



HAL
open science

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

Edouard Kraffe, Réjean Tremblay, Sonia Belvin, Jean-René Lecoq, Yanic Marty, Helga Guderley

► **To cite this version:**

Edouard Kraffe, Réjean Tremblay, Sonia Belvin, Jean-René Lecoq, Yanic Marty, et al.. Effect of reproduction on escape responses, metabolic rates and muscle mitochondrial properties in the scallop *Placopecten magellanicus*. *Marine Biology*, 2008, 156 (1), pp.25 - 38. 10.1007/s00227-008-1062-4 . hal-01580522

HAL Id: hal-01580522

<https://hal.univ-brest.fr/hal-01580522>

Submitted on 5 Feb 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

Edouard Kraffe^{1,2,*}, Réjean Tremblay³, Sonia Belvin³, Jean-René LeCoz⁴, Yanic Marty¹ and Helga Guderley²

¹ Unité mixte CNRS 6521, Université de Bretagne Occidentale, C.S. 93837, 29238 Brest Cedex 3, France

² Département de Biologie, Université Laval, Québec, QC, G1K 7P4, Canada

³ Institut des Sciences de la Mer, 310 allée des Ursulines, Rimouski, QC, G5L 3A1, Canada

⁴ UMR 100 Physiologie et Ecophysiologie des Mollusques Marins, Ifremer, Centre de Brest, B.P. 70, 29280 Plouzané, France

*: Corresponding author : Kraffe E., email address : Edouard.Kraffe@univ-brest.fr

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*.

27 **Introduction**

28

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

38 In scallops, the striated adductor muscle is one of the largest soft tissues and has as its
39 primary role the rapid movement of valves during escape responses. The capacities of the
40 adductor muscle are thus important for survival during encounters with predators. In many
41 scallop species, the energetic reserves of the adductor muscle are depleted during gonadal
42 maturation and spawning (Barber and Blake 1981; Barber and Blake 1991; Brokordt and
43 Guderley 2004). At the same time, the glycolytic and aerobic capacity of the adductor muscle
44 declines, which could explain reductions in the scallops' swimming ability, and particularly in
45 their capacity to recuperate from exhaustive exercise (Brokordt et al. 2000a; Brokordt et al.
46 2000b; Brokordt and Guderley 2004). The impact of the reproductive cycle upon escape
47 response performance could also reflect the instantaneous metabolic costs of reproductive
48 investment. Indeed, the gametogenetic cycle of scallops accentuates seasonal changes in
49 metabolic rate with the highest rates occurring during reproductive months and the lowest
50 rates during winter quiescence (Shumway et al. 1988). Thus, the metabolic costs of

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

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

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

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

77 This study examined potential mechanisms underlying the compromise between
78 locomotor performance and reproductive investment in the giant scallop, *P. magellanicus*, by
79 evaluating changes in the aerobic power budget and mitochondrial capacities with
80 reproductive investment. To this end, we compared scallops sampled in their natural habitat
81 at 3 reproductive stages (pre-spawned, spawned and reproductive-quiescent). We
82 characterized their escape responses, recuperation from exhaustion, aerobic power budget,
83 muscle mitochondrial capacities and mitochondrial composition. To evaluate how respiratory
84 capacity could be associated with swimming ability, particularly in the capacity to recuperate
85 from exhausting exercise, oxygen uptake was measured at rest in non-feeding animals
86 ($VO_2\text{min}$) and during aerobic recovery from exhausting exercise ($VO_2\text{max}$). We further
87 examined properties of adductor muscle mitochondria as possible drivers of whole animal
88 metabolic rate and recuperation from exhausting exercise. We determined maximal rates of
89 glutamate oxidation, levels of adenine nucleotide translocase (ANT), concentrations of
90 cytochromes A, B, C and C_1 , activity of cytochrome C oxidase (CCO) and proportions of the
91 phospholipid classes and subclasses as well as their FA compositions. By examining
92 performance and structure at these levels, we sought to evaluate whether changes in scallop
93 escape response performance with reproductive investment were due to their aerobic power
94 budget or to their muscle mitochondrial properties.

