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Research Article

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Annual variation of the biochemical composition, energy reserves and physiological indices in *Mactra stultorum* tissues from the Tunisian coasts

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Abstract

The present study assessed the consequences of environmental changes on the biochemical quality and the physiological condition of the whole body and others organs *Mactra stultorum* collected from the north coast of Tunisia. Significant variations in the biochemical components, PE and CI have shown between seasons. The depletion of glycogen in all tissues was recorded during spawning periods (late spring and summer). Lipid accumulation in gonad–visceral mass during the early and late gametogenesis stages was associated with the increase in CI and GSI. However, decreases in lipid and protein contents were recorded in all the tested organs during summer when the animals are in the spawning period, suggesting that clams could accumulate proteins and lipids. Regarding the environmental factors, a negative and a significant correlation was recorded between glycogen, lipid contents and water temperature. Based on these results and as *M. stultorum* is appreciated by the consumers, we suggest that the harvesting of *M. stultorum* should be concentrated on February to June when the whole body was characterized by an important amount of the biochemical composition when the seafood is at its highest nutritive value as compared to August to January. Because of its high nutritive value especially it's richness by some important and essentials fatty acids, *M. stultorum* seems to be considered as an important commercial species in many countries and in Tunisia particularly. We suggest that harvesting of *M. stultorum* should be concentrated on the period when the seafood is at its highest nutritive value. survivability rates from malignancy. The greatest variations in the mortality ratios existed within the European countries.

Keywords: *Mactra stultorum*, Seasonal variations, Biochemical composition, Percentage edibility, Condition index and Gonado-somatic index.

Introduction

Over the last few decades, mollusk bivalves became a high nutritious food source in the human diet. They are considered highly nutritional with interesting dietetic properties because of their valuable biochemical constituents such as proteins, glycogen, lipids, fatty acids and minerals. Research on the biochemical and energy reserves of bivalve species was launched in the 19th century. Several authors have studied the seasonal variation of the biochemical compounds of bivalves, the storage cycle of their energy reserves relative to the reproduction of the species and the environmental fluctuations of the environment [1-3].

In bivalves, glycogen constitutes the most prominent reserve, especially during the processes of gametogenesis [4, 5]. Glycogen is a good supplying energy demand during the formation of gametes [6]. Due to their high caloric content, lipids serve as energy storage under critical nutritional conditions [7]. They play an important role in metabolic pathways in the formation of gametes and are considered as the major reserve of oocytes [8, 9]. Protein, one of the abundant biochemical components in bivalves, can be serving also serve as an energy reserve during gametogenesis when other reserves are exhausted [10].

Certain exogenous factors, such as the temperature of the water and the availability of food in the surrounding environment as well as some endogenous conditions such as the reproductive cycle play a key role in the variation of the physiological fitness of many marine organisms [11, 12]. So it has been demonstrated that there is an important and broad relationship between the percentage edibility, the condition index and organic content. Additionally, the condition index, which supported as the health index, considered as a good index to determine the evolution of the physiological processes in relation to the reproduction, growth, mortality, parasitic and other contagions factors [13, 14].

The bivalve *Mactra stultorum* is a benthic species inhabiting sandy beaches of the lower infra-littoral zone. It is widely distributed along the north and south coasts of the Mediterranean Sea, the Black Sea, Senegal and Europe [15]. In Tunisia, it has been reported from the North coasts and from the South in the Gulf of Gabes [16]. Because of its high nutritive value especially it's richness by some important and essentials fatty acids such as eicosapentanoic (EPA, 20:5n-3) and docosahexanoic (DHA, 22:6n-3) acids [17], *M. stultorum* seems to be considered as an important commercial species in many countries such as in Manche (Atlantic coast) [18]. Recently, the reproductive cycle of *M. stultorum* has been determined by Chetoui *et al*. [19]. The assessment of histological studies indicated that its reproductive cycle is synchronous for both sexes. The gametogenesis process is initiated in January. The maturation phase is extended from April to July and the partial spawning started in spring to reach its peak in late summer, indicating a prolonged reproductive activity. To the best of our knowledge, the biochemical composition of *M. stultorum* from the Mediterranean coasts in relation to various endogenous (reproductive cycle…etc) and exogenous conditions (temperature, salinity, food accessibility…etc) have not yet been studied.

The goal of the present work is to investigate the annual dynamic of the biochemical composition (including macromolecules and glycogen) of the whole soft tissue and different organs of *M. stultorum* collected from northern Tunisian coasts and to assess its percentage of edibility (PE) and the condition index (CI) in relation to the total reproductive period and environmental parameters.

Materials and Methods

Sampling site and collection

Specimens of *Mactra stultorum* were collected during 12 months (from June 2008 until May 2009) from the sandy beach of station Kalaat El Andalous located at the Gulf of Tunis (37°03'73''N and 10°10'67''E). The current North-South is reduced in this range through advanced Sidi Ali Mekki. Thus, these waters are usually calm and the bottom consists mainly of fine sand deposited on the front of the delta plain of Oued Medjerda (**Figure 1**).

