

# Storage metabolism in the Pacific oyster (*Crassostrea gigas*) in relation to summer mortalities and reproductive cycle (West Coast of France)

Clothilde Berthelin \*, Kristell Kellner, Michel Mathieu

*Laboratoire de Biologie et Biotechnologies Marines, Université de Caen Basse-Normandie, 14032 Caen Cedex, France*

Received 9 August 1999; received in revised form 3 December 1999; accepted 6 December 1999

## Abstract

We describe seasonal changes in the biochemical composition of digestive gland, adductor muscle and gonad and surrounding mantle area in *Crassostrea gigas* from the Western Atlantic coast of France. Seasonality in histology of storage tissues and glycogen storage capacity in isolated vesicular cells were also studied. Proteins, the main muscle components did not contribute to the gametogenetic effort. Glycogen and lipids were stored in the digestive gland, gonad and surrounding mantle area during the wintering period and the gonad and surrounding mantle area represented the main storage compartment supplying the reproductive effort. Gametogenesis in spring and summer was associated with an increase in lipid and protein contents and took place at the expense of glycogen reserves. Histological study of storage tissue in the gonad led us to define four seasonal stages of storage tissue development. In vitro, glycogen storage capacity in isolated vesicular cells was high from November to March and markedly reduced during gametogenesis, decreasing below detectable levels after spawning. This physiological state should be taken into account with relation to summer mortalities occurring in commercial growing areas. © 2000 Elsevier Science Inc. All rights reserved.

**Keywords:** Bivalve; *Crassostrea gigas*; Gametogenesis; Glycogen; Pacific oyster; Storage tissue; Summer mortalities; Vesicular cells

## 1. Introduction

Significant summer mortalities of Pacific oysters *Crassostrea gigas* have occurred in growing areas of Japan and the United States since the 1960s (Mori, 1979; Perdue et al., 1981). More recently, oyster mortalities have been also noticed in French sea farms. Independent of location, summer mortalities were generally associated with high nutrient levels when water temperature reached 20°C and above and coincided with the

period of sexual ripeness (Lipovsky and Chew, 1972; Beattie et al., 1980; Perdue et al., 1981). The summer period is ordinarily responsible for 10–15% of the mortalities, but high levels of losses reaching up to 80% in some areas have been reported during recent years. Different hypotheses were taken into account to explain these abnormal mortalities. The possible occurrence of pathogens has been investigated. Bacterial infestation of moribund oysters appeared to be a result of a general weakness of the animal rather than a direct cause of mortality (Lipovsky and Chew, 1972). Mortalities associated with herpes-like viruses were also noticed. Elevated temperatures are thought to increase the susceptibility of ani-

\* Corresponding author. Tel.: +33-2-31565474; fax: +33-2-31565346.

E-mail address: kellner@ibba.unicaen.fr (C. Berthelin)

imals to this virus, but viral infections were not always associated with oyster summer mortalities (Renault et al., 1994). The different resistance of oyster strains to the mortality suggests involvement of a genetic factor in these events (Beattie et al., 1980), although the mechanisms of the possible genetic component still remain unknown. The impact of physico-chemical aspects of seawater in culture areas as well as cultural practices were also taken into account (Lodato, 1997). Oysters reared directly on seabed were more affected than oysters maintained on tables. This differential mortality could not, however, be related with quality of sediment or with the quantity of organic matter in the water column and was never correlated with the presence of faecal *Streptococcus* in sediments. Mechanical stress due to handling of oysters during summer is thought to increase the level of mortality. Different studies indicated, finally, a possible correlation between summer mortality and spawning or post-spawning events. At this time, glycogen content in oysters reached its lowest level (Mori et al., 1965; Perdue et al., 1981; Allen and Downing, 1986). This low energetic condition could increase the sensitivity of oysters to different stresses observed at this time of the year (thermal stress, hypoxia, etc.).

Gametogenesis in *C. gigas* occurs on the western Atlantic coast of France from winter to summer resulting in high energetic costs. As in other bivalve species, storage metabolism in oysters is closely associated with reproductive events and represents an important biochemical pathway. Glycogen plays a central role in energetic and metabolic supply of gametogenesis in many bivalves (Gabbott, 1975; Bayne et al., 1982; Ruiz et al., 1992; Mathieu and Lubet, 1993). Whereas biochemical composition and specifically glycogen levels in the whole animal have previously been studied (Walne and Mann, 1975; Robert et al., 1993; Almeida et al., 1997), biochemical mechanisms of glycogen storage and mobilisation in relation to reproductive activity remain poorly documented in comparison with other models like the marine mussel *Mytilus edulis*. In fact, mussel storage cells invading the gonadal area during the sexual resting stage were described extensively (Houtteville, 1974; Pipe, 1987; Lenoir, 1989). Glycogen metabolism of these cells studied in vitro, shows that glycogen storage is closely related to the free circulating glucose concentration all year round (i.e. food supply), whereas glycogen

mobilisation may be subject to regulation by neuroendocrine factors synchronously to reproductive activity (Robbins et al., 1990). In oysters, vesicular cells were found mainly in the labial palps and the gonad and surrounding mantle area (Bargeton, 1942; Mathieu and Lubet, 1993; Berthelin et al., 2000). The mobilisation of glycogen reserves in these storage cells may be different from that in the mussel since their reproductive periods are different: in oysters of the North West European coasts, gametogenesis occurs from winter to summer with a spawning period in July whereas it takes place in the winter period with a spawning event in early spring for mussels in the same location.

The aim of this study is to provide further information concerning glycogen metabolism in *C. gigas* with respect to the energetic costs of gametogenesis. The biochemical composition was studied in different organs of the oyster and histological evolution of the gonad and surrounding mantle area was investigated over a period of 1 year. Annual changes in glycogen storage capacity in isolated vesicular cells exposed to low and high levels of free circulating glucose were investigated in vitro. The possible involvement of glycogen metabolism in the summer mortalities observed in French oyster populations over several years is also discussed.

