J. Mar. Biol. Ass. U.K. (2001), **81**, 663–671
Printed in the United Kingdom

Seasonal digestive gland dynamics of the scallop *Pecten maximus* in the Bay of Brest (France)

Gaël Le Pennec*‡, Marcel Le Pennec* and Peter G. Beninger†

*UMR CNRS 6539, Institut Universitaire Européen de la Mer, 29280–Plouzané, France. †Laboratoire de Biologie Marine, ISOMer, Faculté des Sciences, BP 92208.44322–Nantes Cedex 3, France. ‡E-mail: Gael.Le-Pennec@univ-brest.fr

Cytological and biochemical changes in the digestive gland of *Pecten maximus* throughout a one-year period were investigated in the Bay of Brest (France) relative to lipid storage and glycogen and α-amylase concentrations in tubuloacinar terminations. Seasonal variations in both cell structure and biochemical composition were observed. These changes were independent of the tidal cycle, but correspond to phytoplankton abundance. From November to January, cells of digestive acini were hypertrophied due to the high lipid storage. From February to May, these reserves were markedly reduced, cell size decreased, and the acinar lumen was clearly apparent. From May to September, the RNAs transcribing for α-amylase increased, and a correlation was found between digestion events and lipid storage in acinar cells. The relationships between metabolite transfers from digestive gland to gonad and other tissues are described. Distinct lipid storage sites appear to be associated with maintenance energy and acute demand energy, such as gametogenesis when adductor muscle reserves have been depleted. The digestive gland may thus function as a relay organ during periods of energetic stress, notably during the first gametogenesis in February and March and for shell growth in early spring.

INTRODUCTION

The bivalve digestive gland plays an essential role in digestion/assimilation and detoxification functions (Livingstone & Farrar, 1984; Livingstone, 1985, 1998; Stegeman, 1985; Henry, 1987; Henry et al., 1991; Le Pennec et al., 1991; Livingstone & Pipe, 1992; Yawetz et al., 1992; Wootton et al., 1994; Fitzpatrick et al., 1995). This organ is particularly dynamic, displaying changes in cell types during digestion stages (Henry, 1987) as well as morphofunctional modifications of tubules relative to environmental factors and digestion stages (Morton, 1956; McQuinston, 1969; Mathers, 1973, 1976; Mathers et al., 1979; Robinson & Langton, 1980; Robinson et al., 1981; Robinson, 1983). In terms of the energy budget of bivalves, the digestive gland is involved in the storage of lipids and, to a lesser extent, glycogen. These substances constitute energy reserves, particularly for gametogenesis (Stanley, 1967; Ansell, 1974; Comely, 1974; Pollero & Brenner, 1979; Taylor & Venn, 1979; Barber & Blake, 1981; Robinson et al., 1981; Shafee, 1981; Beninger, 1984; Caers et al., 1998, 1999b; Strohmeier et al., 2000).

Although the digestive gland is of considerable importance in the physiology of bivalves, there are still important gaps in our knowledge about this key organ. Despite the marked dynamism of the digestive gland, its cytological and cytochemical patterns have never been studied throughout an annual physiological cycle. The present study therefore reports long-term cytological and cytochemical changes in the digestive gland within a natural scallop population in the North Atlantic (Bay of Brest, France).

MATERIALS AND METHODS

Histological study

The digestive glands of five 3-y-old animals collected by dredging were fixed monthly during one year for 48 h in aqueous Bouin's fluid upon arrival in the laboratory. These organs were dehydrated in an ascending ethanol-toluene series and embedded in paraffin. Sections 5 μm thick were stained using Masson's trichrome protocol (Gabe, 1968).

Transmission electron microscopy (TEM)

Samples (~1 mm^3) from various areas of the digestive gland of animals were fixed overnight at +4°C by immersion in a 2.5% glutaraldehyde solution in 0.1 M sodium cacodylate buffer, pH 7.3, adjusted to 1100 milliosmoles by addition of sodium chloride. Two rinses (1 h each) were performed in 0.1 M cacodylate buffer before post-fixation for 1 h at 4°C with 1% osmium tetroxide in sodium cacodylate buffer. Samples were dehydrated at room temperature in an ascending ethanol series, then saturated with a mixture of propylene oxide and Epon 812 resin (v/v) for 90 min and then with pure resin for 12 h before embedding in resin. Polymerization was performed at 60°C for 48 h. Semi-thin sections (1 μm) were immediately stained for 30 s by a 0.5% toluidine blue solution in an aqueous solution of 2.5% calcium carbonate (pH 11). Thin sections (60 nm) collected on copper-rhodium grids were contrasted with uranyl acetate and lead citrate (Reynolds, 1963) and observed using a JEOL 100 CX microscope operating at 80 kV.

Scanning electron microscopy (SEM)

Digestive gland fragments were fixed in 2.5% glutaraldehyde solution in sodium cacodylate buffer (0.1 M, pH 7.3, 1100 milliosmoles), dehydrated in an ascending ethanol series, and critical-point dried. They were then sputter coated with silver colloidal extender before being observed using a JEOL JSM 35 scanning electron microscope.

Biochemical assay of glycogen and proteins

Paraffin sections were treated with periodic acid-Schiff (PAS) (Gabe, 1968) to reveal carbohydrates. Negative control sections were first treated with 1% amylase in aqueous solution for 1 h at 37°C.

Fragments of digestive tubules, excluding the stomach and the main digestive ducts, were obtained monthly from tubuloacinar terminations of five *Pecten maximus* L. and immediately immersed in liquid nitrogen before storage at −70°C. These samples were ground using a liquid nitrogen-cooled Potter grinder, and assayed for glycogen using a Glucose (Trinder) enzymatic spectrophotometric assay kit (Sigma, Saint Quentin Fallavier, France). Free glucose was subtracted from total glucose after glycolysis with amyloglucosidase, and the results were expressed as $g\,l^{-1}\,mg^{-1}$ protein used as a reference base. Protein was determined using Coomassie blue (calibration range established with serum bovine albumin) and measured spectrophotometrically at 540 nm (BioRad-Pharmacia-Biotech, France).

Semi-quantitative study of lipids

Prior to the semi-quantitative study of lipids, verification of frozen sections stained with Sudan black (Gabe, 1968) confirmed that the optically empty vacuoles observed after topographic staining of the slides by Masson's trichrome technique (Gabe, 1968) actually corresponded to the lipids extracted during histological preparation of the samples. These 5-μm-thick sections were analysed using a Leica light microscope equipped with a Sony video camera coupled to a computer with image acquistion capacity and software for digitizing and processing Visilog 5.1 images to determine the area occupied by lipids prior to histological processing. All samples were processed in an identical manner.

