UNIVERSITÉ DU QUÉBEC À RIMOUSKI

VARIATIONS SAISONNIÈRES DE PARAMÈTRES PHYSIOLOGIQUES CHEZ LA MOULE BLEUE *MYTILUS* SPP. DANS DIFFÉRENTS SITES D'ÉLEVAGE MYTILICOLE DE L'EST DU QUÉBEC

MÉMOIRE

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AVANT-PROPOS

Le second chapitre de ce mémoire de maîtrise intitulé *Seasonal variations of physiological parameters of the blue mussel Mytilus spp. in farm sites of Eastern Quebec* est présenté sous forme d'un article qui sera soumis prochainement à la revue *Aquaculture*. Cette étude s'inscrit dans le cadre d'un projet de développement d'indicateurs biologiques permettant d'évaluer la qualité nutritionnelle des sites pour l'industrie mytilicole. Ce projet a été réalisé en collaboration avec le Centre Collégial de Transfert des Technologies des Pêches de Grande-Rivière ainsi que des producteurs de l'industrie mytilicole de l'Est du Québec.

RÉSUMÉ

Un suivi saisonnier, de moules indigènes (Baie de Gaspé, Carleton, Havre-Saint-Pierre, les Îles-de-la-Madeleine et Blanc-Sablon) et transplantées (Baie de Gaspé, Carleton, Havre-Saint-Pierre), nous a permis d'étudier les variations de différentes variables physiologiques, en fonction de la température et de la concentration en cellules phytoplanctoniques. Nos résultats ont démontré une influence positive des concentrations en cellules phytoplanctoniques sur la gamétogenèse ainsi qu'une influence positive de la température de l'eau sur la période de ponte. Toutefois, le cycle de maturation sexuelle était trop fragmenté pour établir des corrélations significatives. Une chute de l'indice hépatosomatique a été observée pendant la période de gamétogenèse. L'influence importante de ce processus très énergivore sur la masse de l'hépatopancréas ne nous a pas permis d'établir de corrélation significative avec la croissance et les facteurs du milieu. Nous avons également observé une chute de l'activité phagocytaire au début de la gamétogenèse ainsi qu'une corrélation significative entre l'indice de maturité sexuelle et l'activité phagocytaire. Cette suppression immunitaire intervenant en même temps que les fortes températures estivales peut poser des problèmes d'infection et de mortalité dans les élevages. La maturation sexuelle apparaît comme un processus clef à prendre en compte dans la gestion des sites d'élevage. Nos recherches s'orientent actuellement vers le développement d'outils prédictifs fiables des périodes de gamétogenèse et de ponte. L'influence du cycle de maturation sexuelle sur les mécanismes immunitaires est également à l'étude dans nos laboratoires.

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INTRODUCTION

1.1. La mariculture au Québec

La mariculture au Québec est dominée par l'élevage conchylicole et principalement par la mytiliculture (Cartier *et al.*, 2004). Même si elle est encore peu présente, en comparaison avec d'autres provinces du Canada, comme l'Île-du-Prince-Edouard, c'est un secteur en pleine expansion. Au cours des dernières années, la mytiliculture est l'activité aquacole qui a connu, au Québec, la plus forte croissance en terme de productivité (Scarratt, 1995). Ces résultats montrent que la province du Québec offre un potentiel intéressant de développement, dans le domaine de la mytiliculture, mais qui demande une amélioration des techniques de production et la mise au point d'outils pour estimer la qualité des sites d'élevages.

1.2. Influence du milieu sur le rendement en chair

L'objectif principal dans un élevage mytilicole est d'optimiser le rendement ainsi que la qualité de la chair des organismes. Plusieurs facteurs, tels que la quantité de nourriture disponible dans le milieu, la température de l'eau, la salinité et l'hydrodynamisme, influencent la croissance de la moule bleue (Seed, 1976). La quantité de nourriture semble être le principal facteur influençant le rendement des cultures en suspension (Navarro *et al.*,

1991) et selon les travaux de Incze *et al.* (1980) le phytoplancton apparaît comme la principale source de nourriture. D'autres études ont démontré une corrélation positive entre la quantité de nourriture disponible dans le milieu et la croissance des moules (Fréchette et Bourget, 1985; Fréchette et Grant, 1991; Clausen et Riisgård, 1996). La température influence également la croissance. Incze *et al.* (1980) ont observé chez une population de *Mytilus edulis* (Maine, États-Unis) une diminution de la croissance lorsque la température de l'eau augmentait et dépassait 20°C. Bøhle (1972) a démontré qu'une réduction de la salinité diminuait le taux de croissance des moules. De tels résultats avaient déjà été démontrés par Navarro *et al.* (1988) pour la moule *Choromytilus chorus*. Les auteurs avaient mis en évidence un effet positif des fortes salinités (24 à 30 ‰) sur le taux de filtration corrélé à une augmentation de la masse de tissu sec. Par contre, de faibles salinités (15 à 18 ‰) entraînaient une diminution du taux de filtration et de la masse de tissu sec. Wildish et Miyares (1990) ont démontré que la vitesse du courant influençait les apports de nourriture.

1.3. Influence du milieu sur le cycle reproducteur

Le cycle reproducteur est un phénomène physiologique indispensable pour assurer un bon recrutement de la population. Les facteurs influençant ce phénomène sont donc importants pour un élevage mytilicole. Selon Pieters *et al.* (1980) la qualité nutritive d'un site mytilicole a une influence sur la production de gamètes. Une telle relation a également été observée chez d'autres bivalves comme *Mya arenaria*. Gauthier-Clerc *et al.* (2002) ont observé un délai de la gamétogenèse lié à un manque de nourriture dans le milieu. La

température influence également le cycle reproducteur, comme l'ont montré différentes études menées au Canada (Thompson, 1984) et au Royaume-Uni (Maug Myint et Tyler, 1982).

1.4. Les facteurs de stress

1.4.1. Les paramètres naturels

Les facteurs de stress sont des variables importantes à prendre en compte dans la gestion d'un élevage, car ils peuvent affecter l'état physiologique des organismes et porter atteinte à leur santé et/ou leur survie. Des problèmes de mortalité massive ont déjà été rapportés en Amérique du Nord (Emmett *et al.*, 1987) et plus particulièrement aux Îles-de-la-Madeleine (Myrand et Gaudreault, 1995). Tremblay *et al.* (1998) expliquent ce phénomène par une interaction synergique entre plusieurs facteurs (température de l'eau, manque de nourriture, stress post-ponte et la génétique des stocks). D'autres études appuient l'hypothèse de Tremblay *et al.* (1998). Des événements de mortalité, observés dans l'état du Maine aux États-Unis, ont été expliqués par Insze *et al.* (1980) par une réduction de l'abondance de phytoplancton dans le milieu. Emmett *et al.* (1987) ont rapporté que les épisodes de fortes mortalités coïncidaient avec les périodes de ponte intensives. Enfin, Pellerin-Massicotte (1997) a démontré que l'exposition à l'air et l'augmentation de la température de l'eau induisaient un stress sur les populations de moules.

1.4.2. Les contaminants

Les contaminants représentent des facteurs secondaires pouvant affecter la croissance et le cycle reproducteur des organismes. De hauts niveaux de butylétains ont déjà été observés dans l'estuaire du Saint-Laurent (Saint-Louis, 1997). Selon les travaux d'Alzieu (1986), les butylétains présentent un problème dans les élevages conchylicoles européens. Plusieurs études ont montré que ces contaminants affectent la physiologie de la moule. Widdows et Page (1993) ont observé une diminution de la croissance chez des organismes contaminés. Des résultats similaires ont été rapportés par Salazar et Salazar (1991) et Guolan et Yong (1994). Un délai du développement de la gonade a été observé chez des moules zébrées exposées aux butylétains (Regoli *et al.*, 2001). Les métaux lourds peuvent également affecter la croissance et le cycle reproducteur de la moule bleue. Selon Maug Myint, et Tyler (1982), le cuivre induit une inhibition du développement des ovocytes ainsi que de la vitellogenèse, le zinc cause également une inhibition du développement des ovocytes ainsi qu'une destruction des gamètes à l'intérieur des follicules et le cadmium entraîne une inhibition de la gamétogenèse.

1.5. Le système immunitaire de la moule

Chez les bivalves marins, les hémocytes représentent la composante principale du système immunitaire (Bayne et Newell, 1983). Les hémocytes sont distribués à travers le système vasculaire et puisque le système est ouvert, ils peuvent se retrouver aussi à l'intérieur des tissus (Bayne, 1976). Le système immunitaire des bivalves peut être influencé par les facteurs environnementaux. En effet, l'activité des hémocytes peut être influencée par

la température et la salinité du milieu (Fisher et Tamplin, 1988). Une augmentation de la salinité aura un effet négatif sur l'étalement des hémocytes. Par contre, une augmentation de température stimule leur locomotion. Santarém *et al.* (1994) ont observé chez *Mytilus galloprovincialis* une diminution du nombre d'hémocytes, reliée à une période de post-ponte et à de mauvaises conditions nutritives du milieu.

1.6. Problématique et objectifs

Dans les sites d'élevages mytilicoles de l'Est du Québec, un suivi de la température, de la salinité, de la turbidité et de la concentration en chlorophylle *a* est parfois effectué par les producteurs et les chercheurs gouvernementaux. Malheureusement, ces suivis sont très souvent discontinus dans le temps. De plus, peu d'efforts ont été investis jusqu'à ce jour pour mettre en relation ces paramètres environnementaux avec le rendement en chair des moules et la santé des stocks (Cartier *et al.*, 2004). Or, nous avons vu que de tels paramètres environnementaux sont des variables importantes dans un élevage mytilicole et peuvent avoir une influence sur le rendement en chair, la maturation sexuelle, ainsi que le niveau de stress des organismes. Il est donc important pour un mytiliculteur d'avoir une bonne connaissance des conditions environnementales de ses sites de production, mais aussi une bonne connaissance de la condition physiologique de ses stocks afin d'optimiser le rendement de son élevage.

L'objectif général de cette étude est de développer des outils afin d'améliorer la gestion des sites de production mytilicole dans l'Est du Québec. Plus spécifiquement, nous

voulions établir, dans un premier temps, une base de données sur les variations saisonnières de paramètres environnementaux (température de l'eau et abondance de cellules phytoplanctoniques) et de variables physiologiques de la moule (croissance, maturation sexuelle, compétence immunitaire) dans différents sites de production mytilicole de l'Est du Québec. Il est à noter que parallèlement à cet objectif, nous voulions évaluer les taux d'organoétains et de métaux lourds dans les moules afin de renseigner les producteurs sur la présence ou non de contamination métallique et stanique dans leur élevage. Cet objectif, quoique mineur dans notre étude, entrera dans la caractérisation environnementale des sites de production mytilicole. Dans un deuxième temps nous voulions établir des corrélations entre ces différentes variables pour proposer des indicateurs fiables et utilisables par les producteurs de moules en vue d'une meilleure gestion de leurs stocks.

CHAPITRE II

SEASONAL VARIATIONS OF PHYSIOLOGICAL PARAMETERS IN THE BLUE MUSSEL *MYTILUS* SPP. FROM FARM SITES OF EASTERN QUEBEC

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Cartier, S.

ABSTRACT

A seasonal monitoring of indigenous (Gaspe Bay, Carleton, Havre-St-Pierre, Magdalen Islands and Blanc-Sablon) and transplanted (Gaspe Bay, Carleton, Havre-St-Pierre) mussels from different farm sites of Eastern Quebec allowed us to study mussel physiology as a function of environmental factors. Our results showed a positive influence of phytoplankton cell concentration and temperature on the reproductive cycle even if no significant relationship has been demonstrated due to the fragmented pattern of gametogenesis. A decrease of digestive gland index (DGI) was observed during gametogenesis. This process requires a lot of energy and its great influence on digestive gland weight prevented us to correlate mussel growth with environmental factors. In each site, an immunosuppression was also observed in summer and fitted to the most active spawning period in summer. Our results showed a significant positive correlation between maturity index and phagocytic activity. This immunosuppression could have a direct impact on mussel population viability because it occurred during high temperature period when mussels had to face spawning, bacterial developments and diseases. It appeared in our study that reproductive cycle is a key phenomenon in mussel physiology and important for farmers to follow-up. This study is therefore the beginning of the development of reliable indicators of the reproductive cycle.

INTRODUCTION

For mussel farmers the main objective is to get a better quality and growth of the meat yield. Many factors influence growth such as food supply, temperature, salinity and hydrodynamism (Seed, 1976). Food supply seems to be the main factor that can influence production in suspended cultures (Navarro *et al.*, 1991) and according to Incze *et al.* (1980) phytoplankton is the major source of food. Other studies have also reported a positive correlation between food availability and mussel growth (Fréchette and Bourget, 1985; Fréchette and Grant, 1991; Clausen and Riisgård, 1996). Temperature has also an effect on growth. Insze *et al.* (1980) have observed a decrease of growth when water temperature reached 20°C. Bøhle (1972) has shown that a variation in salinity may have a negative effect on the growth rate of mussels. Similar results were found in another mussel species, *Choromytilus chorus* (Navarro, 1988). The author has observed a positive effect of high salinities (24 and 30 ‰) on filtration rate correlated with an increase of dry tissue weight. Low salinities (15 and 18 ‰) caused a decrease in filtration rate and dry tissue weight. A positive linear relationship was described by Strömgren and Cary (1984) between the final shell growth rate and the phytoplankton cell concentrations within the range 2.5 to 10.0 × 10^7 cells.L^{-1}. Mussels held in batches in filtered seawater with no addition of food showed an immediate decrease in shell growth rate while mussels rapidly showed significant growth responses to major changes in the food conditions. Enhanced growth with

multispecies diets indicated a synergetic effect. Results of Strömgren and Cary (1984) suggested that mussels might adjust to high concentration of phytoplankton cells. Fréchette *et al.* (1989) and Wildish and Miyares (1990) have demonstrated that current velocity modifies food flux and feeding efficiency.

