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Effect of reproduction on escape responses, metabolic rates and muscle mitochondrial properties in the scallop *Placopecten magellanicus*

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Abstract:

In scallops, gametogenesis and spawning can diminish the metabolic capacities of the adductor muscle and reduce escape response performance. To evaluate potential mechanisms underlying this compromise between reproductive investment and escape response, we examined the impact of reproductive stage (pre-spawned, spawned and reproductive quiescent) of the giant scallop, *Placopecten magellanicus*, on behavioural (i.e., escape responses), physiological (i.e., standard metabolic rates and metabolic rates after complete fatigue) and mitochondrial capacities (i.e., oxidative rates) and composition. Escape responses changed markedly with reproductive investment, with spawned scallops making fewer claps and having shorter responses than pre-spawned or reproductive-quiescent animals. After recuperation, spawned scallops also recovered a lower proportion of their initial escape response. Scallop metabolic rate after complete fatigue (VO_{2max}) did not vary significantly with reproductive stage whereas standard metabolic rate (VO_{2min}) was higher in spawned scallops. Thus spawned scallops had the highest maintenance requirements (VO_{2min}/VO_{2max}). Maximal capacities for glutamate oxidation by muscle mitochondria did not change with reproductive stage although levels of ANT and cytochromes as well as cytochrome *C* oxidase (CCO) activity did. Total mitochondrial phospholipids, sterols and the proportion of phospholipid classes differed only slightly between reproductive stages. Few modifications were detected in the fatty acid (FA) composition of the phospholipid classes except in cardiolipin (CL). In this class, pre-spawned and spawned scallops had fairly high proportions of 20:5n-3 whereas this FA in reproductive-quiescent scallops was threefold lower and 22:6n-3 was significantly higher. These changes paralleled the increases in CCO activity and suggest an important role of CL on the modifications of CCO activity in scallops. However, mitochondrial properties could not explain the decreased recuperation ability from exhausting exercise in spawned scallops. Shifts in maintenance requirements (VO_{2min}/VO_{2max}) and aerobic scope ($VO_{2max} - VO_{2min}$) provided the best explanation for the impact of reproduction on escape response performance.

Keywords: mitochondria, reproduction, scallops, muscle, escape response, metabolic rate, cytochrome C oxidase, phospholipids, plasmalogens, fatty acids, sterols, *Placopecten magellanicus.*

Introduction

The requirements of broadcast spawning lead many marine invertebrates to invest so heavily in gametogenesis and spawning that reproduction represents a major stress (Barber and Blake 1991). Given the material and energetic costs of gametogenesis and spawning, metabolic expenditures are likely to change considerably during the reproductive cycle, particularly in species with a high reproductive output. In scallops, gonadal maturation and spawning lead to a negative energetic balance, a generally diminished physiological condition and a decreased capacity to recover from exhausting exercise (Barber and Blake 1985; Brokordt et al. 2000a; Brokordt et al. 2000b). Thus, scallops face a compromise between two major components of their fitness: reproductive success and individual survival.

In scallops, the striated adductor muscle is one of the largest soft tissues and has as its primary role the rapid movement of valves during escape responses. The capacities of the adductor muscle are thus important for survival during encounters with predators. In many scallop species, the energetic reserves of the adductor muscle are depleted during gonadal maturation and spawning (Barber and Blake 1981; Barber and Blake 1991; Brokordt and Guderley 2004). At the same time, the glycolytic and aerobic capacity of the adductor muscle declines, which could explain reductions in the scallops' swimming ability, and particularly in their capacity to recuperate from exhaustive exercise (Brokordt et al. 2000a; Brokordt et al. 2000b; Brokordt and Guderley 2004). The impact of the reproductive cycle upon escape response performance could also reflect the instantaneous metabolic costs of reproductive investment. Indeed, the gametogenetic cycle of scallops accentuates seasonal changes in metabolic rate with the highest rates occurring during reproductive months and the lowest rates during winter quiescence (Shumway et al. 1988). Thus, the metabolic costs of

reproductive investment and spawning could reduce aerobic scope and slow aerobic recuperation.

Rapid valve movements (claps) in scallops are primarily fueled by phosphoarginine, with subsequent recovery using anaerobic glycolysis and then oxidative metabolism. In *Placopecten magellanicus* and *Argopecten irradians concentricus*, 70% of the ATP required for exhaustive escape activity is derived from phosphoarginine, with only 30% arising from anaerobic glycolysis and octopine generation (Thompson et al. 1980; de Zwaan et al. 1980; Livingstone et al. 1981; Chih and Ellington 1986). Phosphoarginine levels decrease as a function of the number of claps (Livingstone et al. 1981; Bailey et al. 2003). The complete restoration of phosphoarginine pools after exhaustiing swimming requires aerobic metabolism (Livingstone et al. 1981), presumably relying upon ATP production by adductor muscle mitochondria (Guderley et al. 1995). Both muscle glycolytic capacities and the oxidative capacities of muscle mitochondria change with the reproductive cycle in *Euvola ziczac* and *Chlamys islandica*, decreasing in animals that have invested extensively in reproduction (Boadas et al. 1997; Brokordt et al. 2000a). Thus, muscle mitochondria can be considered as key organelles in covering the high energy demand of aerobic recuperation in scallops. Any limitation of their oxidative capacity could hinder aerobic metabolism and could explain the impact of reproductive investment on aerobic recuperation.

Mitochondrial capacities can be changed by shifts in their protein and phospholipid components. Alterations in phospholipid head groups and acyl chain compositions modify the molecular activities of respiratory chain components (Hazel 1972a; Hazel 1972b). Mitochondrial membrane proteins can have specific requirements for phospholipid head group arrangements and FA in their proximity (Clandinin et al. 1985; Berger et al. 1993; Schlame et al. 2000). Thus, modifications in membrane lipid composition, even of minor

phospholipid classes during the reproductive cycle, could underlie changes in mitochondrial oxidative capacity with the reproductive cycle.