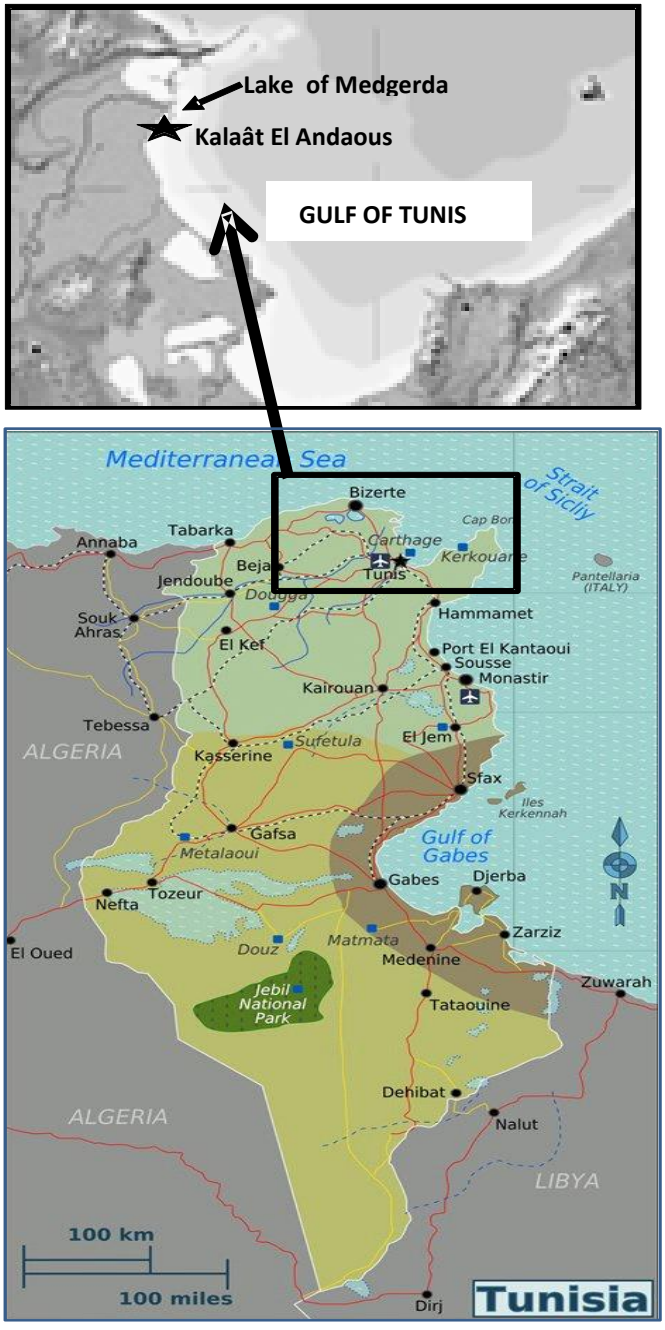

Figure 1. Maps of sampling area in Tunisia (The black full star in the Gulf of Tunis shows the sampling site: Kalaât El Andalous).

Environmental parameters were measured monthly. Surface seawater temperature and salinity were carried out in situ depth of 30cm using a mercury thermometer and a salinometer, respectively. Chlorophyll a was extracted using Whatman GF/F filters with 90% methanol in the laboratory and concentration was evaluated according to the protocol of Aminot and Chaussepied [20]. Samples of *M. stultorum* were transferred to the laboratory for acclimation in filtered seawater in order to eliminate all types of nutrients from their digestive glands. After 72 h, clams of similar size (30 and 57mm) were selected for experimentation. The total Wight and shell dry weight of all *M. stultorum* were analyzed using 0.001 g electronic precision balance. The biometric measurements such as shell length (SL), height (SH) and width (SW) were measured with a digital caliper (0.1mm).

Experimental analysis

After sacrifice, separate organs such as mantle (MT), adductor muscle (AM), foot (FT), gonad-visceral mass (GV) and rest (RE) were dissected for the first group and for the second group we selected the body whole. All selected animals were kept in a freezer at -30 ° C for biochemical analysis. The gonad and visceral mass are taken as a single organ (GV) due to the difficulty of their separation. For biochemical analysis, each month six replicates were used to produce the means with 6–8 *M. stultorum* pooled in each replicate. Total protein content was determined by the method of Lowry *et al.* [21] using the Bovine serum albumin (BSA) as a standard range. Samples were homogenized in a Tris-HCl buffer (20 mM; pH=7.4), then, centrifuged at 10.000 × g for 20 min (4°C). Supernatants were stored in Eppendorf tubes at -80°C for subsequent analyses. A volume of 10 µL supernatants was dissolved in 400 µL of water solution to which we added a 2ml of a mixture composed of three solutions: sodium carbonate hydrate dissolved in a solution of NaOH (0.1N), copper sulfate dissolved in water and sodium hydrate dissolved in water. To the last mixture we added 200 µL of Folin Reagent, then, incubated for 30 min at the darkness. The proteins were quantified using the spectrometric method at 540 nm.

The total glycogens were determined by the enzymatic potocol of Dubois *et al*. [22]. The glycogen was released from the tissues by heating 0.5 g of fresh tissue in the presence of 2 ml of alkaline solution (KOH, 300 g / l) for 2 min in a water bath at 100 ° C. Glycogen was precipitated by the addition of ethanol 95°C. This precipitate was recovered after cooling the tubes in ice and centrifugation at 3000 rpm for 10 min. It was dissolved in 10 ml of water. Then, 1 ml of the last glycogen solution was hydrolyzed by a hydrochloric acid HCl (1.2mol / L) in a bath at 100°C for 2 h. Then, this hydrolyzate was neutralized by the addition of sodium hydroxide solution NaOH (0.4 N) until the color changes to pink. Finally, the glucose oxidase reagent was added to these last mixtures, which were subsequently incubated in a bath at 37 °C for 15 min. The optical density was measured at 520 nm.

Total lipids (TL) were extracted using the method of Folch *et al*. [23]. The flesh was crushed with a solution of chloroform: methanol (2:1, v:v) including 0.01% Butyl-hydroxy-toluene (BHT) as an antioxidant. The extraction volume was estimated by 30 ml of extraction solvent per g of tissue. The mixture of the extract volume and 2 ml of NaCl solution (15%) were centrifuged at 3000 x g for 15 min. The chloroform phase was recovered and evaporated to dryness by Rotavapor® R-300. Finally, the residue was diluted in 2 ml of chloroform solution. The levels of total lipid, glycogen and protein are expressed as mg / g Dry Weight (mg/g DW). The percentage edibility (PE), the condition index (CI) and the gonado-somatic index (GSI) were assessed monthly from May 2008 to Jun 2009. A total of 30 clams were selected each month for the analysis of each index. The flesh and shells of each clam were dried in an oven at 60°C for up to 72 h to determine the constant dry tissue and shell weight. For each index, 30 individuals were collected each month. According to Deslou-Paoli *et al*. [24], the CI was calculated as equation 1 and the GSI was expressed by equation 2: Equation 1: CI= (dry weight of the soft parts/dry weight of shell) *100; Equation 2: GSI= (dry weight of the gonad-visceral mass/dry weight of shell) *100. The percentage edibility (PE) was calculated using Anibal *et al*. method [25] and was estimated using equation 3: Equation 3: PE= (wet meat weight (g)/Total wet weight including shell (g)) *100.