95

96

97

97 **Materials and Methods**

98

99 *Collection and maintenance of experimental animals*

100 The giant scallop, *Placopecten magellanicus*, population we studied is located at the
101 mouth of Baie des Chaleurs near Percé, Québec, in eastern Canada's Gulf of St. Lawrence,
102 (48° 30' N, 65° 15' W). As at this location, spawning events occur between mid-July and
103 early September (Bonardelli et al. 1996), we sampled at three dates: July 5th (before spawning
104 events), September 6th (during or just after spawning) and October 18th 2004 (reproductive
105 quiescence). At each sampling date, 9 scallops (shell height: 106.9±10.9 mm) were collected
106 by SCUBA diving at 30 m and water temperature was noted. Animals were transported on
107 seawater ice in air to the research station of the Ministère de l'Agriculture, des Pêcheries et de
108 l'Alimentation du Québec (MAPAQ) at Grande-Rivière (CAMGR), less than 50 km from the
109 sampling site. Upon arrival at CAMGR, animals were tagged with Bee Tags[™] (labels were
110 glued on the upper valve using a cyanoacrylate adhesive) and placed in three flow-through
111 seawater tanks (100 L) kept at the water temperature measured during collection (7.2°C in
112 July 5th, 7.1°C in September 6th and 6.3°C in October 18th) and held under natural
113 photoperiod. Seawater was filtered (1 µm) and UV-sterilized. Salinity varied between 28.2
114 and 29.1 ‰, as is encountered in the scallops' natural habitat. According to the stage of the
115 experiment, the scallops were either starved or fed with phytoplankton cultures (see below).

116 At the end of each series of metabolic and behavioural analyses at CAMGR, scallops
117 were flown live on frozen seawater (travel time less than 12 h) to Université Laval, Québec
118 for analysis of mitochondrial performance, dissection and gonad sampling. After their arrival,
119 scallops were placed in a 1000 L aquarium containing artificial seawater at 7.0±0.5 °C. After
120 24 h of acclimation and during the subsequent 4-5 days, scallops were sampled to assess
121 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_2 min, 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 ($\approx 7^\circ\text{C}$) 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 ($\text{ml O}_2 \cdot \text{h}^{-1}$) 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 25°C. 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 $5^\circ C$ by a circulating refrigerated water bath. For each assay, around 1 mg of
205 mitochondrial protein ($\sim 100 \mu L$ 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 $5^\circ C$ and $15^\circ C$ 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 $30 \text{ mmol.L}^{-1} K_2HPO_4$, pH 6.8). We used an initial cytochrome C concentration of
219 $100 \mu \text{mol.L}^{-1}$. All assays were run in triplicate using fresh mitochondrial preparations.
220 Activities were calculated using an extinction coefficient of $19.1 \text{ mmol.L}^{-1}.\text{cm}^{-1}$ (first order
221 reaction).

222

223 *Cytochromes, ANT and protein concentrations*

224 Cytochromes A, B, C and C₁ 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% H₂O (Marty et al. 1992). Neutral lipids were eluted with 10 mL of
245 CHCl₃/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 µm 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/BF₃), 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

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

274 *Calculation of amounts of phospholipid classes*

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

280

281 *Statistical analysis*

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

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

297

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

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

324

325 *Aerobic power budget*

326 Metabolic rates after exhaustion ($VO_2\max$) did not vary significantly with
327 reproductive state, but $VO_2\min$ did, with higher values of oxygen consumption in spawned
328 scallops than at the other stages ($P<0.001$) (Figure 3). Whole animal metabolic rates and
329 allometrically corrected rates showed the same trends between reproductive stages.
330 $VO_2\min/VO_2\max$ ratios, indicating the proportion of oxygen consumption needed for
331 maintenance, showed values of 34 and 41% in pre-spawned and reproductive-quiescent
332 animals and significantly higher values in spawned scallops (59%, $P=0.005$) (Figure 3).
333 Aerobic scope, i.e. the difference between maximum and standard VO_2 , tended to be lowest in
334 spawned scallops (Figure 4).