Gametogenesis is crucial for species, since production and fertility of gametes will insure a good recruitment to renew the stock. Factors influencing this process are important in mussel farming. Nutritional quality at farm sites has an influence on mussel gametogenesis (Pieters *et al.*, 1980). A similar relationship has also been observed in other bivalves such as *Mya arenaria*. Gauthier-Clerc *et al.* (2002) have observed a delay of gametogenesis linked to a lack of food. Other studies have reported an effect of temperature on gametogenesis in Canada (Thompson, 1984) and in United Kingdom (Maug Myint and Tyler, 1982).

Stress factors are also important parameters in mussel farms management since they could affect the condition of the stock and could lead to diseases and mortality. In Magdalen Islands (Myrand and Gaudreault, 1995) and North America (Emmett *et al.*, 1987) problems of massive mortality in mussel populations have been reported. A possible cause could be a synergetic interaction between temperature, lack of food, post-spawning stress and the genetics of the stocks (Tremblay *et al.*, 1998). Insze *et al.* (1980), who studyied mussels from a northern estuary (Main, USA), also observed a peak of mortality in summer. The authors suggested that mortality was triggered by reduction in phytoplankton

concentration and starvation. Emmett *et al.* (1987) have reported that the time of high mortality coincided with intense spawning activity. Pellerin-Massicotte (1997) has shown an effect of air exposure and increased temperatures to induce oxidative stress in the blue mussel.

Contaminants represent factors that could affect growth and reproduction of many organisms. Moderate levels of butyltins have been measured in the St. Lawrence River (Saint-Louis *et al.*, 1997). Butyltins have already been a problem in European shellfish farms (Alzieu, 1986). Numerous studies have shown butyltin effects on mussel physiology. Widdows and Page (1993) have observed a decreased growth rate in contaminated mussels. Similar results were observed by Salazar and Salazar (1991) and Guolan and Yong (1994). A delay in gonad development was observed in zebra mussels exposed to butyltins (Regoli *et al.*, 2001). Heavy metals also had effects on mussel growth and reproductive cycle. According to Maug Myint and Tyler (1982), copper induced an inhibition of oocyte development and vitellogenesis, zinc, an inhibition of oocyte development and gametes breakdown inside follicles and cadmium an inhibition of gametogenesis.

In marine bivalves, hemocytes are the main component of the immune system (Bayne and Newell, 1983). Hemocytes are distributed all over tissues by the vascular circulation (Bayne, 1976). Immune system of marine bivalves could be affected by environmental factors. Indeed, phagocytic activity could be influenced by water temperature and salinity

(Fisher and Tamplin, 1988). Santarém *et al.* (1994) observed a decrease of hemocytes number correlated with post-spawning period and low nutritive quality.

We will study the seasonal variations of water temperature, phytoplankton abundance at various farm sites in eastern Quebec, as well as physiological aspects of both indigenous and transplanted mussels. We will examine correlations between exogenous factors and the reproductive cycle, growth rate and immunocompetence of mussels. Seasonal pattern of morphological indices, digestive gland, metabolic reserves and hemocytes' phagocytic activity have been surveyed. The objective of this study was to examine relationship between environmental factors and mussel physiology to improve mussel-farming practices.

MATERIALS AND METHODS

Site selection and sampling period

One-year juvenile blue mussels (*Mytilus* spp.) were collected in May 2002 in the Gaspe Bay (Gaspe Peninsula, Eastern Quebec) and transplanted in cages in three farm sites: Havre-Saint-Pierre (50°14'N 63°36'W, North Shore of the Gulf of St. Lawrence), Carleton (48°06'N 64°30'W, Bay of Chaleur) and Gaspe (48°46'N 64°17'W, inside Gaspe Bay) as the reference site (Figure 1). Mussels from Gaspe Bay were chosen for the safe bacteriological conditions of the site and the lack of toxic algae bloom. A monitoring of indigenous (cultivated) mussels was also realized on each transplantation site and on two other farm sites: Havre-aux-Maisons lagoon in Magdalen Islands (47°25'N 61°48'W) and Blanc-Sablon (51°28'N 57°27'W, North Shore of the Gulf of St. Lawrence). We were not allowed to transplant mussels from Gaspe Bay to Magdalen Islands and Blanc-Sablon sites due to genetic differences between populations. Physiological condition of mussels from Gaspe site was used as a reference pattern to compare responses between transplanted mussels and to give farmers information about their stock performance. After a first successful sampling period in 2002 (Cartier *et al.*, 2003) another six-month sampling period was realized every two weeks, from June to November 2003.

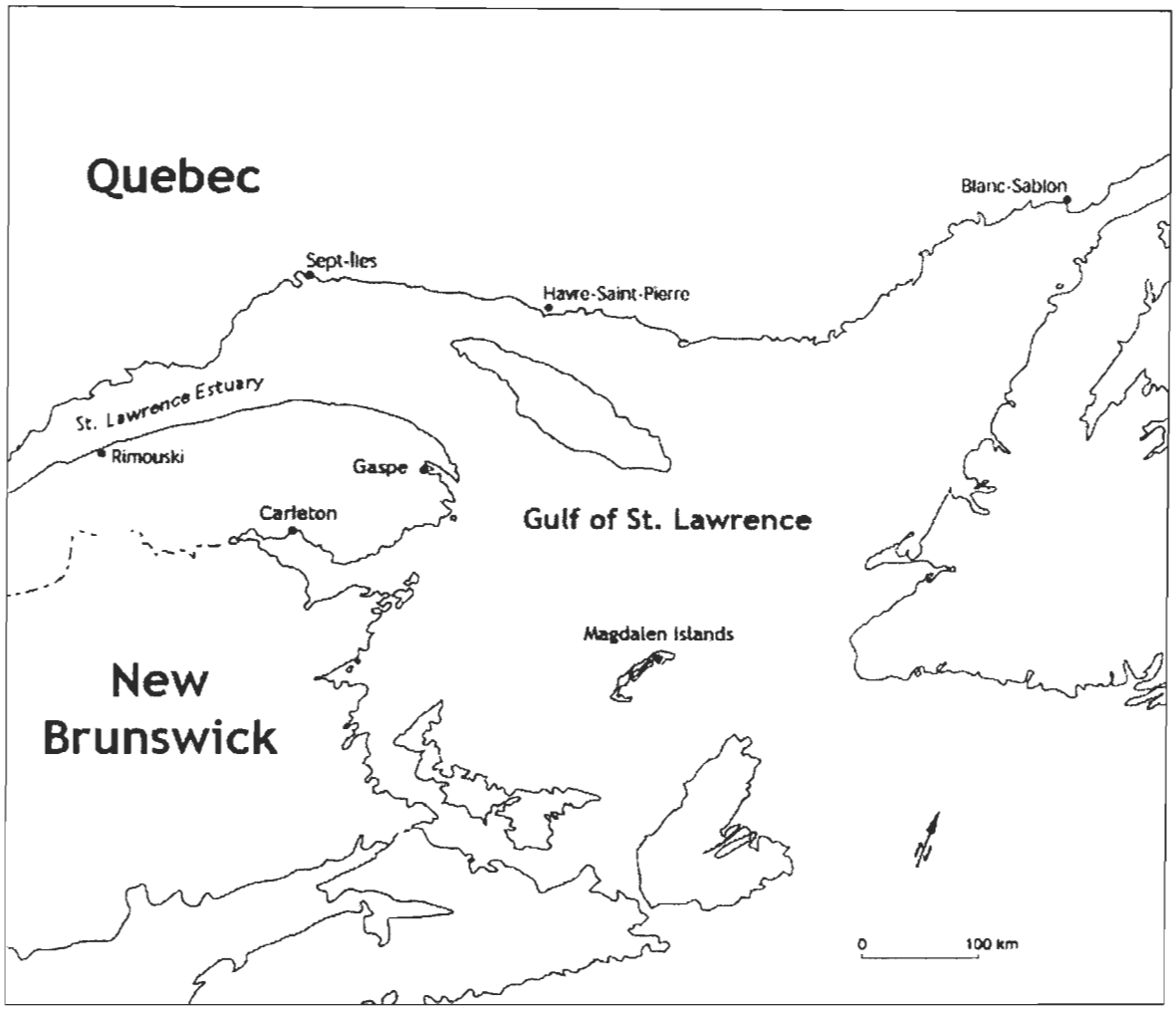

Figure 1. Location of the five sampling sites in the Gulf of St. Lawrence (Eastern Canada). Mussels collected in Gaspe Bay were caged in Havre-St-Pierre, Carleton and Gaspe Bay. Indigenous mussels were collected at each transplantation site, as well as at Magdalen Islands and Blanc-Sablon.

Morphological measurements

For each sampling date and site, shell length and wet weights of total flesh, digestive gland, mantle and gonad were measured on both indigenous and transplanted mussels (N=25). Mantles dedicated to histology examination were quickly frozen in acetone (-50°C) using a Histobath® (Thermo® Shandon®) to preserve tissue integrity. Digestive gland and mantle samples were kept at -80°C until analysis.

Phagocytosis

For each sampling site and date, phagocytic activity of hemocytes was assessed on both indigenous and transplanted mussels (N=15) according to the method described by Cartier *et al.* (2004).

Histological examination

After tissue dissection, mantle smears of transplanted mussels (N=25) were directly observed with a light microscope (Hund-Wetzlar V300) to determine sex and calculate sex-ratio. Frozen mantle (N=12) sections of 5 µm were cut at -30°C with a cryostat (Thermo® Shandon® Cryotome® 0620) and stained with Lee's methylene blue-basic fuchsin. Gonad maturation stage was identified with a light microscope (Leitz Ortholux II) and classified according to Gauthier-Clerc *et al.* (2002). Each maturation stage was characterized by a maturity factor and used to calculate a maturity index separately for male and female according to Siah *et al.* (2003):

$$MI = \sum_{i=1}^{6} (PS \times MF)$$

MI Maturity Index

PS Proportion of each maturation stage

MF Maturity Factor

1: for indifferent

2: for development I (male) or pre-vitellogenic (female)

3: for development II (male) or vitellogenic (female)

4: for mature (male) or post-vitellogenic (female)

5: for spawning

6: for spent

Biochemical analyses

For each sampling date and transplantation site, metabolic reserves were determined on transplanted mussel's digestive gland (N=12). Tissues were homogenized in phosphate buffer (0.1 M pH 7). Total protein content was determined according to the protein-Coomassie blue dye binding reaction (Bradford, 1976) adapted to micromethod and using bovine serum albumin (Sigma, fraction V) as standard. Samples were analyzed with a microplate reader (Teacan SpectraFluor Plus). Glycogen content was determined after enzymatic digestion of digestive gland homogenate with amyloglucosidase and analyzed according to the colorimetric method described by Carr and Neff (1984). Glycogen from

oyster (Sigma, type II) was used as standard. Samples were analyzed with a spectrophotometer (UV-VIS Beckman DU 640).

Mussel contamination assessment

Analysis of organotins and heavy metals were performed on the digestive gland of indigenous mussels from each site at the first and last sampling dates (June and November 2003) to assess contamination on sampling sites.

Organotins analysis

Organotin extraction (tri-, di- and monobutyltin) was adapted from the method developed by Chau *et al.* (1997). For each sampling site and date, triplicate of freeze-dried digestive gland pool (N=3) was digested with tetramethyl ammonium hydroxide (TMAH). Then, organotin species were ethylated with sodium tetraethyl borate ($NaBEt_4$) and after centrifugation the organic fraction was removed and cleaned on a silica-gel micro-column. The volume of the extract was reduced to 200 µl under a nitrogen stream at room temperature. Organotin species were quantified on a Finnigan POLARIS Q Ion Trap GC/MS according to Viglino *et al.* (2004).

Heavy metal analysis

For each sampling site and date, freeze-dried digestive gland pools (N=6) were digested in ultrapure nitric acid at high temperature and high pressure and then diluted in demineralised water. Cadmium, copper, zinc and lead levels were measured using a

ICP/MS (Inductively coupled plasma mass spectrometry). Mercury levels were measured with the cold vapour atomic absorption method. Quality control was performed using reference materials certified by the National Research Council Canada. Analyses were carried out by the Centre de Toxicologie de Québec (Sainte-Foy, Quebec, Canada).

Environmental factors

Water samples were collected at each site and at each sampling date with a 5 liter Niskin bottle. Water was immediately transferred into a one liter dark High Density Poly Ethylene bottle and fixed with a fresh paraformaldehyde solution (20% w/w; 0.2 µm prefiltered). Samples were stored on ice in an insulate container and sent to the laboratory the same day. Three 5 ml subsamples were collected, placed in cryovials and quickly frozen in acetone (-50°C) with a Histobath® (Thermo® Shandon®) and stored at -80°C until analysis. Phytoplankton concentration, size of phytoplankton cells (<2 – 20 µm) and their fluorescence signature (chlorophyl *a* and phycoerythrin) were determined with a FACScan (Becton Dickinson). Fluorescent microbeads of 2, 10 and 20 µm diameter (Molecular Probes Inc.), used as an internal calibration standard for the determination of particle size, were mixed to 1,5 ml of each sample before analysis. Sheath fluid used for the analysis was FACSflow (Becton Dickinson). Thermographs (model ONSET H08-001-02, Hoskin Ltd. Montreal) were immersed on each sampling site and water temperature was monitored three times per day during the whole sampling period.

Statistical treatments

Pearson correlations were used to verify the influence of environmental factors on mussel physiology and within physiological variables. Correlations were tested between maturity index versus abundance of phytoplankton cells, water temperature and metabolic reserves. Correlation was also tested between abundance of phytoplankton cells versus growth and digestive gland index. Furthermore, the relationship between growth versus digestive gland index and protein concentration was verified. Correlations between data were analyzed with SPSS 13.0 for Windows. Differences between maturity index of males and females were tested at each transplanted site by a T-Test for independent samples or a Mann-Whitney Rank Sum Test when no normal distribution of data was observed. The same statistic procedure was used to test differences between indigenous and transplanted populations at each transplanted sites. A One-Way ANOVA was performed on condition index to test significant differences between sites for both indigenous and transplanted mussels. To isolate sites that differed from the others, Tukey or Games-Howell (when variance was heterogenous) post-hoc tests were performed. When ANOVA assumptions were not respected, a Kruskal-Wallis One Way Analysis of Variance on Ranks was performed followed by a Dunn's post-hoc test. Comparisons of means were performed with SigmaStat software version 3.11 from Systat.