Statistical analysis

Data analysis was performed using the software Statistica version 5.0. The biochemical composition of each separate tissue and whole body was compared between months. Normality was assessed for all datasets using the Shapiro-Wilcoxon test. The significant differences between variables were analyzed using one-way analysis of variance (ANOVA) followed by posthoc Tukey's test ($p<0.05$). When the conditions for ANOVA were not satisfied, nonparametric Kruskal–Wallis's test was used ($p<0.05$). The differences between samples were deemed to be significant at $p<0.05$. Pearson correlation matrix and principal component analysis (PCA) were used to exhibit the association between biochemical, biological indices, percentage ediblity and environmental factors among the studied periods

Results

Environmental parameters

The temperature of the seawater varied between 8°C in January to 28 °C in July and August. Salinity was highest (38.2 psu) in July, dropping to <35psu from December to

March with a minimum value noted in January (31 psu). This decrease in salinity is generally due to the relatively high pluviometry (84.14mm in January) and to discharge from the river Medjerda. Chlorophyll *a* values ranged from 1.28 mg/l (November) to 6.35mg/l (April) (**Figure 2**).

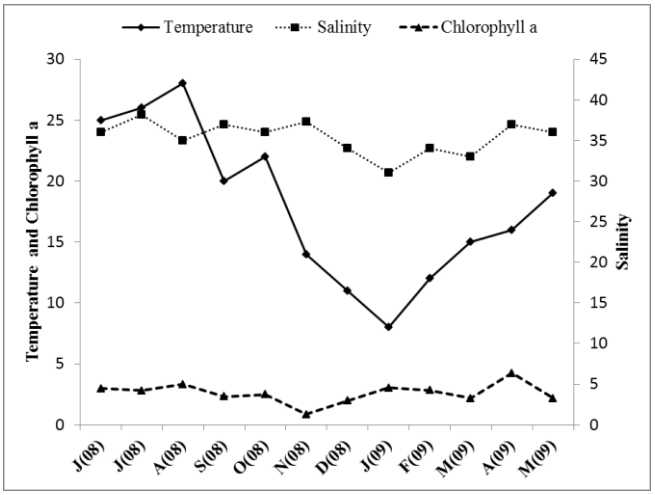

Figure 2. Physicochemical parameters of Kalaât El Andalous during the sampling period (Jun 2008–May 2009).

Biometric analyses

The biometric data (shell length, height and width) are elucidated in **Table 1**. The shell length (SL) ranged from 32.68 to 47.82 mm; the shell height (SL) ranged from 25.72 to 39.83mm, the shell width (SW) varied from 12.88 to 25.58 mm and the shell dry weight ranged from 3.57 to 10.5g.

Table1: Biometric measurements of *Mactra stultorum* during one year.

Month	SL (mm)		SH (mm)		SW (mm)		SDW (g)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
J(08)	40.15	6.39	33.81	7.67	20.94	4.35	5.69	1.7
J(08)	47.52	4.04	39.41	4.81	25.22	2.68	10.5	2.43
A(08)	46.25	3.16	38.09	3.84	24.52	2.2	8.73	2.21
S(08)	47.97	2.84	39.2	3.44	25.48	2.1	9.16	2.31
O(08)	43.55	4.83	36.3	5.9	23.54	3.9	8.08	2.26
N(08)	40.95	3.68	33.69	4.88	21.83	4.02	5.31	1.99
D(08)	36.82	3.32	30.8	3.86	19.32	2.38	3.57	1.41
J(09)	32.68	7.04	25.72	7.79	12.88	5.64	6.34	2.5
F(09)	46.71	5.64	39.35	6.59	25.56	3.78	8.18	1.74
M(09)	45.49	4.56	37.99	5.63	24.31	3.22	7.03	2.47
A(09)	46.97	3.49	39.83	6.22	25.58	3.8	9.78	2.07
M(09)	40.54	8.08	33.63	9.1	19.8	7.02	9.46	2.85

Results are given as mean ± **SD**. n=50 individuals each month. **SL**: shell length; **SH**: shell height; **SW**: shell width and **SDW**: shell dry weight.

Biochemical composition

Seasonal variations in the protein, glycogen and total lipid contents of different body parts (foot, gonad-visceral mass, mantle, adductor muscle and rest) are shown in (**Table 2)**. The biochemical constituents showed a significant variation between seasons and tissues ($p<0.001$). In all tissues, the highest protein content (235.±8.42 mg/g DW) was noted in the foot, while, the minimal amount of protein (31.2±3.4 mg/g DW) was noted in the adductor muscle. Using to HSD Tukey's tests ($F=3.35$, $p = 0.001$), significant differences were recorded for a protein of foot which varied from 140 to 235 mg/g DW ($p <0.05$). It marked by tow peaks in September (220.78±10.20 mg/g DW) and December (175.07±11.53 mg/g DW).In the gonad-visceral mass, the seasonal evolution of protein has shown a significant variation throughout the year.

The minimum level was observed in July (54.18±19.97 mg/g DW), then; increased significantly to reach two peaks in September (231.84±23.13mg/g DW) and April (250.94±22.60 mg/g DW). For the mantle, the level was relatively stable from June to December, then it dropped in January (39.67 ± 9.38 mg/g DW) and enhanced in March (126.84 ± 14.48mg/g DW). The protein level in the adductor muscle remained stable between 120 and 130mg /g DW throughout the study year except October (31.17 ± 8.38 mg/g DW) and February (60.41 ± 9.40 mg/g DW) in which protein level decreased significantly. The protein content in the rest part was comprised between 24.3± 0.37 mg/g DW in January to 144.9± 1.35 mg/g DW in March.

The gradient of the glycogen content in *M stultorum* body parts has shown that the FT tissue has the high level, followed by those in the tissues from the AM, MT and GV. In contrast, lower glycogen content was recorded in the RE tissues. The glycogen contents varied similarly in the four examined tissues (foot, gonad-visceral mass, mantle, adductor muscle). They were lower from July to September (<20 mg/g DW) and peaked in April, and then declined considerably in May. The lowest values of glycogen in the rest part (RE) were detected during autumn and winter in particular in November and January with (3.54±1.17mg/g DW) and (4.94±1.28 mg/g DW). However, similar to other parts of the body, the quantity of glycogen peaked in April (17.64±3.9 mg/g DW) One-way ANOVA analysis (HSD Tukey's tests, $F=6.51$, $p = 0$) indicated that glycogen levels in all examined tissues showed significant monthly variations.