## 2. Materials and methods

### 2.1. Animals

Oysters provided by IFREMER were obtained from an oyster farm (La Tremblade, Marennes Oléron, France). Samples of 60 oysters from homogeneous pools of commercial size animals (3 years old) were collected monthly during the year 1997. Thirty animals were used for the biochemical analysis, six for the histological study and 24 to carry out the in vitro bioassays.

### 2.2. Biochemical analysis

Lipid, protein and glycogen contents were determined in freeze-dried samples of adductor muscle, digestive gland and gonad and surrounding mantle area. Each sample consisted of a pool of total tissues from five animals and each data point was the mean of six replicates. Protein determina-

tion was based on the binding of Coomassie Brilliant Blue G-250 to protein (Bradford, 1976), using bovine serum albumin as a reference. Lipids were extracted and purified according to the protocol of Bligh and Dyer (1959) and the analytical procedure was from Marsh and Weinstein (1966) with tripalmitate as a standard. Glycogen was extracted according to Pinheiro and Gomes (1994). Briefly, after protein elimination by trichloroacetic acid, glycogen was precipitated with absolute ethanol, dissolved in water before being quantified using the procedure of Dubois et al. (1956). Purified oyster glycogen was used as a standard. Biochemical results were expressed as percentage of the total organ dry weight.

### 2.3. Histology

Transverse sections were cut from an oyster at the vicinity of the gills–palps junction. Transverse sections from six oysters were fixed in Bouin's fluid (75% picric acid, 20% formaldehyde, 5% acetic acid). Pieces were then dehydrated in serial dilutions of ethanol, transferred to butanol and embedded in paraffin wax. Seven micrometre sections were stained according to the trichrome method of Prenant–Gabe Gabe (1968).

### 2.4. Dissociation of gonad and surrounding mantle area

The protocol of dissociation was derived from Lenoir (1989) for the study of the mussel *M. edulis* glycogen cells.

Gonad and surrounding mantle area were dissected and minced with sterile scissors in 30 ml of sterile culture medium (Leibovitz L15; NaCl 340 mM, KCl 50 mM, Hepes 20 mM, pH 7.4, 1100 mOsm, filter sterilised (0.22 µm)) containing collagenase (0.1% w/v). The sample was allowed to dissociate for 30 min at 15°C with gentle stirring before filtration through a 100-µm nylon mesh. Retained material was dissociated again for a further 30 min in fresh enzyme solution and then filtered. The two filtrates of dissociated cells were pooled, centrifuged (80 × g, 30 min, 15°C) and fresh medium was added to the pellet to wash off the enzyme. After subsequent centrifugation (80 × g, 30 min, 15°C), cells were resuspended in 5 ml of fresh culture medium.

### 2.5. Separation of storage cells

Percoll discontinuous density gradient was performed with dilutions in culture medium (1100 mOsm; pH 7.4; 10, 20, 30, 40, 50, 60% of Percoll). The density of each Percoll fraction was determined with a densitometer. Cell suspension was carefully layered on the density gradient and this gradient was centrifuged (400 × g, 30 min, 15°C). The 20–30% interface fraction (1.043 g ml<sup>-1</sup>), containing storage cells, was saved. Cells were finally rinsed in 30 ml of fresh medium for Percoll elimination and then collected by centrifugation (80 × g, 30 min, 15°C). The resulting cell suspension was adjusted to a final concentration of 5 × 10<sup>6</sup> cell ml<sup>-1</sup>. Cellular viability was estimated with a MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) reduction assay (Mosmann, 1983; Coulon, 1993).

### 2.6. In vitro study of [U-<sup>14</sup>C] glucose incorporation into glycogen

Five hundred-µm aliquots of the storage cell suspension in culture medium were incubated in 5 ml sterile tubes with 50 µl [U-<sup>14</sup>C] glucose (0.5 µCi) (ICN) and 50 µl of unlabelled D-glucose at a final concentration of 0.5 or 1.5 mM. Both the D-glucose and the [U-<sup>14</sup>C] glucose solutions were prepared in Leibovitz medium.

After a 7-h incubation at 15°C, the reaction was stopped by centrifugation (8000 × g, 10 min). Three hundred µm of the supernatant were transferred to a 5 ml tube containing 10 mg unlabelled oyster glycogen; then 4 ml absolute ethanol were added for glycogen precipitation. After overnight precipitation, glycogen was collected by centrifugation (2500 × g, 10 min) and the pellet was washed three times with absolute ethanol containing D-glucose (0.1 M). Glycogen pellets were dried and resuspended in 500 µl distilled water. Radioactivity was determined with a scintillation counter on 200-µl aliquots of sample diluted in 4.5 ml scintillation fluid. Controls were stopped immediately after [U-<sup>14</sup>C] glucose addition and controls without cells or radioactive labelled glucose were also performed.

### 2.7. Data analysis

Results were expressed as mean ± S.D. Each value is the mean of six replicates. A non para-

metric test (Kruskal–Wallis test) followed by a multiple comparison test (S.N.K.) was also applied to determine the significance of differences between samples (Scherrer, 1984).

### 3. Results

#### 3.1. Seasonal changes of the total dry weight per organ

Total dry weight of three organs was measured during one complete year (Fig. 1). Muscle and digestive gland dry weight showed low but regular increase leading to a doubling of the dry weight in December compared to the previous January. In contrast, gonad and surrounding mantle area dry weight increased significantly in July ( $P < 0.05$ ) during the maturing period and then decreased after spawning ( $P < 0.05$ ). Except for this summer peak, this fraction showed a continuous progress in weight over the year.

