experimental (S-NS)	1.627	0.2065	−0.352 to 0.077
	Pre-experimental $\times$ Experimental	5.427	0.0228	−0.930 to −0.072

3.3. Total Lipids and Fatty Acid Analysis

The FA composition of *R. decussatus* after the 40-day experimental period is shown in Table 4, and the results of the two-way ANOVA are summarized in Table 5. In clams initially reared with substrate, substrate deprivation (NS) did not significantly affect the relative abundance of any individual FAs or FA class compared to the control group (CTRL). Likewise, no significant differences were observed in total lipid content between the CTRL and NS groups.

In clams initially reared without substrate, transfer to substrate (S) significantly reduced the relative abundance of palmitic acid (16:0) and total saturated fatty acids (SFAs) compared with the CTRL group. Conversely, the levels of arachidonic acid (20:4n-6),

adrenic acid (22:4n-6), n-6 docosapentaenoic acid (22:5n-6), and docosaheptaenoic acid (22:6n-3) were significantly increased in the S group. Accordingly, the proportions of total n-3 polyunsaturated fatty acids (n-3 PUFAs), total PUFAs, and total unsaturated fatty acids (UFAs) were significantly higher in clams transferred to the substrate (S) than in the CTRL group. No significant differences were detected in the remaining individual FAs, total monounsaturated fatty acids (MUFAs), total n-6 PUFAs, or total lipid content between the two experimental groups.

Table 4. Fatty acid composition (% of total fatty acids) of the clam *R. decussatus* reared with or without substrate for 15 months and subsequently exposed for 40 days to the opposite substrate conditions (presence or absence of substrate). The results are expressed as mean $\pm$ SEM (n = 6). Asterisks (*) indicate significant differences (Student's *t*-test with Bonferroni correction, $p \leq 0.05$) among clams maintained under their original substrate conditions (CTRL) and substrate-deprived clams (NS) or clams transferred to substrate (S).