Total lipids contents are unevenly distributed between tissues of *M. stultorum* and varied significantly ($p<0.05$) between seasons except for foot, which varied from 14 to 22 mg/g. In the gonad-visceral mass, TL content started at (50.38± 8.58 mg/g DW) in June and decreased in August (25.16± 8.78 mg/g DW). Then, it gradually increased in autumn and stabilized in winter.

Table 2. Seasonal variations in the contents of total protein, glycogen and lipids (mg/g DW) of the body parts and whole body of clam *Mactra stultorum.*

Total protein contents in mg/g dry weight tissue												
	J(08)	**J(08)**	**A(08)**	**S(08)**	**O(08)**	**N(08)**	**D(08)**	**J(09)**	**F(09)**	**M(09)**	**A(09)**	**M(09)**
FT	150±9.48^{be}	142±8.65^{ab}	152.1±9.80^{bc}	221±12^{f}	170±8.07^{de}	147±11.2^{abc}	175.1±10.8^{e}	138±10.1^{ab}	130.4±11.41^{a}	142.3±7.828^{ab}	162.9±12.62^{dca}	235±8.42^{f}
GV	112±10.1^{b}	54.2±9.99^{a}	164±10.77^{c}	232±11.6^{e}	117±11.1^{bd}	97±10.9^{b}	157±10.94^{c}	146±12.3^{cd}	95.2±11.36^{b}	177.8±10.21^{c}	250.9±11.3^{c}	172±10.9^{c}
MT	96.8±6.14^{d}	83.4±8.55^{be}	70.82±13.65^{b}	77.2±9.66^{b}	79.6±6.35^{b}	81±10.6^{b}	77.82±5.7^{b}	39.7±5.0^{a}	96.22±7.27^{ad}	126.8±5.35^{f}	97.92±8.47^{de}	97.6±10.^{ce}
AM	136±8.21^{f}	137±7.57^{f}	142.5±6.63^{f}	126±3.7^{ed}	31.2±3.4^{a}	73.9±3.8^{c}	135±8.19^{ef}	138±9.7^{f}	60.43±6.36^{b}	70.6±6.6^{bc}	121.4±6.9^{d}	134±4.58^{ef}
RE	93.4±3.08^{c}	83.8±4.01^{c}	79.76±1.075^{c}	97.9±2.3^{d}	53.9±4.08^{b}	57.5±7.87^{b}	98.11±1.71^{d}	24.3±0.37^{a}	84.37±5.52^{c}	144.9±1.35^{f}	121.2±1.38^{e}	97.2±2.1^{d}
WB	114±4.89^{c}	97.4±9.2^{b}	114.8±3.9^{c}	142±9.2^{e}	84.2±6.7^{a}	85.8±4.5^{a}	123.5±5.56^{d}	85.2±2.52^{a}	91.84±6.16^{ab}	134.6±3.11^{f}	145.9±2.98^{e}	139±2.11^{ef}
Total glycogen contents in mg/g dry weight tissue												
	J(08)	**J(08)**	**A(08)**	**S(08)**	**O(08)**	**N(08)**	**D(08)**	**J(09)**	**F(09)**	**M(09)**	**A(09)**	**M(09)**
FT	14.6±1.96^{cd}	10.06±1.5^{acd}	6.45±1.56^{ab}	5.15±1^{a}	15.4±1.49^{d}	11.5±1.64^{bca}	28.84±2^{ef}	26.8±1.41^{eg}	29.7±2.82^{ef}	33.4±1.17^{fh}	35.5±3^{h}	23±2.4^{g}
GV	9.04±2.93^{bc}	6.22±1.06^{b}	3.47±1.68^{a}	4.18±1.02^{ab}	11.88±1^{cd}	16.56±1.89^{de}	20.05±2.96^{eg}	14.84±1.02^{df}	18.5±1.9^{ef}	23.81±1.1^{g}	35.46±1.2^{h}	8.53±0.81^{ab}
MT	14.11±1.57^{c}	9.51±1.05^{b}	8.37±0.75^{a}	7.55±2.03^{b}	8.86±1.83^{b}	10.45±1.51^{bc}	22.37±1.4^{d}	12±1.31^{bc}	10.25±1.28^{c}	24.78±2.21^{d}	27.71±1.5^{e}	20.54±1.34^{d}
AM	12.71±0.92^{b}	10.54±1.1^{a}	6.03±1.25^{a}	7.01±1.76^{a}	10.81±1.61^{ab}	19.84±2.36^{ab}	17.20±0.82^{bc}	19.55±1.35^{cd}	21.61±2.09^{cd}	33.8±1.9^{e}	34.55±1.5^{e}	22.47±2.3^{d}
RE	7.07±1.17^{c}	10.8±1.78^{d}	7.71±1.44^{c}	6.24±0.6^{b}	5.77±1.43^{b}	3.54±1.17^{a}	8.94±2.3^{f}	4.94±1.28^{b}	13.69±3.8^{e}	17.64±3.9^{f}	10.94±2.5^{d}	5.21±1.3^{b}
WB	10.8±3.97^{be}	10±2.91^{be}	6.12±2.54^{a}	5.73±2.44^{a}	11.6±3.71^{cd}	12.7±4.57^{cd}	21.05±5.41^{f}	13.8±4.67^{d}	18.65±5.86^{e}	25.19±5.91^{g}	27.34±5.42^{g}	15.6±3.7^{d}
Total lipid contents in mg/g dry weight tissue												
	J(08)	**J(08)**	**A(08)**	**S(08)**	**O(08)**	**N(08)**	**D(08)**	**J(09)**	**F(09)**	**M(09)**	**A(09)**	**M(09)**
FT	14.7±0.93	16.4±1.67	16.8±1.80	19±0.93	17.2±2.03	18.9±1.15	20.89±1.95	21.4±1.14	20.13±2.08	21.12±1.13	22.62±1.9	20.2±2.7
GV	50.4±17.8^{e}	45.2±12.6^{d}	25.17±8.78^{a}	28.9±8.58^{a}	43.7±5.25^{cd}	43.8±5.15^{cd}	40.58±5.64^{c}	43.9±16.5^{cd}	34.47±12.02^{b}	57.07±11.42^{f}	72.46±14.64^{h}	64±7.8^{g}
MT	36.6±7.8^{b}	32.1±4.57^{acd}	30.84±8.5^{ac}	35.1±6.42^{bd}	30.9±3.44^{a}	35.5±8.63^{bd}	35.56±9.87^{bd}	36.1±6.13^{bd}	28.27±8.89^{a}	35.35±3.3^{b}	41.25±3.25^{e}	283.23^{a}
AM	13.8±2.4^{ab}	14.6±4.97^{b}	9.82±2.02^{a}	24.1±7.82^{c}	25.4±3.51^{c}	30.8±4.68^{d}	32.86±8.16^{e}	28±3.43^{cde}	26.06±5.64^{c}	16.51±3.63^{b}	15.25±5.52^{b}	13.6±2.3^{ab}
RE	55.5±4.38^{f}	44.9±4.94^{d}	31.64±3.47^{cb}	34.3±3.63^{cb}	24.2±3.09^{b}	55.6±3.94^{f}	58.33±3.57^{f}	24.3±2.6^{b}	28.24±3.352^{b}	39.73±4.48^{d}	25.22±3.7^{b}	20.3±3.3^{a}
WB	37.8±8.2^{c}	33±7.61^{c}	24.32±6.12^{a}	29.3±6.93^{b}	30.9±4.56^{b}	40.1±6.44^{d}	41.09±7.2^{d}	28.8±6.99^{b}	28.07±7.55^{b}	34.92±7.2^{c}	33.67±6.86^{c}	27.7±5.4^{b}