335

336 *Oxidative capacities and composition of muscle mitochondria*

337 Maximal rates of glutamate oxidation (State 3) ($\text{nmol O}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$
338 mitochondrial protein) did not change with reproductive stage (Table 2). State 4 rates (nmol
339 $\text{O}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$ mitochondrial protein) and the phosphorylation capacity of mitochondria,
340 expressed as the molar ratio between added ADP and consumed oxygen (ADP/O), also
341 changed little between reproductive stages.

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

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

357

358 *Cytochrome C oxidase activity*

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

368

369 *Composition of mitochondrial membrane lipids with reproductive stage*

370 The HPLC method used in the present study allowed the separate analysis of
371 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-

397 spawned scallops, ($P=0.02$) whereas 20:5n-3 represented 10% of the FA in pre-spawned and
398 spawned scallops and decreased threefold in reproductive-quiescent animals ($P<0.001$).
399 Levels of 18:0 were also lower in reproductive-quiescent scallops compared to pre-spawned
400 scallops ($P=0.02$). The decreases in 20:5n-3 and 18:0 accounted for the increase of 22:6n-3.
401
402

402 **Discussion**

403

404 *Metabolism and behavioural responses*

405 Standard metabolic rates (SMR) of *P. magellanicus* varied with reproductive stage. In
406 numerous bivalves, oxygen uptake varies seasonally in relation to ambient temperature and
407 food availability, both of which are linked with the energy demands of gametogenesis
408 (MacDonald and Thompson 1986; Shumway et al. 1988; Delgado and Pérez Camachao 2007
409 Tran et al. 2008). The influence of temperature on SMR found for scallops *P. magellanicus* in
410 our study cannot explain the differences in SMR since temperature varied little between
411 sampling dates and thus, during metabolic rate measurements. Standard metabolic rates were
412 higher in spawned scallops, than in pre-spawned or reproductive-quiescent scallops. The
413 lower SMR in pre-spawned scallops is not expected for bivalves during gametogenesis
414 (Shumway et al. 1988; Delgado and Pérez Camachao 2007). Nevertheless, O₂-consumption in
415 the oyster *C. gigas* was shown to be lower in mature pre-spawned animals than after they had
416 spawned (Soletchnik et al. 1997, Tran et al. 2008). In our study, the increase in SMR after
417 spawning remains to be explained. The scallops sampled in July were likely close to
418 spawning and had probably completed gametogenesis, as reflected by oocytes maturity. At
419 Percé, in the Baie des Chaleurs, spawning takes place 2 or 3 months after the spring
420 phytoplankton bloom in May/June (Bonardelli et al. 1996). Once the gametes are mature,
421 scallops may have lower energy demands than during periods of gonad development, which
422 require high rates of food ingestion and mobilization of energy reserves. Tran et al. (2008)
423 suggested that cardiac output could be limited by mechanical pressure on the ventricle, since
424 the large gonadal volume in pre-spawning bivalves could limit blood flow and oxygen
425 consumption. In spawned scallops, the increased SMR may reflect enhanced energetic needs
426 during and after spawning, including the needs for tissue restoration.

427 While we observed a marked increase in standard metabolism of spawned scallops, no
428 significant changes in maximum metabolic rate with reproductive stage were found.
429 Consequently, VO_{2min}/VO_{2max} ratios (proportion of VO_2 required for maintenance
430 requirements) differed between reproductive stages, indicating a greater maintenance demand
431 in spawned scallops. Similarly, aerobic scope (i.e., the difference between maximum and
432 standard VO_2) tended to be lower in spawned than in pre-spawned and reproductive-quiescent
433 scallops (Figure 4). The weakness of spawned scallops in their initial escape test as well as in
434 the capacity for recuperation may reflect this aerobic power budget. Indeed, spawned scallops
435 had a shortened total escape time and made fewer claps before exhaustion than pre-spawned
436 and reproductive-quiescent scallops. Even with this weaker initial escape response, spawned
437 scallops recovered a lower proportion of their initial status after a 30 min rest. Given the
438 patterns of metabolic support for swimming, the reduced initial escape response in spawned
439 scallops could reflect reduced arginine kinase activity or arginine phosphate levels in the
440 adductor muscle of spawned animals, such as seen in *Chlamys islandica* (Brokordt et al.
441 2000a) and *Euvola ziczac* (Brokordt et al. 2000b). In *Chlamys islandica*, *Euvola ziczac* and
442 *Argopecten purpuratus*, gametogenesis and spawning also reduce recuperation from
443 exhausting exercise (Brokordt et al. 2000a; Brokordt et al. 2000b; Brokordt and Guderley
444 2004). Since complete recuperation of phosphoarginine levels after exhausting exercise
445 requires aerobic metabolism (Livingstone et al. 1981), the increased maintenance
446 requirements of spawned scallops would slow their metabolic recuperation.