Image analysis was performed on the digestive glands of five animals sampled monthly throughout the year. Ten fields per section and animal were randomly analysed. Four parameters were measured: T, the total area of the measured field; S, the area occupied by interacinar spaces; L, the area occupied by lumina of digestive acini; and Vl, the area occupied by lipid vacuoles. The following ratio gave the percentage of lipid occupation within digestive acini:

$$\frac{Vl}{(T - S + L)} \times 100 \qquad (1)$$

The mean and standard deviation were calculated for each month (Figure 3). Analysis of variances (one-way ANOVA) and mean discrimination (Fisher's least significant difference procedure) tests of the procedure for measuring lipid area indicated that the technique was consistent and reproducible at the 95% confidence level using STATGRAPHICS Plus 2.1.

Study of the accumulation rates for RNAs transcribed from α-amylase

The RNAs from biopsies of tubuloacinar terminations were extracted (RNABle, Eurobio, France) and transferred by capillarity onto a nylon membrane (Hybond N+, Amersham-Pharmacia-Biotech) after electrophoresis in denaturing conditions. The RNAs transferred were irreversibly fixed to the membrane by UV light exposure. An α-amylase probe, the cDNA homologue of *P. maximus*, provided by the Brest IFREMER Centre (X99729, Le Moine et al., 1997), was hybridized for 12 h at 62°C with the RNAs. After two 30-min rinses at 62°C with 3SSC, 0.1 SDS, and a 30-min rinse at 62°C with 1 SSC, 0.1 SDS, the membrane was exposed on autoradiographic film for 12 h at −70°C and developed.

RESULTS

Depending on the period of the year, interstitial connective and muscle tissue was more or less lacunar and of variable thickness. Histological sections showed digestive ducts (secondary or general) lined with a single layer of prismatic and ciliated epithelium. Their terminations consisted of two distinct parts: the first was a tubular zone in which epithelial cells with basophilic cytoplasm sometimes showed apical protrusions often containing numerous globules. The second, an acinar part, showed varying features according to the period of the year (Figure 1A,B). In section, the acinar part presented a rounded lumen with no crypts. Cells could have a hypertrophied appearance because of the presence of one or more large optically empty vacuoles (30 to 50 μm) corresponding to extracted lipids.

Histological monitoring of the digestive gland throughout the year showed morphological changes in the terminations. During the winter period (November to January), acinar cells were filled with lipids, giving them an adipocyte-like appearance. This was particularly true during January when lipid reserves were most abundant (Figure 1A). The acinar lumen was almost occluded due to the hypertrophy of these cells. The tubular part of the junction, in which epithelial cells had a more basophilic cytoplasm, was reduced in size, and interacinar spaces were tiny.

From the month of February, lipids were less abundant, reaching a minimum at the beginning of April (Figure 1B). The acinus was reduced in size. Conversely, in the tubular junction zone, digestive cells accumulated lipid-like droplets, and the acinar lumen was clearly visible. In the tubular zone, apical protrusions were apparent in the lumen, and connective tissue was also markedly thicker than in winter.

During the second half of April, lipids began to accumulate in digestive acini, and the number and volume of adipocyte-like cells increased. From May to September, the digestive gland recovered its lipid reserves, although at a lower level than that of November to January. At this period of the year, lipid-rich cells were not preponderant among the other cell types.