This study examined potential mechanisms underlying the compromise between locomotor performance and reproductive investment in the giant scallop, *P. magellanicus*, by evaluating changes in the aerobic power budget and mitochondrial capacities with reproductive investment. To this end, we compared scallops sampled in their natural habitat at 3 reproductive stages (pre-spawned, spawned and reproductive-quiescent). We characterized their escape responses, recuperation from exhaustion, aerobic power budget, muscle mitochondrial capacities and mitochondrial composition. To evaluate how respiratory capacity could be associated with swimming ability, particularly in the capacity to recuperate from exhausting exercise, oxygen uptake was measured at rest in non-feeding animals (VO_2min) and during aerobic recovery from exhausting exercise (VO_2max). We further examined properties of adductor muscle mitochondria as possible drivers of whole animal metabolic rate and recuperation from exhausting exercise. We determined maximal rates of glutamate oxidation, levels of adenine nucleotide translocase (ANT), concentrations of cytochromes A, B, C and C_1, activity of cytochrome *C* oxidase (CCO) and proportions of the phospholipid classes and subclasses as well as their FA compositions. By examining performance and structure at these levels, we sought to evaluate whether changes in scallop escape response performance with reproductive investment were due to their aerobic power budget or to their muscle mitochondrial properties.

Materials and Methods

Collection and maintenance of experimental animals

The giant scallop, *Placopecten magellanicus*, population we studied is located at the mouth of Baie des Chaleurs near Percé, Québec, in eastern Canada's Gulf of St. Lawrence, (48° 30' N, 65° 15' W). As at this location, spawning events occur between mid-July and early September (Bonardelli et al. 1996), we sampled at three dates: July 5th (before spawning events), September 6th (during or just after or spawning) and October 18th 2004 (reproductive quiescence). At each sampling date, 9 scallops (shell height: 106.9±10.9 mm) were collected by SCUBA diving at 30 m and water temperature was noted. Animals were transported on seawater ice in air to the research station of the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ) at Grande-Rivière (CAMGR), less than 50 km from the sampling site. Upon arrival at CAMGR, animals were tagged with Bee Tags™ (labels were glued on the upper valve using a cyanoacrylate adhesive) and placed in three flow-through seawater tanks (100 L) kept at the water temperature measured during collection (7.2°C in July 5th, 7.1°C in September 6th and 6.3°C in October 18th) and held under natural photoperiod. Seawater was filtered (1 µm) and UV-sterilized. Salinity varied between 28.2 and 29.1 ‰, as is encountered in the scallops' natural habitat. According to the stage of the experiment, the scallops were either starved or fed with phytoplankton cultures (see below).

At the end of each series of metabolic and behavioural analyses at CAMGR, scallops were flown live on frozen seawater (travel time less than 12 h) to Université Laval, Québec for analysis of mitochondrial performance, dissection and gonad sampling. After their arrival, scallops were placed in a 1000 L aquarium containing artificial seawater at 7.0±0.5 °C. After 24 h of acclimation and during the subsequent 4-5 days, scallops were sampled to assess properties of muscle mitochondria, take gonad samples and measure tissue-wet masses.

122

123 *Determination of reproductive status*

124 For each individual, part of the gonad was rinsed with filtered seawater and then fixed
125 in a 10% Helly fixative (Shaw and Battle 1957). Tissues were dehydrated through an
126 ascending ethanol series and embedded in paraffin wax. Four 5µm thick sections were cut
127 through the entire piece of gonad and then stained with Ehrlich's haematoxylin and eosin
128 (H&E). Reproductive condition was assessed from one field per section (four fields per
129 scallop) using stereological analysis. For females, the proportions of normal and atresic
130 oocytes per field (gamete volume fraction, GVF, and atresic volume fraction, AVF) were
131 estimated on histological slides examined at 400x magnification with an image capture kit
132 CoolSnap-Pro digital kit 4.1 (Pernet et al. 2003). Oocytes with atretic (abnormal shape or
133 colour) or lytic (denaturated) characteristics were considered to be in resorption (Lubet 1959).
134 For males and females, the gametogenic stage (indifferent, development, mature, spawning or
135 spent) of each individual was determined by microscopic observation of slides as described
136 by Lowe et al. 1982. We also estimated the gonadosomatic index (GSI: gonad wet mass/total
137 wet mass of soft tissues) as a quantitative criterion of reproductive stage.

138

139 *Evaluation of escape responses*

140 Individual scallops were placed in 60 x 60 x 12 cm trays containing ~ 15 L of filtered
141 (1 µm) seawater at the habitat temperature and escape responses were measured following
142 Brokordt et al. (2000a) and Lafrance et al. (2003). After 2 min in the trays and when they
143 were ventilating normally, scallops were stimulated to swim and escape by touching them
144 with an arm of a sea star, *Asterias vulgaris*. The time before the first reaction of the scallop,
145 the maximal number of valve adductions (claps) in a series, the total number of claps before
146 exhaustion, and the escape time (total time until exhaustion minus the time before the

147 scallop's first reaction) were noted. The observation was stopped if no claps occurred after 2
148 min of stimulation. After 30 min of recuperation (during which metabolic rate was measured),
149 each scallop underwent a second escape response test (again followed by 30 min of metabolic
150 rate measurement).

151 Sea stars were obtained from the same site as the scallops and were maintained in a
152 flow-through seawater tank (100 L) at temperatures similar to those of the scallop tanks. To
153 standardize their hunger level, sea stars were starved for 24 h before the experiments.

154

155 *Metabolic rate measurements*

156 VO_2min, or standard metabolic rate (SMR), estimates maintenance requirements of
157 resting, unstressed organisms that are not digesting food and are at a stable temperature within
158 their optimal range (Rolfe and Brown 1997). After being acclimated to maintenance
159 conditions and starved for three days, the oxygen consumption of each scallop was measured
160 at habitat temperature (≈ 7°C) to estimate SMR. Animals were transferred individually into 1
161 L metabolic chambers that were maintained open for 60 min before starting measurements.
162 Four chambers were used simultaneously, which allowed us to measure three animals and a
163 blank with an empty shell. The oxygen consumption of individual animals was determined by
164 sealing the chamber and measuring the reduction in percent dissolved O_2 with a YSI (5331)
165 polarographic electrode and analyzer. Seawater in the metabolic chamber was well mixed
166 with a magnetic stirrer. The output signal was monitored continuously starting from 100%
167 saturation and until at least a 20% decrease in saturation was reached. Immediately after
168 measurements, scallops were re-introduced into the flow-through seawater tanks and fed a 1:1
169 mixture of *Chaetoceros muelleri* and *Isochrysis galbana*. The diet was supplied continuously,
170 maintaining a concentration of ~30 cells.μL^{-1} in the tanks, allowing the scallops to feed to
171 satiety for two days (Pernet et al. 2003; Pernet et al. 2005).