#### 3.2. Seasonal biochemical changes in adductor muscle

Protein, lipid and glycogen contents were quantified in the adductor muscle and results are presented in Fig. 2a. This organ is mainly constituted by protein (up to 85% in April) (Fig. 2a1). The protein content presented a 20% increase in early spring ( $P < 0.05$ ) and then declined gradually down to its basal level in December (about 60%). In adductor muscle, lipid content represented a variable portion of the total dry weight throughout the year (5–30%) with an increase in autumn ( $P < 0.05$ ) (Fig. 2a2). Similarly, glycogen

content always remained at a very low level ( $P < 0.05$ ) (Fig. 2a3).

#### 3.3. Seasonal biochemical changes in digestive gland

Biochemical composition of the digestive gland is presented in Fig. 2b. Protein content of this organ varied between 35 and 55%. A significant increase ( $P < 0.05$ ) was observed in April, and the protein content remained at this highest level until August ( $P < 0.05$ ) (Fig. 2b1). Lipid content was about 30% from January to August and then increased significantly in September (65%) ( $P < 0.05$ ) (Fig. 2b2). Glycogen content represented about 5% of the total dry weight of this organ from January to July (Fig. 2b3). This component subsequently increased markedly in summer ( $P < 0.05$ ) reaching 15% of the total dry weight in autumn and then declined significantly to 0.5% in December.

#### 3.4. Seasonal biochemical changes in gonad and surrounding mantle area

Results concerning the biochemical composition of this compartment are presented in Fig. 2c. Protein represented 30% of the dry weight from January to March (Fig. 2c1). This percentage increased significantly in April ( $P < 0.05$ ), reached its maximal value (65%) in June and then declined to an approximately constant level from July to December ( $P < 0.05$ ). A slight summer increase was observed in lipid content from April to July ( $P < 0.05$ ) concomitant with the final stage of gonad ripening (Fig. 2c2). An additional major lipid peak was noticed in September ( $P < 0.05$ ). Glycogen content increased significantly during autumn and early winter (Fig. 2c3). At this time, the glycogen content in the gonadal area and surrounding mantle reached about 30% of the dry weight, whereas the lowest value observed in July represented only 3% of the dry weight.

#### 3.5. Histology

Storage cells were usually clear, polygonal in shape and represented easily identified areas interspersed by numerous lacunae containing circulatory cells. Gonadal tubule ducts were located between mantle and gonad, delineated by a thin epithelial layer. Both the storage cells and the

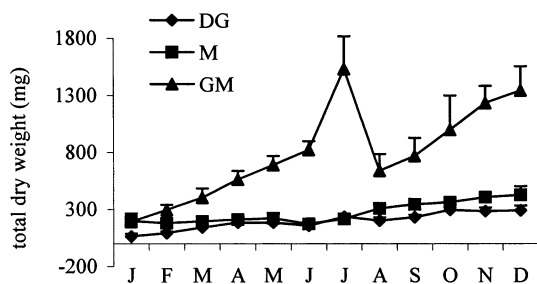
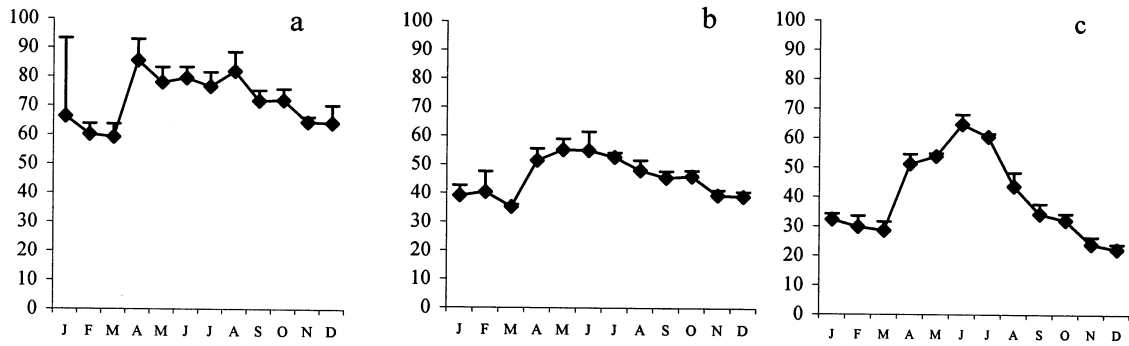
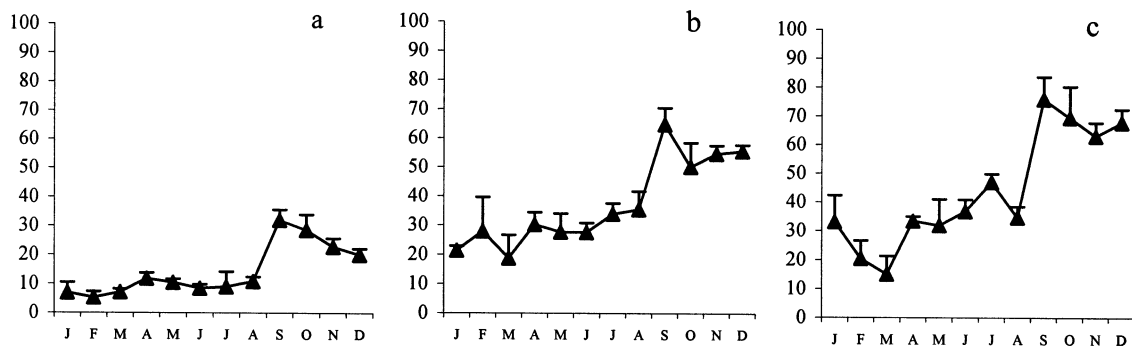


Fig. 1. Seasonal variation of the total dry weight (mg per organ) in digestive gland (DG), adductor muscle (M) and gonadal area and surrounding mantle (GM). Data points represent the mean ( $\pm$  S.D.) of six determinations.