RESULTS

Environmental factors

Phytoplankton abundance

Abundance of phytoplanktonic cells has been quantified for three size classes: <2, 2-10 and 10-20 µm. Results have been pooled in two size classes: <20 and 2-20 µm and presented by abundance curves (Figure 2) and cumulative abundance curves (Figure 3). On each site, there was a dominance of small size (<2 µm) phytoplanktonic cells. In Gaspe Bay, no major bloom was observed (Figure 2A). Cumulative abundance curves (Figure 3 A,B) have shown a regular input of cells in this site, all along the sampling period, except for some seasonal variations. In Carleton (Figure 2B), a bloom was observed at the end of August and early September. This important input of phytoplanktonic cells is well illustrated by a break (⬅①) in the slope of cumulative abundance curves (Figure 3A,B). In Havre-St-Pierre (Figure 2C), concentrations of 2-20 µm cells were most important during the first part of the sampling period (from early June to the end of August). Two blooms of small size (<2 µm) cells were observed at the end of July and the end August. The first bloom was twice less important than the second one in term of abundance. These inputs in small size cells were characterized by a break in the slope (⬅②, ⬅③) of the cumulative abundance curve (Figure 3B). In Magdalen Islands (Figure 2D), the seasonal pattern of phytoplankton abundance was characterized by an important bloom at the end of August,

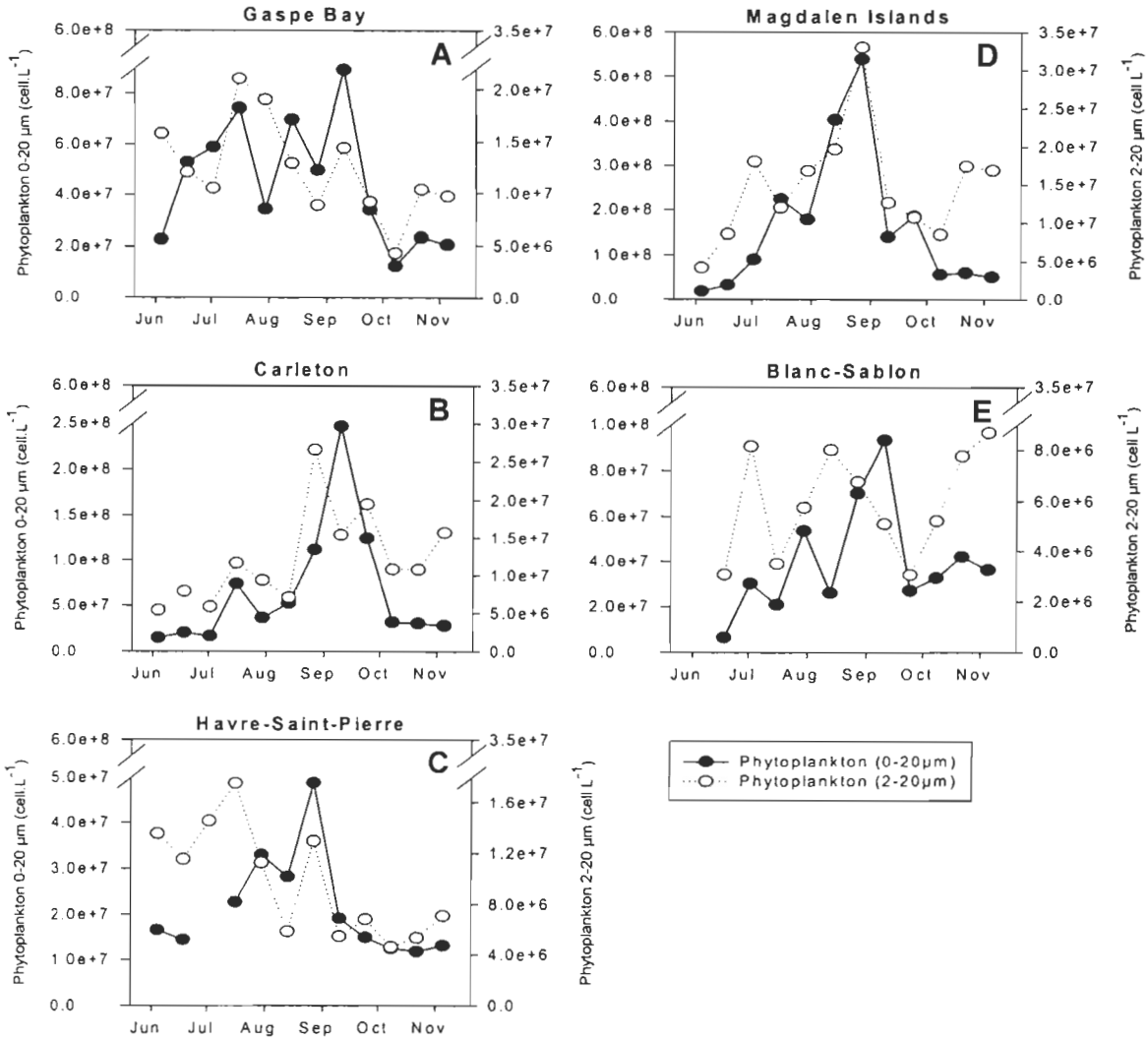

Figure 2. Variations of the abundance of phytoplankton cells. Samplings were done from June to November 2003 in five sites: Gaspe Bay (A), Carleton (B), Havre-St-Pierre (C), Magdalen Islands (D) and Blanc-Sablon (E). Concentrations of phytoplankton cells are presented in a range from 0 to 20 µm (●) and excluding cells below 2 µm (○). As in Cartier *et al.* (2004), standard deviations were not put on graphics to get a better view of results.

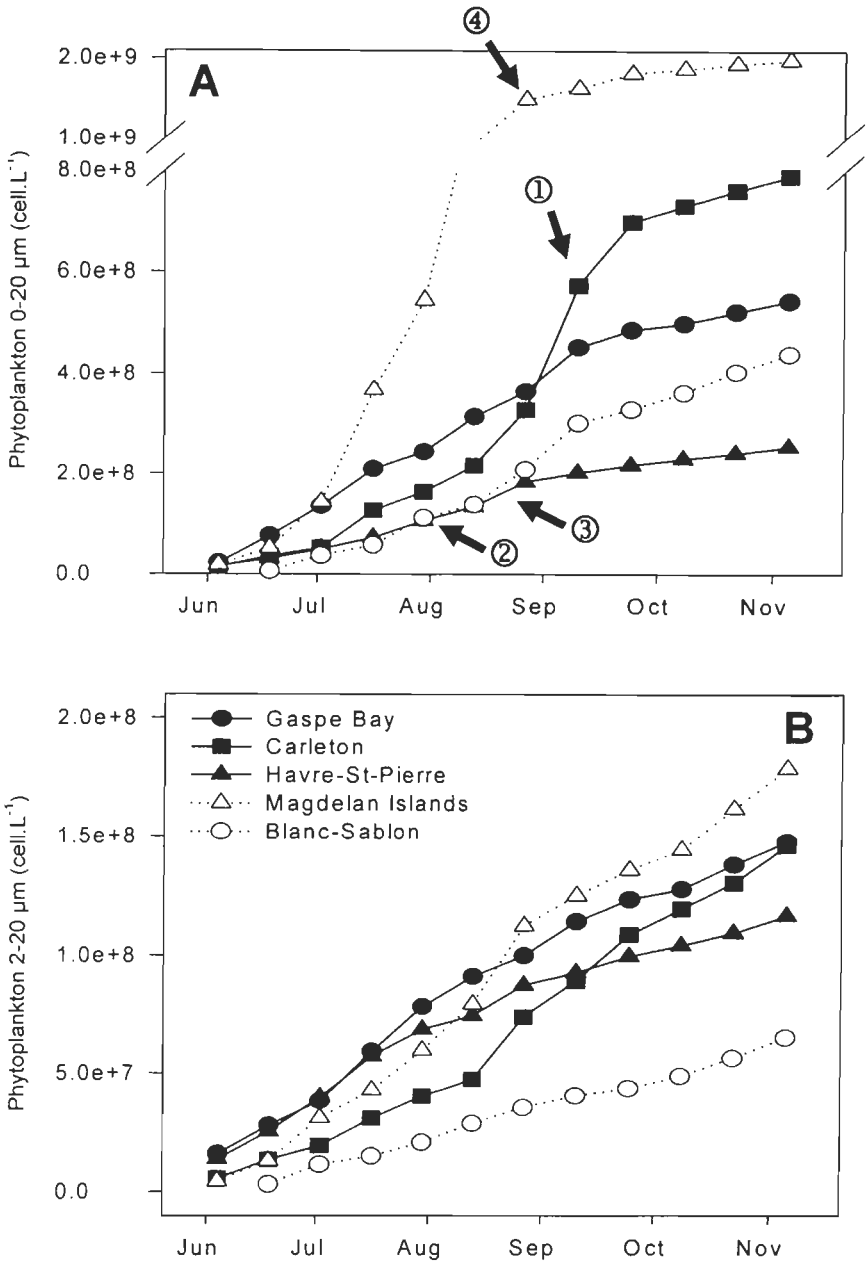

Figure 3. Cumulative abundance of phytoplankton cells from June to November 2003. Sampling was done in the five experimental sites: Gaspe Bay, Carleton, Havre-St-Pierre, Magdalen Islands and Blanc-Sablon. Graphic A represents concentrations of 0-20 µm cells and graphic B, 2-20 µm cells. (➡) spots a break in the slope of cumulative curve.

characterized by a break in the slope (⬅ ④) of the cumulative abundance curves (Figure 3A,B). In Blanc-Sablon (Figure 2E), there was no major bloom of cells. Phytoplankton inputs were almost regular as illustrated by the linear pattern of cumulative abundance curves (Figure 3A,B).

Water temperature

Water temperatures are illustrated in Figure 4. In Gaspe Bay (Figure 4A), temperature increased of about 2°C from early June to September and then decreased until 0°C in early November. Temperature pattern in Carleton (Figure 4B) was characterized by numerous seasonal variations that could reach 13°C of magnitude (at the end of July). The minimum temperature (4°C) was observed in early July and the maximum (20°C) at the end of July. Havre-St-Pierre site (Figure 4C) was also characterized by seasonal variations (10°C magnitude for highest values in August). Two rises of temperature were observed in this site in early August (14°C) and at the end of October (9°C). In Magdalen Islands (Figure 4D), temperature rose from early June to July (reaching a maximum of 21°C) and then decreased until early November to reach a minimum of 5°C. Missing values for Blanc-Sablon are explained by technical problems encountered with the thermograph. However, previous results recorded from June to November 2002 have shown seasonal variations in a range from 4°C to 13°C with maximum values between July and mid-September.

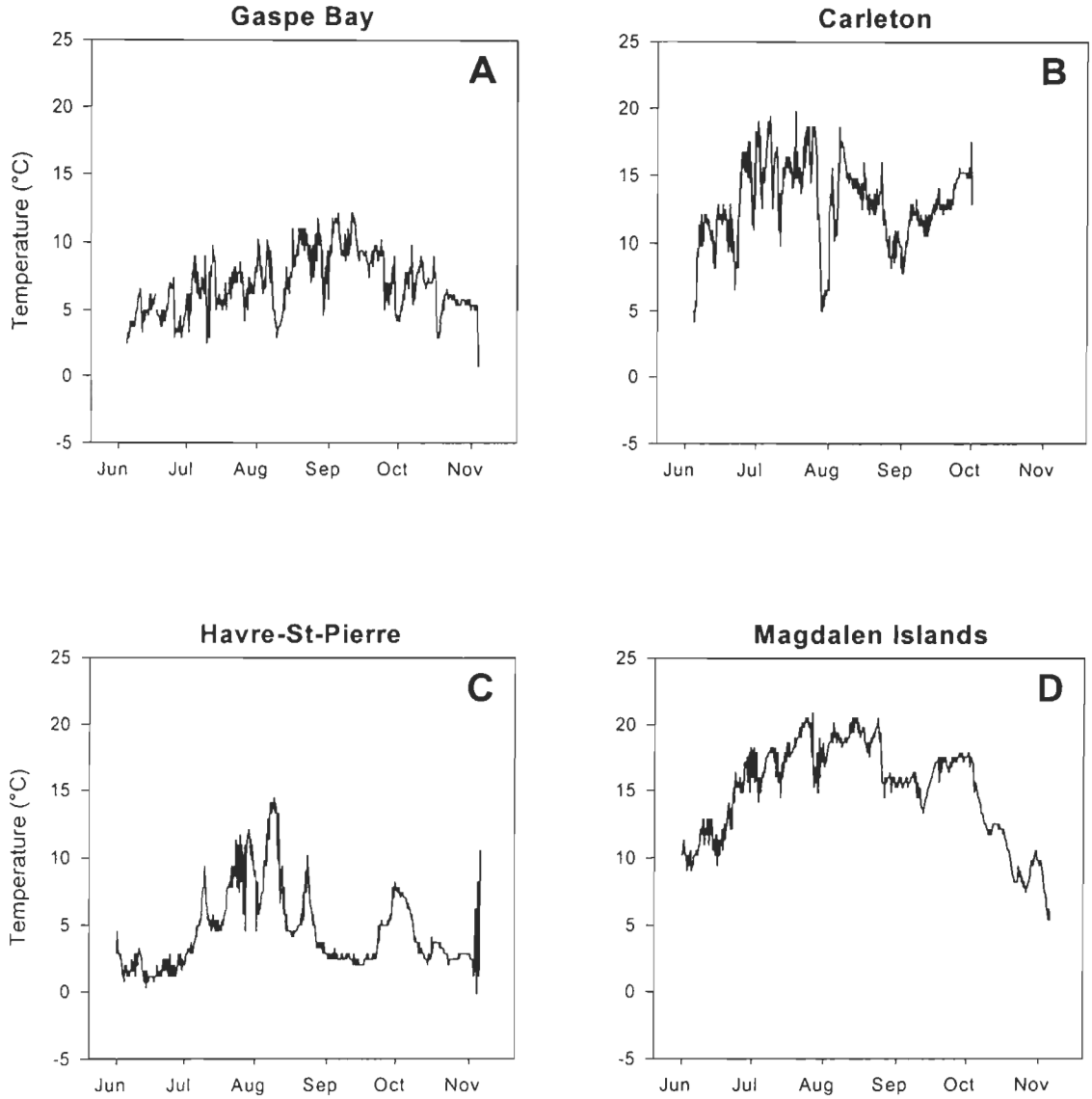

Figure 4. Seasonal variations of water temperature in Gaspe Bay (A), Carleton (B), Havre-St-Pierre (C) and Magdalen Islands (D) from June to November 2003. Thermographs were installed at the same depth as the cages (9-10 m for all sites except for Magdalen Islands lagoon: 4 m) and water temperature was measured three times per day (8h00, 16h00 and 24h00).