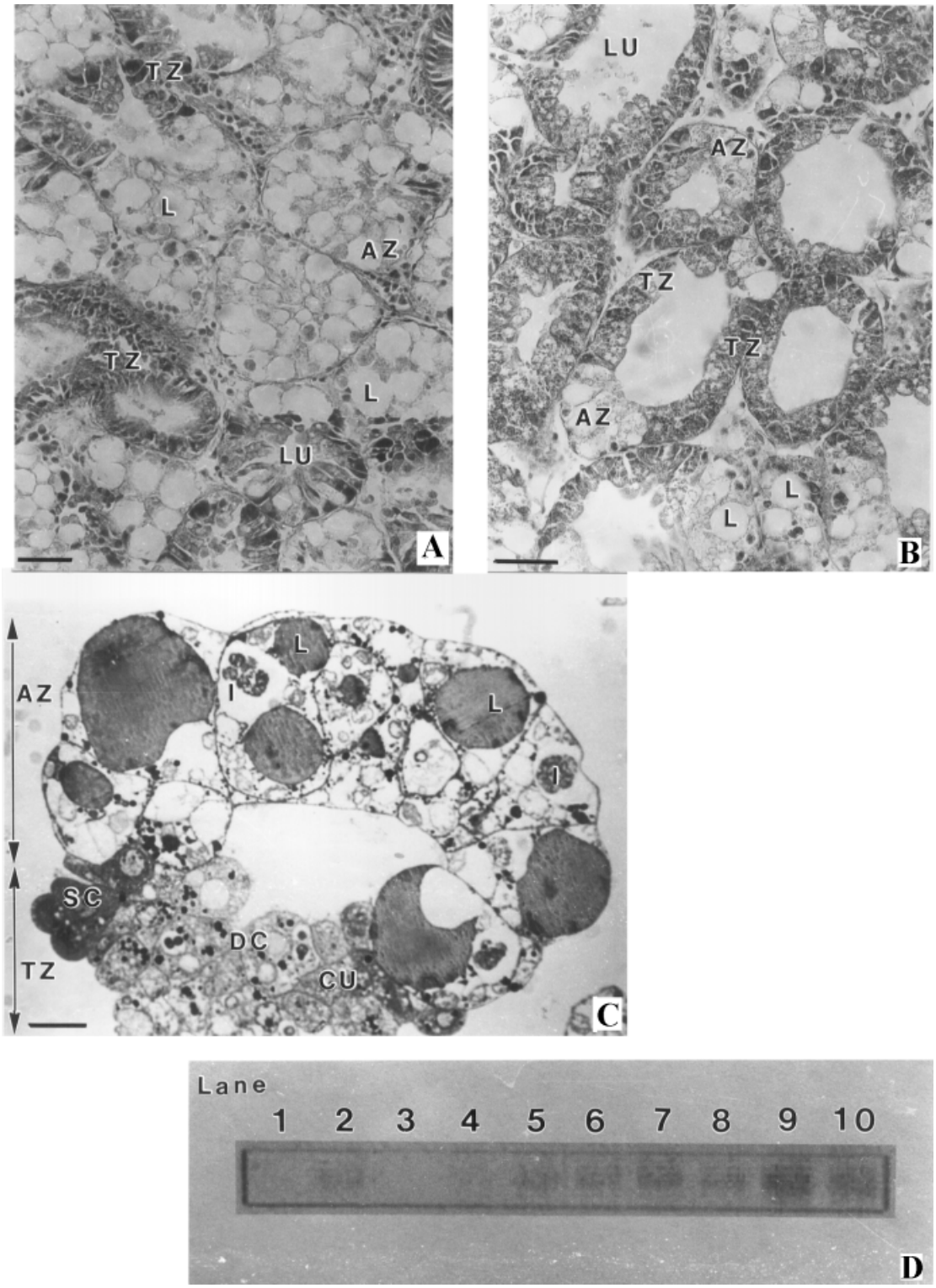

Figure 1. Changes in tubuloacinar terminations of the digestive gland of *Pecten maximus* during two key periods of the accumulation/consumption cycle for energy reserves. (A) Tubuloacinar terminations in December. The acinar zone is hypertrophied because of the abundance of lipids. The lumen is nearly lacking. The tubular zone is greatly reduced. (B) Tubuloacinar terminations at the end of April. The acinar zone is markedly reduced and lipids are not abundant, whereas the tubular zone and the lumina are clearly visible. (C) Tubuloacinar termination of the digestive gland of *P. maximus* observed in a semi-thin section stained with toluidine blue. The acinar and tubular zones are clearly stained. The digestive cells of the acinar zone are hypertrophied due to the presence of a large lipid inclusion and a vacuole containing digestion residues. Secretory cells and undifferentiated cells can be seen in the acinar and tubular zones. (D) Accumulation of α-amylase RNA transcripts in the digestive gland of *P. maximus* during a year period. Lane 1, December; Lane 2, January; Lane 3, early February; Lane 4, late February; Lane 5, early April; Lane 6: late April; Lane 7, May; Lane 8, June; Lane 9, July; Lane 10, September. AZ, acinar zone; CU, undifferentiated cells; DC, digestive cells; L, lipids; LU, lumina; SC, secretory cells; TZ, tubular zone. Scale bars: A&B, 42 μm; C, 12 μm.

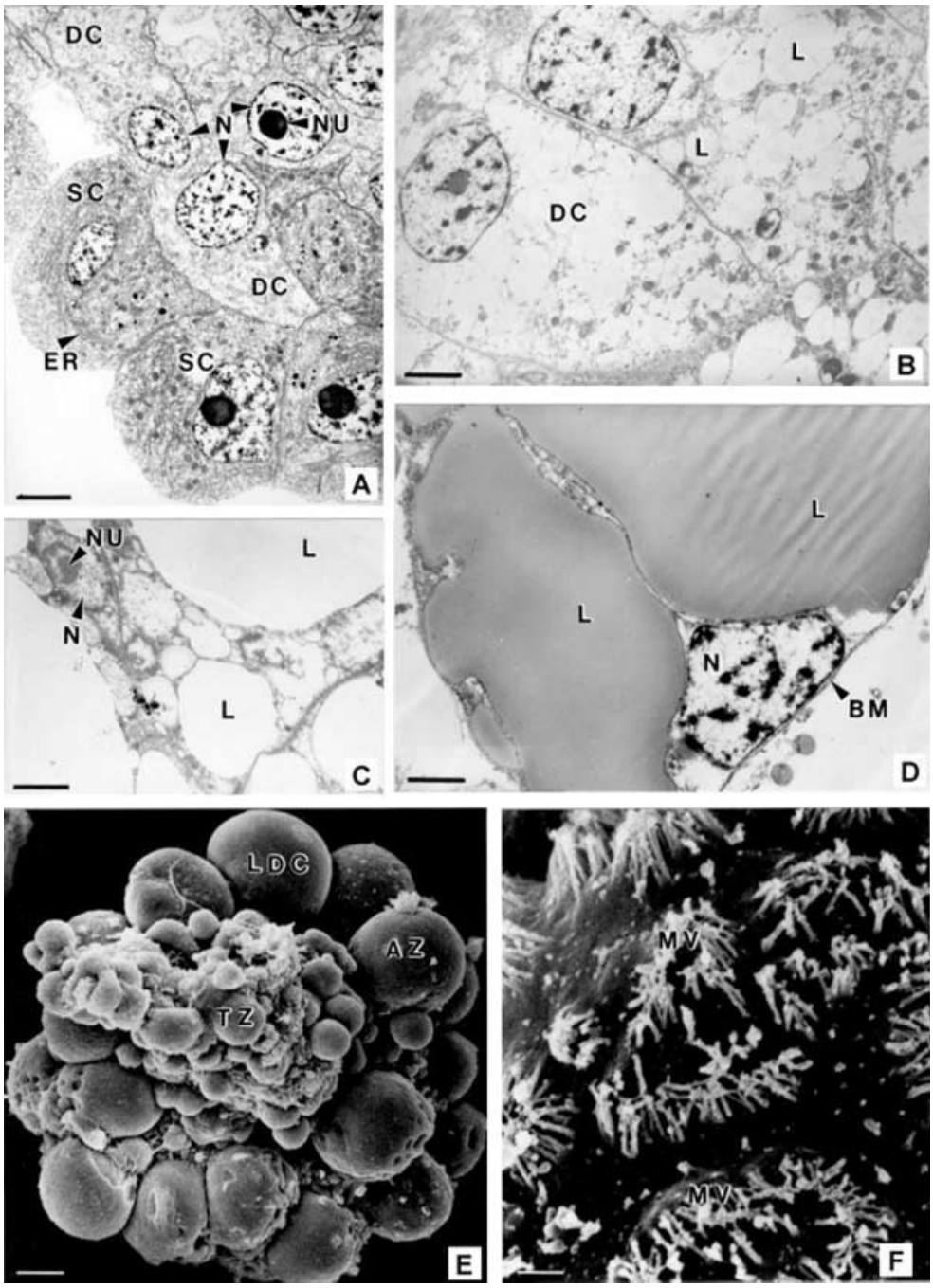

Figure 2. Cells constitutive of tubuloacinar terminations of digestive glands of *Pecten maximus*—tubuloacinar terminations. (A) Secretory cell situated mainly at the interface of the tubular and acinar zones of the tubuloacinar termination and displaying an abundant granular reticulum. Digestive cells still without lipids can be seen in the tubular zone. Their nuclei are large, and the nucleolus is clearly visible. (B) Digestive cell in the tubular zone accumulating lipid droplets. (C) Digestive cells in the acinar zone showing lipid droplets heterogeneous in both size and number. (D) Digestive cell in the acinar zone displaying a large lipid inclusion occupying all of the cytoplasmic space and pushing the nucleus out to the periphery of the cell against the basal membrane. (E) Tubuloacinar termination of the digestive gland observed in scanning electron microscopy. Two distinct parts can be seen: the tubular zone connected to the secondary digestive duct and the acinar zone composed essentially of lipid-containing digestive cells. (F) Apical area of digestive cells in the tubular zone showing clusters of microvilli probably in relation to cytoplasmic protrusion phenomena in the lumen of the tubular zone. AZ, acinar zone; BM, basal membrane; DC, digestive cell; ER, granular reticulum; L, lipid droplets; LDC, lipid-containing digestive cell; MV, microvilli; N, nucleus; NU, nucleolus; SC, secretory cell; TZ, tubular zone. Scale bars: A, 3 μm; B, 2 μm; C, 1 μm; D, 2 μm; E, 20 μm; F, 2 μm.