447 In contrast to responses observed for *C. islandica* and *E. ziczac*, but similar to results
448 for *Argopecten purpuratus* (Brokordt et al. 2006), escape response behaviours measured at
449 different points in the reproductive cycle varied both in initial tests and during repeat escape
450 tests. In *P. magellanicus* as in *A. purpuratus*, various parameters, including the time to first
451 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 (VO₂max). 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

477 follow the same patterns as rates of glutamate oxidation expressed over proteins, cytochromes
478 or ANT. This suggests that in scallop muscle, maximal rates of glutamate respiration (state 3
479 rates) are not dictated, solely or in part, by changes in CCO capacity. The calculated
480 proportion of maximal CCO capacity used by the mitochondria oxidizing glutamate (State
481 3/CCO activity expressed in mU) was lower in reproductive-quiescent scallops than in pre- or
482 spawned scallops. These calculated values are high compared to those for fish red muscle
483 (Blier and Lemieux 2001; Kraffe et al. 2007) and may be due to differing assay conditions.

484

485 *Membrane lipid composition*

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

496 Control of membrane-bound protein complexes in mitochondria (CCO, cytochrome
497 *b_c1* complex, ADP-ATP translocase) is exerted partly at the level of CL (Schlame et al. 2000;
498 Paradies et al. 2002; Schlame and Ren 2006), with changes in the FA composition of CL
499 influencing, at least in part, the activity of these membrane complexes. In particular,
500 alterations in the CL acyl composition are suggested to modulate the CCO activity in
501 mammals (Yamaoka et al. 1988; Robinson et al. 1990; Berger et al. 1993; Watkins et al.

502 1998) as well as in fish (Wodtke 1981; Kraffe et al. 2007). We found that changes in CCO
503 activity in scallop muscle mitochondria with reproductive stage paralleled FA modifications
504 of this annular phospholipid. We propose that the increase of CCO activity in reproductive-
505 quiescent scallops is due, at least in part, to decreased levels of 20:5n-3 in CL concomitant
506 with increases in 22:6n3.

507

508 *Standard metabolic rate and muscle mitochondrial properties*

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

522

523 **Conclusion**

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

527 spawned scallops, suggesting that increased maintenance requirements and decreased aerobic
528 scope limit their capacity to escape and to recover from exhaustion. While examining muscle
529 mitochondrial properties, we found that glutamate oxidation capacity did not change with
530 reproductive status, although CCO did. Even though our examination at these multiple levels
531 did not seem to draw a single causal chain between the molecular and organismal levels, links
532 were apparent between some levels of organization. The stability of maximal organismal VO_2
533 paralleled the lack of change of maximal capacities for glutamate oxidation by phasic muscle
534 mitochondria. This suggests a possible role of muscle mitochondria in setting organismal
535 VO_{2max} . CCO activity modification was associated with changes in the FA composition of
536 CL. In keeping with the regulatory role of CL for major complexes in oxidative
537 phosphorylation, the marked changes in 20:5n-3 and 22:6n-3 of CL suggest that the specific
538 FA composition of CL may modify CCO activity in scallop muscle.

539

540 **Acknowledgements**

541 This research was supported by a grant from NSERC to HG. EK received post-doctoral
542 support from the Réseau Aquacole du Québec and from the Université de Bretagne
543 Occidentale. The direction of innovation and technology of the Ministère de l'Agriculture,
544 des Pêcheries et de l'Alimentation du Québec (MAPAQ) allowed accessibility and metabolic
545 measurements at CAMGR.