Results are given as mean ± SD. n=8 replicates each month. FT: foot. GV: gonad-visceral mass. MT: mantle. AM: adductor muscle. RE: rest. WB: whole body. Different letters indicate significant monthly difference for each organ (HSD Tukey's tests. $p < 0.05$).

TL in the gonad-visceral mass reached a maximum value during April (72.45 ± 5.73 mg/g DW). In mantle, TL content presented low values in October (30.86 ± 3.44 mg/g DW) and February (28.27± 8.89mg/g DW). For adductor muscle, the content of lipids seems to be low from Jun to August and from March to May (below 20mg/g DW). However, the high values were observed in autumn and winter in order of 25 and 32 mg/g DM, respectively. In the rest part the TL contents fluctuated between 20.3 mg/g DW and 58 mg/g DW throughout the study year. One-way ANOVA analysis (HSD Tukey's tests, F=18.11, p = 0) indicated that the TL contents in GV, AM, MT and RE showed significant monthly variations. Seasonal variations in the content of proteins, total lipids and glycogen of the *M. stultorum* whole body are shown in (**Table 2)** expressed as mg per g of dry weight. Protein content was the major biochemical compound and underwent significant variations during the experimental period (HSD Tukey's tests, F =146.42, p = 0). It was in minimum value (84.2±6.7 mg/g DW) in October and reached a maximum (145.9±2.98mg/g DW) in April. The total lipid contents, which are the second major biochemical constituents in the whole body, it were comprised between 24 and 37 mg/g DW during the experimental period. Glycogen content varied in a similar way to that observed in GV. It was in a lowest value during August and September and increased gradually during winter and spring to reach a maximum in April. This compound has demonstrated significant changes among month (HSD Tukey's tests, F =270.20, p = 0).

Condition index (CI) and gonado-somatic (GSI) index

The CI and GSI indexes showed a synchronous evolution. Lowest values of CI (4.43 ± 1.24%) and of GSI (1.26±0.32%) were recorded in August (08). However, high percent of CI and GSI were observed in April with values of (14.41± 2.09%) and (3.97±0.52%) respectively. These indexes have shown significant differences among months (ANOVA, (p< 0.05) (**Figure 3**).

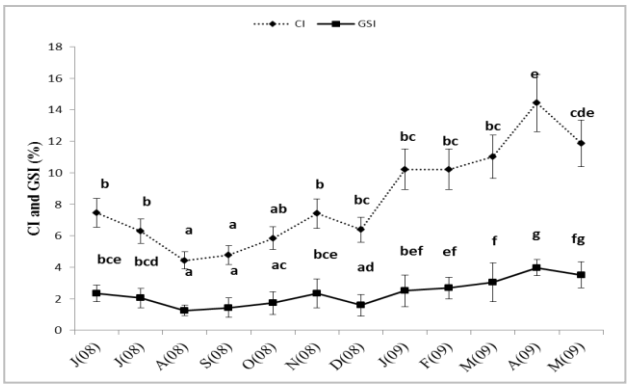

Figure 3: Seasonal variation of the condition index (CI) and gonado-somatic index (GSI) of *Mactra stultorum* throughout the study period.Results are given as mean ± SD, n=30 replicates each month. Different letters indicate significant monthly difference for each index (HSD Tukey's tests, p < 0.05).

Percentage edibility (PE)

During the reproductive cycle, the percentage of edibility showed a significant fluctuation from 25%-60%, with a sharp enhancement in the percentage edibility during April (59.64±1.93%). During the summer, autumn and winter, it decreased considerably, with the lowest of the edibility percent (25.34±0.71%) being noted in August (**Figure 3**). The percentage edibility has shown significant differences among months (ANOVA, (p< 0.05) (**Figure 4**).

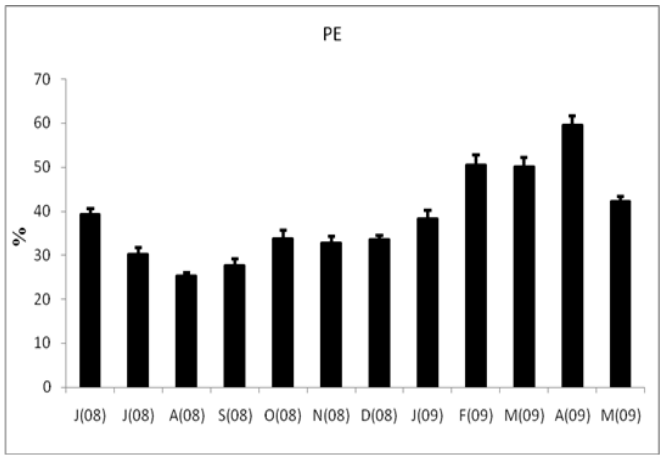

Figure 4: Seasonal variation of percentage edibility (%) in *Mactra stultorum* throughout the study period. Results are given as mean ± SD, n=30 replicates each month. Different letters indicate significant monthly difference for PE (HSD Tukey's tests, p < 0.05).