Observations of semi-thin (Figure 1C) and ultrathin sections (Figure 2A–D) allowed a more precise description of the cells in tubuloacinar zones. Cells in the tubular zone were 10 to 15 μm high and had a nucleus with a large basal nucleolus. Their nucleocytoplasmic ratio was high, and their apical area presented microvilli. Scanning electron microscopy showed that these microvilli were typically grouped in rings in each zone (Figure 2F). Two

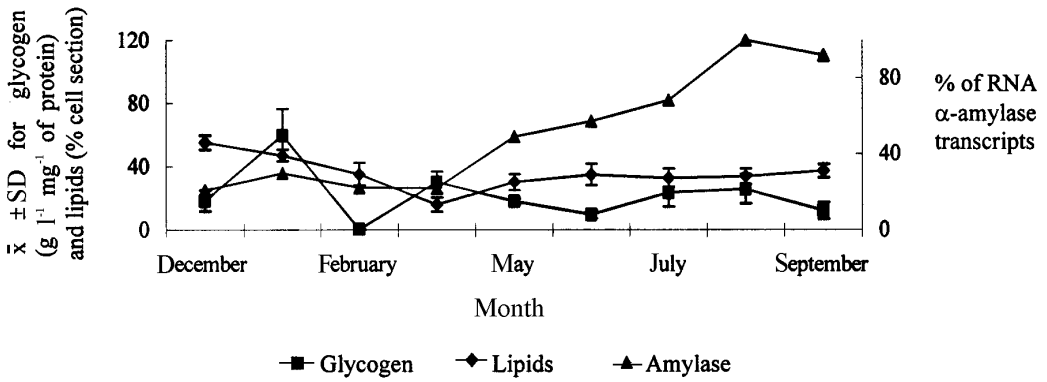

Figure 3. Evolution of glycogen (g l^{-1} mg^{-1} of proteins), lipids (occupation levels) contents and α-amylase RNA transcripts (%) in the tubuloacinar terminations of the digestive gland of *Pecten maximus*.

types of cells could be distinguished: those with highly basophilic cytoplasm and those with clearer cytoplasm filled with globules.

Cells with clear cytoplasm

Cells with clear cytoplasm showed vacuoles that were optically empty or contained one or more lipid-like globule(s), residual bodies, and small amounts of glycogen characterized by PAS-positive staining which disappeared on negative amylase-treated control sections (Figure 2A–D). A few mitochondria were scattered among these various organelles. The apical part of the cells often showed outgrowths into the acinus lumen, which then became detached, releasing fragmentation spherules (Figure 1B,C).

In the acinar zone, the cells showed different features depending on the period of the year and the variability of their lipid content. Their height in sections ranged from ~20 μm when they contained few or no lipids, to 40 to 50 μm when lipid content was maximal, resembling adipocytes (Figures 1A & 2B,C). The ultrastructural features of these cells in the acinar zone showed a marked annual cycle. In the spring, during the pre-adipocyte-like stage, no lipids or vacuoles were present (Figure 1B). During the summer and autumn, they were filled with lipid droplets (Figures 1A,C & 2B,C). The cell nucleus was pushed out to the periphery by a large lipid area occupying most of the cytoplasm (Figure 2D). In addition to a few optically empty vacuoles of variable size situated near the apical area (Figure 2B), another large vacuole situated at the base had a heterogeneously-dense inclusion (suggestive of digestive residues) (Figure 1C), and glycogen-like inclusions could be seen at the periphery of the lipid area (Figure 2C). Small undifferentiated cells (Figure 1C) were sometimes apparent below and between the adipocyte-like cells.

Basophilic cells

The second type of cells (Figure 2A), mainly situated at the tubuloacinar interface and not in crypts, was characterized by an abundant granular reticulum, numerous ribosomes, Golgi apparatus, numerous secretory vesicles and mitochondria, indicating intense protein synthesis activity. These cells corresponded to non-flagellated mature basophilic cells observed in semi-thin (Figure 1C) and paraffin (Figure 1A,B) sections. Flagellated basophilic cells were not observed.

Glycogen assays

The results of the glycogen assay (expressed in g l^{-1} mg^{-1} of proteins) reported in Figure 3 indicate that maximum glycogen storage was reached in January (0.6 ± 0.2 g l^{-1} mg^{-1} of proteins). Afterwards, glycogen decreased rapidly to almost zero by the end of February (0.005 ± 0.003 g l^{-1} mg^{-1} of proteins). For the remainder of the year, glycogen showed a variation reciprocal to that of lipids (Figure 3). During April, glycogen reserves recovered rapidly, reaching a level close to that of January. A second reduction occurred between April and mid-June. The reserves were then reconstituted, though with a slight but significant drop in September.

Semi-quantitative study of lipids

Image analysis results from paraffin sections (Figure 3) showed that the lipid index was maximal from December to January. It decreased significantly (*F* value 0.05) from February on, reaching a minimal value in April. The rate of lipid occupation in tubuloacinar terminations then increased progressively, reaching practically the same levels in June as those observed at the end of the year.

Study of the accumulation of α-amylase RNA transcripts

The rate of accumulation of α-amylase RNA transcripts was minimal in winter (December to February) (Figure 3) and then increased progressively from the end of April, becoming maximal at the end of August and September.

DISCUSSION

Ultrastructural characteristics of cells within a digestive tubule

Digestive cells

In the tubulous part of the digestive gland, no evident descriptive differences were noted between our description of clear cells and those pertaining to digestive cells by

Henry (1987) and Henry et al. (1991) in *Pecten maximus* and by other authors in non-Pectinid species (i.e. *Cerastoderma edule*, Owen, 1970; *Crassostrea virginica*, Langdon & Newell, 1996). The progressive changes in cell size and the appearance of phagolysosomes, small lipid droplets, etc. indicate that these cells follow a rapid turn-over. A certain synchronism was observed within this group of digestive cells in tubules. Such short-term, synchronized turn-over has been related to tidal rhythms (see Morton, 1977).