172 Maximal metabolic rates (VO_2max) were assessed using scallops immediately after
173 the escape response tests. Scallops were transferred into metabolic chambers and oxygen
174 consumption was measured for 30 min. Scallops were open and ventilating after a few
175 minutes in the chambers thus ensuring that animals were well into their aerobic recovery
176 phase. Therefore, their oxygen consumption most likely reflected the maximum O_2
177 consumption rate (Tremblay et al. 2006). The escape response was then quantified a second
178 time, followed by a second determination of oxygen consumption rates. The higher of the two
179 oxygen uptake rates following fatigue was considered as the maximum metabolic rate
180 (VO_2max) (Tremblay et al. 2006). Oxygen consumption (ml O_2. h^{-1}) was expressed as the rate
181 expected for a standard animal (soft tissue wet mass of 50 g) using the allometric correction,
182 $y=aM^b$, where y is the oxygen consumption and b the scaling coefficient (Davies and Moyes
183 2007). We calculated a scaling coefficient of 0.8 using our data. We used wet instead of dry
184 mass as the denominator for oxygen uptake rates as the use of the muscle for subsequent
185 biochemical measurements prevented assessment of dry mass.
186
187 *Mitochondrial isolation and measurement of substrate oxidation*
188 Isolation procedures and measurements of substrate oxidation followed Guderley et al.
189 (1995) and Brokordt et al. (2000a). For mitochondrial isolation, all manipulations were
190 carried out on ice except the centrifugations, which were performed at 4°C. Phasic muscles
191 were rinsed in 5 mL isolation medium and then minced. The minced muscle was then
192 homogenized in 8 volumes of ice-cold isolation buffer containing 480 mM sucrose, 30 mM
193 HEPES, 230 mM KCl, 3mM Na_2EDTA, 6 mM EGTA, and 5 mM $MgCl_2$, pH 7.0 at 25ºC. On
194 the day of the experiment, 0.1% fatty acid-free bovine serum albumin (BSA) was added. The
195 homogenate was centrifuged at 900g for 10min and the superficial lipid layer was removed.
196 The remaining supernatant was again centrifuged at 900g for 10min. The resulting

197 supernatant was considered free of unbroken cells or cell debris and was centrifuged at 9000g.
198 To optimize the purity of mitochondrial pellets and to remove $MgCl_2$, the resulting pellet was
199 rinsed once by resuspension in isolation buffer free of $MgCl_2$ and recentrifuged at 9000g. The
200 mitochondrial pellet was re-suspended in a volume of reaction buffer corresponding to one-
201 tenth of the mass of muscle used.

202 Mitochondrial oxygen uptake was measured polarographically using a water–jacketed
203 O_2 monitoring system (Qubit System, Kingston, Ontario, Canada). Temperature was
204 maintained at 5°C by a circulating refrigerated water bath. For each assay, around 1 mg of
205 mitochondrial protein (~100 µL mitochondrial preparation) was added to 1 mL assay medium
206 containing 480 mM sucrose, 70 mM HEPES, 100 mM KCl, 10 mM KH_2PO_4, 50 mM taurine
207 and β-alanine, pH 7.0. On the day of the experiment, 0.5% BSA was added to the assay
208 medium (Guderley et al. 1995). To obtain maximal rates of respiration (state 3), glutamate
209 was added to a final concentration of 24 $mmol.L^{-1}$ and ADP to a final concentration of 0.4
210 $mmol.L^{-1}$. The ADP/O ratio was measured according to Chance and Williams (1956). Only
211 mitochondrial preparations with respiratory control ratios (RCR; state 3 / state 4, when ADP
212 was depleted) ≥ 3 were used. Preliminary experiments established that glutamate was
213 oxidized at higher rates than pyruvate or succinate.
214
215 *Cytochrome C oxidase activity*
216 CCO activity was measured at 5°C and 15°C according to Kraffe et al. (2007). Fresh
217 mitochondrial suspensions were diluted in phosphate buffer without Triton-X (45 $mmol.L^{-1}$
218 KH_2PO_4 and 30 $mmol.L^{-1}$ K_2HPO_4, pH 6.8). We used an initial cytochrome C concentration of
219 100 $\mu mol.L^{-1}$. All assays were run in triplicate using fresh mitochondrial preparations.
220 Activities were calculated using an extinction coefficient of 19.1 $mmol.L^{-1}.cm^{-1}$ (first order
221 reaction).

222

223 *Cytochromes, ANT and protein concentrations*

224 Cytochromes A, B, C and C_1 in the mitochondrial preparations were quantified by
225 difference spectra (Kraffe et al. 2007). The electron transport chain components in 2%
226 deoxycholate-dispersed mitochondria were reduced by 5 mmol.L^{-1} ascorbate and oxygen in
227 the solution was eliminated by the addition of dithionite (Williams 1964). The reduced
228 samples were read against samples oxidized with 5 mmol.L^{-1} ferricyanide. The concentration
229 of adenine nucleotide translocase (ANT) in mitochondrial suspensions was measured by
230 titration with its non-competitive irreversible inhibitor, carboxyatractyloside (CAT) (Guderley
231 et al. 2005). The quantity of ANT in mitochondrial suspensions corresponded to the amount
232 of CAT needed for inhibition. The protein concentration in mitochondrial suspensions was
233 determined by the bicinchoninic acid method (Smith et al. 1985) using BSA.

234

235 *Membrane lipid analysis*

236 The membrane lipids of mitochondrial suspensions were extracted according to Folch
237 et al. (1957) as modified for mitochondrial preparations (Kraffe et al. 2007). Separation of
238 polar and neutral lipids on silica gel micro-columns, separation of membrane lipid classes, FA
239 and sterol analyses, and calculation of amounts of phospholipid classes followed Kraffe et al.
240 (2007).

241 *Separation of polar lipids on silica gel micro-columns*

242 An aliquot of the lipid extract was deposited at the top of a silica gel micro-column (30
243 x 5 mm i.d., packed with Kieselgel 60 (70-230 mesh, Merck) previously heated at 450°C and
244 deactivated with 6 wt% H_2O (Marty et al. 1992). Neutral lipids were eluted with 10 mL of
245 $CHCl_3$/MeOH (98:2, vol/vol) and stored at –20°C for later sterols analysis. The polar lipid
246 fraction was recovered with 20 mL of MeOH and stored at –20°C for later phospholipid class

247 separation by high performance liquid chromatography (HPLC) and FA composition analysis
248 by gas chromatography (GC).