546

547

547 **References**

548

- 549 Bailey DM, Peck LS, Bock C, Pörtner HO (2003) High-energy phosphate metabolism during
550 exercise and recovery in temperate and Antarctic scallops: an in vivo ³¹P-NMR study.
551 *Physiol Biochem Zool* 76: 622-633.
- 552 Barber BJ, Blake NJ (1981) Energy storage and utilization in relation to gametogenesis in
553 *Argopecten irradians concentricus* (Say). *J Exp Mar Biol Ecol* 52: 121-134.
- 554 Barber BJ, Blake NJ (1985) Substrate catabolism related to reproduction in the bay scallop
555 *Argopecten irradians concentricus*, as determined by O/N and RQ physiological
556 indexes. *Mar. Biol.* 87: 13-18.
- 557 Barber BJ, Blake NJ (1991) Reproductive physiology. In: Shumway SE (ed) *Scallops:*
558 *biology, ecology and aquaculture.* Elsevier, Amsterdam, pp 377-428.
- 559 Berger A, German JB, Gershwin ME (1993) Biochemistry of CL: sensitivity to dietary fatty
560 acids. In: Kissela JE (ed) *Advances in food and nutrition research*, vol 37. Academic
561 Press, San Diego, pp 259-338.
- 562 Blier PU, Lemieux H (2001) The impact of the thermal sensitivity of cytochrome *c* oxidase on
563 the respiration rate of Arctic charr red muscle mitochondria. *J Comp Physiol B* 171:
564 246-253.
- 565 Boadas MA, Nusetti OA, Mundarain F, Lodeiros C, Guderley H (1997) Seasonal variation in
566 the properties of muscle mitochondria from the tropical scallop *Euvola (Pecten) zizac*.
567 *Mar Biol* 128: 247-255.
- 568 Bonardelli JC, Himmelman JH, Drinkwater K (1996) Relation of spawning of the giant
569 scallop, *Placopecten magellanicus*, to temperature fluctuations during downwelling
570 events. *Mar Biol* 124: 637-649.
- 571 Brand MD, Pakay JL, Ocloo A, Kokoszka J, Wallace DC, Brookes PS, Cornwall EJ (2005)
572 The basal proton conductance of mitochondria depends on adenine nucleotide
573 translocase content. *Biochem J* 392: 353-362.
- 574 Brokordt KB, Himmelman JH, Guderley HE (2000a) Effect of reproduction on escape
575 responses and muscle metabolic capacities in the scallop *Chlamys islandica* Müller
576 1776. *J Exp Mar Biol Ecol* 251: 205-225.
- 577 Brokordt KB, Himmelman JH, Nusetti OA, Guderley HE (2000b) Reproductive investment
578 reduces recuperation from exhaustive escape activity in the tropical scallop *Euvola*
579 *zizac*. *Mar Biol* 137: 857-865.

580 Brokordt KB, Guderley H (2004) Energetic requirements during gonad maturation and
581 spawning in scallops: sex differences in *Chlamys islandica* (Müller 1776). J Shell Res
582 23: 25-32.

583 Brokordt KB, Fernandez M, Gaymer C (2006) Domestication reduces the capacity to escape
584 from predators. J. Exp. Mar. Biol. Ecol. 329: 11-19.

585 Chance B, Williams GR (1956) The respiratory chain and oxidative phosphorylation. Adv
586 Enzymol 17: 65-134.

587 Chih PC, Ellington WS (1986) Control of glycolysis during contractile activity in the phasic
588 adductor muscle of the bay scallop, *Argopecten irradians concentricus*: identification
589 of potential sites of regulation and a consideration of the control of octopine
590 dehydrogenase activity. Physiol Zool 59: 563-573.

591 Clandinin MT, Field CJ, Hargraves K, Morson L, Zsigmond E (1985) Role of diet fat in
592 subcellular structure and function. Can J Physiol Pharmacol 63: 546-556.