Saturated Fatty Acid	Substrate		No Substrate	
	Ctrl	NS	Ctrl	S
14:0 (myristic acid)	4.21 $\pm$ 0.05	3.90 $\pm$ 0.25	2.36 $\pm$ 0.51	1.50 $\pm$ 0.14
15:0 (pentadecanoic acid)	0.98 $\pm$ 0.06	1.03 $\pm$ 0.07	0.76 $\pm$ 0.04	0.88 $\pm$ 0.04
16:0 (palmitic acid)	23.45 $\pm$ 0.80	23.11 $\pm$ 0.87	20.81 $\pm$ 0.58	18.90 $\pm$ 0.29 *
17:0 (margaric acid)	1.32 $\pm$ 0.03	1.33 $\pm$ 0.05	1.26 $\pm$ 0.11	1.41 $\pm$ 0.04
18:0 (stearic acid)	7.14 $\pm$ 0.25	6.81 $\pm$ 0.17	7.47 $\pm$ 0.44	7.47 $\pm$ 0.20
20:0 (arachidic acid)	0.15 $\pm$ 0.01	0.15 $\pm$ 0.02	0.12 $\pm$ 0.02	0.09 $\pm$ 0.01
Total SFAs	36.55 $\pm$ 1.37	36.30 $\pm$ 1.17	32.79 $\pm$ 0.59	30.25 $\pm$ 0.28 *
Unsaturated Fatty Acid				
16:1n-7 (palmitoleic acid)	5.01 $\pm$ 0.40	5.02 $\pm$ 0.68	4.05 $\pm$ 0.53	2.90 $\pm$ 0.19
18:1n-7 (vaccenic acid)	4.73 $\pm$ 0.32	4.57 $\pm$ 0.34	4.18 $\pm$ 0.21	3.73 $\pm$ 0.23
18:1n-9 (oleic acid)	7.31 $\pm$ 0.18	7.70 $\pm$ 0.36	4.18 $\pm$ 0.20	3.84 $\pm$ 0.20
18:2n-6 (linoleic acid)	0.67 $\pm$ 0.06	0.74 $\pm$ 0.03	0.79 $\pm$ 0.08	0.63 $\pm$ 0.04
18:3n-3 (α -linolenic acid)	1.24 $\pm$ 0.17	1.21 $\pm$ 0.14	1.32 $\pm$ 0.10	1.21 $\pm$ 0.06
18:3n-6 (γ -linolenic acid)	0.20 $\pm$ 0.02	0.22 $\pm$ 0.02	0.22 $\pm$ 0.02	0.18 $\pm$ 0.01
18:4n-3 (stearidonic acid)	0.92 $\pm$ 0.02	1.01 $\pm$ 0.10	1.27 $\pm$ 0.18	0.93 $\pm$ 0.02
20:1n-7 (paullinic acid)	1.73 $\pm$ 0.07	1.59 $\pm$ 0.07	1.83 $\pm$ 0.17	1.96 $\pm$ 0.05
20:1n-9 (gondoic acid)	1.99 $\pm$ 0.09	1.83 $\pm$ 0.11	1.62 $\pm$ 0.19	1.98 $\pm$ 0.09
20:2n-6 (eicosadienoic acid)	1.44 $\pm$ 0.09	1.40 $\pm$ 0.10	1.61 $\pm$ 0.15	1.91 $\pm$ 0.09
20:3n-3 (docosatrienoic acid)	0.14 $\pm$ 0.02	0.16 $\pm$ 0.01	0.34 $\pm$ 0.05	0.36 $\pm$ 0.03
20:3n-6 (dihomo- γ -linolenic acid)	0.26 $\pm$ 0.01	0.25 $\pm$ 0.02	0.31 $\pm$ 0.02	0.35 $\pm$ 0.04
20:4n-3 (eicosatetraenoic acid)	0.20 $\pm$ 0.02	0.19 $\pm$ 0.02	0.30 $\pm$ 0.01	0.30 $\pm$ 0.02
20:4n-6 (arachidonic acid)	2.09 $\pm$ 0.18	2.13 $\pm$ 0.19	1.83 $\pm$ 0.16	2.22 $\pm$ 0.05 *
20:5n-3 (eicosapentaenoic acid)	3.61 $\pm$ 0.14	3.83 $\pm$ 0.49	8.81 $\pm$ 0.23	8.56 $\pm$ 0.24
22:1n-9 (erucic acid)	0.48 $\pm$ 0.03	0.47 $\pm$ 0.03	0.39 $\pm$ 0.05	0.42 $\pm$ 0.01
22:2n-6 (docosadienoic acid)	5.15 $\pm$ 0.40	5.07 $\pm$ 0.37	4.18 $\pm$ 0.36	4.65 $\pm$ 0.36
22:4n-6 (adrenic acid)	0.82 $\pm$ 0.10	0.87 $\pm$ 0.15	0.98 $\pm$ 0.12	1.29 $\pm$ 0.06 *
22:5n-3 (n-3 docosapentaenoic acid)	1.27 $\pm$ 0.07	1.34 $\pm$ 0.09	2.12 $\pm$ 0.24	2.39 $\pm$ 0.06
22:5n-6 (n-6 docosapentaenoic acid)	1.53 $\pm$ 0.13	1.58 $\pm$ 0.19	1.40 $\pm$ 0.17	1.91 $\pm$ 0.10 *

Table 4. Cont.

Saturated Fatty Acid	Substrate		No Substrate	
	Ctrl	NS	Ctrl	S
22:6n-3 (docosaheptaenoic acid)	6.83 ± 0.46	7.22 ± 0.79	10.09 ± 0.82	12.34 ± 0.35 *
n-3 PUFAs	14.07 ± 0.50	15.18 ± 1.04	24.26 ± 0.61	26.12 ± 0.24 *
n-6 PUFAs	12.15 ± 0.79	12.28 ± 0.98	11.34 ± 0.71	13.15 ± 0.46
Total MUFAs	21.25 ± 0.77	21.20 ± 0.97	16.25 ± 0.62	14.83 ± 0.52
Total PUFAs	26.22 ± 1.22	27.26 ± 1.87	35.60 ± 1.26	39.27 ± 0.56 *
Total UFAs	47.47 ± 0.59	48.47 ± 1.21	51.85 ± 0.84	54.10 ± 0.49 *
Total lipids (%)	7.71 ± 0.36	8.02 ± 0.24	8.47 ± 0.50	7.54 ± 0.25

Total lipids (%): percentage of lipids with respect to the total dry weight of the samples analyzed. MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids; UFAs: Unsaturated fatty acids; SFAs: Saturated fatty acids.

