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Seasonal and diurnal variability in carbon respiration, calcification and excretion rates of the abalone *Haliotis tuberculata L.*

Chapperon Coraline [2, 3], Clavier Jacques [3], Dugué Clément [3], Amice Erwan [1], Le Goff Marion [3], Roussel Sabine [3, *]

[1] Laboratoire des Sciences de l'Environnement Marin (LEMAR), UMR 6539 UBO, CNRS, IRD, IFREMER, Institut Universitaire Europe´en de la Mer (IUEM), rue Dumont d'Urville, 29280 Plouzane´, France
[2] School of Biological Sciences, Flinders University, Sturt Rd, Bedford Park, Adelaide, 5042, South Australia

* Corresponding author : Sabine Roussel, email address : sabine.roussel@univ-brest.fr

Abstract :

Abalone (*Haliotis spp*.) are commercially important marine shellfish species worldwide. Knowledge about the physiology of abalone that impacts life-history traits is important for a better understanding of the biology of the species and the impact of stressful husbandry procedures at different seasons. The present study quantified the seasonal and diurnal variations in four physiological parameters of the European species *Haliotis tuberculata*, i.e. carbon aerial and aquatic respiration, calcification and excretion rates, and the effect of prolonged aerial exposure upon abalone aerial respiration. We also investigated the effect of individual size upon these physiological parameters. Aquatic respiration and calcification rates showed an allometric relationship with biomass. All parameters showed lower rates in cool season and higher rates in warmer season. Temperature was assumed to be the primary driver of the reported seasonal variability in physiological parameters, although reproductive needs and nutrition may also contribute to the observed patterns. Importantly, abalone did not stop calcifying in winter, and calcified more at night than during the day. Abalone did not respire more underwater at night-time than at daytime, however they excreted more overnight. The low air:aquatic ratio (0.2) is likely to be an energy-saving strategy for emerged *H. tuberculata* individuals. This study highlights the temporal heterogeneity in physiological rates of *H. tuberculata*, which constitutes a species

Keywords : ormer, metabolism, carbon fluxes, physiology

INTRODUCTION

Abalone (Haliotis species) are commercially important marine gastropod species worldwide. Whilst abalone fisheries declined from 19 720 to 7 486 metric tonnes between 1970 and 2013, farming production exploded from 50 to 103 464 metric tonnes over the same period (Cook, 2014). The decrease in abalone fisheries has been triggered by overexploitation, illegal catches and disease that lessened wild abalone stocks (Cook, 2014). *Haliotis tuberculata* L. is the only species present in Europe (van Wormhoudt *et al.*, 2011). This species is relatively abundant on the Channel Islands and the Atlantic coast of France (Clavier & Richard, 1982; Gaty & Wilson, 1986). In the wild, *H. tuberculata* is sedentary (Forster, 1967) and found at the subtidal and low intertidal levels in shallow rocky habitats (Hayashi, 1983). Their biology and ecology such as their reproductive cycle (Hayashi, 1980a), population structure and growth (Hayashi, 1980b; Clavier & Richard, 1986; Roussel *et al.*, 2011) have been well studied. Respiration rates of *H. tuberculata* from hatcheries (oxygen consumption rates in mg or ml O_2 h^{-1}) have previously been investigated in regards to factors such as *e.g.* body size (10-50mm, Gaty & Wilson, 1986; 10-90g, Basuyaux *et al.*, 2001) and simulated temperature (8, 16 and 24°C in Gaty & Wilson, 1986, and 12, 15, 18, 21, 28°C in Basuyaux *et al.*, 2001). Nonetheless, the natural variations (day-night and season cycles) in the metabolism of abalone from the natural habitat and the effect of body size upon respiratory rates in mature individuals (*i.e.* size over 50mm, Hayashi, 1980a), are still not well understood and represent important biological information for the recently developed industry in Europe (Roussel *et al.*, 2013; Cook, 2014; Lachambre *et al.*, 2017). Indeed, many abalone farms rely on coastal seawater and hence factors such as seawater temperature or dissolved oxygen which vary according to season, day-night and tidal cycles are likely to impact abalone physiology (Morash & Alter, 2015). A better understanding of the natural temporal variations in physiological and metabolic rates which affect life-history traits like growth and reproduction

can thus contribute to improving fisheries management and aquaculture development (Young *et al.*, 2006; Cooke *et al.*, 2014; Ragg & Watts, 2015; Gao *et al.*, 2016).