### 1. Protein (% of the DW)



### 2. Lipid (% of the DW)



### 3. Glycogen (% of the DW)

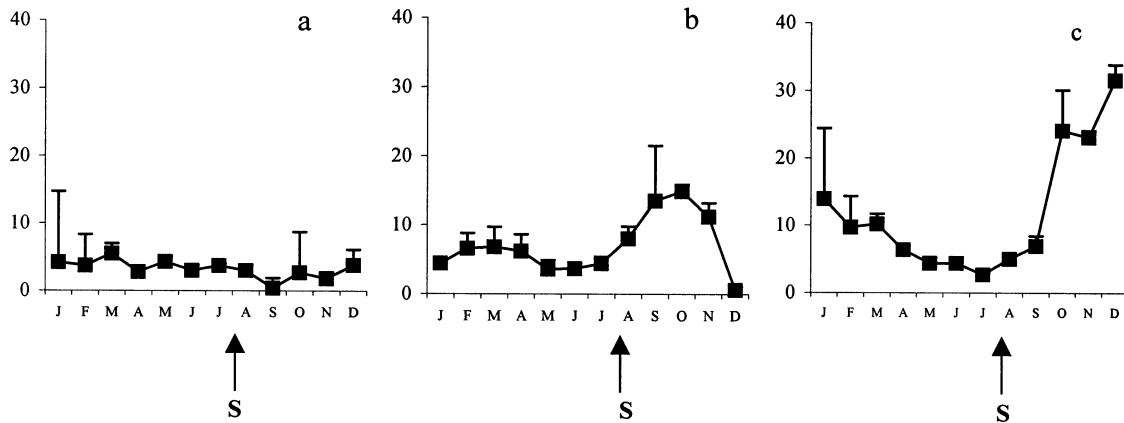


Fig. 2. Seasonal biochemical variation in protein (1), lipid (2) and glycogen (3) content (% of the dry weight (DW)): (a) in adductor muscle, (b) in digestive gland, (c) in gonad and surrounding mantle area. S: time of spawning.

gonadal tubules in the gonad showed large systematic variations throughout the year. Therefore, storage tissue evolution can be divided into different histological stages. This evolution followed the same pattern for both female and male animals.

### 3.5.1. Stage A

In the typical post-spawn oysters (August and

September), gonadal tubules were largely collapsed, containing only primordial germinal cells. Genital ducts were extensively dilated along the peripheral ciliated margin and sometimes contained residual ripe gametes. The gonad appeared empty, interfollicular connective tissue had been invaded by numerous hemocytes and few macrophages. Storage tissue initiated its restruc-

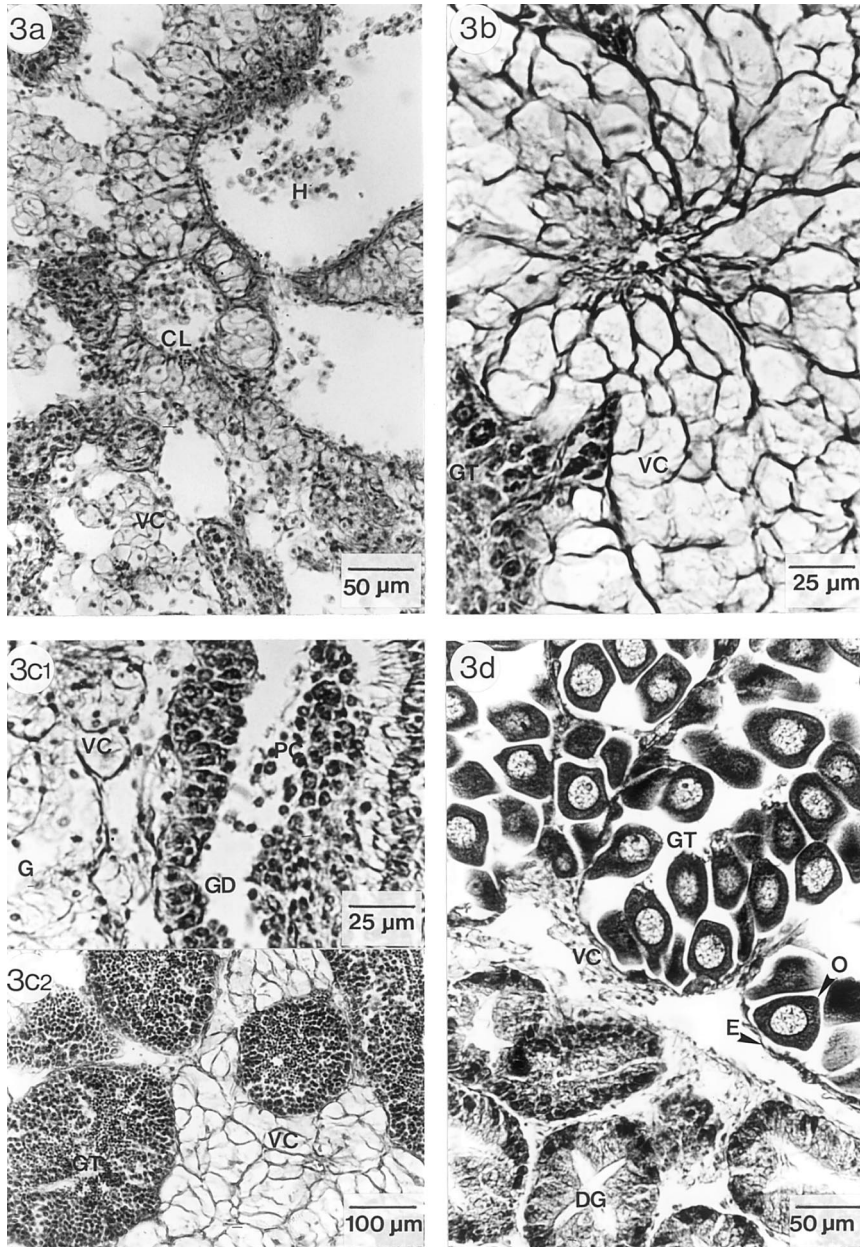


Fig. 3. Histology: (a) stage A of storage tissue, CL: circulatory lacunae, H: hemocytes, VC: vesicular cells; (b) stage B of storage tissue, GT: gonadal tubules, VC: vesicular cells; (c) stage C of storage tissue, (1) G: gaps, GD: genital ducts, PC: phagocytic cells, VC: vesicular cells, (2) GT: male gonadal tubules, VC: vesicular cells; (d) stage D of storage tissue, DG: digestive gland, E: epithelium, GT: female gonadal tubules, O: oocytes, VC: vesicular cells.