249 *Cholesterol analysis*

250 Free sterols (membrane components) and esterified sterols (sterol reservoir and
251 reserves) from the neutral lipid fraction were not previously separated since sterols are mostly
252 in the free form in scallop muscle (Napolitano and Ackman 1992; Palacios et al. 2007). An
253 aliquot of the neutral lipid fraction was transesterified with methoxide (MeONa) for 90 min at
254 ambient temperature (Soudant et al. 1996). The sterols thus released were extracted in hexane
255 and injected directly into GC. Sterols were analyzed in a Chrompak 9002 gas chromatograph
256 equipped with a RTX65 (65% diphenyl, 35% dimethylpolysiloxane) fused silica capillary
257 column (50 m x 0.32 mm, 0.2 pm film thickness) using an on-column injection system and
258 hydrogen as carrier gas, with a thermal gradient from 60 to 280°C. Quantification of sterols
259 was achieved by adding a known quantity of cholestane to samples.

260 *Separation of membrane lipid classes and FA analysis*

261 Separation of phospholipid classes and subclasses used two successive HPLC
262 separations with two different mobile phases. This method allowed the separate analysis of
263 plasmalogen (1-alkenyl-2-acyl-) and diacyl subclasses of phosphatidylethanolamine (PE),
264 phosphatidylcholine (PC) and phosphatidylserine (PS) in bivalves along with cardiolipin
265 (CL), phosphatidylinositol (PI), and natural lysophosphatidylcholine (LysoPC) (Kraffe et al.
266 2004). Each fraction was collected and, after transesterification ($MeOH/BF_3$), analyzed by GC
267 for FA composition. Fatty acid methyl esters (FAME) obtained were identified and quantified
268 using both polar (CPWAX 52 CB – 50m x 0.25 mm i.d.; 0.2 µm thickness) and non-polar
269 (CP-Sil 8 CB – 25m x 0.25 mm i.d.; 0.25µm thickness) capillary columns and C23:0 FA as an
270 internal standard. FA were expressed as the molar percentage of the total FA content of each
271 class or subclass. For plasmalogen subclasses, the total percentage was adjusted to 50% to

take into account the absence of alkenyl chains of the *sn*-1 position hydrolyzed by the acid mobile phase.

Calculation of amounts of phospholipid classes

The quantities of each phospholipids class and subclass of were determined from their respective FA spectrum obtained by GC. To obtain the molar content of each analyzed fraction, a correction factor was applied to their respective total FA molar contents: x1 for plasmalogen fractions and for the natural lysoPC fraction; x1/2 for PI and the diacyl fractions of PE, PC and PS, and x1/4 for the CL fraction.

Statistical analysis

Statistical comparisons were carried out with StatGraphics Plus 5.1 (Sigma Plus Inc., Toulouse, France). Initial two-factor analysis of variance (ANOVA) (reproductive stage and sex as factors) established that sex was not a significant factor for the measured parameters, thus values for males and females were pooled. None of the variables we measured showed any significant tank effect or interaction between tank and reproductive state. One-way ANOVA followed by *a posteriori* Bonferoni multiple comparisons was used for analysis of the effect of reproductive stage on behaviour, metabolic measurements and mitochondrial characteristics. Differences were considered significant when $P<0.05$.

For lipid compositions, expressed as mol%, two-way factorial multiple analysis of variance (MANOVA) using SPSS 13.0 (Lead Technologies, Chicago, Illinois, USA) was applied with reproductive stage as the factor. Data were log+1 or 1/sqrt (x) transformed before the analysis. Where differences were detected, least-square means multiple comparison tests were used to determine which means were significantly different. Residuals were screened for normality using expected normal probability plots and homogeneity of variance was assessed with Levene's Test.

297 **Results**

298 *Reproductive status*

299 The gamete volume fraction (GVF) and gonadosomatic index (GSI) both showed a
300 significant effect of date (Figure 1, $P<0.001$). The highest values were observed on July 5th
301 and represented the mature (or ripe) gametogenic stage; lower values were observed on
302 September 6th and October 18th. Maturation stage as determined by microscopy indicated that
303 all male (2) and female (7) scallops in July had mature gonads (pre-spawned). Although GVF
304 and GSI did not discriminate between scallops in the September and October samples,
305 maturation stages did. In September, all female gonads (3) were spent while 4 of the 6 males
306 analyzed were virtually spent and 2 were spent. In October, all male gonads (3) were spent, 1
307 of the 5 female gonads was spent and 4 showed small primary oocytes. Thus, we identified
308 scallops sampled in early September as spawned and those in October as reproductive
309 quiescent.

310

311 *Escape response behaviour*

312 All behavioural responses except for the maximum number of claps in a series,
313 changed with reproductive stage (Figure 2). Specifically, reproductive-quiescent scallops took
314 more time to respond to the predator both for their initial test ($P<0.001$) and after 30 min of
315 recuperation ($P=0.03$) (Figure 2a). Spawned scallops made fewer claps before exhaustion
316 than did pre-spawned and reproductive-quiescent scallops ($P=0.03$) (Figure 2c). Total escape
317 time was shorter for spawned scallops during their initial response and after 30 min of
318 recuperation ($P<0.001$) (Figure 2d). Spawned scallops tended to have a lower capacity for
319 recovery, only performing 56% of their initial claps after recovery whereas scallops in the
320 other reproductive stages responded with 68-75% of their initial claps (Table 1). After 30 min
321 of recuperation, the escape responses of pre-spawned and reproductive-quiescent scallops

lasted 82-85% of their initial duration while spawned animals only responded for 53% of their initial escape duration (P=0.02) (Table 1).

Aerobic power budget

Metabolic rates after exhaustion (VO_2max) did not vary significantly with reproductive state, but VO_2min did, with higher values of oxygen consumption in spawned scallops than at the other stages (P<0.001) (Figure 3). Whole animal metabolic rates and allometrically corrected rates showed the same trends between reproductive stages. VO_2min/VO_2max ratios, indicating the proportion of oxygen consumption needed for maintenance, showed values of 34 and 41% in pre-spawned and reproductive-quiescent animals and significantly higher values in spawned scallops (59%, P=0.005) (Figure 3). Aerobic scope, i.e. the difference between maximum and standard VO_2, tended to be lowest in spawned scallops (Figure 4).

Oxidative capacities and composition of muscle mitochondria

Maximal rates of glutamate oxidation (State 3) (nmol $O.min^{-1}.mg^{-1}$ mitochondrial protein) did not change with reproductive stage (Table 2). State 4 rates (nmol $O. min^{-1}.mg^{-1}$ mitochondrial protein) and the phosphorylation capacity of mitochondria, expressed as the molar ratio between added ADP and consumed oxygen (ADP/O), also changed little between reproductive stages.