Statistical analyses

The interaction between biochemical compositions in each separate tissue and in the whole body of *M. stultorum* (1); environmental parameters (water temperature, salinity and chlorophyll *a*) (2); reproductive indices of *M. stultorum* (CI and GSI) (3), percentage edibility (4) and seasons (5) was tested using Principal components analysis (PCA) and a Pearson correlation (**Figure 5** and **Table 3**).

Results of PCA allowed us to retain the first two factorial axes that explain 57.32% of the total variance. Factor 1 displayed 41.30% of the total variance, defined by CI; GSI; PE and different biochemical's compounds in each organ and whole body except the LIPWB; LIPMA; LIPRE; PROMT; PROFT, which characterized factor 2 (14.5%). In addition, the environmental parameters (T °C, S, and Chlo a) described the factor 2 (**Figure 5A**). Analyze of biochemical's composition reveled significant seasonal fluctuations marked by two phases the first one is the storage phase and the second is the depletion of reserves. During late winter and spring, glycogen was accumulated in high quantities in all tissues and whole body, which was positively and highly correlated with CI and GSI (**Table 3**).

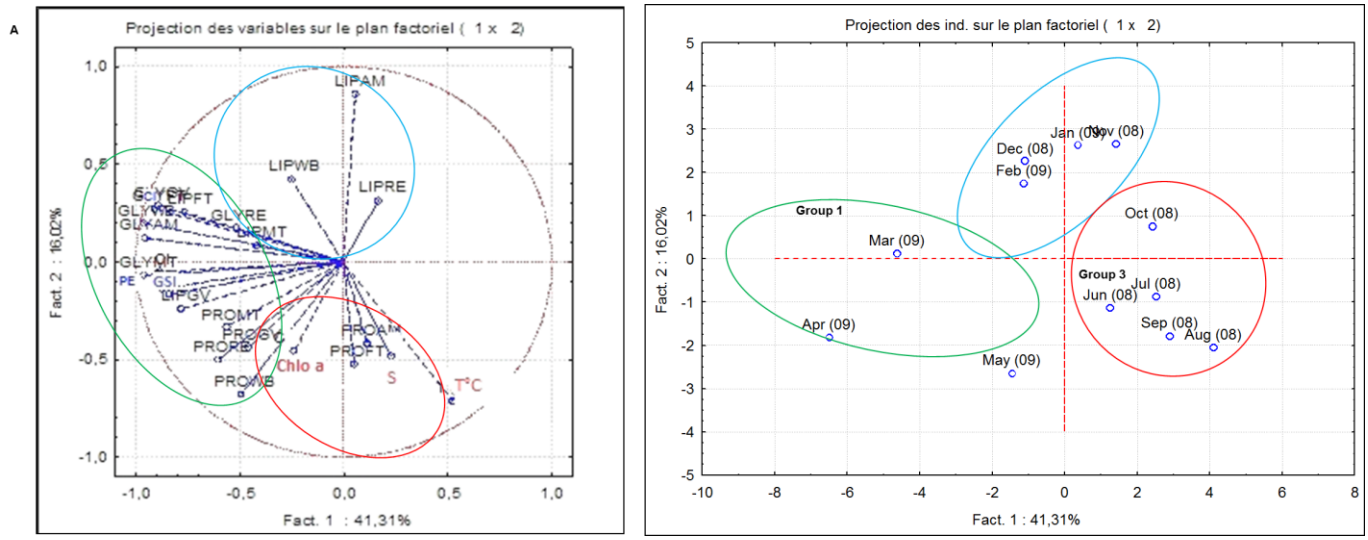

Figure 5. Principal analysis component (PCA) represented by two factors F1 and F2 and produced by the biochemical parameters and reproductive indices of *Mactra stultorum* and the environmental condition. Projection of the variables on the factor-plane (1-2) (A) and projection of the cases on the factor-plane (1-2) (B) PRO: protein, GLY: glycogen, LIP: total lipid, FT : foot , GV: gonad-visceral mass, MT: mantle, AM : adductor muscle, RE: rest, BW: whole body, CI: condition index, GSI: gonado-somatic index, T°C: water temperature, Chlo *a*: chlorophyll *a*, S: salinity.

This reserve seems to be lower during summer when the temperature is higher which presented a negative correlation with GLYGV and GLYFT. The total lipid in GV, MT, FT increased significantly especially during spring when the CI, GSI and PE are higher (positive correlation with LIPGV). While the AM deplete their lipid reserves during this same period and when the water temperature increased (negative correlation).In other hand the position of LIPWB in the middle of the two factors of PCA can be explained by the insignificant correlation with all parameters except with LIPRE. The major biochemical compound in all tissues, proteins significantly fluctuated with seasons and varied with tissues.

Additionally, the protein in the gonad-visceral mass was negatively correlated with chlorophyll *a*, especially during autumn and winter when CI and Chlo a values were lower (p <0.05). PE showed a positive and high correlation with the accumulation of protein and carbohydrate (glycogen) in the tissue of *M. stultorum* and with CI and GSI when increased. Only the accumulation of lipid in GV is positively correlated with PE index of physiological fitness. The salinity did not correlate with the biochemical compound of *M. stultorum*. A synergy affirmed by a positive correlation was observed between seasonal evolutions of glycogen contents of body parts and those of total lipid levels in foot and gonad-visceral mass of *M. stultorum*. Nevertheless, the changes in protein contents of *M. stultorum* did not demonstrate a significant correlation with the biochemical compounds (lipid and glycogen) except for PRORE. The projection of individuals (each sample from each month) on the same factorial plan (1:2) showed that the different cases could be clustered into three groups. It has been demonstrated that are an interactions between groups 1 and 2, which each individuals accumulated high levels of biochemical's compounds in each tissue during winter and spring corresponding to gametogenetic and maturity stages and they are characterized by a high level of PE. The third group represents the animals how depleted their reserve when environmental parameters such as sea water temperature and chlorophyll a increased signaling to spawning period (**Figure 5B**).