Table 5. Full Type III two-way ANOVA summary for fatty acids measured in *R. decussatus*. The table shows the effects of pre-experimental rearing conditions, experimental rearing conditions, and their interaction on selected fatty acids, fatty acid groups, and total lipids. Columns report the F-statistic, *p*-value, and 95% confidence interval (95% CI). S: Substrate; NS: No substrate; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids; UFAs: Unsaturated fatty acids; SFAs: Saturated fatty acids.

Variable	Effect	F	<i>p</i>	95% CI
20:4n-6 (ARA)	Pre-experimental (S-NS)	0.2447	0.6315	−0.291 to 0.459
	Experimental (S-NS)	2.295	0.1607	−0.539 to 0.103
	Pre-experimental × Experimental	1.367	0.3154	−0.295 to 0.989
20:5n-3 (EPA)	Pre-experimental (S-NS)	207.4	<0.001	−5.752 to −4.211
	Experimental (S-NS)	0.0015	0.9702	−0.572 to 0.592
	Pre-experimental × Experimental	1.753	0.1949	−1.601 to 0.728
22:6n-3 (DHA)	Pre-experimental (S-NS)	42.69	<0.001	−5.616 to −2.759
	Experimental (S-NS)	4.275	0.0655	−2.744 to 0.103
	Pre-experimental × Experimental	1.007	0.4960	−0.988 to 4.705
n-3 PUFAs	Pre-experimental (S-NS)	282.3	<0.001	−11.97 to −9.163
	Experimental (S-NS)	4.510	0.0597	−3.048 to 0.073
	Pre-experimental × Experimental	0.8057	0.6304	−2.373 to 3.870
n-6 PUFAs	Pre-experimental (S-NS)	0.0009	0.9757	−1.869 to 1.817
	Experimental (S-NS)	2.008	0.1869	−2.489 to 0.5539
	Pre-experimental × Experimental	1.468	0.2776	−1.364 to 4.721
Total MUFAs	Pre-experimental (S-NS)	58.94	<0.001	4.037 to 7.338
	Experimental (S-NS)	0.9911	0.3429	−0.9110 to 2.383
	Pre-experimental × Experimental	1.005	0.4972	−4.665 to 1.922
Total PUFAs	Pre-experimental (S-NS)	55.04	<0.001	−13.77 to −7.411
	Experimental (S-NS)	4.303	0.0648	−5.092 to 0.182
	Pre-experimental × Experimental	1.455	0.2821	−2.844 to 7.704
Total UFAs	Pre-experimental (S-NS)	24.43	<0.001	−7.114 to −2.693
	Experimental (S-NS)	7.495	0.0209	−3.120 to −0.320
	Pre-experimental × Experimental	2.494	0.0828	−1.746 to 3.853
Total SFAs	Pre-experimental (S-NS)	16.27	0.0024	2.192 to 7.604
	Experimental (S-NS)	5.284	0.0444	0.04307 to 2.764
	Pre-experimental × Experimental	3.957	0.0203	−5.034 to −0.4072
Total lipids (%)	Pre-experimental (S-NS)	0.1687	0.6900	−0.9022 to 0.6214
	Experimental (S-NS)	2.766	0.1273	−0.2086 to 1.437
	Pre-experimental × Experimental	0.8571	0.5939	−1.024 to 2.267

4. Discussion

The present study demonstrates that *R. decussatus* exhibits remarkable physiological plasticity in response to changes in substrate availability. Despite the recognized importance of sediment for the ecology and behavior of infaunal bivalves [17–19], the removal or addition of substrate for 40 days produced only limited effects on growth performance, whereas more pronounced responses were detected in metabolic and lipid-related parameters. These findings suggest that adult *R. decussatus* can rapidly adjust to temporary changes in their physical environment while maintaining their somatic growth. However, such acclimation appears to rely on modifications in energy metabolism rather than changes in tissue production.