The denominator typically used to standardize mitochondrial rates is the protein content in the mitochondrial preparation. To evaluate rates of oxygen uptake relative to parameters that are exclusively located in mitochondrial membranes, we used the concentrations of ANT and cytochromes A, B and C_1 as denominators. Cytochrome levels (expressed relative to the mg of proteins in the mitochondrial preparations) did not change

significantly between reproductive stages except that cytochrome B was higher in spawned and reproductive-quiescent scallops compared to pre-spawned animals (P=0.02) (Table 3). The protein-specific levels of ANT exceeded those of the cytochromes and were significantly higher (P<0.001) in reproductive-quiescent scallops compared to pre-spawned and spawned scallops. Modifications in the maximal rates of glutamate respiration were more apparent when mitochondrial membrane proteins were used as the denominator. When State 3 rates were expressed over cytochrome B, they were higher in pre-spawned scallops than in the other reproductive states (P=0.07). When expressed per nmol of ANT, mitochondrial rates were significantly lower in reproductive-quiescent scallops than in the other reproductive stages (P=0.02) (Figure 5).

Cytochrome C oxidase activity

CCO activity in mitochondrial suspensions was nearly two-fold higher in reproductive-quiescent animals than in the other reproductive stages at both 5 and 15°C (P<0.001) (Figure 6). The same pattern was found when CCO activity was expressed over cytochrome A levels, except that spawned scallops showed an intermediate catalytic capacity. The Q_{10} for CCO remained between 1.7 and 1.85 for the different reproductive stages. Whereas all of the measured CCO activity was used by mitochondria oxidizing glutamate (State 3/CCO activity expressed in mU) in pre-spawned and spawned scallops (100.9% ± 10.3 and 109.9% ± 3.5, respectively), the proportion decreased to 71.8% ± 6.4 in reproductive-quiescent scallops (P=0.006).

Composition of mitochondrial membrane lipids with reproductive stage

The HPLC method used in the present study allowed the separate analysis of plasmalogen (1-alkenyl-2-acyl-) and diacyl subclasses of phosphatidylethanolamine (PE),

372 phosphatidylcholine (PC) and phosphatidylserine (PS) found in scallops, along with
373 phosphatidylinositol (PI), cardiolipin (CL), and natural lysophosphatidylcholine (LysoPC).
374 The diacyl forms of phosphatidylcholine (diacylPC), phosphatidylethanolamine (diacylPE),
375 and phosphatidylserine (diacylPS), as well as phosphatidylinositol (PI) and cardiolipin (CL)
376 were the predominant phospholipid classes in the mitochondrial fraction from scallop muscle
377 (Figure 7). Plasmalogen forms were found in PE (PlsmPE) while they were only present in
378 trace amounts in PC and PS. LysoPC was also always found in trace amounts.

379 The relative levels (mol%) and content (nmol.mg^{-1} protein) of glycerophospholipids
380 (total, classes and subclasses) remained stable between the three reproductive stages (Figure 7
381 and Table 4). Total sterol content did not change significantly with reproductive stage and
382 constituted one third of total lipids (glycerophospholipids + sterols) in the mitochondrial
383 preparations. Sterols were principally composed of cholesterol (28%), 24-
384 methylenecholesterol (18%), brassicasterol (15%), 22-dehydrocholesterol (9%),
385 norcholesterol (5%) and β-sitosterol (5%) and the relative levels did not change between the
386 reproductive stages (data not shown).

387

388 *Fatty acyl chain composition of membrane phospholipid classes and subclasses*
389 *between reproductive stages*

390 The total phospholipids were dominated by 16:0 for the saturated fatty acids (SFA)
391 and by 20:5n-3 and 22:6n-3 as the main unsaturated FA. The other major FA were 18:0 and
392 18:1n-7. The FA composition of total phospholipids was remarkably stable between the three
393 reproductive stages (Table 5). Few modifications were detected in the FA compositions of
394 the specific phospholipid classes and subclasses of mitochondrial fractions (data not shown).
395 A major exception was the FA composition of the minor phospholipid class CL (Figure 8).
396 The predominant FA, 22:6n-3, was higher in reproductive-quiescent scallops than in pre-

spawned scallops, ($P=0.02$) whereas 20:5n-3 represented 10% of the FA in pre-spawned and spawned scallops and decreased threefold in reproductive-quiescent animals ($P<0.001$). Levels of 18:0 were also lower in reproductive-quiescent scallops compared to pre-spawned scallops ($P=0.02$). The decreases in 20:5n-3 and 18:0 accounted for the increase of 22:6n-3.

Discussion

Metabolism and behavioural responses

Standard metabolic rates (SMR) of *P. magellanicus* varied with reproductive stage. In numerous bivalves, oxygen uptake varies seasonally in relation to ambient temperature and food availability, both of which are linked with the energy demands of gametogenesis (MacDonald and Thompson 1986; Shumway et al. 1988; Delgado and Pérez Camachao 2007 Tran et al. 2008). The influence of temperature on SMR found for scallops *P. magellanicus* in our study cannot explain the differences in SMR since temperature varied little between sampling dates and thus, during metabolic rate measurements. Standard metabolic rates were higher in spawned scallops, than in pre-spawned or reproductive-quiescent scallops. The lower SMR in pre-spawned scallops is not expected for bivalves during gametogenesis (Shumway et al. 1988; Delgado and Pérez Camachao 2007). Nevertheless, O_2-consumption in the oyster *C. gigas* was shown to be lower in mature pre-spawned animals than after they had spawned (Soletchnik et al. 1997, Tran et al. 2008). In our study, the increase in SMR after spawning remains to be explained. The scallops sampled in July were likely close to spawning and had probably completed gametogenesis, as reflected by oocytes maturity. At Percé, in the Baie des Chaleurs, spawning takes place 2 or 3 months after the spring phytoplankton bloom in May/June (Bonardelli et al. 1996). Once the gametes are mature, scallops may have lower energy demands than during periods of gonad development, which require high rates of food ingestion and mobilization of energy reserves. Tran et al. (2008) suggested that cardiac output could be limited by mechanical pressure on the ventricle, since the large gonadal volume in pre-spawning bivalves could limit blood flow and oxygen consumption. In spawned scallops, the increased SMR may reflect enhanced energetic needs during and after spawning, including the needs for tissue restoration.