593 Davies R, Moyes CD (2007) Allometric scaling in centrarchid fish: origins of intra- and inter-
594 specific variation in oxidative and glycolytic enzyme levels in muscle. J Exp Biol 210:
595 3798-3804.

596 de Zwaan A, Thompson RJ, Livingstone DR (1980) Physiological and biochemical aspects of
597 valve snap and valve closure responses in the giant scallop *Placopecten magellanicus*.
598 II. Biochemistry. J Comp Physiol 137: 105-114.

599 Delgado M, Pérez Camachao A (2007) Influence of temperature on gonadal development of
600 *Ruditapes philippinarum* (Adams and Reeve, 1850) with special reference to ingested
601 food and energy balance. Aquaculture 264: 398-407.

602 Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and
603 purification of total lipides from animal tissues. J Biol Chem 226: 497-509.

604 Groen AK, Wanders RJA, Westerhoff HV, Van der Meer R, Tager JM (1982) Quantification
605 of the contribution of various steps to the control of mitochondrial respiration. J Biol
606 Chem 257: 2754-2757.

607 Guderley HE, Rojas FM, Nusetti OA (1995) Metabolic specialization of mitochondria from
608 scallop phasic muscles. Mar Biol 122: 409-416.

609 Guderley H, Turner ND, Else PL, Hulbert AJ (2005) Why are some mitochondria more
610 powerful than others: Insights from comparisons of muscle mitochondria from three
611 terrestrial vertebrates. Comp Biochem Physiol B 142: 172-180.

- 612 Hazel JR (1972a) The effect of temperature acclimation upon succinic dehydrogenase activity
613 from the epaxial muscle of the common goldfish (*Carassius auratus* L.) - I. Properties
614 of the enzyme and the effect of lipid extraction. *Comp Biochem Phys* 43B: 837-861.
- 615 Hazel JR (1972b) The effect of temperature acclimation upon succinic dehydrogenase activity
616 from the epaxial muscle of the common goldfish (*Carassius auratus* L.) - II. Lipid
617 reactivation of the soluble enzyme. *Comp Biochem Phys* 43B: 863-882.
- 618 Hulbert AJ, Else PL (2005) Membranes and the setting of energy demand. *J Exp Biol* 208:
619 1593-1599.
- 620 Kraffe E, Soudant P, Marty Y, Kervarec N, Jehan P (2002) Evidence of a
621 tetradocosaheptaenoic cardiolipin in some marine bivalves. *Lipids* 37: 507-514.
- 622 Kraffe E, Soudant P, Marty Y (2004) Fatty acids of serine, ethanolamine and choline
623 plasmalogens in some marine bivalves. *Lipids* 39: 59-66.
- 624 Kraffe E, Marty Y, Guderley H (2007) Changes in mitochondrial oxidative capacities during
625 thermal acclimation of rainbow trout *Oncorhynchus mykiss*: roles of membrane
626 proteins, phospholipids and their fatty acid compositions. *J Exp Biol* 210: 149-165.
- 627 Lafrance M, Cliche G, Haugum G, Guderley H (2003) Comparison of cultured and wild sea
628 scallops, *Placopecten magellanicus* (Gmelin, 1791), using behavioral responses,
629 morphometric and biochemical indices. *Mar Ecol Prog Ser* 250: 183-195.
- 630 Livingstone DR, de Zwaan A, Thompson RJ (1981) Aerobic metabolism, octopine production
631 and phosphoarginine as sources of energy in the phasic and catch adductor muscles of
632 the giant scallop *Placopecten magellanicus* during swimming and the subsequent
633 recovery period. *Comp Biochem Phys* 70B: 35-44.
- 634 Lowe DM, Moore MN, Bayne BL (1982) Aspects of gametogenesis in the marine mussel
635 *Mytilus edulis* L. *Mar Biol Ass U.K.* 62: 133-145.
- 636 Lubet P (1959) Recherches sur le cycle sexuel et l'émission des gamètes chez les mytilidés et
637 les pectinidés. *Rev Trav Inst Pêches Marit* 23: 389-548.
- 638 MacDonald BA, Thompson RJ (1986) Influence of temperature and food availability on the
639 ecological energetics of the giant scallop *Placopecten magellanicus*. *Mar Biol* 93:37-
640 48.
- 641 Marty Y, Delaunay F, Moal J, Samain JF (1992) Changes in the fatty acid composition of the
642 scallop *Pecten maximus* (L.) during larval development. *J. Exp. Mar. Biol. Ecol.* 163:
643 221-234