Previous studies have shown that the presence and composition of sediment strongly influence burrowing efficiency, filtration activity, feeding performance, and ultimately growth in several commercially important bivalve species, including *Paphia undulata* [7], *Macra chinensis* [9] and *Sinonovacula constricta* [10]. Likewise, natural sediment disturbances caused by storms, tidal currents, and wave action, together with aquaculture operations, may temporarily alter habitat suitability and modify the physiological performance of buried clams [14–16]. Nevertheless, most previous studies have focused on long-term culture conditions or juvenile stages [34,35]. In contrast, information on the capacity of adult clams to cope with abrupt changes in substrate availability is scarce.

One of the most relevant findings of the present study is that the short-term removal of substrate from clams previously maintained under sediment conditions did not impair growth performance. Similarly, individuals previously cultured without substrate for 15 months did not exhibit measurable growth improvements after sediment was introduced. These observations suggest that adult *R. decussatus* possesses sufficient physiological flexibility to maintain somatic growth over relatively short periods, despite abrupt changes in habitat conditions. This pattern is consistent with the slow growth strategy of adult venerid clams, in which shell formation and tissue accretion are gradual processes that reflect long-term environmental conditions rather than transient environmental fluctuations [36,37]. Likewise, the lack of growth improvement after transferring clams from bare-bottom conditions to sediment indicates that long-term acclimation to substrate deprivation cannot be rapidly reversed. After 15 months without substrate, clams likely developed physiological adjustments that maintained growth under those conditions, preventing any immediate compensatory response following substrate addition [38,39]. Consequently, the 40-day experimental period may have allowed the detection of metabolic adjustments to changes in substrate availability, whereas the effects on biometric parameters may require longer exposure periods to become detectable. Although growth performance remained largely unaffected by short-term changes in substrate availability, the metabolic profile revealed a more rapid physiological response. Clams maintained with substrate for 15 months showed significantly lower cholesterol, triglyceride, and glucose concentrations, together with higher lactate levels, after substrate removal, whereas glycogen content remained unchanged. In contrast, the introduction of substrate to clams previously reared without sediment did not significantly modify any of the metabolic parameters evaluated in this study. These contrasting responses suggest that substrate withdrawal represents a stronger physiological challenge than addition, highlighting the importance of previous acclimation history in determining the metabolic response of *R. decussatus*.

Glucose is the main circulating carbohydrate in bivalves and a key indicator of energy metabolism during environmental stress [27,40]. The significant reduction in glucose levels observed after substrate removal suggests an increase in metabolic demand under altered culture conditions. Similar decreases have been reported in *R. decussatus* exposed to high-temperature stress, which have been interpreted as reflecting increased metabolic activity

associated with acclimation [8,41]. Comparable responses in other aquatic animals further support the role of glucose as an early indicator of shifts in energy allocation [24].

In contrast, glycogen concentrations remained unchanged, regardless of the substrate conditions. As the principal long-term energy reserve in bivalves, glycogen is generally mobilized under prolonged or severe stress, whereas moderate environmental disturbances often alter circulating metabolites before causing measurable depletion of glycogen stores [8,42,43]. Accordingly, the stable glycogen levels observed in the present study suggest that substrate removal increased metabolic demand without compromising long-term energy reserves during the experimental period.