In the acinar part of the digestive gland, digestive cells always appear very voluminous in size; only their number vary depending on the time of year. The ultrastructural profile of these cells (a large single lipid inclusion pushing back a large vacuole containing residual digestive-like material) is suggestive of long-term storage cells. To our knowledge, such a cell type has not previously been reported in bivalves, even in previous studies of the digestive gland of *P. maximus* (Henry et al., 1991).

Basophilic cells

Basophilic cells were found at the interface between the tubular and the acinar portion of the digestive gland, but since the lumen was rounded in cross-section, they cannot be considered as occupying well-defined crypts, as has been described for many bivalves species (Summer, 1966; McQuinston, 1969; Owen, 1970, 1973; Pal, 1971; Pal et al., 1990). The basophilic cells contain all the specialized material for production of extracellular enzymes and are considered to be secretory cells (although enzyme excretion has yet not been proven, see Weinstein, 1995). No flagellae were found associated with any basophilic cells in the present study, even under TEM and SEM, although they have been encountered in *P. maximus* (Henry et al., 1991). Flagellated basophilic cells have been reported in various bivalve species such as *Crassostrea virginica* (Weinstein, 1995), *Meretrix meretrix* (Pal et al., 1990) or *Mya arenaria* (Pal, 1971). Such cells may be facultative in *P. maximus*, as none were observed over the entire 12-month sampling period of the present study.

Cytological dynamics of the digestive gland over an annual cycle

Our observations show cytological and cytochemical changes in the digestive gland of *P. maximus* over one year within a natural population in the North Atlantic. Morphological changes in the digestive gland have previously been observed and attributed, in particular, to occasional events such as tidal movements and food inputs (Morton, 1956; McQuinston, 1969; Mathers, 1976; Mathers et al., 1979; Robinson & Langton, 1980; Robinson et al., 1981). Mathers (1976) reported that digestive gland cells of *P. maximus* follow a cyclical rhythm from low to high tide. Different morphological features appear to be affected, leading notably to an increase in their height and a corresponding reduction in acinar lumina. Our study revealed long-term modifications during the year that were independent of tidal movements. In April, cell size was smallest and acinar lumina were largest, while from May to September cell height increased and lumina decreased. From November to January, the cell apices reunited, occulting the acinar lumina.

Storage and release of reserves

The cytological modifications observed in the present study appear to relate to the physiology of reserve storage and utilization, essentially of lipids that accumulate in the acinar part of the terminations of digestive ducts, as shown by monitoring with image analysis. Other compounds such as glycogen are also accumulated. Long-term monitoring allowed us to define the precise periods of the year for the storage and utilization of these energy reserves in the acinar zone. Thus, the progressive storage of lipids takes place from May to December, being more rapid from August to December when reserves are highest. The period of intense utilization of these reserves extends from December to April.

Digestive gland and phytoplankton

Numerous examples of the relation between food availability and the constitution of energy reserves have been documented in various bivalve families, including Mytilidae (*Mytilus edulis*, Gabbott, 1975), Veneridae (*Tapes philippinarum* and *Ruditapes decussates*, Beninger & Lucas, 1984), and Pectinidae (*Chlamys tehuelcha*, Pollero & Brenner, 1979; *Argopecten irradians concentricus*, Barber & Blake, 1981; *Chlamys septemradiata*, Ansell, 1974; *Pecten maximus*, Comely, 1974; *Chlamys opercularis*, Taylor & Venn, 1979; *Mercenaria mercenaria*, *Ostrea edulis*, *Mytilus edulis*, Robinson et al., 1981). A comparison of our observations with the studies of phytoplankton bloom abundance in the Bay of Brest (Chauvaud, 1998, cumulative data from 1988 to 1997; E. Nézan-Concarneau IFREMER Station, France; personal communication) indicates that the digestive gland accumulates lipids progressively beginning in May, i.e. during the period with the highest phytoplankton densities. Few studies have examined the effect of algal lipids on quantities of lipids in specific bivalves tissues; however, algae rich in eicosapentaenoic acid promote higher growth in *Tapes philippinarum* (Caers et al., 1998) and dietary lipid supplementation also promotes growth (Caers et al., 1999a).

Role of α-amylase

Alpha-amylase has been detected in different regions of the digestive tract of *P. maximus*, particularly in the stomach (Henry et al., 1993). In the digestive gland, this enzyme is also present in secretory granules (Benlimane-Mrini, 1993). Alpha-amylase is then incorporated into the crystalline style where it is involved in extracellular digestion of food (Beninger & Le Pennec, 1991). The variation in the number of secretory granules accumulated in glandular cells suggests that enzymes are released according to the continuous or discontinuous digestion related to feeding processes (Henry et al., 1991, 1993). In fact, our results concerning the rates of accumulation of α-amylase mRNA transcripts show a resurgence of amylase activity in the spring (April), which progressively intensifies until autumn (September), i.e. over a six-month period during which environmental conditions (notably temperature and food) are more favourable to the growth and reproduction of bivalves in the Bay of Brest (Paulet et al., 1997).

Utilization of reserves

The role of reserves in gametogenesis and the development of somatic tissues has been described for numerous Pectinidae. Transfers to the reproductive compartment have usually been studied, e.g. for *Chlamys tehuelcha* (Pollero & Brenner, 1979), *Argopecten irradians concentricus* (Barber & Blake, 1981), *Chlamys septemradiata* (Ansell, 1974), *Pecten maximus* (Comely, 1974), *Chlamys opercularis* (Taylor & Venn, 1979), *Mercenaria mercenaria*, *Ostrea edulis*, *Mytilus edulis* (Robinson et al., 1981), *Placopecten magellanicus* (Thompson & McDonald, 1991), *Argopecten purpuratus* (Caers et al., 1999b) and *Pecten maximus* (Strohmeier et al., 2000). The present study, together with independent data on spawning and shell growth, provides a more detailed description of the probable transfer schedule throughout an annual cycle.