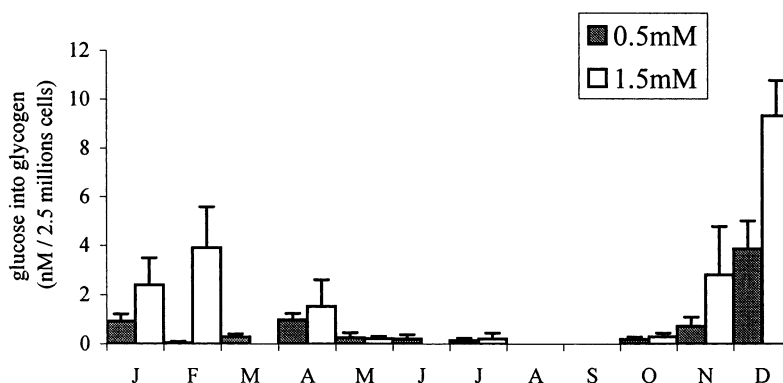


Fig. 4. Seasonal variation of glycogen storage capacity in isolated storage cells. [ $U^{14}C$ ] glucose incorporation into glycogen. Incubation time is 7 h, exogenous glucose concentration is 0.5 or 1.5 mM. Results expressed as nM of glucose incorporated per  $2.5 \times 10^6$  cells ( $\pm$  S.D.),  $n = 6$ .

turing: vesicular cells, located preferentially around the circulatory lacunae, showed typical rosette shapes (Fig. 3a). Few other storage cells were arranged in clusters of stacked elements (Fig. 3a). At the same time, storage tissue was more or less restructured. In all cases, replenishment occurred initially on the digestive area side and progressed throughout the gonad towards genital ducts.

### 3.5.2. Stage B

This stage was characteristic of the wintering oysters (October to February). Vesicular cells consisted of dense storage tissue showing a distinctive paved pattern (Fig. 3b). Although gonadal mitosis started again in November, gonadal tubules remained reduced from October to February and the main part of the gonad appeared full of large storage cells. Circulating hemocytes were restricted to the hemolymph lacunae.

### 3.5.3. Stage C

This stage, observed from March to April, was associated with the regression of storage tissue in gonad (Fig. 3c). Depending on the individuals, different storage tissue patterns were observed. In some cases, storage tissue showed large gaps disrupting the pavement of the cells (Fig. 3c1). In other cases, extension of gonadal tubules appeared concomitantly to the storage tissue regression and few gaps were found (Fig. 3c2). This storage tissue dismantling progressed from the genital ducts to the digestive area as

gametogenesis progressed. At the end of this stage, storage tissue was reduced to a thin belt of cells around the digestive area.

### 3.5.4. Stage D

Storage tissue of the pre-spawned and spawned oysters (May to July) was reduced to one or two cell layers surrounding circulatory lacunae. The whole gonad is invaded from genital ducts to digestive area by ripe gametes (Fig. 3d). The epithelium of gonadal tubules appeared thin and disrupted in spawning oysters.

### 3.6. Seasonal evolution of glycogen storage capacity in isolated storage cells

Glucose incorporation into glycogen was evaluated in vesicular cell suspensions at two extracellular glucose concentrations (0.5 and 1.5 mM) in order to mimic physiological conditions under a low or high nutrient supply. The rate of glucose incorporation expressed in nM per 2.5 millions of cells was evaluated monthly over a year (Fig. 4). The rate was very low from March to October with undetectable values during August and September just after spawning. In November, glucose incorporation into glycogen increased gradually (significant at 1.5 mM,  $P < 0.05$ ) with a winter peak in December ( $P < 0.05$  at 0.5 and 1.5 mM) and then decreased from January to March ( $P < 0.05$ ). From November to April, glucose incorporation into vesicular cell glycogen was found to be higher as exogenous glucose concentration increased.

#### 4. Discussion

This study gives the average seasonal changes in biochemical composition of muscle, digestive gland and gonad and surrounding mantle area in the oyster *C. gigas* of the Western Atlantic French coast in 1997. The analysis of body parts is often more instructive than analysis of the whole animal when studying biochemical composition in relation to growth and reproductive cycles. In fact, measurement of the total dry weight of the different organs shows that they do not experience the same growth rate. Muscle and digestive gland increase linearly, doubling over 1 year, whereas gonad and surrounding mantle area shows an important increase (six fold initial weight in December), with a summer peak reflecting sexual ripeness and spawning. For marine bivalves, reproductive activity is supported by mobilisation of energetic reserves stored at different times of the year depending on the species and its localisation. Storage form of these reserves varies for one group to another. In Pectinidae, proteins are stored in unspecialised cells of the adductor muscle (Barber and Blake, 1981; Epp et al., 1988). In Veneridae, reserves can be retained in unspecialised as well as in specialised storage cells (Mathieu and Lubet, 1993). In other groups, like Mytilidae, reserves are retained only in specialised cells: adipogranular cells contain lipids, proteins and glycogen (Houtteville, 1974; Pipe, 1987) whereas glycogen cells contain mainly glycogen reserves (Lenoir, 1989; Mathieu and Lubet, 1993).