Reproductive cycle

Reproductive cycle was studied in transplanted mussels by studying the proportion of males and females at each stage of the sexual maturation (Figure 5) and by the evolution of the maturity index (Figure 6). In Gaspe Bay, males (Figure 5A) showed a delay in gametogenesis in comparison to females (Figure 5B). Indeed, maturity index was always lower in males than in females (Figure 6A). This trend was observed in the two other transplanted mussel's sites (Carleton: Figure 6B and Havre-St-Pierre: Figure 6C). In Gaspe Bay, ripe stage was observed for males in mid-July (Figure 5A) while 80% of females were at this stage in early June (Figure 5B). Except for a partial spawning in July (20% of organisms), males have spawned from early September to the end of October (Figure 5A). Female spawning began in July until the end of the sampling period in November (Figure 5B). At the end of August and beginning of September, a resumption of gametogenesis was observed for 40-60% of females (Figure 5B). In early November, 30% of males were in indifferent stage (Figure 5A) comparatively to 80% of females (Figure 5B). In Carleton, a partial spawning was observed for 40-60% of males from mid-June to mid-July (Figure 5C). Another spawning period was also observed for females in July (Figure 5D). This delay in the reproductive cycle between males and females was characterized by a higher maturity index for males than females from early June to mid-July (Figure 6B). Males remained in ripe stage until the end of October where 50% of mussels were observed in spawning or spent stage (Figure 5C). For females, a second spawning period has been observed in September. From the end of October, 80% of females were in spent or indifferent stage (Figure 5D). In Havre-St-Pierre, a delay in gametogenesis of females was

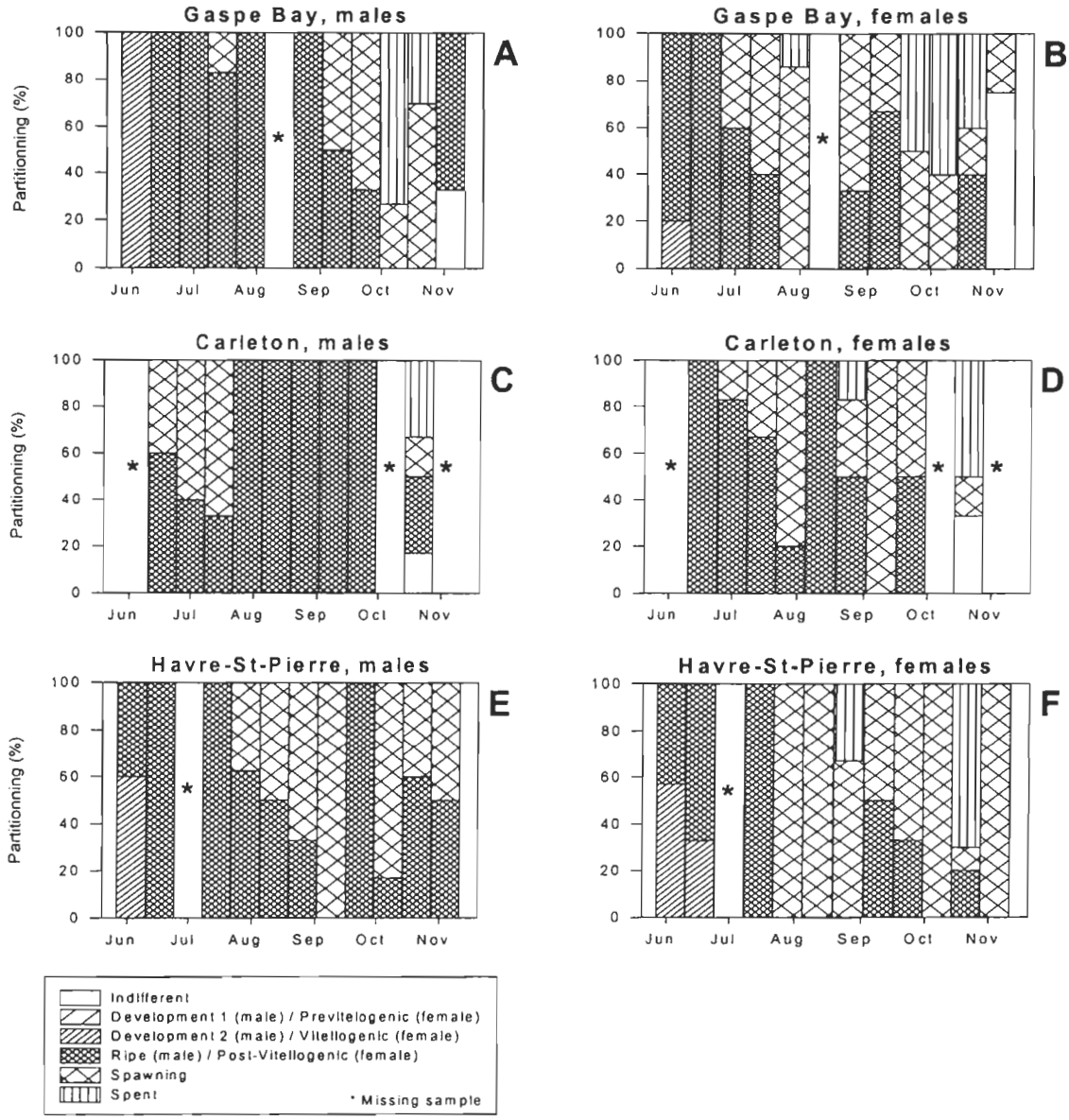

Figure 5. Proportions of males (A: Gaspe Bay, C: Carleton, E: Havre-St-Pierre) and females (B: Gaspe Bay, D: Carleton, F: Havre-St-Pierre) for each stage of the sexual maturation of transplanted *Mytilus* spp. from June to November 2003.

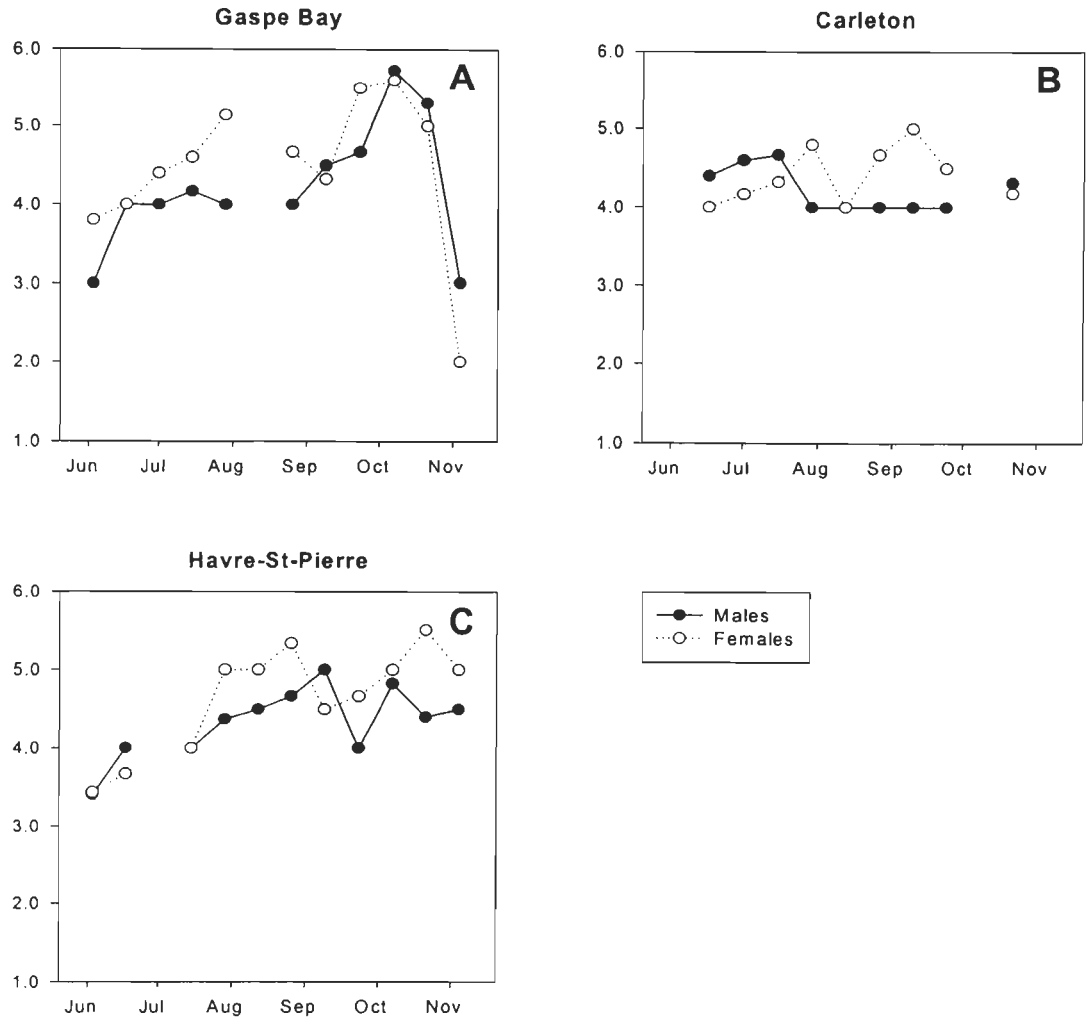

Figure 6. Variations of maturity index (MI) during reproductive cycle of transplanted Mytilus spp. in Gaspe Bay (A), Carleton (B) and Havre-St-Pierre (C) from June to November 2003. MI=Σ (% of each sexual state×maturity factor) and calculated with the different maturity factors: 1=indifferent; 2=development I for males and previtellogenic for females; 3=development II for males and vitellogenic for females; 4=ripe for males and postvitellogenic for females; 5=spawning; 6=spent.

observed in June as illustrated by a lower maturity index than males (Figure 6C). All the males were in ripe stage in mid-June (Figure 5E) whereas 30% of females were still in development stage (Figure 5F). A first spawning period was observed for males from the end of July (30% of organisms) to early September (100% of organisms). After a resumption of gametogenesis, another spawning period was observed from early October to early November (Figure 5E). Most of the females were in spawning stage from the end of July to early November. A partial resumption of gametogenesis was observed in September (Figure 5F). None of the males or females was observed in indifferent stage in Havre-St-Pierre. Despite of delays observed between males and females reproductive cycle, no significant difference was observed between males and females maturity indices in each transplantation site.

Growth and morphometric indices

Condition indices are presented in Figure 7 for both indigenous and transplanted mussels from the different sampling sites. The general trend in transplanted sites (Figure 7A, 7B and 7C) was a decrease of the condition index (CI) all along the sampling period. In Magdalen Islands (Figure 7D), no variations of CI were observed while in Blanc-Sablon (Figure 7E) condition index slightly increased during the sampling period. A significant difference of condition index was observed for indigenous sites except for Gaspe Bay and Blanc-Sablon. In transplanted mussels, CI was significantly different between Carleton and the two other transplanted sites. However, CI of mussels in Gaspe Bay and Havre-St-Pierre