Transfer to the gonad

Metabolite transfers from the digestive gland to the gonad have been demonstrated by the use of ^{14}C-leucine in *Argopecten irradians* (Sastry & Blake, 1971) and lipid ^{14}C-labelled *Chlorella* extract in *Chlamys hericia* (Vassalo, 1973). For *P. maximus* in the Bay of Brest, the first spawning takes place at the beginning of spring (mid-March to the end of April) (Paulet et al., 1997: cumulative data from 1989 to 1994). During this period, environmental concentrations of chlorophyll are low (6-y mean and standard deviation of $1.23 \pm 0.05\ \mu g\, l^{-1}$, Lorrain et al., 2001). The energy required during this early phase of gamete maturation in February and March comes from the glycogen accumulated in the digestive gland which, according to our observations, disappears nearly totally from this organ at the end of February. The second spawning lasts from May to July (Paulet et al., 1997) when phytoplankton blooms are maximal, thereby allowing the digestive gland to reconstitute its lipid and glycogen reserves (see previous results). During July and August, when gamete emissions stop, glycogen and lipid concentrations remain at about the same level. At the end of August, glycogen decreases again, probably because of a transfer to the gonad where it will contribute to the last gamete productions at the end of September or at the start of October. Subsequently, there is a period of sexual inactivity until the beginning of December. The digestive gland progressively recovers its lipid and glycogen reserves during this period, and the tubuloacinar terminations have the same appearance as they did in December and January.

In the Tinduff *P. maximus* hatchery (Bay of Brest), June spawnings provide the best hatching rates of the year (measured two days after fertilization), i.e. 54%, vs 17–18% in April and 6% in September (Paulet et al., 1997).

Significant decreases in digestive gland reserve components may indicate transfer to developing gametes (Caers et al., 1999b) However, it is uncertain whether this energy transfer from the digestive gland involves a 'transfer' of lipids to the gonad, whose oocytes are known to be lipid-rich (Besnard et al., 1989; Caers et al., 1999b), or whether there is a metabolic conversion of lipids into carbohydrates. The transfer mechanism of metabolites, notably that of lipids, from the digestive gland to the gonad is still unknown. The mechanism could improve the bursting of lipid-containing cells in the acinar lumen where they would enter into the larger digestive tract. Histological and ultrastructural observations have lent support to the hypothesis of a direct transfer of metabolites from the intestine to developing gametes (Le Pennec et al., 1991). In the present case, and such a mechanism may also be involved in lipid transfer to the gonad from the digestive gland. Another possibility is the catabolism of lipids to a carbohydrate pathway within the digestive gland, and subsequent transfer to gonad via haemocytes with lipid reconstitution in the oocytes.

Transfer to shell growth and shell matrix

Shell growth begins at the end of March, i.e. ~1 month before the spring phytoplankton bloom at the end of April and beginning of May (Chauvaud, 1998: cumulative data from 1988 to 1997). Depending on the year, shell growth increases from $40\ \mu m\, d^{-1}$ at the beginning of April to $120–180\ \mu m\, d^{-1}$ at the end of April (Lorrain et al., 2001). This growth appears related to the increased photoperiod, and to a lesser extent to higher temperature, but is independent of the trophic quality of the environment (Chauvaud, 1998). Since muscle weight reaches its minimum value in March (Paulet et al., 1997), it would thus seem likely that the energy required for this resumption of shell growth comes from metabolite transfers originating in the digestive gland. The higher concentrations of phytoplankton lead to a deceleration of growth, which drops to $40\ \mu m\, d^{-1}$. Once phytoplankton concentrations become lower, growth resumes. The possibility of an alteration of filtration due to a very high density of phytoplankton cells has been suggested by Bricelj et al. (1987). Similarly, an alteration of oxygen consumption due to a gas deficiency caused by bacterial degradation of the phytoplankton at the water-sediment interface is also possible (Lewis & Cerrato, 1997).

Maintenance and acute demand lipid storage sites

The digestive gland and adductor muscle of *Mercenaria mercenaria*, *Ostrea edulis* and *Mytilus edulis* constitute the two storage organs for lipids and carbohydrates (Robinson et al., 1981). However, the main source of energy for gamete maturation in bivalves is the adductor muscle (Sastry & Blake, 1971; Vassalo, 1973; Robinson et al., 1981; Lubet et al., 1987; Epp et al., 1988). For *P. maximus* in the Bay of Brest, the weight loss of muscle begins in October and continues until early March, the month in which muscle reaches its minimum mass. From mid-March to the end of September, muscle mass increases, with a more intense period from the end of April to the end of June (Paulet et al., 1997). Our study shows that digestive gland plays a significant role in this process, notably as a relay organ in supplying energy in the form of lipids and glycogen for a two-week period during a month in which adductor muscle glycogen reserves are exhausted.

Our SEM observations showed that a large lipid inclusion and glycogen clusters co-exist within adipocyte-like cells in the acinar part of the digestive gland. Glycogen was consumed very rapidly at the beginning of the year (January and February) and in preference to lipids during spring gamete maturation. After gamete emission,

glycogen was stored again in the digestive gland and remained at a nearly constant level throughout the rest of the year.

On the basis of histological studies and observations made during the utilization of energy reserves, it appears that two types of cells accumulate lipids in the tubulo-acinar terminations of *P. maximus* digestive gland. These cells have different morphological features which may correspond to different functions. In the tubular part, forming the junction between the digestive duct and the acini, the cells accumulate numerous lipid droplets. In fact, these cells protrude into the tubule lumen, releasing fragmentation spherules. They could correspond to those related to tidal movements and described by Morton (1956), McQuinston (1969), Mathers (1976), Mathers et al. (1979), Robinson & Langton, 1980 and Robinson et al. (1981). The small size of these droplets could indicate use in daily metabolism, including somatic growth and gametogenesis. In the acinar part, the large size of the lipid inclusion suggests storage for the long-term energy requirement of the animal at critical physiological times, including the maturation of gametes.

Numerous studies have shown structural modifications of the digestive gland mainly associated with tidal rhythms. Herein, we have demonstrated long-term cytological and cytochemical changes in the digestive gland within a natural population of *P. maximus* associated with nutrition and reproductive effort and shell growth. Changes are mostly related to lipid content in this organ which may thus be considered as a lipid storage organ. Whereas the adductor muscle glycogen reserve has hitherto been considered as the only reserve energy source available for gametogenesis, the present study shows that the digestive gland also participates in this effort, at least as a relay-organ when muscle reserve has been depleted.

The authors thank Dr Monique Henry (Faculté des Sciences St Jérôme, Marseille) for her comments and suggestions.

REFERENCES

Ansell, A.D., 1974. Seasonal changes in biochemical composition of the bivalve *Chlamys septemradiata* from the Clyde Sea area. *Marine Biology*, **25**, 85–99.

Barber, B.J. & Blake, N.J., 1981. Energy storage and utilization in relation to gametogenesis in *Argopecten irradians concentricus* (Say). *Journal of Experimental Marine Biology and Ecology*, **52**, 121–134.

Beninger P.G., 1984. Seasonal variations of the major lipid classes in relation to the reproductive activity of two species of clams raised in a common habitat: *Tapes decussatus* L. (Jeffreys, 1863) and *T. philippinarum* (Adams & Reeve, 1850). *Journal of Experimental Marine Biology and Ecology*, **79**, 79–90.