For *C. gigas*, biochemical composition of muscle shows that proteins are the major component throughout the year (60–80% of the dry weight), whereas glycogen and lipid remain always at a low level (less than 6% for glycogen and about 10–20% for lipids). Seasonal increase observed in the protein content in spring (20% increase in April) is concomitant with a period of intense energetic demand in the gonad due to gamete development. It could be concluded that muscle does not represent a storage compartment supplying the energetic cost of reproduction, in agreement with previous data concerning other oyster species like *Ostrea puelchana* (Fernandez Castro and de Vido de Mattio, 1987). Lipid content of the digestive gland increases regularly over the year with a peak in September. This marked increase is also observed in the gonad and surrounding mantle area. In the same way, glycogen

content presents the same autumnal increase in both tissues. These data show that these organs are involved in glycogen storage during autumn and early winter allowing accumulation and release of glycogen as previously observed in *M. edulis* (De Zwaan and Zandee, 1972). In molluscs, however, the digestive gland is implicated not only in nutrient storage but also in the transfer of assimilated food to the body tissues (Sastry and Blake, 1971; Vassalo, 1973). In *C. gigas* we observed that glycogen content in the digestive gland never exceeds 15% of the total dry weight leading to the conclusion that this organ has a transitory and minor storage role. Moreover, histological study of this organ indicated the existence of a peripheral layer of vesicular cells all around the digestive area (Berthelin et al., 2000). Consequently, dissected digestive gland is always contaminated by storage cells leading to overestimation of the glycogen content of this fraction. In bivalves, lipid reserves are considered to be used principally in gametogenesis and lost during spawning (Gabbott, 1983). In our study, the summer peak of lipids in gonad and surrounding mantle area is masked by the very high protein content at this time, because of the expression of the results in percent of dry weight. An additional lipid increase in September concomitant with storage of glycogen in this compartment leads to the conclusion that lipid reserves are probably built up in early winter. Seasonal changes of the glycogen stored in gonad and surrounding mantle area is inversely related to the pattern of gonadal tubule development with 3% of glycogen in July at the spawning time and 32% in December while gonad is restricted to reduced tubules constituted by protogonia and few gonia initiating a new gametogenetic cycle. These results are in agreement with previous investigations of glycogen seasonal changes in whole oysters from different locations. Perdue and Erickson (1984), have studied two different populations of *C. gigas* that exhibit a maximal glycogen level (about 33%) in April just before gonad volume increase. Glycogen level is minimal for these two populations in early August concomitant with the spawning event and remains at about 6% of the total dry weight. Ruiz et al. (1992) have confirmed these data with their study of a Spanish *C. gigas* population that exhibits glycogen accumulation followed by a period of gametogenesis. Glycogen as well as stored lipids constitute a wintering form



of energetic reserves in oyster. Protein is the major structural material of the gonad and surrounding mantle area during gametogenetic development. Different authors suggested that proteins could serve as an energy reserve supporting the end of gametogenesis (Gabbott and Bayne, 1973; Barber and Blake, 1981; Ruiz et al., 1992). In this study, however, the protein changes in gonad and surrounding mantle area is closely related to gonad maturation and may therefore reflect protein accumulation in oocytes, in addition to protein contribution to energetic maintenance in periods of reduced glycogen levels.

The histological study of storage cells in the gonad led us to define four histological stages of storage tissue development in this compartment. Stage A corresponds to the structuring phase of the storage tissue; stage B is associated with a dense and structured storage tissue; stage C is characterised by the regression of the storage tissue and stage D is related to the completely dismantled storage tissue. The course of these four stages of development is strictly related to the gametogenetic events. Storage tissue development is maximal (stage B) from October to February during the sexual resting stage and early gametogenesis events. In contrast, regressed storage tissue (stage D) is related to the ripe gonad from May to July in pre-spawned and spawned oysters. These results are in agreement with biochemical data especially concerning glycogen level in this compartment. Even if the storage period is totally different from those observed in the mussel *M. edulis* from the Atlantic coasts, it must be noticed that the storage tissue cycle in relation to gametogenesis is similar in these two species. Indeed in mussels, storage tissue also paved all the gonadal area during the sexual resting stage in spring and summer and it is reduced to scarce islets in ripe animals during the wintering period (Lubet et al., 1976; Bayne et al., 1982; Pipe, 1987).

The in vitro approach developed in this study leads to an evaluation of the storage capacity of vesicular cells over an entire sexual cycle in *C. gigas*. Glucose incorporation into glycogen was found to be high from November to March and markedly reduced over the gametogenesis course with undetectable values in August and September in the cells obtained from post-spawn oysters. This annual pattern of glucose incorporation into storage cell glycogen must be related to the glycogen level in storage cells of gonad and surround-

ing mantle area and to histological observations of these cells. During sexual ripeness, storage cells are scarce, moreover their metabolism is turned towards glycogen mobilisation, leading to very low glycogen levels at this period. By contrast, in wintering oysters, whereas gonadic tubules are reduced in size, storage cells are numerous and their storage capacity is maximal leading to an optimal glycogen storage function. In addition, in *M. edulis*, Zaba and Davies (1981) have studied the seasonal changes in utilisation of labelled glucose in a mantle tissue slice preparation. In their work, the rate of glucose utilisation increases in the spring and summer during the sexual resting stage of mussels and declines in the winter. In mussels, glucose incorporation into glycogen appears directly dependent on the free circulating glucose concentration all year round. This is not the case in oysters, where maximal glycogen storage capacity occurs in winter. These results suggest that storage function in oysters is probably subject to endocrine regulation.

In oysters, glycogen synthesis is closely related to extracellular glucose concentration. The same results were previously obtained in *M. edulis* mantle tissue slices (Gabbott et al., 1979; Whittle and Gabbott, 1986) and in mantle organ cultures (Whittle et al., 1983). These authors showed that increased glucose concentration in the external medium stimulates glycogen synthase by interconversion of the D and I kinetic forms.