While we observed a marked increase in standard metabolism of spawned scallops, no significant changes in maximum metabolic rate with reproductive stage were found. Consequently, VO_2min/VO_2max ratios (proportion of VO_2 required for maintenance requirements) differed between reproductive stages, indicating a greater maintenance demand in spawned scallops. Similarly, aerobic scope (i.e., the difference between maximum and standard VO_2) tended to be lower in spawned than in pre-spawned and reproductive-quiescent scallops (Figure 4). The weakness of spawned scallops in their initial escape test as well as in the capacity for recuperation may reflect this aerobic power budget. Indeed, spawned scallops had a shortened total escape time and made fewer claps before exhaustion than pre-spawned and reproductive-quiescent scallops. Even with this weaker initial escape response, spawned scallops recovered a lower proportion of their initial status after a 30 min rest. Given the patterns of metabolic support for swimming, the reduced initial escape response in spawned scallops could reflect reduced arginine kinase activity or arginine phosphate levels in the adductor muscle of spawned animals, such as seen in *Chlamys islandica* (Brokordt et al. 2000a) and *Euvola ziczac* (Brokordt et al. 2000b). In *Chlamys islandica*, *Euvola ziczac* and *Argopecten purpuratus*, gametogenesis and spawning also reduce recuperation from exhausting exercise (Brokordt et al. 2000a; Brokordt et al. 2000b; Brokordt and Guderley 2004). Since complete recuperation of phosphoarginine levels after exhausting exercise requires aerobic metabolism (Livingstone et al. 1981), the increased maintenance requirements of spawned scallops would slow their metabolic recuperation.

In contrast to responses observed for *C. islandica* and *E. ziczac*, but similar to results for *Argopecten purpuratus* (Brokordt et al. 2006), escape response behaviours measured at different points in the reproductive cycle varied both in initial tests and during repeat escape tests. In *P.magellanicus* as in *A. purpuratus*, various parameters, including the time to first respond to the sea star, the number of initial claps and the initial clapping time changed with

452 reproductive investment. In *A. purpuratus*, the impact of reproductive investment differed
453 between domesticated and wild scallops (Brokordt et al. 2006). Both in *P. magellanicus* and
454 *A. purpuratus*, the most marked changes were in the time to first respond to the predator. It is
455 unlikely that this parameter is linked with the changed aerobic power budget. However, the
456 decreased escape performance in spawned scallops could have a metabolic basis. We suggest
457 that, for the various species of scallops for which an impact of reproductive investment upon
458 escape response performance has been noted, limitations in the aerobic power budget may be
459 a central mechanism affecting performance.
460
461 *Mitochondrial rates of glutamate oxidation and CCO activity*
462 Mitochondrial rates of glutamate oxidation (expressed over mg mitochondrial protein)
463 changed little between sampling periods, as found in other scallops (Boadas et al. 1997;
464 Brokordt et al. 2000a; Brokordt and Guderley 2004). This lack of change in maximal
465 capacities of muscle mitochondria for glutamate parallels the lack of change of organismal
466 maximal aerobic capacities (VO_2max). The limited changes in the capacities for glutamate
467 oxidation of muscle mitochondria (per mg protein) do not preclude modifications in the
468 capacity for oxidation of other substrates, such as pyruvate. Indeed, during gonadal
469 maturation and immediately after spawning, *Chlamys islandica* have lower mitochondrial
470 capacities for pyruvate oxidation (Brokordt et al. 2000a). Pyruvate being the principal
471 substrate to be oxidized after muscular activity, this reduced capacity for pyruvate oxidation
472 was suggested to slow the aerobic recovery of scallops from exhausting escape responses
473 (Guderley et al. 1995; Brokordt et al. 2000a).
474 Numerous enzymatic complexes participate in oxidative phosphorylation, with
475 complex IV (CCO) having significant control over mitochondrial respiration rates (Groen et
476 al. 1982; Blier and Lemieux 2001). In scallop muscle mitochondria, CCO activity did not

follow the same patterns as rates of glutamate oxidation expressed over proteins, cytochromes or ANT. This suggests that in scallop muscle, maximal rates of glutamate respiration (state 3 rates) are not dictated, solely or in part, by changes in CCO capacity. The calculated proportion of maximal CCO capacity used by the mitochondria oxidizing glutamate (State 3/CCO activity expressed in mU) was lower in reproductive-quiescent scallops than in pre- or spawned scallops. These calculated values are high compared to those for fish red muscle (Blier and Lemieux 2001; Kraffe et al. 2007) and may be due to differing assay conditions.

Membrane lipid composition

Proportions of phospholipid classes and subclasses as well as those of sterols did not change with reproductive state. Few modifications were apparent for the FA composition of total phospholipids or of specific phospholipid classes and subclasses. However, among the isolated classes, marked differences were found in the FA composition of CL. This minor phospholipid class, predominantly constituted of 22:6n-3 in scallops (Kraffe et al. 2002), showed fairly high proportions (10 mol% of the total FA in CL) of 20:5n-3 in pre-spawned and spawned scallops whereas this FA in reproductive-quiescent scallops was three fold less concentrated and 22:6n-3 was present at a significantly higher level. Interestingly, the decrease in 20:5n-3 (and the increase in 22:6n-3) in CL coincided with the increase in CCO activity.

Control of membrane-bound protein complexes in mitochondria (CCO, cytochrome bc_1 complex, ADP-ATP translocase) is exerted partly at the level of CL (Schlame et al. 2000; Paradies et al. 2002; Schlame and Ren 2006), with changes in the FA composition of CL influencing, at least in part, the activity of these membrane complexes. In particular, alterations in the CL acyl composition are suggested to modulate the CCO activity in mammals (Yamaoka et al. 1988; Robinson et al. 1990; Berger et al. 1993; Watkins et al.

1998) as well as in fish (Wodtke 1981; Kraffe et al. 2007). We found that changes in CCO activity in scallop muscle mitochondria with reproductive stage paralleled FA modifications of this annular phospholipid. We propose that the increase of CCO activity in reproductive-quiescent scallops is due, at least in part, to decreased levels of 20:5n-3 in CL concomitant with increases in 22:6n3.

Standard metabolic rate and muscle mitochondrial properties

Basal mitochondrial proton conductance (or proton leak) may account for 20-25% of SMR (Rolfe et al. 1999). Although quantitative estimates of the contribution of mitochondrial proton leak to standard metabolic rate are not available for scallops, the mitochondrial carrier protein, ANT, and the FA composition of inner-membrane phospholipids can explain much of the variation in basal proton conductance in many animals (Brand et al. 2005; Hulbert and Else 2005). If ANT were an important determinant of basal proton conductance in scallop muscle mitochondria, then mitochondria in reproductive quiescent scallops, with their higher ANT contents, would have a higher proton leak. However, the lack of change of state 4 rates with reproductive stage suggests that proton leak does not increase with ANT levels. Furthermore, the minor changes in the FA composition of total phospholipids in muscle mitochondria suggest that they would contribute little to shifts in proton conductance between reproductive stages. Thus, the increased SMR in spawned scallops is more likely associated with other energetic needs or mechanical routes.