Beninger, P.G. & Le Pennec, M., 1991. Functional anatomy of scallops. In *Scallops: biology, ecology and aquaculture* (ed. S.E. Shumway), pp. 133–223. New York: Elsevier.

Beninger P.G. & Lucas, A., 1984. Seasonal variations in condition, reproductive activity, and gross biochemical composition of two species of adult clam reared in a common habitat: *Tapes decussatus* (Jeffreys) and *Tapes philippinarum* (Adams & Reeve). *Journal of Experimental Marine Biology and Ecology*, **79**, 19–37.

Benlimane-Mrini, N., 1993. *L'estomac de la coquille Saint Jacques* Pecten maximus *(L): caractérisations morphologiques, ultrastructurales, histochimiques et immunocytochimiques*. PhD thesis, Université d'Aix-Marseille III, France.

Besnard, J.Y., Lubet P. & Nouvelot, A., 1989. Seasonal variations of the fatty acid content of the neutral lipids and phospholipids in the female gonad of *Pecten maximus* L. *Comparative Biochemistry and Physiology*, **93B**, 21–26.

Bricelj, V.M., Epp, J.M. & Malouf, R.E., 1987. Intraspecific variation in reproductive and somatic growth cycles of bay scallop, *Argopecten irradians* Lamarck. *Marine Ecology Progress Series*, **36**, 123–137.

Caers, M., Coutteau, P. & Sorgeloos, P., 1999a. Dietary impact of algal and artificial diets, fed at different feeding ratios, on the growth and fatty acid composition of *Tapes philippinarum* (L.) spat. *Aquaculture*, **170**, 307–322.

Caers, M., Coutteau, P., Cure, K., Morales, V., Gajardo, G. & Sorgeloos, P., 1999b. The Chilean scallop *Argopecten purpuratus* (Lamarck, 1819). I. Fatty acid composition and lipid content of six organs. *Comparative Biochemistry and Physiology*, **123B**, 89–96.

Caers, M., Coutteau, P., Lombedia, P. & Sorgeloos, P., 1998. The effect of lipid supplementation on growth and fatty acid composition of *Tapes phillipinarum* (L.) spat. *Aquaculture*, **162**, 287–299.

Chauvaud, L., 1998. *La coquille Saint-Jacques en Rade de Brest: un modèle biologique d'étude des réponses de la faune benthique aux fluctuations de l'environnement*. PhD thesis, Université de Bretagne Occidentale, France.

Comely, C.A., 1974. Seasonal variations in the fresh weights and biochemical content of the scallop *Pecten maximus* (L.) in the Clyde Sea area. *Journal du Conseil*, **35**, 281–295.

Epp, J., Bricelj, V.M. & Malouf, R.E., 1988. Seasonal partitioning and utilization of energy reserves in two age classes of the bay scallop *Argopecten irradians irradians* (Lamarck). *Journal of Experimental Marine Biology and Ecology*, **121**, 113–136.

Fitzpatrick, P.J., Krag, T.O.B., Hojrup, P. & Sheehan, D., 1995. Characterization of a glutathione S-transferase and a related glutathione-binding protein from gill of the blue mussel, *Mytilus edulis*. *Biochemistry Journal*, **305**, 145–150.

Gabbott, P.A., 1975. Storage cycles in marine bivalve molluscs: a hypothesis concerning the relationship between glycogen metabolism and gametogenesis. In *Proceedings of the Ninth European Marine Biology Symposium* (ed. H. Barnes), pp. 191–211. Aberdeen, Scotland: Aberdeen University Press.

Gabe, M., 1968. *Techniques histologiques*. Paris: Masson.

Henry, M., 1987. La glande digestive de la palourde méditerranéenne *Ruditapes decussatus* L. Recherches ultrastructurales, cytochimiques, écophysiques et écotoxicologiques. *Vie Marine*, **1 & 2**, 1–407.

Henry M., Benlimane, N., Boucaud-Camou, E., Mathieu, M., Donval, A. & Van Wormhoudt, A., 1993. The amylase-secreting cells of the stomach of the scallop, *Pecten maximus*: ultrastructural, immunohistochemical and immunocytochemical characterizations. *Tissue and Cell*, **25**, 537–548.

Henry, M., Boucaud-Camou, E. & Lefort, Y., 1991. Functional micro-anatomy of the digestive gland of the scallop *Pecten maximus* (L.). *Aquatic Living Resource*, **4**, 191–202.

Langdon, C.J. & Newell, R.I.E., 1996. Digestion and nutrition in larvae and adults. In *The eastern oyster* Crassostrea virginica (ed. V.S. Kennedy and R.I.E Newell), pp. 231–269. Maryland, USA: Maryland Sea Grant College.

Le Moine, S., Sellos, D., Moal, J., Daniel, J.Y., San Juan Serrano, F., Samain, J.F. & Van Wormhoudt, A., 1997. Amylase in *Pecten maximus* (Mollusca, bivalves): protein and cDNA characterization; expression in the digestive gland. *Molecular Marine Biology and Biotechnology*, **6**, 228–237.

Le Pennec, M., Beninger, P.G., Dorange, G. & Paulet, Y.M., 1991. Trophic sources and pathways to the developing gametes of *Pecten maximus* (Bivalvia: Pectinidae). *Journal of the Marine Biological Association of the United Kingdom*, **71**, 451–463.

Lewis, D.E. & Cerrato, R.M., 1997. Growth uncoupling and relationship between shell growth and metabolism in the soft shell clam *Mya arenaria*. *Marine Ecology Progress Series*, **158**, 177–189.

Livingstone, D.R., 1985. Response of the detoxification/toxication enzymes systems of molluscs to organic pollutants and xenobiotics. *Marine Biological Bulletin*, **16**, 158–164.

Livingstone, D.R., 1998. The fate of organic xenobiotics in aquatic ecosystems: quantitative and qualitative differences in bio transformation by invertebrates and fishes. *Comparative Biochemistry and Physiology*, **120A**, 43–49.

Livingstone, D.R. & Farrar, S.V., 1984. Tissue and subcellular distribution of enzyme activities of mixed-function oxygenase and benzo[a]pyrene metabolism in the common mussel *Mytilus edulis* L. *Science of the Total Environment*, **39**, 209–235.

Livingstone, D.R. & Pipe, R.K., 1992. Mussels and environmental contaminants: molecular and cellular aspects. *Development of Aquaculture and Fishery Science*, **25**, 424–464.

Lorrain, A., Paulet, Y.M., Chauvaud, L., Savoye, N., Nizan, E. & Guérin, L., 2001. Growth anomalies in *Pecten maximus* from coastal waters (Bay of Brest, France): relationship with diatom blooms. *Journal of the Marine Biological Association of the United Kingdom*, in press.