The extrapolation of in vitro results presented in this paper to the whole animal remains, of course, difficult since variations in temperature, salinity and substrate concentration in natural conditions must be taken into account. The in vitro results provide, however, an assessment of the way in which glycogen metabolism of this cell type is turned to storage or mobilisation according to the season. Pre-spawned and spawned oysters have depleted glycogen content, storage tissue reduced to scarce islets in the gonadal area and an impossibility of glycogen storage in the vesicular cells. Moreover, lipid and glycogen contents of the digestive gland are low at this time of the year. This situation may not reflect a low level of food in seawater because of phytoplankton abundance in summer but the extensive energetic demand during gametogenesis. Not only is the ingested food consumed, but also the stored reserves are mobilised. Therefore, the animals are in a fragile condition at this time with regard to the

energetic cost due to the spawning and post-spawning events. Results presented in this paper are in agreement with the observations of Mori et al. (1965) concerning oyster summer mortality. They pointed out that oysters in areas of low mortality exhibited relatively high levels of glycogen and less extensive gonadal development, while oysters from high mortality areas exhibited extremely low glycogen levels and more extensive gonadal development. These results were supported by Perdue et al. (1981), who reported that mortalities are concomitant with the post-spawning period while glycogen level in oyster reaches its lowest level. Results from this study show that there was no relationship between absolute levels of carbohydrate and either high or low mortality. Perdue et al. (1981) hypothesise, however, that the timing of mortality coincides with a change in carbohydrate metabolism to a storage phase.

### Acknowledgements

This project is sustained by European Union (DG XIV) and C. Berthelin by a grant from FEDER and SMEL (Conseil Général de la Manche). The authors are grateful to J. Barbier for her technical assistance in the preparation of specimens for microscopy, to C. Costil for essential advice on all the statistical aspects of this study and to I. Probert for his expert linguistic guidance.

### References

- Allen, S.K., Downing, S.L., 1986. Performance of triploid pacific oysters *Crassostrea gigas* (Thunberg). I. Survival, growth, glycogen content and sexual maturation in yearlings. *J. Exp. Mar. Biol. Ecol.* 102, 197–208.
- Almeida, M.J., Machado, J., Coimbra, J., 1997. Growth and biochemical composition of *Crassostrea gigas* (Thunberg) at three fishfarm earthen ponds. *J. Shellfish Res.* 16 (2), 455–462.
- Barber, B.J., Blake, N.B., 1981. Energy and utilisation in relation to gametogenesis in *Argopecten irradians concentricus* (Say). *J. Exp. Mar. Biol. Ecol.* 52, 121–134.
- Bargeton, M., 1942. Les variations saisonnières du tissu conjonctif vésiculeux de l'huître. *Bull. Biol. Fr. Belg.* 76, 175–191.
- Bayne, B.L., Bubel, A., Gabbott, P.A., Livingstone, D.R., Lowe, D.M., Moore, M.N., 1982. Glycogen utilisation and gametogenesis in: *Mytilus edulis* (L.). *Mar. Biol. Lett.* 3, 89–105.
- Beattie, J.H., Chew, K.K., Hershberger, W.K., 1980. Differential survival of selected strains of Pacific oysters *Crassostrea gigas* during summer mortality. *Proc. Nat. Shellfish. Assoc.* 70, 184–189.
- Berthelin, C., Kellner, K., Mathieu, M., 2000. Isolation and characterisation of storage cells of the Pacific oyster *Crassostrea gigas*, *Mar. Biotech.*, submitted for publication.
- Bligh, E.G., Dyer, W.F., 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 47, 911–917.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of the protein–dye binding. *Anal. Biochem.* 72, 248–254.
- Coulon, I., 1993. Mise au point d'un système contrôlé de culture de cellules de coeur d'huître *Crassostrea gigas*. Application au test de la toxicité des produits chimiques en milieu aquatique. Thèse de doctorat es Sciences. Institut National Agronomique Paris-Grignon.
- De Zwaan, A., Zandee, D.I., 1972. Body distribution and seasonal changes in the glycogen content of the common sea mussel *Mytilus edulis*. *Comp. Biochem. Physiol.* 43A, 53–58.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28, 350–356.
- Epp, J., Bricelj, V.M., Malouf, R.E., 1988. Seasonal partitioning and utilisation of energy reserves in two age classes of the bay scallop *Argopecten irradians irradians* (Lamarck). *J. Exp. Mar. Biol. Ecol.* 121, 113–136.
- Fernandez Castro, N., de Vido de Mattio, N., 1987. Biochemical composition, condition index, and energy value of *Ostrea puelchana* (D'Orbigny): relationships with the reproductive cycle. *J. Exp. Mar. Biol. Ecol.* 108, 113–126.
- Gabbott, P.A., Bayne, B.L., 1973. Biochemical effects of temperature and nutritive stress on *Mytilus edulis* (L.). *J. Mar. Biol. Assoc. U.K.* 53, 269–286.
- Gabbott, P.A., Cook, P.A., Whittle, M.A., 1979. Seasonal changes in glycogen synthetase activity in the mantle tissue of the mussel *Mytilus edulis* (L.): Regulation by tissue glucose. *Biochem. Soc. Trans.* 7, 896–898.
- Gabbott, P.A., 1975. Storage cycle in marine bivalve molluscs: a hypothesis concerning the relationship between glycogen metabolism and gametogenesis. In: Barnes, H. (Ed.), *Proceedings of the 9th European Marine Biology Symposium*. Aberdeen University Press, pp. 191–211.