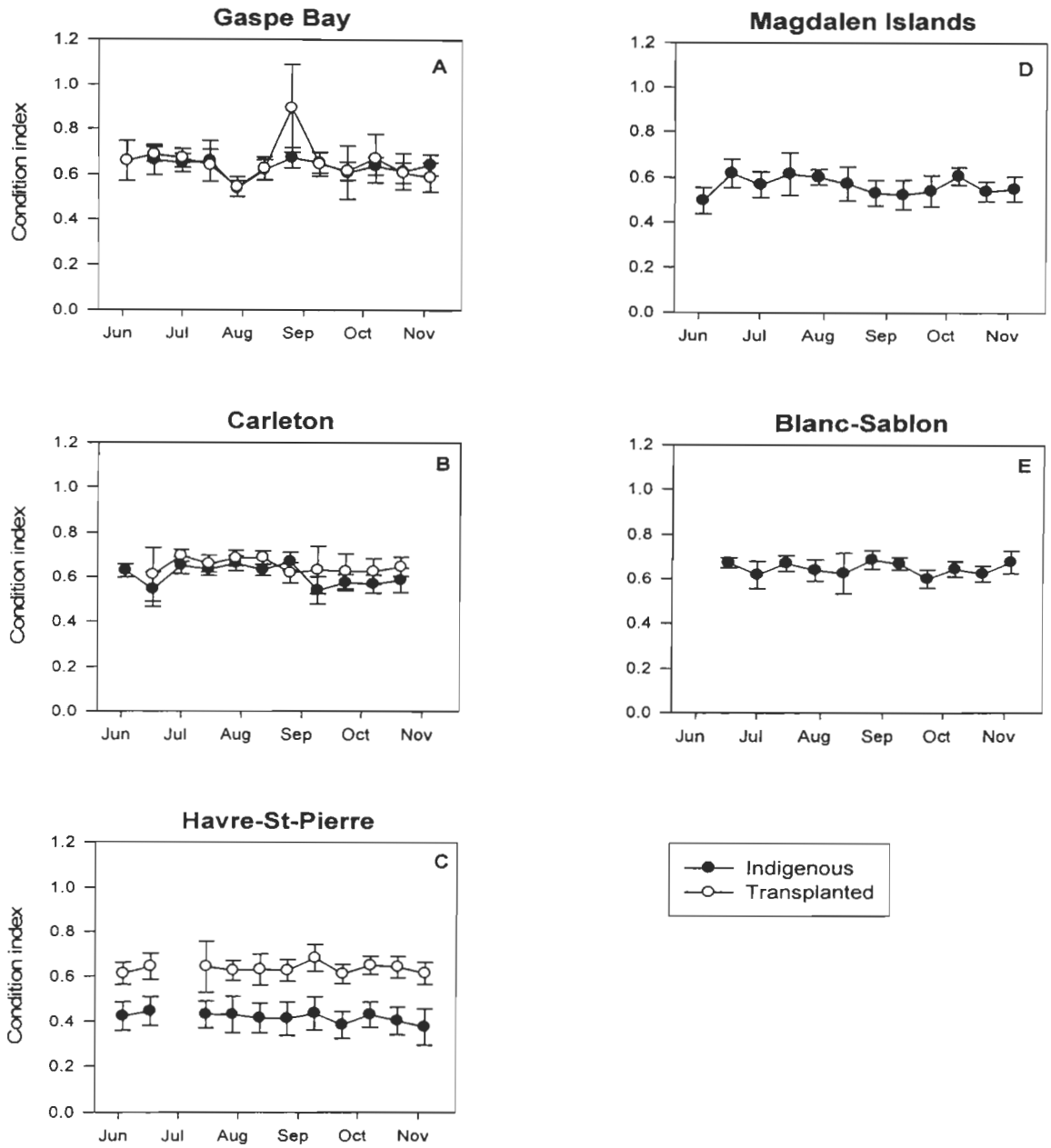

Figure 7. Seasonal variations of condition index of *Mytilus* spp. in each experimental sites: Gaspe Bay (A), Carleton (B), Havre-St-Pierre (C), Magdalen Islands (D) and Blanc-Sablon (E) from June to November 2003. Data are presented for indigenous (●) and transplanted (○) mussels.

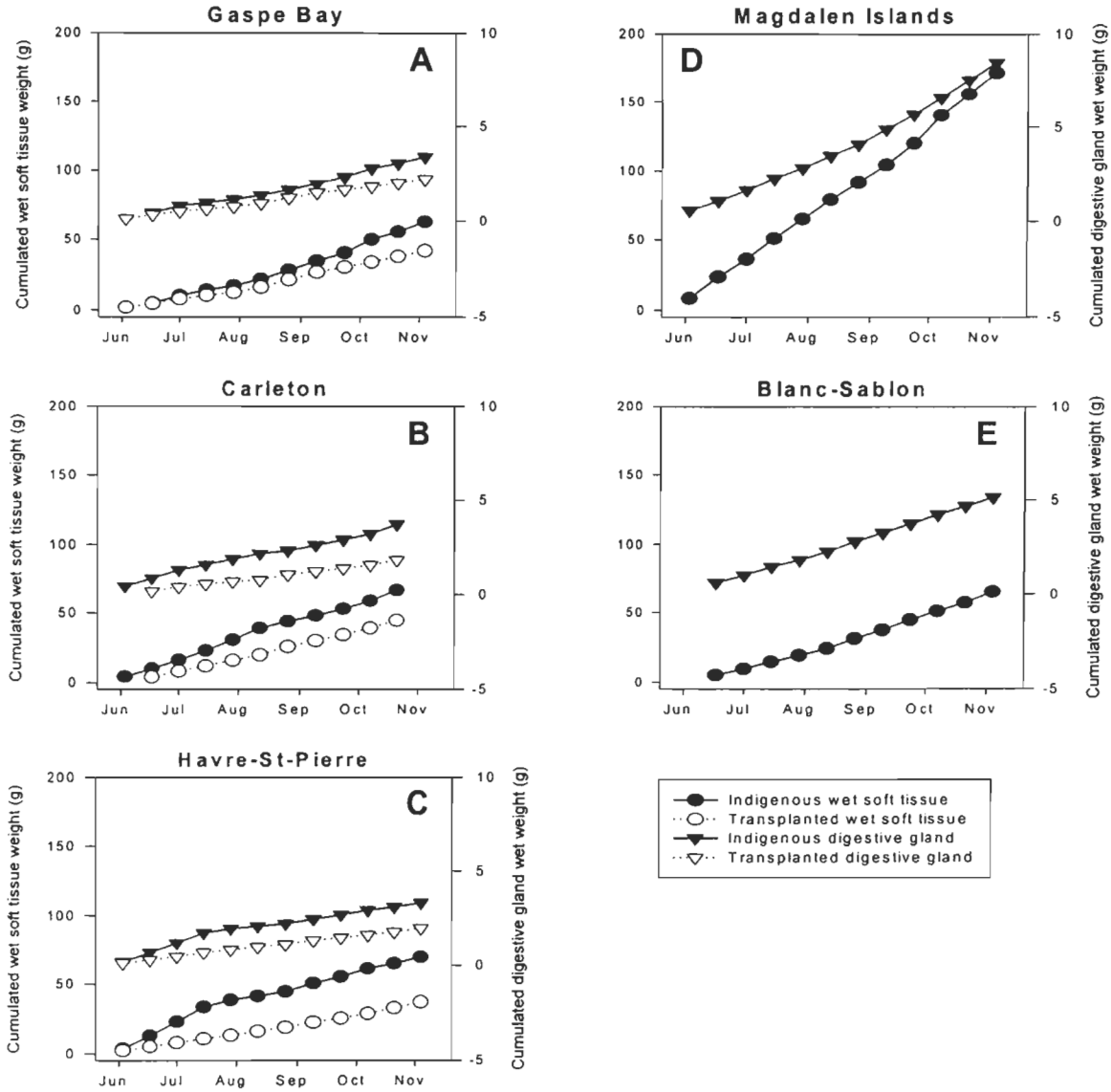

Figure 8. Cumulative weights of total wet soft tissue (indigenous=● and transplanted=○) and digestive gland (indigenous=▼ and transplanted=▽) of *Mytilus* spp. at each experimental site: Gaspe Bay (A), Carleton (B), Havre-St-Pierre (C), Magdalen Islands (D) and Blanc-Sablon (E) from June to November 2003.

were not significantly different. The digestive gland index (DGI) decreased all along the sampling period. In Carleton (Figure 7B), DGI decreased mainly in summer, from June to September. Only the indigenous mussels of Magdalen Islands showed an increase of DGI. Cumulative weights of digestive glands demonstrated a similar pattern for each site for both indigenous and transplanted mussels (Figure 8) except for Magdalen Islands where soft tissue growth was most important than the other sites. Generally, at each site, digestive gland and soft tissue weights of transplanted mussels were lower than those of indigenous mussels (Figure 8). Except in Blanc-Sablon (Figure 8E), a meat yield variation was observed in each site. This variation was characterized by a break in the slope of cumulative growth curve (Figure 8). In Gaspe Bay (Figure 8A) and Magdalen Islands (Figure 8D), we observed an increase of the meat yield from September whereas in Carleton (Figure 8B) and Havre-St-Pierre (Figure 8C) we observed a decrease of the meat yield.

Digestive gland metabolic reserves

Total Protein

Concentrations in total protein were quantified in digestive gland of transplanted mussels (Figure 9) and a significant seasonal variation was observed (See Figure 10 for post-hoc tests results). Except for some seasonal variations, concentrations in total protein global increased at each site during the sampling period. In Gaspe Bay (Figure 9A), numerous variations of total protein concentrations were observed. Total protein levels in

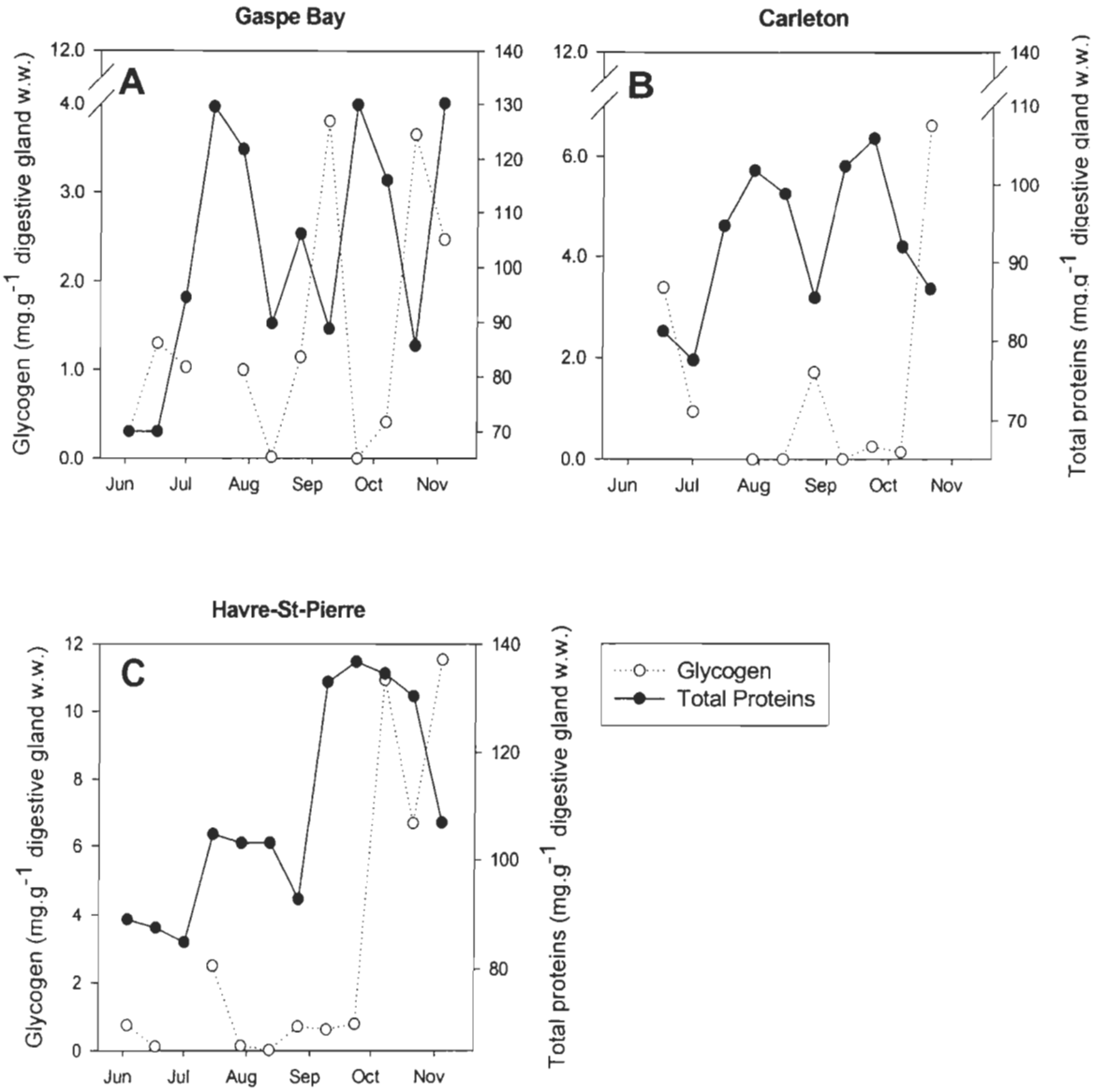

Figure 9. Seasonal variations of glycogen (○) and total protein (●) concentrations in the digestive gland tissue of transplanted *Mytilus* spp. at Gaspe Bay (A), Carleton (B) and Havre-St-Pierre (C) from June to November 2003. As Cartier *et al.* (2004), standard deviations were not put on graphics to get a better view of results.

digestive gland ranged from 70 to 130 $mg.g^{-1}$ w.w (wet weight). In Carleton (Figure 9B), a first increase of total protein concentrations was observed in July (100 $mg.g^{-1}$ w.w.) and was followed by a decrease to 90 $mg.g^{-1}$ w.w. in August and a second increase in September (110 $mg.g^{-1}$ w.w.). In Havre-St-Pierre (Figure 9C), the increase of total protein levels seemed to occur by successive steps. Concentrations have reached 90 (in June) to 100 (in August) and finally 140 $mg.g^{-1}$ w.w. in September.

Glycogen

Concentrations in glycogen were analyzed in the digestive gland of transplanted mussels and a significant seasonal variation was observed (See Figure 10 for post-hoc tests results). Results are illustrated in Figure 9. Generally, at each site, glycogen concentrations were low in spring and summer and higher in autumn. In Gaspe Bay (Figure 9A), glycogen concentrations varied from 1.0 (in early June) to 4.0 (in early September) $mg.g^{-1}$ w.w. then decreased to very low values at the end of September and finally increased to 4.0 $mg.g^{-1}$ w.w. in October. In Carleton (Figure 9B) glycogen concentrations decreased from 3.5 $mg.g^{-1}$ w.w. in early June to very low values in July and remained to this level until early October. However, a slight increase to 2.0 $mg.g^{-1}$ w.w. was observed in August. Glycogen level increased rapidly to reach 6.0 $mg.g^{-1}$ w.w. in mid-October. In Havre-St-Pierre (Figure 9C), glycogen concentrations decreased from 2.0 $mg.g^{-1}$ w.w. to undetectable levels during July. Level remained to these low values until the end of September where a rapid and important increase of glycogen concentration (10.0 $mg.g^{-1}$ w.w.) was observed.