Conclusion

This study examined mechanisms that could explain changes in escape response performance of giant scallop, *Placopecten magellanicus*, with reproductive status. We found a rise in standard metabolic rate without significant changes in maximum metabolic rate in

spawned scallops, suggesting that increased maintenance requirements and decreased aerobic scope limit their capacity to escape and to recover from exhaustion. While examining muscle mitochondrial properties, we found that glutamate oxidation capacity did not change with reproductive status, although CCO did. Even though our examination at these multiple levels did not seem to draw a single causal chain between the molecular and organismal levels, links were apparent between some levels of organization. The stability of maximal organismal VO_2 paralleled the lack of change of maximal capacities for glutamate oxidation by phasic muscle mitochondria. This suggests a possible role of muscle mitochondria in setting organismal VO_2max. CCO activity modification was associated with changes in the FA composition of CL. In keeping with the regulatory role of CL for major complexes in oxidative phosphorylation, the marked changes in 20:5n-3 and 22:6n-3 of CL suggest that the specific FA composition of CL may modify CCO activity in scallop muscle.

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705

FIGURE LEGENDS

Figure 1: Gamete volume fraction (GVF) and gonadosomatic index (GSI; gonad wet mass/ total tissue mass) of scallops sampled at different reproductive stages. GSI was determined for males and females while GVF was only determined for females. Values are means ± s.e.m. (GVF: N=7 for pre-spawned scallops, N=3 for spawned and N=5 for reproductive-quiescent scallops; GSI: N=8 for pre-spawned scallops, N=7 for spawned and reproductive-quiescent scallops). Different letters indicate values that differ between dates and hence reproductive stages (ANOVA and *a posteriori* test, $P<0.05$).

Figure 2: Escape responses performance as a function of reproductive status of *Placopecten magellanicus*. A) Time before first reaction, B) maximum number of claps in a series, C) total number of claps and D) total escape time before exhaustion for scallops at the different reproductive stages. Values are means ± s.e.m. (N=9 for pre-spawned and spawned scallops, N=8 for reproductive-quiescent scallops). Different letters indicate values that differ between reproductive stages (ANOVA and *a posteriori* test, $P<0.05$).

Figure 3: Standard metabolic rates (VO_2min) and metabolic rates after exhaustion (VO_2max) for scallops at different reproductive stages. Values are means ± s.e.m. (N=9 for pre-spawned and spawned scallops, N=8 for reproductive-quiescent scallops). Different letters indicate values that differ between reproductive stages (ANOVA and *a posteriori* test, $P<0.05$).

Figure 4: Ratio of standard metabolic rate to maximal VO_2 and maximum minus standard metabolic rates (VO_2max - VO_2min) for scallops at different reproductive stages. Values are means ± s.e.m. (N=9 for pre-spawned and spawned scallops, N=8 for reproductive-quiescent scallops). Different letters indicate values that differ between reproductive stages (ANOVA and *a posteriori* test, $P<0.05$).

Figure 5: State 3 respiratory rates (nmol $O.min^{-1}$) expressed over concentrations of cytochromes and ANT ($nmol^{-1}$) in mitochondria isolated from the muscle of scallops at different reproductive stages (assay temperature, 5°C). Values are means ± s.e.m. (N=8 for pre-spawned scallops, N=7 for spawned and reproductive-quiescent scallops). Different

738 letters indicate rates that differ between reproductive stages (ANOVA and *a posteriori* test,
739 $P<0.05$).
740
741 **Figure 6**: Cytochrome *C* oxidase (CCO) activity in muscle mitochondria isolated from
742 scallops at different reproductive stages, expressed in U_{CCO} mg^{-1} mitochondrial protein and
743 U_{CCO} $nmol^{-1}$ cytochrome A, measured at 5°C (white bars) and 15°C (grey bars)
744 (U_{CCO}=µmol cytochrome *c* reduced min^{-1}). Values are means ± s.e.m. *(*N=8 for pre-spawned
745 and spawned scallops and N=7 for reproductive-quiescent scallops). Different letters indicate
746 rates that differ between reproductive stages at a given assay temperature (ANOVA and *a*
747 *posteriori* test, $P<0.05$).
748
749 **Figure 7**: Classes and subclasses of phospholipids from the mitochondrial fraction isolated
750 from scallop muscle at different reproductive stages. Phospholipids are expressed as mol% of
751 total moles of glycerophospholipids. Values are means ± s.e.m. (N=4 for each stage). No
752 significant differences were noted between reproductive stages (two-way factorial
753 MANOVA, least square means multiple comparison tests, $P<0.05$).
754 *diacyl form of phosphatidylserine (PS), phosphatidylethanolamine (PE) and
755 phosphatidylcholine (PC).
756 **plasmalogen (1-alkenyl-2-acyl-) form of phosphatidylethanolamine (PE). The plasmalogen
757 forms of PC and PS were also detectable but only in trace amounts (< 0.5mol%).
758
759 **Figure 8**: Main fatty acid composition of cardiolipin (CL) in mitochondrial fractions isolated
760 from scallop muscle at different reproductive stages. Values are means ± s.e.m. *(*N=4 for each
761 stage). Different letters indicate values that differ between reproductive stages (two-way
762 factorial MANOVA, least square means multiple comparison tests, $P<0.05$).
763
764

764 **Table 1:** Recuperation (%) of total claps and escape time after 30 min following exhausting
765 exercise for scallops in the different reproductive states. Values are means ± s.e.m. (N=9 for
766 pre-spawned and spawned scallops, N=8 for reproductive-quiescent scallops). Different
767 superscripts indicate differences between reproductive stages (ANOVA and *a posteriori* test,
768 $P<0.05$).
769

% Recovery	Pre-spawned	Spawned	Reproductive quiescent
Total claps	75.2 ± 9.8	56.2 ± 7.6	67.8 ± 8.1
Escape time	84.5 ± 11.1[a]	53.3 ± 7.3[b]	81.7 ± 5.1[a]

770
771

771 **Table 2:** Oxidative capacities at 5°C of muscle mitochondria from scallops in different
772 reproductive stages. State 3 and state 4 rates of glutamate oxidation are expressed as nmol
773 $O.min^{-1}.mg^{-1}$ mitochondrial protein. Values are means ± s.e.m. (N=8 for pre-spawned
774 scallops, N=7 for spawned and reproductive-quiescent scallops). No significant differences
775 were noted between reproductive stages.