The increase in lactate concentration provides further evidence of metabolic adjustments. Lactate accumulation is widely regarded as an indicator of increased reliance on anaerobic pathways in bivalves, reflecting a greater contribution of glycolytic ATP production when aerobic energy metabolism may not fully meet cellular energy demands [44,45]. Although dissolved oxygen remained within the optimal range throughout the experiment, the absence of sediment may have affected burrowing behavior and potentially altered the energetic demands of the clams [46,47]. Such changes could contribute to a greater reliance on anaerobic pathways; however, this mechanism was not directly assessed in the present study and should therefore be considered a possible physiological explanation rather than a definitive mechanism. Consistent with the present findings, elevated lactate concentrations have been widely reported in bivalves exposed to environmental stressors, including hypoxia, acidification, and thermal stress [48–50], highlighting lactate as a sensitive biomarker of short-term physiological disturbance in species such as *R. decussatus* [8]. Changes in lipid metabolites further support a shift in energy allocation after substrate removal. Both triglyceride and cholesterol concentrations decreased significantly in clams deprived of substrate following prolonged culture under sediment conditions. Triglycerides are the principal neutral storage lipids in aquatic invertebrates and constitute an important energy reserve that can be mobilized during periods of increased metabolic demand [51–53]. Therefore, their depletion suggests enhanced lipid utilization to support physiological processes during acclimation to altered culture conditions [54–56]. In addition to its structural role in biological membranes, cholesterol contributes to membrane integrity and cellular function, and variations in its concentration have been associated with physiological acclimation in marine invertebrates [26,57]. Together, these findings indicate that substrate removal altered energy allocation and lipid metabolism without producing measurable changes in growth performance.

Importantly, these metabolic alterations were only observed in clams subjected to substrate removal, whereas individuals cultured without sediment for 15 months remained metabolically stable after substrate addition. This asymmetrical response suggests that long-term acclimation may enhance the capacity of *R. decussatus* to cope with changes in substrate availability by reducing the magnitude of subsequent physiological adjustments.

Despite the marked changes observed in metabolic parameters, substrate removal did not significantly alter the FA profile of clams previously maintained under sediment conditions. In contrast, clams reared without substrate for 15 months exhibited selective changes in FA composition following substrate addition, including a reduction in total SFAs and increases in total UFAs, PUFAs, n-3 PUFAs, and key LC-PUFAs 20:4n-6 and 22:6n-3. Although FA analysis was performed on whole soft tissue rather than on isolated membrane phospholipid fractions, the observed changes suggest that substrate introduction promoted a gradual remodeling of tissue FA composition in clams previously acclimated to sediment-free conditions. In contrast, substrate removal appeared to have a more limited effect on tissue FA composition than on intermediary metabolism. These findings indicate that substrate availability may influence tissue lipid composition, although

specific effects on membrane lipid remodeling cannot be directly inferred from the present whole-tissue analysis. Membrane lipid remodeling is one of the primary mechanisms by which aquatic ectotherms maintain membrane function under changing environmental conditions [24,52,56]. By modulating the relative proportions of SFAs and UFAs, organisms regulate membrane fluidity, permeability, and the activity of membrane-associated proteins, thereby preserving cellular homeostasis during acclimation [20,25,26]. Accordingly, alterations in FA composition have been widely reported in marine bivalves exposed to environmental stressors, such as temperature, salinity, and chemical contaminants, reflecting adaptive physiological responses rather than simple dietary effects [8,20,25,58–60]. However, changes in FA composition measured in whole soft tissue may reflect alterations across multiple lipid pools and cannot be directly attributed to membrane phospholipid remodeling unless membrane-specific fractions are analyzed. Nevertheless, tissue-level changes in FA composition may provide an indication of broader lipid metabolic and acclimation responses to environmental conditions.

Among the SFAs, palmitic acid (16:0) was the most abundant fatty acid in all experimental groups. As the predominant SFA in marine bivalves, it is a major structural component of membrane phospholipids [61–63]. Its high abundance is also consistent with a diatom-based diet, since elevated proportions of 16:0 are widely recognised as biomarkers of diatom-derived nutrition in bivalves [61,64]. This interpretation is supported by the microalgal diet used in the present study, in which *Skeletonema costatum*, a diatom species, was the principal dietary component. Therefore, the reduction in 16:0 and total SFAs following substrate addition, together with the concomitant increase in UFAs, suggests a selective adjustment of membrane lipid composition to maintain membrane function during acclimation. The replacement of SFAs by UFAs, particularly LC-PUFAs, represents a conserved adaptive strategy that enhances membrane fluidity while preserving structural stability and the function of membrane-associated proteins under changing environmental conditions [25,26,60]. Although substrate availability does not directly modify temperature or salinity, the restoration of natural burrowing behavior may alter oxygen availability, sediment-water interactions, and the energetic requirements associated with life within the sediment, creating physiological conditions that favor FA remodeling [65,66]. Comparable responses have been described in bivalves exposed to salinity fluctuations and low temperatures, in which increased membrane unsaturation helps maintain optimal membrane properties and cellular function despite environmental stress [67,68].