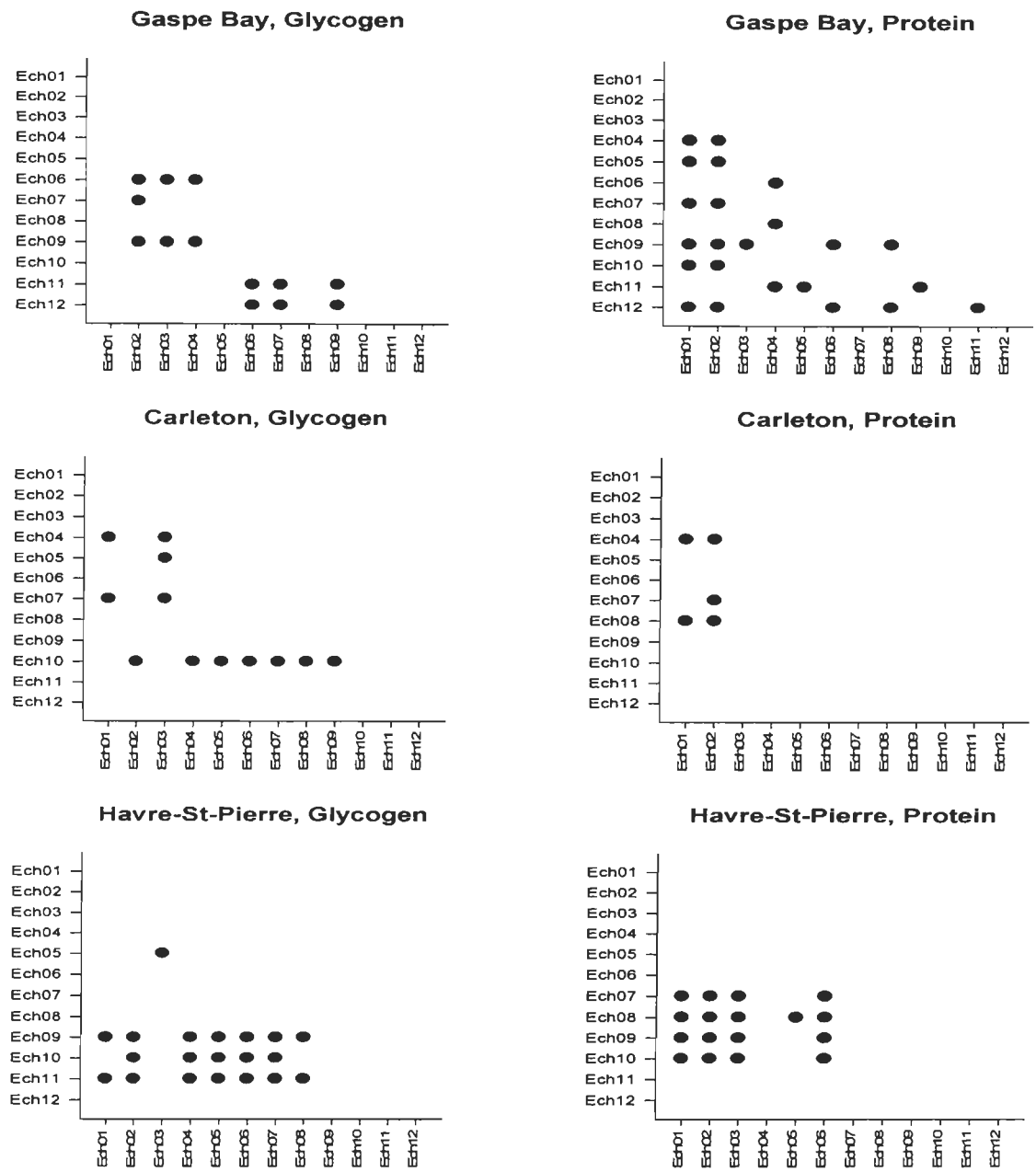

Figure 10. Matrix results of post-hoc tests (Dunn's method) following compared means analysis on metabolic reserves. Symbols (●) indicate significant differences (P=0.05) between two sampling dates. Label from Ech01 to Ech12 represent the twelve sampling dates.

Phagocytic activity

Phagocytic activity was assessed on hemocytes from hemolymph of both indigenous and transplanted mussels. Results are illustrated in Figure 11 and Figure 12. In Gaspe Bay, phagocytic activity pattern of transplanted mussels (Figure 11A1) showed significant differences with indigenous mussels (Figure 11A2). This result was also observed in Carleton (Figure 11B1 and Figure 11B2). In Havre-St-Pierre (Figure 11C1 and Figure 11C2) no significant difference was shown between indigenous and transplanted mussels. A period of immunosuppression was observed at each site for both indigenous and transplanted mussels. This period extended through two or four weeks depending on the site and was predominant in July (Figure 11 and Figure 12).

Contamination level in organisms

Butyltins

Concentrations in tri- (TBT), di- (DBT) and monobutyltins (MBT) in the digestive gland of indigenous mussels were determined at the beginning (June) and the end (November) of the sampling period. Results are presented in Figure 13. Butyltins levels range from limit detection to 25.0 $ng.g^{-1}$ d.w. (dry weight). in each site. In Gaspe Bay (Figure 13A), a decrease of TBT concentrations was observed (from 20.0 to 5.0 ng.g-1 d.w.) whereas DBT and MBT levels remained stable (respectively 15.0 and 7.0 ng.g-1 d.w.). In Carleton (Figure 13B), we observed a decrease of TBT (from 10.0 to 3.0 ng.g-1 d.w.) and DBT (from 14.0 to 10.0 ng.g-1 d.w.) concentrations. MBT levels remained stable at 10.0 ng.g-1 d.w. In Havre-St-Pierre (Figure 13C), butyltins concentrations remained

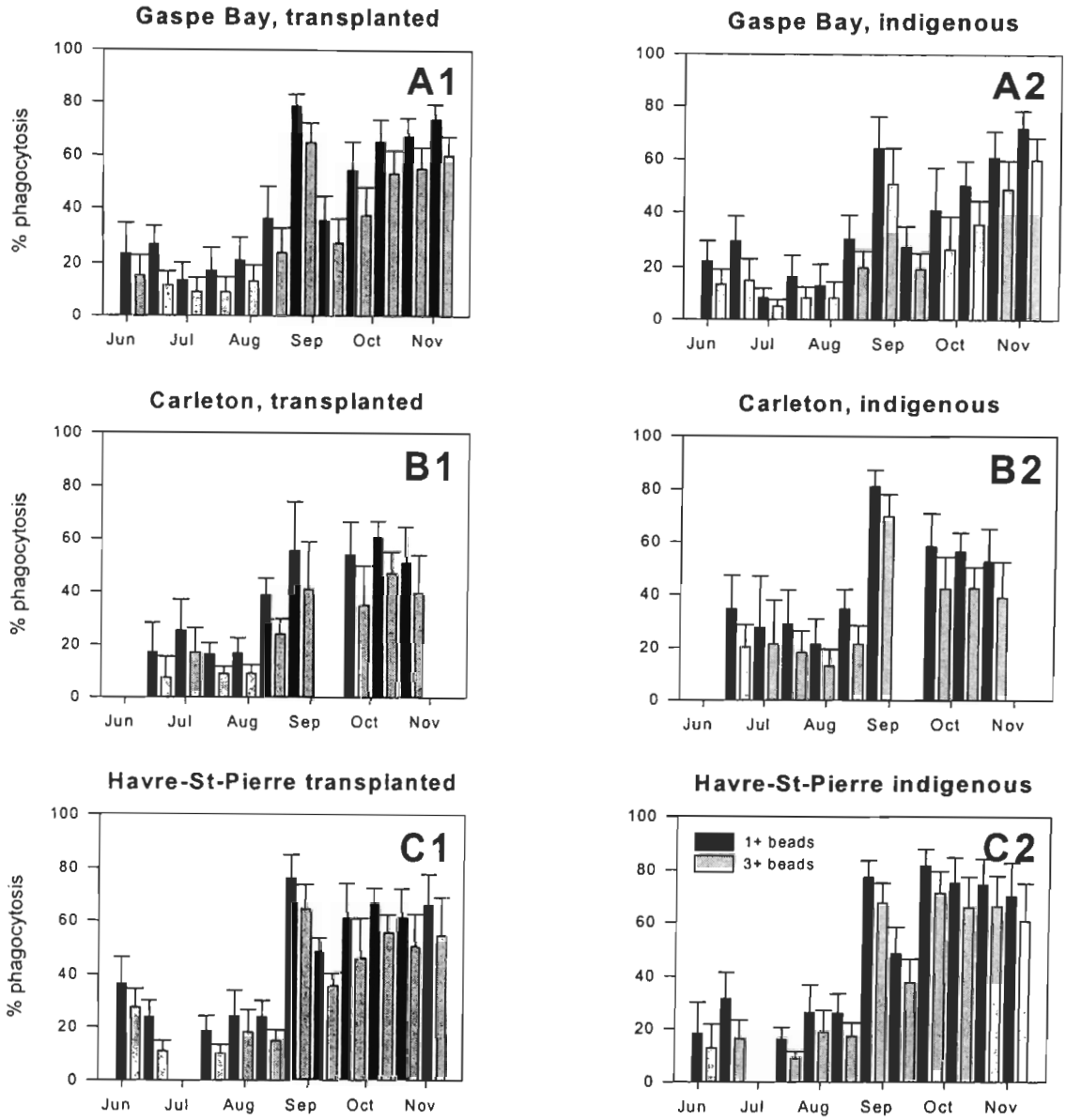

Figure 11. Phagocytic activity of hemocytes from hemolymph of transplanted (1) and indigenous (2) mussels at transplanted sites: Gaspe Bay (A), Carleton (B) and Havre-St-Pierre (C) from June to November 2003. Data are presented in two categories. "One bead and more" represents hemocytes with phagocytosis of at least one bead. "Three beads and more" represents more efficient hemocytes which engulfed three beads or more.

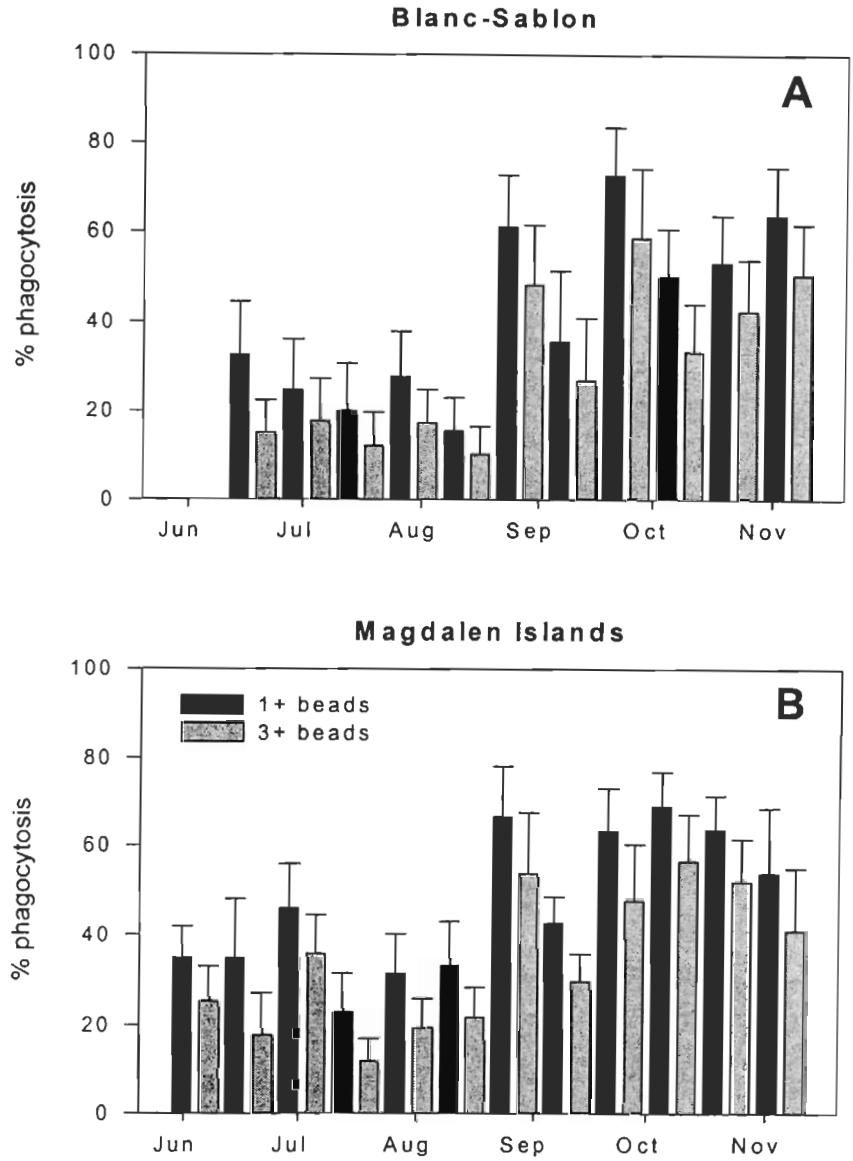

Figure 12. Phagocytic activity of hemocytes from hemolymph of indigenous mussels in Magdalen Islands (A) and Blanc-Sablon (B) from June to November 2003. Data are presented in two categories. "One bead and more" represents hemocytes with phagocytosis of at least one bead. "Three beads and more" represents more efficient hemocytes which engulfed three beads or more.

stable during the sampling period. TBT levels were near limit detection. DBT and MBT concentrations were near 10.0 ng.g-1 d.w. In Magdalen Islands (Figure 13D), concentrations of TBT were near limit detection (1.0 $ng.g^{-1}$ d.w.). A slight decrease was observed during the sampling period for DBT (from 15.0 to 12.0 $ng.g^{-1}$ d.w.) and MBT (from 13.0 to 9.0 $ng.g^{-1}$ d.w.) levels. In Blanc-Sablon (Figure 13E), concentrations in TBT were near or beyond limit detection during the sampling period. As DBT levels remained stable at 10.0 $ng.g^{-1}$ d.w. during the sampling period, MBT concentrations decreased from 10.0 $ng.g^{-1}$ d.w. in June to values near limit detection in November.