644 Napolitano GE, Ackman RG (1992) Anatomical distributions and temporal variations of lipid
645 classes in sea scallops *Placopecten magellanicus* (Gmelin) from Georges Bank (Nova
646 Scotia). *Comp Biochem Phys* 103: 645-650.

647 Palacios E, Racotta IS, Arjona O, Marty Y, Le Coz JR, Moal J, Samain JF (2007) Lipid
648 composition of the pacific lion-paw scallop, *Nodipecten subnodosus*, in relation to
649 gametogenesis 2. Lipid classes and sterols. *Aquaculture* 266: 266-273.

650 Paradies G, Petrosillo G, Pistolese M, Ruggiero FM (2002) Reactive oxygen species affect
651 mitochondrial electron transport complex I activity through oxidative cardiolipin
652 damage. *Gene* 286: 135-141.

653 Pernet F, Tremblay R, Bourget E (2003) Biochemical indicator of sea scallop (*Placopecten*
654 *magellanicus*) quality based on lipid class composition. Part I: Broodstock
655 conditioning and young larvae performance. *J Shell Res* 22: 365-376.

656 Pernet F, Bricelj VM, Parrish CC (2005) Effect of varying dietary levels of w6
657 polyunsaturated fatty acids during the early ontogeny of the sea scallop, *Placopecten*
658 *magellanicus*. *J Exp Mar Biol Ecol* 327: 115-133.

659 Robinson NC, Zborowski J, Talbert LH (1990) Cardiolipin-depleted bovine heart cytochrome
660 *c* oxidase: Binding stoichiometry and affinity for cardiolipin derivatives. *Biochemistry*
661 29: 8962-8969.

662 Rolfe DFS, Brown GC (1997) Cellular energy utilization and molecular origin of standard
663 metabolic rate in mammals. *Physiol Rev* 77: 731-758.

664 Rolfe DFS, Newman JM, Buckingham JA, Clark MG, Brand MD (1999) Contribution of
665 mitochondrial proton leak to respiration rate in working skeletal muscle and liver and
666 to SMR. *Am J Physiol* 276: C692-C699.

667 Schlame M, Rua D, Greenberg ML (2000) The biosynthesis and functional role of cardiolipin.
668 *Prog Lipid Res* 39: 257-288.

669 Schlame M, Ren M (2006) Barth syndrome, a human disorder of cardiolipin metabolism.
670 *FEBS Lett* 580: 5450-5455.

671 Shaw BL, Battle HI (1957) The gross microscopic anatomy of the digestive tract of the oyster
672 *Crassostrea virginica* (Gmelin). *Can J Zool* 35: 325-346.

673 Shumway SE, Barter J, Stahlnecker J (1988) Seasonal changes in oxygen consumption of the
674 giant scallop, *Placopecten magellanicus* (Gmelin). *J Shell Res* 7: 77-82.

675 Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto
676 EK, Goeke NM, Olson BJ, Klenk DC (1985) Measurement of protein using
677 bicinchoninic acid. *Anal Biochem* 150: 176-185.