Among the individual UFAs, the increases in 20:4n-6 (ARA), 22:4n-6, 22:5n-6, and 22:6n-3 (DHA) following substrate addition suggest a selective enrichment of LC-PUFAs in membrane lipids. DHA is a major component of structural phospholipids in biological membranes and plays a central role in maintaining membrane fluidity and cellular function under fluctuating environmental conditions [56,69]. Likewise, the increase in ARA may have important physiological implications beyond membrane structure, as it serves as a precursor of numerous eicosanoids involved in immune regulation, inflammation, and cellular signaling [70,71]. Variations in ARA abundance have frequently been associated with environmental acclimation in marine invertebrates [8,20]. Together with the concomitant increases in 22:4n-6 and 22:5n-6, these results suggest that substrate addition promoted a selective remodeling of structural lipids rather than a simple accumulation of lipid reserves.

The findings of the present study have important implications for the culture and management of *R. decussatus*. Although short-term substrate removal did not compromise survival or growth in adult clams, it induced marked metabolic adjustments without significantly altering tissue FA composition. In contrast, substrate introduction promoted tissue FA remodeling, highlighting the remarkable physiological plasticity of clams in response to changes in their benthic environment. These contrasting responses may reflect

the combined influence of substrate manipulation and the physiological state established during the pre-experimental rearing period. These results indicate that growth performance alone may underestimate the physiological consequences of temporary substrate loss resulting from natural disturbances or routine aquaculture operations, including harvesting, grading, transport, and handling. Therefore, integrating metabolic and lipid biomarkers with conventional growth indicators may provide a more comprehensive assessment of animal welfare, physiological status, and adaptive capacity of cultured bivalves. Likewise, several limitations should be considered when interpreting these results. The experiment was conducted on adult clams under controlled environmental conditions and evaluated the short-term effects of substrate manipulation. Moreover, baseline measurements of metabolic parameters and tissue FA composition were not available at the beginning of the experimental period, preventing the relative contributions of pre-experimental rearing history, baseline physiological condition, and substrate manipulation from being fully disentangled. Consequently, the responses observed may differ in juvenile stages or under more complex farming conditions involving multiple environmental stressors. Future research should investigate the interactive effects of multiple environmental factors and characterize the molecular mechanisms underlying the metabolic and tissue FA responses identified in the present study.

5. Conclusions

The present study demonstrates that substrate availability influences the physiology of adult *R. decussatus* primarily through metabolic and biochemical adjustments, rather than immediate changes in growth or survival. Short-term substrate removal induced significant alterations in glucose, lactate, triglyceride, and cholesterol concentrations without affecting tissue FA composition, whereas substrate introduction promoted tissue FA remodeling, characterized by reduced SFAs and increased unsaturated FAs, particularly LC-PUFAs. These findings reveal a high degree of physiological plasticity, enabling adult *R. decussatus* to maintain growth despite substantial metabolic reorganization following changes in substrate availability. From an aquaculture perspective, the results indicate that conventional growth indicators alone may not fully reflect the physiological status of cultured clams and highlight the value of integrating metabolic and lipid biomarkers into culture assessments to support improved management practices, animal welfare, and the sustainability of clam aquaculture.

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Abbreviations

The following abbreviations are used in this manuscript:

FAs	Fatty acids
FAMEs	Fatty Acid Methyl Esters
SFAs	Saturated Fatty Acids
UFAs	Unsaturated Fatty Acids
PUFAs	Polyunsaturated Fatty Acids
LC-PUFAs	Long-Chain Polyunsaturated Fatty Acids
n-3 PUFAs	n-3 Polyunsaturated Fatty Acids
n-6 PUFAs	n-6 Polyunsaturated Fatty Acids
ARA	Arachidonic Acid
EPA	Eicosapentaenoic Acid
DHA	Docosahexaenoic Acid
CTRL	Control
S	Substrate
NS	No Substrate
SEM	Standard Error of the Mean

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