Lubet, P., Besnard, J.Y., Faveris, R. & Robbins, I., 1987. Physiologie de la reproduction de la coquille Saint Jacques (*Pecten maximus* L.). *Oceanis*, **13**, 265–290.

Mathers, N.F., 1973. A comparative histochemical survey of enzymes associated with the processes of digestion in *Ostrea edulis* and *Crassostrea angulata* (Mollusca: Bivalvia). *Journal of Zoology*, **169**, 169–179.

Mathers, N.F., 1976. The effects of tidal currents on the rhythm of feeding and digestion in *Pecten maximus* L. *Journal of Experimental Marine Biology and Ecology*, **24**, 271–283.

Mathers, N.F., Smith, T. & Colins, N., 1979. Monophasic and diphasic cycles in *Venerupis decussata* and *Chlamys varia*. *Journal of Molluscan Studies*, **45**, 68–81.

McQuinston, R.W., 1969. Cyclic activity in the digestive diverticula of *Lasaea rubra* (Montagu) (Bivalvia: Eulamellibranchia). *Proceedings of the Malacological Society of London*, **38**, 483–495.

Morton, B.S., 1977. The tidal rhythm of feeding and digestion in the Pacific oyster, *Crassostrea gigas* (Thunberg). *Journal of Experimental Marine Biology and Ecology*, **26**, 135–151.

Morton, J.E., 1956. The tidal rhythm and action of the digestive system of the lamellibranch *Lasaea rubra*. *Journal of the Marine Biological Association of the United Kingdom*, **35**, 563–583.

Owen, G., 1970. The fine structure of the digestive tubules of the marine bivalve *Cardium edule*. *Philosophical Transactions of the Royal Society* B, **258**, 245–260.

Owen, G., 1973. The fine structure and histochemistry of the digestive diverticula of the protobranchiate bivalve *Nucula sulcata*. *Proceedings of the Royal Society* B, **183**, 249–264.

Pal, S.G., 1971. The fine structure of the digestive tubules of *Mya arenaria* L. I. Basophil cell. *Proceedings of the Malacological Society of London*, **39**, 303–309.

Pal, S.G., Ghosh, B. & Modak, S., 1990. Fine structure of the digestive tubules of *Meretrix*. In *The Bivalvia—Proceedings of a Memorial Symposium in honour of Sir Charles Maurice Yonge, Edinburgh 1986* (ed. B. Morton), pp. 167–175. Hong Kong: University Press.

Paulet, Y.M., Bekhadra, F., Devauchelle, N., Donval, A. & Dorange, G., 1997. Cycles saisonniers, reproduction et qualité des ovocytes chez *Pecten maximus* en rade de Brest. *Annales de l'Institut Océanographique, Paris*, **73**, 101–112.

Pollero, R.J. & Brenner, R.R., 1979. Seasonal changes of the lipids of the mollusc *Chlamys tehuelcha*. *Comparative Biochemistry and Physiology*, **64A**, 257–263.

Robinson, W.E., 1983. Assessment of bivalve intracellular digestion based on direct measurements. *Journal of Molluscan Studies*, **49**, 1–8.

Robinson, W.E. & Langton, R.W., 1980. Digestion in a subtidal population of *Mercenaria mercenaria* (Bivalvia). *Marine Biology*, **58**, 173–179.

Robinson, W.E., Pennington, M.R. & Langton, R.W., 1981. Variability of tubules types within the digestive glands of *Mercenaria mercenaria* (L.), *Ostrea edulis* (L.), and *Mytilus edulis* (L.). *Journal of Experimental Marine Biology and Ecology*, **54**, 265–276.

Sastry, A.N. & Blake, N.J., 1971. Regulation of gonad development in the bay scallop, *Aequipecten irradians* Lamarck. *Biological Bulletin. Marine Biological Laboratory, Woods Hole*, **140**, 274–283.

Shafee, M.S., 1981. Seasonal variation in the biochemical composition and calorific value of the black scallop, *Chlamys varia* (L.) from Lanveoc, Bay of Brest. *Oceanologica Acta*, **4**, 331–341.

Stanley, C.A., 1967. *The commercial scallop (*Pecten maximus *(L.)) in northern Irish waters*. PhD thesis, The Queen's University of Belfast, Ireland.

Stegeman, J.J., 1985. Benzo[a]pyrene oxidation and microsomal enzyme activity in the mussel (*Mytilus edulis*) and other bivalve mollusc species from the western North Atlantic. *Marine Biology*, **89**, 21–30.

Strohmeier, T., Duinker, A. & Lie O., 2000. Seasonal variations in chemical composition of the female gonad and storage organs in *Pecten maximus* (L.) suggesting that somatic and reproductive growth are separated in time. *Journal of Shellfish Research*, **19**, 741–747.

Summer, A.T., 1966. The cytology and histochemistry of the digestive gland cells of *Anodonta*. *Journal of the Royal Microscopical Society*, **85**, 417–423.

Taylor, A.C. & Venn, T.J., 1979. Seasonal variation in weight and biochemical composition of the tissues of the queen scallop, *Chlamys opercularis*, from the Clyde Sea area. *Journal of the Marine Biological Association of the United Kingdom*, **59**, 605–621.

Thompson, R.J. & McDonald, B.A., 1991. Physiological integrations and energy partitioning. In *Scallops: biology, ecology and aquaculture* (ed. S.E. Shumway), pp. 347–376. New York: Elsevier.

Vassalo, M.T., 1973. Lipid storage and transfer in the scallop *Chlamys hericia* Gould. *Comparative Biochemistry and Physiology*, **44A**, 1169–1175.

Weinstein, J.E., 1995. Fine structure of the digestive tubule of the eastern oyster, *Crassostrea virginica* (Thunberg). *Journal of Shellfish Research*, **14**, 97–103.

Wootton, A.N., Herring, C., Spry, J.A., Wiseman, A., Livingstone, D.R. & Goldfarb, P.S., 1994. Evidence for the existence of cytochrome P450 gene families (CYP1A1, 3A, 4A1, 11A1) and modulation of gene expression (CYP1A1) in the mussel *Mytilus* sp. *Marine Environmental Research*, **28**, 57–60.

Yawetz, A., Manelis, R. & Fishelson, L., 1992. The effects of arochlor 1254 and petrochemical pollutants on cytochrome p-450 from the digestive gland microsomes of four species of mediterranean molluscs. *Comparative Biochemistry and Physiology*, **103C**, 607–614.

Submitted 28 September 2000. Accepted 18 May 2001.