- 678 Soletchnik P, Razet D, Geairon P, Faury N, Goulletquer P (1997) Ecophysiology of
679 maturation and spawning in oyster (*Crassostrea gigas*): metabolic (respiration) and
680 feeding (filtration and absorption rates) responses at different maturation stages. *Aquat*
681 *Living Resour* 10: 177-185.
- 682 Soudant P, Marty Y, Moal J, Robert R, Quéré C, Le Coz JR, Samain JF (1996) Effect of food
683 fatty acids and sterol quality on *Pecten maximus* gonad composition and reproduction
684 process. *Aquaculture* 143: 361-378.
- 685 Thompson RJ, Livingstone DR, de Zwaan A (1980) Physiological and biochemical aspects of
686 valve snap and valve closure responses in the giant scallop *Placopecten magellanicus*.
687 I. Physiology. *J Comp Physiol* 137: 97-104.
- 688 Tran D, Massabuau JC, Vercelli C (2008) Influence of sex and spawning status on oxygen
689 consumption and blood oxygenation status in oysters *Crassostrea gigas* in a
690 Mediterranean lagoon (Thau, France). *Aquaculture* 277: 58-65.
- 691 Tremblay I, Guderley H, Fréchette M (2006) Swimming performance, metabolic rates, and
692 their correlates in the iceland scallop *Chlamys islandica*. *Physiol Biochem Zool* 79:
693 1046-1057.
- 694 Watkins SM, Carter LC, German JB (1998) Docosahexaenoic acid accumulates in cardiolipin
695 and enhances HT-29 cell oxidant production. *J Lipid Res* 39: 1583-1588.
- 696 Williams JN (1964) A method for the simultaneous quantitative estimation of cytochromes A,
697 B, C1 and C in mitochondria. *Arch Biochem Biophys* 107: 537-543.
- 698 Wodtke E (1981) Temperature adaptation of biological membranes. The effects of
699 acclimation temperature on the unsaturation of the main neutral and charged
700 phospholipids in mitochondrial membranes of the carp (*Cyprinus carpio L.*). *Biochim*
701 *Biophys Acta* 640: 698-709.
- 702 Yamaoka S, Urade R, Kito M (1988) Mitochondrial function in rats is affected by
703 modification of membrane phospholipids with dietary sardine oil. *J Nutr* 118: 290-
704 296.
- 705

705 **FIGURE LEGENDS**

706

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

714

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

721

722 **Figure 3:** Standard metabolic rates (VO_2 min) and metabolic rates after exhaustion (VO_2 max)
723 for scallops at different reproductive stages. Values are means \pm s.e.m. (N=9 for pre-spawned
724 and spawned scallops, N=8 for reproductive-quiescent scallops). Different letters indicate
725 values that differ between reproductive stages (ANOVA and *a posteriori* test, $P<0.05$).

726

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

732

733

734 **Figure 5:** State 3 respiratory rates ($\text{nmol O} \cdot \text{min}^{-1}$) expressed over concentrations of
735 cytochromes and ANT (nmol l^{-1}) in mitochondria isolated from the muscle of scallops at
736 different reproductive stages (assay temperature, 5°C). Values are means \pm s.e.m. (N=8 for
737 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 $U_{CCO} \text{ mg}^{-1}$ mitochondrial protein and
743 $U_{CCO} \text{ nmol}^{-1}$ cytochrome *A*, measured at 5°C (white bars) and 15°C (grey bars)
744 ($U_{CCO} = \mu\text{mol cytochrome } c \text{ reduced min}^{-1}$). Values are means \pm 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 \pm 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 ($< 0.5 \text{ mol}\%$).

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 \pm 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 \pm 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 \pm 9.8	56.2 \pm 7.6	67.8 \pm 8.1
Escape time	84.5 \pm 11.1 ^a	53.3 \pm 7.3 ^b	81.7 \pm 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

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

782

	Pre-spawned	Spawned	Reproductive quiescent
A	0.23 \pm 0.03	0.18 \pm 0.01	0.22 \pm 0.03
B	0.1 \pm 0.01 ^a	0.16 \pm 0.02 ^b	0.15 \pm 0.01 ^{ab}
C ₁	0.16 \pm 0.02	0.11 \pm 0.01	0.15 \pm 0.01
C	0.05 \pm 0.01	0.05 \pm 0.01	0.04 \pm 0.01
ANT	0.66 \pm 0.07 ^a	0.75 \pm 0.02 ^a	1.05 \pm 0.05 ^b

783

784

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

789

790

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 \pm 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 dimethylacetals (mainly 16:0DMA, 18:0DMA and 20:1DMA).

799

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

800