Discussion

This study provides new data about the seasonal evolution of the biochemical composition on the surf clam *M. stultorum* from Tunisian coasts. Previous studies on marine invertebrates such as bivalves showed that the changes in their biochemical composition are mostly affected by endogenous factors (the reproductive cycle) and exogenous factors (environmental conditions) [1, 26].

Glycogen is considered as the major energy reserve in bivalves. It may be used concurrently as an energy source for growth and also stored in specific cells as an energetic reserve during gametogenesis process when the energy demands are high [27, 28]. In our study, the glycogen content in all tissues and the whole body of *M. stultorum* was lower during summer corresponding with the spawning phase. This compound increased gradually during winter and during spring and reached a maximum in April coinciding with the maturation of gonads. However, in May, they decreased considerably when 40% of the populations are partially spawned. The seasonal variation of glycogen seems to be related to the reproductive cycle of *M. stultorum*, as mentioned previously by Chetoui *et al*. [19], which was also confirmed by a positive correlation with CI and GSI physiological indices.

Table 3: Correlation matrix of the biochemical parameters and reproductive indices of *Mactra stultorum* and the environmental condition.

	PROFT	PROGV	PROMT	PROAM	PRORE	GLYFT	GLYGV	GLYMT	GLYAM	GLYRE	LIPFT	LIPGV	LIPMT	LIPAM	LIPRE	PROWB	GLYWB	LIPWB	CI	GSI	PE	T (°C)	Chlo a
PROGV	ns																						
PROMT	ns	ns																					
PROAM	ns	ns	ns																				
PRORE	ns	ns	0,85	ns																			
GLYFT	ns	ns	ns	ns	ns																		
GLYGV	ns	ns	ns	ns	ns	0,85																	
GLYMT	ns	ns	ns	ns	0,66	0,86	0,82																
GLYAM	ns	ns	ns	ns	ns	0,87	0,88	0,88															
GLYRE	ns	ns	ns	ns	0,63	0,59	ns	ns	ns														
LIPFT	ns	ns	ns	ns	ns	0,78	0,73	0,69	0,76	ns													
LIPGV	ns	ns	ns	ns	ns	0,61	0,62	0,80	0,77	ns	ns												
LIPMT	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns											
LIPAM	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns										
LIPRE	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns									
PROWB	0,60	0,81	ns	ns	0,81	ns	ns	ns	ns	ns	ns	ns	ns	ns	-0,09								
GLYWB	ns	ns	ns	ns	ns	0,94	0,93	0,93	0,93	0,65	0,75	0,68	ns	ns	-0,06	ns							
LIPWB	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0,83	ns	ns						
CI	ns	ns	ns	ns	ns	0,82	0,74	0,80	0,91	ns	0,70	0,82	ns	ns	-0,41	ns	0,79	ns					
GSI	ns	ns	ns	ns	ns	0,71	0,66	0,77	0,87	ns	ns	0,88	ns	ns	-0,35	ns	0,72	ns	0,97				
PE	ns	ns	0,71	ns	0,61	0,76	0,77	0,80	0,83	ns	ns	0,73	ns	ns	ns	ns	0,83	0.75	0,84	0,85			
T (°C)	ns	ns	ns	ns	ns	-0,69	-0,61	ns	ns	ns	-0,81	ns	ns	-0,72	0,04	ns	ns	ns	ns	ns	ns		
Chlo a	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-0,48	ns	ns	ns	ns	ns	ns	ns	
S	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0,20	ns	ns	ns	ns	ns	ns	0,60	ns

Correlation coefficients statistically significant (p <0.05); ns: not significant (p <0.05); the positive correlation is presented with yellow color and the negative one is presented with red color. PRO: protein, GLY: glycogen, LIP: total lipid, FT : foot , GV: gonad-visceral mass, MT: mantle, AM : adductor muscle, RE: rest, BW: whole body, CI: condition index, GSI: gonado-somatic index, T°C: water temperature, Chlo *a*: chlorophyll *a*, S: salinity.

We suggested that the accumulation of glycogen at the end of the cycle (autumn) serves as energy for the start of a new reproductive cycle in winter and support the essential metabolic maintenance during this season food scarcity. Likewise, during gametogenesis development (in winter) and the maturation phase (in spring), the storage of glycogen can be used as precursors for lipid synthesis needed for the formation of gametes for mollusks [6] and as an immediate available energy source during spawning period [29, 30]. Similar results were recorded for *M. veneriformis* [31]. However, some authors demonstrated that glycogen levels in *M. chinensis* and *R. decussatus* decreased considerably during the sexual maturity process [11, 32].

The biochemical composition of bivalve seems to be affected by many environmental parameters such as temperature, salinity and food accessibility [33]. A negative correlation between glycogen content in *M. stultorum* and the water temperature can be related to the direct or indirect effects of the energy reserves fluctuations (storage and exploitation) [34]. However, the accumulation of glycogen in gonad-visceral mass of *M. stultorum*, especially during March and April, could be explained by its positive correlation with food availability expressed by the peak of the chlorophyll *a* [31]. For that reason, the glycogen content represents a good characteristic of nutritional conditions [35].

Our results showed that protein was the major compound in all organs and whole body of *M. stultorum*. In the gonad-visceral mass of *M. stultorum*, the content of protein was higher during ripening (March and April) and during spawning (July and August). By contrast, this compound in other organs (FT, AM and MT) decreased during the same periods. From another point, the whole body seems to accumulate this organic constituent during the same periods. It should be noted that probably *M. stultorum* used protein in the mantle and foot as a source of energy during the ripening phase and partially the spawned and those of adductor muscles during spawning phase. Furthermore, the glycogen quantity of these muscular organs decreased considerably when gametes are released. Our result can be interpreted by the main role of protein to serve as an energy reserve in adult bivalves in periods of reduced glycogen levels [9, 10] and particularly to support the end of gametogenesis [3, 30]. The negative correlation between chlorophyll *a* and protein level in gonad-visceral mass of *M. stultorum* during autumn and winter suggested that the deficiency of food availability (low levels of chlorophyll *a*) has reduced the protein levels [9]. Unuma *et al*. [32] and Qi *et al*. [36] have suggested that the lower level of protein during the same period was interpreted by its role as a nutrient source for the proliferation and differentiation of cell germ in the gametogenesis process (in particularly during spermatogenesis of *M. chinensis* and *Pseudocentrotus depressus*).