	Pre-spawned	Spawned	Reproductive quiescent
State 3	46.3 ± 4.6	53.5 ± 3.8	53.0 ± 3.6
State 4	8.9 ± 1.3	7.7 ± 0.7	9.1 ± 0.6
ADP/O	2.5 ± 0.2	2.6 ± 0.1	2.3 ± 0.1

776
777

Table 3: Concentration of cytochromes and ANT in scallop muscle mitochondria at different reproductive stages. Cytochrome and ANT concentrations were normalized to the protein content in the mitochondrial preparations. Values are means ± s.e.m. *(*N=9 for pre- and spawned scallops, N=8 for reproductive-quiescent scallops). Different superscripts indicate values that differ between reproductive stages (ANOVA and *a posteriori* test, P<0.05).

	Pre-spawned	Spawned	Reproductive quiescent
A	0.23 ± 0.03	0.18 ± 0.01	0.22 ± 0.03
B	0.1 ± 0.01 [a]	0.16 ± 0.02 [b]	0.15 ± 0.01 [ab]
C_1	0.16 ± 0.02	0.11 ± 0.01	0.15 ± 0.01
C	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
ANT	0.66 ± 0.07 [a]	0.75 ± 0.02 [a]	1.05 ± 0.05 [b]

784 **Table 4**: Content of cholesterol, total phospholipids and phospholipid classes and subclasses
785 in mitochondrial fractions isolated from scallop muscle at different reproductive stages.
786 Values are means ± s.e.m. (N = 4 for each stage). Different superscripts indicate values that
787 differ between reproductive stages (ANOVA and *a posteriori* test, P<0.05)
788

	Pre-spawned	Spawned	Reproductive quiescent
Total glycerophospholipids (µmol.mg^{-1} prot)	0.31 ± 0.03	0.37 ± 0.02	0.38 ± 0.08
Sterols (µmol.mg^{-1} prot)	0.16 ± 0.03	0.18 ± 0.02	0.19 ± 0.01
% Sterols	34.1 ± 1.3	33.1 ± 2.8	31.8 ± 4.1
Classes and subclasses (nmol.mg^{-1} prot)			
CL	1.7 ± 0.2	2.1 ± 0.2	1.9 ± 0.3
PS	12.9 ± 0.9	15.9 ± 1.2	14.2 ± 3.4
DiacylPE	34.8 ± 2.9	35.9 ± 2.2	39.4 ± 8.7
PlsmPE	15.9 ± 3.5	16.4 ± 1.0	19.7 ± 3.6
DiacylPC	90.9 ± 12.9	114.8 ± 7.5	104.9 ± 22.9
PI	17.0 ± 1.0^{a}	7.9 ± 1.1^{b}	9.5 ± 2.7^{b}

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790 **Table 5**: FA composition of total phospholipids in mitochondrial fractions isolated from
791 scallop muscle at the different reproductive stages. Results are expressed as mol%. Values are
792 means ± s.e.m. (N=4 for each stage). Different superscripts indicate values that differ between
793 reproductive stages (two-way factorial MANOVA, least square means multiple comparisons,
794 $P<0.05$).

795 *Others : Total of 19 detectable fatty acids (iso17:0, ant17:0, 15:0, 17:0, 16:1n-5, 18:1n-5, 18:2n-4, 18:3n-6,
796 18:3n-3, 20:2nmi(5,11), 20:2nmi(5,13), 20:3nmi(5,11,14), 20:2n-6, 20:3n-6, 20:4n-3, 21:5n-3, 22:2nmi(7,13),
797 22:2nmi(7,15), 22:3nmi(7,13,16), 22:4n-6), none of which exceeded 1.0%.

798 **Total dimetylacetals (mainly 16:0DMA, 18:0DMA and 20:1DMA).

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	Total Phospholipids		
	Pre-Spawned	Spawned	Reproductive quiescent
Fatty acids			
14:0	2.0 ± 0.4	2.3 ± 0.2	2.0 ± 0.1
16:0	18.7 ± 0.5	19.6 ± 0.2	19.1 ± 0.9
18:0	4.9 ± 0.2^{a}	4.6 ± 0.1^{a}	6.2 ± 0.5^{b}
16:1n-7	2.8 ± 0.3	3.2 ± 0.2	2.6 ± 0.2
18:1n-9	1.8 ± 0.1	1.8 ± 0.1	1.8 ± 0.1
18:1n-7	5.7 ± 0.2	6.0 ± 0.2	5.5 ± 0.2
20:1n-11	0.4 ± 0.1^{a}	0.5 ± 0.1^{ab}	0.6 ± 0.1^{b}
20:1n-9	0.5 ± 0.05	0.5 ± 0.02	0.6 ± 0.02
20:1n-7	0.6 ± 0.1	0.6 ± 0.04	0.6 ± 0.02
18:2n-6	0.6 ± 0.03	0.9 ± 0.1	0.9 ± 0.04
18:4n-3	3.0 ± 0.2^{a}	2.0 ± 0.2^{b}	2.0 ± 0.2^{b}
20:4n-6	1.0 ± 0.1^{a}	1.2 ± 0.1^{ab}	1.4 ± 0.1^{b}
20:5n-3	24.5 ± 0.7	22.8 ± 0.7	22.7 ± 0.4
22:5n-6	0.3 ± 0.01^{a}	0.4 ± 0.01^{b}	0.4 ± 0.02^{b}
22:5n-3	0.9 ± 0.1	0.8 ± 0.02	0.8 ± 0.05
22:6n-3	21.3 ± 0.3	21.8 ± 0.3	21.3 ± 0.6
Others*	6.4 ± 0.7	6.4 ± 0.2	5.5 ± 0.2
DMA**	4.6 ± 0.7	4.3 ± 0.5	5.0 ± 0.2
Total SFA	27.8 ± 1.1	29.2 ± 0.3	30.3 ± 1.6
Total MUFA	13.4 ± 0.4	13.9 ± 0.3	12.5 ± 0.6
Total PUFA	54.2 ± 1.0	52.7 ± 0.5	52.2 ± 1.1
UI	295.9 ± 5.0	288.9 ± 2.9	284.2 ± 6.4

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