- Gabbott, P.A., 1983. Developmental and seasonal metabolic activities in marine molluscs. In: Hochachka, P.W. (Ed.), *The Mollusca*, vol. 2. Academic Press, New York, pp. 165–217.
- Gabe, M., 1968. *Techniques histologiques*. Masson et C<sup>ie</sup>, Paris-VI.
- Houtteville, P., 1974. Contribution à l'étude cytologique et expérimentale du cycle annuel du tissu de réserve du manteau de *Mytilus edulis* (L.). Thèse de 3<sup>ème</sup> cycle, Université de Caen.
- Lenoir, F. (1989) Mise au point de techniques de dissociation, de purification et de culture cellulaires chez la moule *Mytilus edulis* (L.). Application à l'étude des régulations du métabolisme du glycogène dans les cellules à glycogène (= cellules vésiculeuses). Thèse de doctorat ès sciences Université de Caen.
- Lipovsky, V., Chew, K.K., 1972. Mortality of Pacific oyster *Crassostrea gigas*: the influence of temperature and enriched seawater on oyster survival. *Proc. Nat. Shellfish. Assoc.* 62, 72–82.
- Lodato, M.I., 1997. Mortalités estivales de l'huître creuse *Crassostrea gigas* sur les bancs ostréicoles de Perquis et Ronce (Bassin de Marennes-Oléron): étude des pratiques culturales et des caractéristiques biologiques et spatiales des élevages. Thèse de doctoral d'Etat de l'Ecole Nationale Vétérinaire de Nantes.
- Lubet, P., Herlin, P., Mathieu, M., Collin, F., 1976. Tissu de réserve et cycle sexuel chez les Lamellibranches. *Haliotis* 7, 59–62.
- Marsh, J.B., Weinstein, D.B., 1966. Simple charring method for determination of lipids. *J. Lipid Res.* 7, 574–576.
- Mathieu, M., Lubet, P., 1993. Storage tissue metabolism and reproduction in marine bivalves — a brief review. *Inv. Repr. Dev.* 23 (2–3), 123–129.
- Mori, K., Tamate, H., Imai, T., Itikawa, O., 1965. Studies on the mass mortality of the oyster in Matsushima Bay. V. Changes in the metabolism of lipids and glycogen of the oyster during the stages of sexual maturation and spawning. *Bull. Tohoku Reg. Fish. Res. Lab.* 25, 66–68 In Japanese with English summary.
- Mori, K., 1979. Effects of artificial eutrophication on the metabolism of the Japanese oyster *Crassostrea gigas*. *Mar. Biol.* 53, 361–369.
- Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and toxicity assays. *J. Immunol. Methods* 65, 55–63.
- Perdue, J., Erickson, G., 1984. A comparison of the gametogenetic cycle between the Pacific oyster *Crassostrea gigas* and the suminoe oyster *Crassostrea rivularis* in Washington state. *Aquaculture* 37, 231–237.
- Perdue, J., Beattie, J.H., Chew, K.K., 1981. Some relationships between gametogenetic cycle and summer mortality phenomenon in the Pacific oyster *Crassostrea gigas* in the Washington state. *J. Shell Res.* 1, 9–16.
- Pinheiro, J., Gomes, E.M., 1994. A method for glycogen determination in molluscs. *Arq. Biol. Tecnol.* 37 (3), 569–576.
- Pipe, R.K., 1987. Ultrastructural and cytochemical study on interactions between nutrient storage cells and gametogenesis in the mussel *Mytilus edulis*. *Mar. Biol.* 96, 519–528.
- Renault, T., Le Deuff, R.M., Cochenne, N., Maffart, P., 1994. Herpesviruses associated with mortalities among Pacific oyster *Crassostrea gigas* in France — comparative study. *Rev. Med. Vet.* 145 (10), 735–742.
- Robbins, I., Lenoir, F., Mathieu, M., 1990. A putative neuroendocrine factor that stimulates glycogen mobilisation in isolated glycogen cells from the marine mussel *Mytilus edulis*. *Gen. Comp. Endocrinol.* 79, 123–129.
- Robert, R., Trut, G., Borel, M., Maurer, D., 1993. Growth, fatness and gross biochemical composition of the Japanese oyster *Crassostrea gigas* in stanway cylinders in the bay of Arcachon, France. *Aquaculture* 110, 249–261.
- Ruiz, C., Abad, M., Sedano, F., Garcia-Martin, L.O., Sanchez Lopez, J.L., 1992. Influence of seasonal environmental changes on the gamete production and biochemical composition of *Crassostrea gigas* (Thunberg) in suspended culture in El Grove, Galicia, Spain. *J. Exp. Mar. Biol. Ecol.* 155, 249–262.
- Sastry, A.N., Blake, N.J., 1971. Regulation of gonad development in the bay scallop, *Aequipecten irradians* (Lamarck). *Biol. Bull. Mar. Biol. Lab. Woods Hole.* 140, 274–283.
- Scherrer, B., 1984. *Biostatistiques*. Gaëtan Morin Canada, Quebec, p. 850.
- Vassallo, M.T., 1973. Lipid storage and transfer in the scallop *Chlamys hericia* (Gould). *Comp. Biochem. Physiol.* 44A, 1169–1175.
- Walne, P.R., Mann, R., 1975. Growth and biochemical composition in *Ostrea edulis* and *Crassostrea gigas*. In: Barnes, H. (Ed.), *Proceedings of the 9th European Marine Biology Symposium*. Aberdeen University Press, pp. 587–607.
- Whittle, M.A., Gabbott, P.A., 1986. Glycogen synthetase in the sea mussel *Mytilus edulis* (L.). II. Regulation by glucose in a mantle tissue slice preparation. *Comp. Biochem. Physiol.* 83B, 209–214.
- Whittle, M.A., Mathieu, M., Gabbott, P.A., Lubet, P., 1983. The effect of glucose and neuroendocrine factors on the activity ratio of glycogen synthetase in organ cultures of the mantle of *Mytilus edulis*. In: Lever, J., Boer, H.H. (Eds.), *Molluscan Neuroendocrinology*. North-Holland, Amsterdam, p. 183.
- Zaba, B.N., Davies, J.I., 1981. Carbohydrate metabolism in isolated mantle tissue of *Mytilus edulis* (L.). Isotopic studies on the activities of the Embden-Meyerhof and pentose phosphate pathways. *Mol. Physiol.* 1, 97–112.