Heavy metals

Concentrations in cadmium, copper, zinc, lead and mercury in the digestive gland of indigenous mussels were determined at the beginning (June) and at the end of the sampling period (November). Results are presented in Figure 14. Cadmium levels (Figure 14A) ranged from 0.0 to 7.0 $\mu g.g^{-1}$ d.w. (dry weight). The highest concentration was observed in Carleton and the lowest in Magdalen Islands. The general trend was a decrease of cadmium levels from the beginning to the end of the sampling period. Copper concentrations (Figure 14C) ranged from 5.0 to 12.0 $\mu g.g^{-1}$ d.w. and remained stable during the sampling period. Similar results were observed for zinc levels (Figure 14E) for a range from $\approx$ 45.0 to 80.0 $\mu g.g^{-1}$ d.w. Lead concentrations (Figure 14B) remained stable during the sampling period and ranged from 0.5 to 0.7 $\mu g.g^{-1}$ d.w. except for Carleton (1.6 $\mu g.g^{-1}$ d.w.) and Blanc-Sablon (0.2 $\mu g.g^{-1}$ d.w.). Mercury levels (Figure 14D) remained stable in each site except

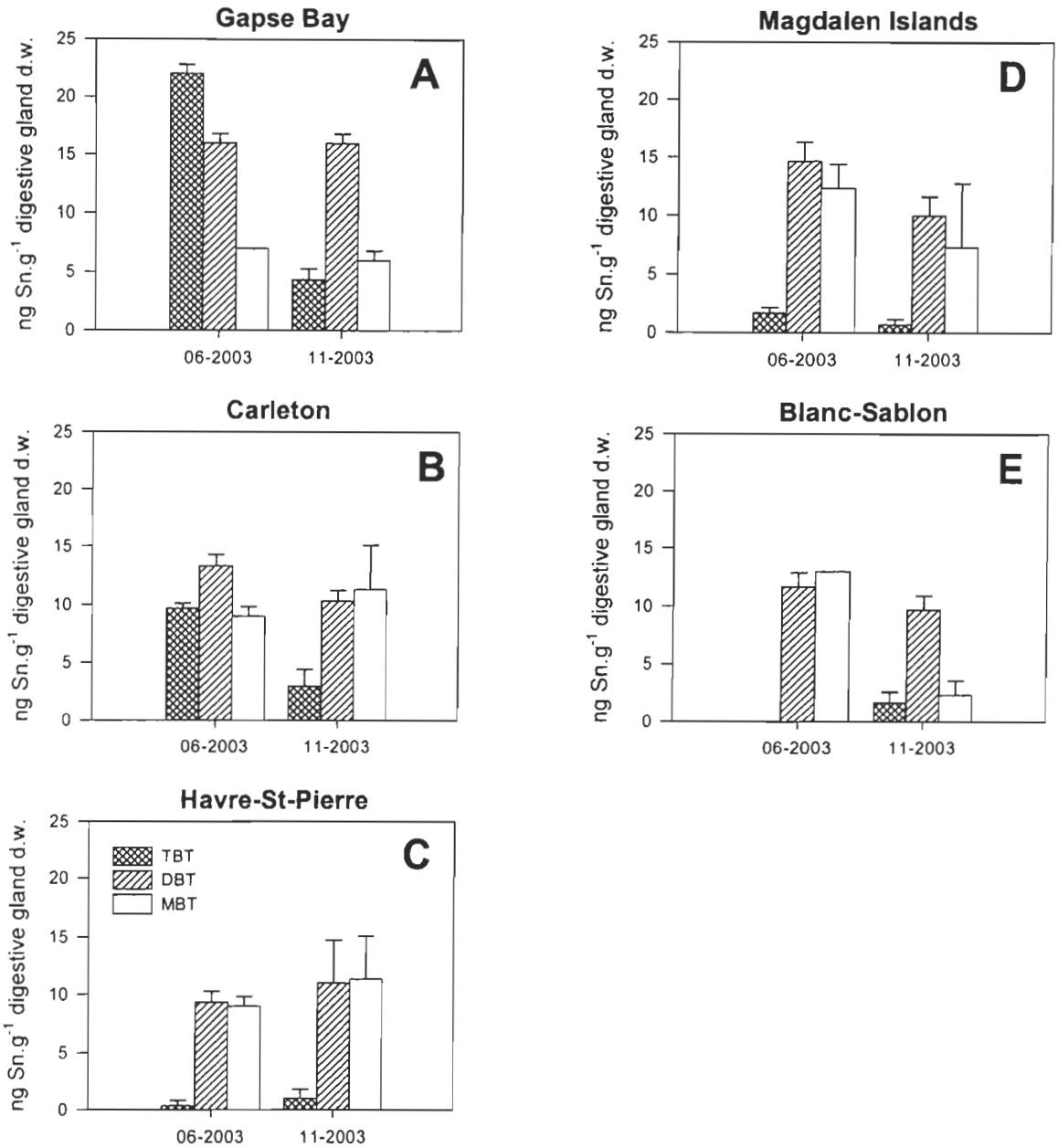

Figure 13. Concentrations of tri- (TBT), di- (DBT) and monobutyltin (MBT) in the digestive gland of indigenous *Mytilus* spp. for Gaspe Bay (A), Carleton (B), Havre-St-Pierre (C), Magdalen Islands (D), and Blanc-Sablon (E) in June and November 2003. d.w.: dry weight.

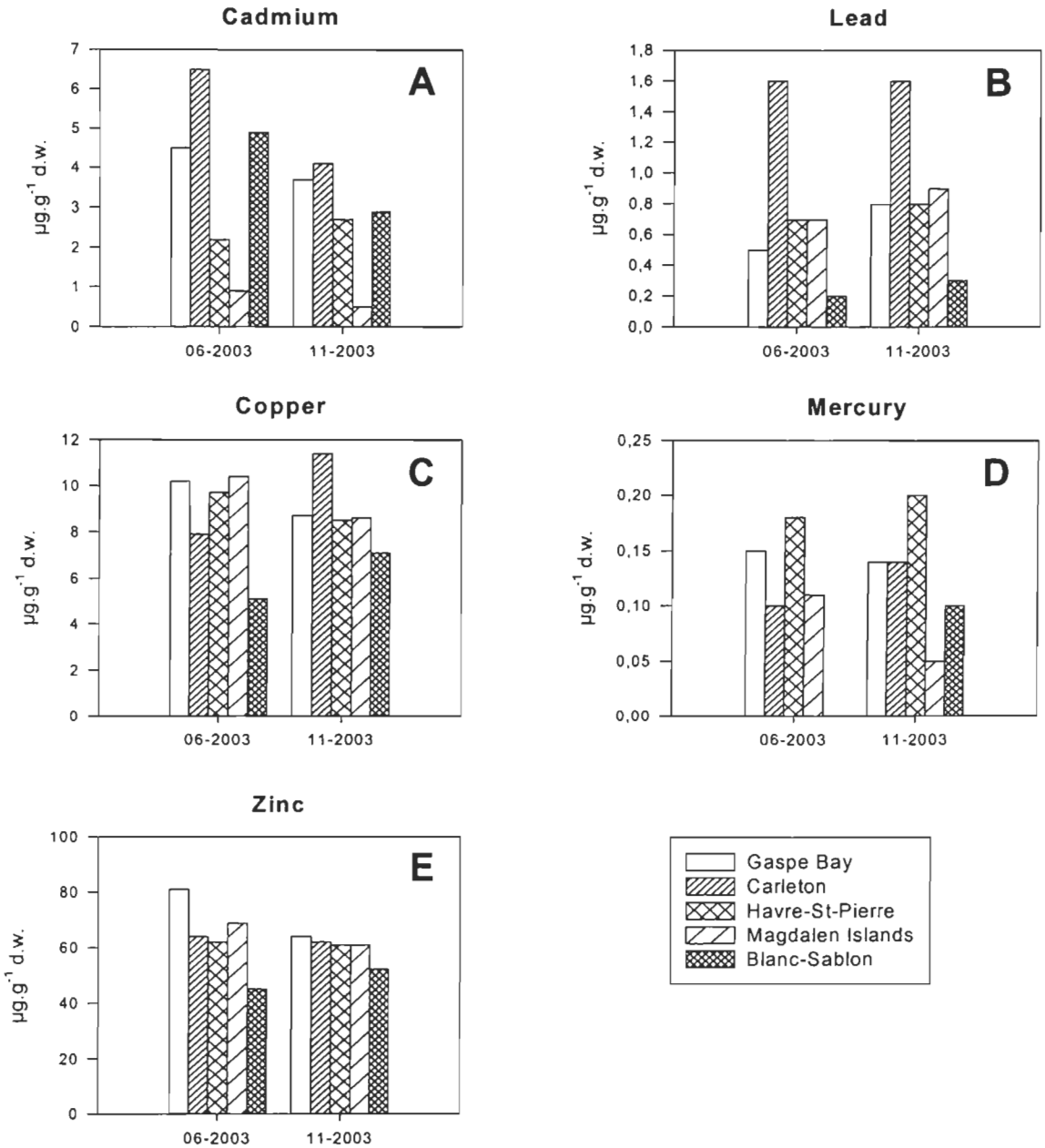

Figure 14. Concentrations of cadmium (A), lead (B), copper (C), mercury (D) and zinc (E) in the digestive gland of indigenous *Mytilus* spp. for Gaspe Bay, Carleton, Havre-St-Pierre, Magdalen Islands and Blanc-Sablon. Metals were analysed in June and November 2003.

for Blanc-Sablon. Concentrations were below the limit of detection in June and increased to 0.1 $\mu g.g^{-1}$ d.w. in November. Highest levels were observed in Havre-St-Pierre (2.0 $\mu g.g^{-1}$ d.w.).

Relationships between environmental and physiological factors

Correlations have been performed to assess relationships between environmental and physiological factors. Pearson correlation values are presented in Table 1 and Table 2. No correlation between the soft tissue weight and the phytoplankton abundance was observed except for Gaspe Bay indigenous mussels. No correlation was found between phytoplankton abundance and digestive gland weight except in Havre-St-Pierre. Results show a significant negative correlation between soft tissue weight and digestive gland index except for transplanted mussels from Carleton. No correlation was observed between soft tissue weight and total protein content of digestive gland except for transplanted mussels from Havre-St-Pierre. A Significant positive correlation between water temperature and the maturity index was observed at each site except for females from Carleton where correlation was negative. We observed a positive correlation between the maturity index and the phagocytic activity for males from Gaspe Bay and both sexes from Havre-St-Pierre. In Carleton, the correlation was significant for males but negative. No correlation was observed between the maturity index and the glycogen content of the digestive gland. No correlation between maturity index and phytoplankton abundance was observed except for females from Carleton.

Table 1. Pearson correlation between maturity index and environmental or physiological factors (phytoplankton abundance, temperature, glycogen and phagocytosis).

		Maturity index					
		Gaspe Bay		Carleton		Havre-St-Pierre	
		Males	Females	Males	Females	Males	Females
Phytoplankton	0-20 µm	-0.066	-0.007	-0.485	+0.739*	-0.221	-0.299
	2-20 µm	-0.414	-0.089	-0.483	-0.567	-0.585	-0.553
Temperature		+0.367**	+0.522**	+0.523**	-0.490**	+0.342**	+0.503**
Glycogen		-0.297	-0.112	-0.439	-0.597	+0.385	+0.449
Phagocytosis		+0.263**	-0.072	-0.360**	+0.102	+0.386**	+0.555**

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

Table 2. Pearson correlation between phytoplankton abundance, growth, digestive gland index (DGI) and total protein content.

		Gaspe Bay		Carleton		Havre-St-Pierre		Magdalen Islands (I)	Blanc-Sablon (I)
		(I)	(T)	(I)	(T)	(I)	(T)		
Phytoplankton vs. growth	0-20 µm	-0.413	+0.263	-0.475	-0.019	-0.316	-0.536	-0.160	+0.231
	2-20 µm	-0.833*	-0.510	-0.428	+0.576	-0.424	-0.487	-0.070	+0.190
Phytoplankton vs. DGI	0-20 µm	+0.116	+0.049	-0.180	-0.060	-0.070	+0.243	+0.354	-0.477
	2-20 µm	+0.040	+0.336	-0.444	+0.149	+0.757	+0.816	+0.138	-0.410
Growth vs. DGI		-0.288**	-0.426**	-0.406**	-0.123	-0.307**	-0.210**	-0.126*	-0.423**
Growth vs. Total protein		n.d.	+0.040	n.d.	-0.076	n.d.	-0.264**	n.d.	n.d.

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

(I) = Indigenous mussels; (T) = Transplanted mussels.

n.d.: non determined

DISCUSSION

Water temperature and food abundance are environmental factors in field influencing timing and duration of the reproductive cycle of the blue mussel. Kautsky (1982) studied *Mytilus edulis* population from the Baltic Sea. He observed in winter when temperatures were generally below 1°C and food availability very low, an important reduction of gonad development that resumed in spring with phytoplankton bloom and leading to maturation. Our results support those reported by Kautsky (1982). A positive relationship between phytoplankton cell concentration and water temperature, on the one hand, and the reproductive cycle, on the other hand, has been observed. Phytoplankton cells abundance and maturity index varied independently, because of the fragmented pattern of gametogenesis. Such fragmented pattern has already been reviewed by Bayne (1976). A positive influence of phytoplankton cell concentration on gametogenesis was also observed. A delay of gametogenesis was observed in relation to low food supply. In Havre-St-Pierre, where the lowest concentrations were observed, gametogenesis was delayed in comparison to Gaspe Bay or Carleton where food supply was more abundant. Cartier *et al.* (2004) also reported such results for the first year of the sampling period. If we compare the two years of sampling a delay of gametogenesis was observed in each site for the first year related to lower phytoplankton cell concentration. A delay of gametogenesis caused by lower food supply was also reported in the soft shell clam *Mya arenaria* by Gauthier-Clerc *et al.*

(2002). Influence of temperature was clearly observed in most of the sampling sites and particularly in Havre-St-Pierre. Two spawning periods were observed that fitted with peaks of temperature. Such results have been already observed in the first year of experiment and reported by Cartier *et al.* (2004). Other studies have also reported that temperature is a factor inducing spawning (Seed, 1976; Maung Myint and Tyler, 1982; Thompson, 1984). Results have shown a delay in maturation of males vs. females. Kautsky (1982) have also reported a differential maturation as function of sex. He observed that males seemed to mature faster, spawning later than females, and explained this observation by a more rapid maturation of spermatozoids. This pattern was only observed in Havre-St-Pierre while in the other sites a general delay of male maturation was noted.