The total lipid in *M. stultorum* gonad-visceral mass was higher than in the other organs, suggesting that GV is the major lipid storage site [31, 32]. Based on the reproductive cycle of *M. stultorum*, the lipid content of the gonad-visceral mass presented a positive correlation with CI and GSI. They begin to accumulate progressively during the earlier and late developmental stages, reached a high level when the gametes mature during the ripening stage (March and April) and finally decreased considerably when the spawning occurred from May to August. It is necessary to mention that the total lipid accumulated in GV has been considered as good indicators of oocyte value and larva viability (1). In this context, it may be admitted as a good index of gonad maturity [34]. Our results are in concordance with those found for *M. chinensis, Ruditapes philippinarum* and *Scapharca broughtonii* [27, 32].

Although mantle and adductor muscles were not really considered as lipid organs storage. In the present study, the lipid reserves stocked in mantle decreased during spawning stage. Nevertheless, those of adductor muscle declined dramatically during the ripening and spawning periods. These decreases indicate that the conversion of lipids accumulated in these tissues could be used to provide the necessary energy for the gametogenesis process of *M. stultorum*. However, the total lipid of foot did not show any changes throughout the year which was affirmed by a positive correlation with CI and a negative correlation with water temperature. It can be suggested that the lipid located in this tissue did not contribute to the energy conversion utilization process of *M. stultorum*. Comparable results have been observed in *M. veneriformis* during sexual maturity and spawning periods [31]. However, dissimilar results were also described by Qiaozhen and Qi, [32] and Qi *et al*. [31]. With regards to the annual changes in total lipid of whole body, it showed significant alterations with seasons.

Bayne, [37] has described two reproductive patterns of marine bivalves based on the relationship between energy storage and gonad development cycles. In a conservative pattern, gametogenesis used the energy stored in various organs in forms of lipids and glycogen. However, in an opportunistic pattern, gametogenesis occurs using the energy supplied by the nutrients when the food in water is abundant. Therefore, we can suggest that *M. stultorum* adopts an intermediate pattern closer to *M. veneriformis* [31]. They used at the beginning of their reproductive cycle during the winter the energy reserves stored previously. Nevertheless, during the spring season, they exploited at the same time the energy stored and energy provided by food availability. Several studies have demonstrated that PE and CI are the main tools for the commercialization of bivalves, which varied seasonally and geographically, depending generally on exogenous and endogenous conditions such as food accessibility and the gametogenic cycle, respectively [11, 38].

The percentage edibility showed significant differences among seasons. It seems to be higher during early summer (08) and during spring (09) which corresponds to the beginning of the gametogenetic cycle. In this period the animals accumulate biochemical's constituents for their gamete development when food in water is available and/ or abundant. Therefore, this index of physiological fitness was positively correlated firstly with accumulation of protein and carbohydrate (glycogen) in all tissues of *M. stultorum;* secondly with the CI and GSI indexes and thistly with lipid contents in GV. While, during late summer, autumn and early winter, it decreased considerably, with the lowest value recorded in August. Some decline in PE coincided with spawning and inactive periods. *M. stultorum* during the last periods has depleted their reserve during the spawning process. Our results are in agreement with those in clams *R. decussatus* and *Paphiamalabarica* [25, 39].

Moreover, CI is considered as a good tool widely used to measure the physiological state and to identify the spawning seasons of bivalves [13]. This indirect method of analysis [40] is necessarily confirmed by histological sectioning and microscopic evaluation of the gonads [41]. Our data of seasonal variation of CI in *M. stultorum* during the period (Jun 2008 to May 2009) was studied and confirmed by the determination of different stages of the gametogenic cycle of *M. stultorum* [19]. According to Chetoui *et al*. [19], CI increased from winter to spring. These seasons represent the start of gametogenic development and ripe periods for both sexes [19]. However, the decrease of CI coincides with spawning and inactive stages [19]. CI was positively correlated with other reproductive index as gonad-somatic index which was specific for the assessment of physiological state of gonad.

In the Mediterranean Sea, mainly in Tunisia, bivalves are considered as a rich food source and a healthy proportionate diet [3, 42]. Since, they are known for their nutritious and essential energy reserves (e.g., protein, vitamins and minerals). In addition, bivalves are the best source of polyunsaturated fatty acids n-3 family (PUFA (n-3)) mainly the two essentials fatty acids EPA and DHA which play crucial roles in human nutrition and health [43].These elements present a dissimilar organization and concentration depending on the bivalve tissues that can be commonly associated with external climate fluctuations. The variation of the nutritional value could be assessed using percentage of edibility and the condition index; however, information about them is crucial for the cultivation and harvesting strategy of bivalves [44]. In other hand, not only the biochemical compounds, PE and CI have influences on the flesh quality during cultivation and harvesting process. The supplement information's about the feeding strategy and the quality of the food in aquaculture are crucial for to keep the good nutritional value of the animals flesh as well for human consumption as for manufacturing animal foodstuff. Consequently, all these data allow adopting a good management of the breeding cost of bivalves.

Conclusion

In conclusion, this study reported, at the first time, the assessment of the biochemical composition in several organs and the whole body of *M. stultorum* in relation to the environmental conditions and the reproductive cycle. A decrease of glycogen levels in all tissues during the spawning periods (from May to August) suggesting it important role as energy in the gametogenesis process. Lipid and protein varied significantly and played an important role as fuel for gametogenesis when stored glycogen was exhausted and to support the basic metabolic maintenance cost in periods of food insufficiency. Additionally, the best nutritive season for *M. stultorum* was noted during the beginning of gametogenic cycle and ripe periods (from February to Jun) associated with the highest contents of the biochemical composition, percentage of edibility and condition index and when the food availability was abundant. However, from July to January, it is not advisable to consume it due to its lowest nutritional value represented by low levels of the organic constituents and the percentage edibility.

Based on these results, we suggest that the harvesting of *M. stultorum* should be concentrated on the period when the seafood is at its highest nutritive value. The percentage of edibility and the condition index are very important tools in physiological studies providing valuable information required for assessing larvae development and promoting successful restocking actions in aquaculture management. Further studies of other aquaculture parameters such as food quality and pathological tests seem to be necessary in order to avoid disease and increase the larva growth and bivalve's quality.

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