Results have also shown a great influence of the reproductive cycle on the mussel physiology. The process of gametogenesis requires a lot of energy for the formation of gametes and has a direct influence on the pattern of metabolic reserves (Thompson, 1984). From July to September, a general decrease of glycogen concentration in digestive gland of transplanted mussels was observed. Storage of glycogen recovered in October when spent stages were observed. This loss of glycogen fitted with gametogenesis period but no significant correlation was observed in reason of the fragmented pattern of gametogenesis. Cartier *et al.* (2004) reported that protein concentrations followed an inverse pattern compared to the digestive gland index and suggested that mussels had sufficient energy for reproductive cycle and did not need protein to achieve gametogenesis. Similar results observed in our study led us to the same conclusion. In general, an increase of protein level

was observed in the digestive gland that seemed to follow the growth pattern previously observed by Cartier *et al.* (2004). However, no significant correlation was observed except for the transplanted mussels from Havre-St-Pierre. The protein pattern in digestive gland could therefore be considered closely as an index of mussel growth. Such relation requires however further studies.

Growth is correlated to food supply available in the environment (Page and Hubbard, 1987; Smaal and van Stralen, 1990). Our results agree in part with this statement. In Magdalen Islands, the highest phytoplankton concentrations were observed and the better growth in comparison to the other sampling sites. In general, we observed a higher growth rate in 2003 than in 2002. This year was shown to have less phytoplankton concentrations (Cartier *et al.*, 2004). Nevertheless, despite of those evidences no significant correlation was found between food supply and mussel growth. The reproductive cycle leads to important variations of body weight due to storage and further use of metabolic reserves and by the production and release of gametes. Kautsky (1982) showed that reproductive tissues account for 50 % of the total soft body weight. A number of studies (Kautsky and Wallentinus, 1980; Rodhouse *et al.*, 1984) have demonstrated that most of the energy goes into gamete production (over 90 % in large mussels). Thus, a positive effect of food supply on mussel growth was covered up by gametogenesis process. Brake et al. (2004) studied gametogenesis and growth of diploid vs. triploid *Mytilus edulis* to bring evidence of reproductive cycle on growth. Their results showed significant growth advantages of triploid mussels (known for their sterility). For the authors as in our study, differential

growth may be the result of sexual maturity in the diploids allowing for the reproductive energy in triploids to be allocated into growth.

Digestive gland is an organ of glycogen storage and is largely influenced by gametogenesis cycle. Results in this study have shown a decrease of digestive gland index during gametogenesis period which could be explained by the important energetic cost to form germ cells. Consequently, no significant correlation was found between DGI and food supply except in Havre-St-Pierre. Both DGI and soft tissue masses were influenced by gametogenesis but not in the same way. DGI was influenced by the variations of energetic reserves used by the gametogenesis process. Body mass was also influenced by variations of metabolic reserves but also by the production and release of gametes (Thompson, 1984). Moreover, results in this study and in Cartier *et al.* (2004) have shown that soft tissues had a better growth rate than digestive gland. Therefore, DGI could not be used as an indicator of nutritive quality and growth potential of mussels.

Shell length has often been used as a measure of mussel growth. It is a non-destructive and practical measure for farmers (Strömgren and Cary, 1984). However, as reported by Allunno-Bruscia *et al.* (2001) and as seen in this study, shell growth is not always representative of soft tissue growth. This study reported a decrease of CI for all indigenous and transplanted mussels except for Magdalen-Islands lagoon, discovering an asynchronous growth in shell and soft tissues. Shell length for that reason does not reflect

temporary biomass loss due to starvation or to the release of germ cells (Strömgren and Cary, 1982).

In each site for both indigenous and transplanted mussels, an immunosuppression was observed in summer and synchronous with the main spawning period as established with the significant correlation between maturity index and phagocytosis activity. Similar results were reported by Ishikawa *et al.* (1998) in hemocytes of *Crassostrea gigas* where phagocytic activity increased with gonad development and decreased after spawning. Watanuki *et al.* (2002) have shown that sex steroids like β-estradiol, progesterone and 11-ketotestosterone exert suppressive effects on phagocytic cells of the common carp *Cyprinus carpio* suggesting that immunosuppression could be driven by hormonal regulation. This immunosuppression has a direct impact on mussel population viability because it occurs during high temperature periods in summer when mussels have to face bacterial challenges and diseases. Mussel handling should therefore be restricted during this period of immunosuppression to avoid immune stress or mortality in stock.

Concerns about the quality of marine farm products have led us to assess metal and butyltin levels in cultured mussels. In each sampling site, the concentrations in butyltins in indigenous mussels were below 25 $ng.g^{-1}$ d.w. and according to previous studies (Salazar and Salazar, 1991; Widdows and Page, 1993), such concentrations do not present any risk or effects on mussel physiology. Salazar and Salazar (1991) in San Diego Bay have demonstrated a significant negative relationship between seawater TBT and mussel growth

and they have fixed to 0.5 $\mu g.g^{-1}$ w.w. (3.33 $\mu g.g^{-1}$ d.w. for 85 % of wetness in tissue) the NOEC (no observed effect concentration) of TBT on mussel growth. Widdows and Page (1993) for their part, observed effects of TBT and DBT on the rate of feeding and oxygen uptake at concentrations above 1.0 $\mu g.g^{-1}$ d.w. According to French and Canadian regulations for shellfish healthiness, legal upper limit for heavy metal concentration is 0.5 ppm w.w. (3.33 ppm d.w. for 85 % of wetness in tissue) for mercury and 2.0 ppm w.w. (13.33 ppm d.w. for 85 % of wetness in tissu) for cadmium and lead. In each sampling site, mercury, cadmium and zinc concentrations were below legal limit.. Moreover, those legal limits are fixed for soft tissue and in our study, heavy metal concentrations were measured in the digestive gland. According to Adami et al. (2004) bioaccumulation of heavy metal is higher in digestive gland than in soft tissue. Therefore, the dilution effect of soft tissue on digestive gland concentration brings heavy metal level even more below legal limit. In conclusion, there is no problem of butyltins and heavy metal contamination in mussel farm sites of Eastern Quebec. Concentrations measured in organisms along our study could be considered as background levels greatly below legal limits.

We demonstrated in this study that reproductive cycle is a key phenomenon in mussel physiology and a physiological process important for farmers. Since spontaneous spawning occurs frequently when mussels are stored and shipped to customers, reliable indicators of the reproductive cycle have to be developed. Gonadosomatic index (GSI) was designed to follow growth of gonad proportionally to somatic growth (Etchian *et al.*, 2004) and often used to assess sexual maturation cycle. Nevertheless, spawning could occur simultaneously

with the renewal of glycogen reserves (Emmett *et al.*, 1987). In this case gonad mass represents both the release of gametes and the beginning of glycogen storage. Therefore, the use of the maturity index is a more precise way to assess the stage of gametogenesis and informs farmers of the evolution of the gametogenic process (Etchian *et al.*, 2004). Since partial spawning occurred throughout the sampling and commercialisation periods, and was not significantly related to phytoplankton concentrations, it is not possible at this stage of the research to determine a threshold of food abundance sufficient to support both reproductive cycle and growth energetic needs. Influences of the food abundance on reproductive cycle were observed in this study and demonstrated in other studies (Kautsky, 1982; Fréchette and Bourget, 1987; Fréchette and Grant, 1991; Clausen and Riisgard, 1996). Newell *et al.* (1982) proposed two physiological strategies used by mussels at the onset of gametogenesis in relation to food availability: (1) mussels may initiate their gametogenic cycle once sufficient energy reserves are accumulated; (2) individuals develop gametes and spawn as soon as sufficient reserves are gathered. Future work in our laboratory will deal with the development of reliable indicators of the reproductive cycle and particularly to determine if there is a threshold concentration of phytoplankton cells to induce spawning.

This study brings to mussel farmers of Eastern Quebec information about the environment of their farm and the physiology of their stocks. To improve mussel culture, management farmers can combine measures to determine the patterns of growth, reproductive cycle and immunology to perform their operational schedule.

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CHAPITRE III

CONCLUSION

Cette étude nous a permis de récolter des informations qui seraient utiles pour les mytiliculteurs de l'Est du Québec. La base de données que nous avons constituée sur les facteurs environnementaux des sites d'élevage et sur la physiologie des moules en culture (croissance, maturation sexuelle et compétence immunitaire) pourra être utilisée par les producteurs dans le but d'optimiser la gestion de leurs élevages et d'ajuster leur calendrier d'opérations.

Nos résultats ont démontré une forte influence du cycle reproducteur sur la croissance, les concentrations en réserves énergétiques et la compétence immunitaire des moules. Ces paramètres physiologiques ont une incidence directe sur le rendement et la viabilité des stocks (résistance aux stress et aux pathogènes). Nos résultats soulignent l'importance pour les mytiliculteurs de pouvoir prévoir les périodes de gamétogenèse et de ponte. C'est pourquoi nous orientons nos recherches vers le développement d'indicateurs du cycle reproducteur fiables et accessibles d'un point de vue technique et financier pour les entreprises mytilicoles.

Nos résultats ont confirmé également une influence positive de la qualité nutritionnelle du milieu sur la gamétogenèse. Il apparaît donc que l'abondance en cellules phytoplanctoniques pourrait être un bon indicateur du cycle de maturation sexuelle. Toutefois nous n'avons pas pu observer de corrélation significative pour valider le lien observé entre ces deux variables. La gamétogenèse, étant trop fragmentée, a masqué l'effet de la qualité nutritive du milieu sur le processus de maturation sexuelle. Une autre explication serait une trop grande variabilité des apports de phytoplancton en milieu naturel. C'est pourquoi des études ultérieures pourraient valider ce facteur de variabilité par le développement d'un indicateur du cycle de maturation sexuelle basé sur l'abondance de cellules phytoplanctoniques. Un protocole expérimental en mésocosme serait donc indiqué pour ces futurs projets de recherches car il permettrait de contrôler les apports de phytoplancton.

Selon Newell *et al.* (1982), la moule aurait deux stratégies pour gérer son cycle reproducteur en fonction de la quantité de nourriture disponible dans le milieu : (1) la moule initierait le processus de gamétogenèse dès qu'une quantité suffisante de réserve serait accumulée ; (2) la moule développerait ses gamètes et pondrait dès qu'une quantité suffisante de réserve serait accumulée. Il serait donc intéressant de déterminer s'il existe une concentration seuil de phytoplancton qui induirait la gamétogenèse et/ou la ponte chez la moule. La réalisation de cet objectif nous permettrait d'affiner nos connaissances sur la façon dont ce paramètre influence le cycle de maturation sexuelle. De plus, par un suivi des concentrations en phytoplancton dans le milieu, nous pourrions (1) obtenir des indications

sur le cycle de maturation sexuelle et (2) prévoir les périodes de ponte qui sont des événements clef modulant la gestion des élevages mytilicoles. Toutefois, l'analyse de ce paramètre est difficile et coûteuse et de ce fait peu abordable pour les entreprises mytilicoles. Afin de répondre au besoin des mytiliculteurs, il serait intéressant de corréler des aspects visuels de l'organisme, observables lors de la dissection d'échantillons, avec son cycle reproducteur afin de développer des indicateurs techniquement plus accessibles pour les producteurs. La gonade et le manteau étant les organes d'entreposage des gamètes chez la moule, l'épaisseur du manteau et sa couleur, la taille de la gonade ainsi que l'aspect « coulant » de ces deux organes seraient des indicateurs potentiels du stade de maturation des organismes. Des observations au sein de notre laboratoire suggèrent que la couleur de la glande digestive témoignerait de sa concentration en réserves énergétiques. Comme le cycle bioénergétique chez la moule est intimement lié au cycle reproducteur, la couleur de la glande digestive serait un autre indicateur potentiel du stade de maturation sexuelle.

Notre projet a mis en évidence un phénomène d'immunosuppression, pendant la période estivale, qui semble coïncider avec la période principale de ponte des organismes. Cette baisse de la compétence immunitaire peut mettre en danger l'élevage et il est donc important d'approfondir aussi nos recherches sur les mécanismes physiologiques qui lient le cycle de maturation sexuelle à la compétence immunitaire. Watanuki *et al.* (2002) ont démontré chez la carpe, une action des hormones stéroïdiennes sur les mécanismes immunitaires. Une telle relation, chez la moule, est actuellement à l'étude dans les équipes des docteurs Michel Fournier et Jocelyne Pellerin. Il faut rappeler toutefois que Delaporte

et al. (2003) ont démontré chez l'huître *Crassostrea gigas* et la palourde *Ruditapes philippinarum* que les fonctions immunitaires peuvent également être influencées par la qualité du phytoplancton. Il sera donc important dans les études futures de pouvoir « départager » l'influence de chacun des facteurs.

Les résultats obtenus lors de ce projet ont mis en évidence des relations entre différents facteurs du milieu et paramètres physiologiques chez la moule, mais également des interactions entre certaines variables physiologiques de ce bivalve. Ces résultats soulignent l'importance de l'approche multiparamétrique pour des études en milieu naturel. Toutefois, une telle approche peut s'avérer limitante. En effet, comme nous l'avons observé au cours du projet, l'étude de l'interaction entre deux facteurs particuliers de notre système organisme-environnement peut se trouver « parasitée » par les nombreux autres facteurs environnementaux et/ou biologiques. Il est donc impératif que le protocole expérimental soit bien ciblé lors de futurs projets de recherche.

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