Several factors can modify abalone metabolism such as light quality (*H. discus discu*s, Gao *et al.*, 2016), infection (*H. rufescens* and *H. discus hannai*, González *et al.*, 2012; *H. diversicolor,* Lu *et al.*, 2017)**,** farm stressors such as density, high temperature and ammonia concentration (see review from Morash & Alter, 2015). To increase our understanding of the effects of environmental and farm stressors upon abalone physiology, it is first necessary to better comprehend the natural temporal variability in abalone metabolism and biology. In particular, seasons have a strong effect on chemical constituents in the muscle and viscera (*H. diversicolor,* Chiou *et al.*, 2001; *H. laevigata* and *Haliotis rubra*, Su *et al.*, 2006; farmed Jade Tiger hybrid abalone, Mateos *et al.*, 2010), metabolic activity of digestive-gland cells (*H. kamtschatkana*, Carefoot *et al.*, 1998), textural proprieties (*Hariltis discus*, Hatae *et al.*, 1995) and even immunity parameters of abalone (*H. tuberculata*, Travers *et al.*, 2008). These variations are partly related to reproductive cycle and energy transfer. However, to our knowledge, the season effect upon abalone (including *H. tuberculata*) metabolism has been poorly investigated to date.

Abalone metabolism might also vary according to diurnal rhythm. Indeed, abalone are nocturnal gastropods. They are active, move and feed mainly over-night in laboratory conditions, and in their natural habitat (*H. discus hannai*, Momma & Sato, 1970; *H. laevigata*, *H. roei, H. ruber, H. cyclobates, H. scalaris*, Shepherd, 1973). One can thus expect that abalone (and thus *H. tuberculata*) metabolism is greater at nighttime in order to fulfill the metabolic needs related to individuals' nocturnal behavior.

Finally, others stressors related to commercial practices such as high water ammonia concentration, high density, and air exposure (see review from Morash & Alter, 2015) are likely to impact the physiological rates of abalone. Nitrogenous waste can be toxic and limit

86 production in aquaculture (Ahmed *et al.*, 2008) and thus likely impact the physiology of
87 abalone. Both fished abalone (*H. iris,* Wells & Baldwin, 1995; Ragg & Watts, 2015, *H.*
88 *australis*, Wells & Baldwin, 1995) and farmed abalone (*H. tuberculata*, Lachambre *et al.*,
89 2017) can be exposed to air during handling procedures. Abalone have been demonstrated to
90 remain metabolically active during air exposure at a much lower rate than during immersion
91 (*H. iris*, Baldwin *et al.*, 1992). *H. tuberculata* can be found at low shore levels on rocky
92 shores during extreme spring tides or even at higher levels in large rockpools (Crofts, 1929).
93 It is thus expected that *H. tuberculata* is physiologically adapted to aerial exposure.
94 Nevertheless, periods of exposure to air longer than those experienced in natural conditions,
95 and at different temperature, can occur especially during live shipments. A greater
96 understanding of abalone (and hence *H. tuberculata*) metabolic capacities under aerial
97 conditions is therefore important to improve the transport and handling of live animals.
98
99 This study proposed to study the natural temporal variations in physiological parameters of *H.*
100 *tuberculata.* We quantified the seasonal, diurnal variations as well as the effect of individual
101 size on four physiological parameters *i.e.* carbon aerial and aquatic respiration, calcification
102 and excretion rates which are key parameters to understand abalone physiology (Morash &
103 Alter, 2015). In addition, aerial respiration was studied during a 6-h exposure and in different
104 temperature conditions to study the effect of temperature on the aerial metabolic rate.

105 MATERIALS AND METHODS

106 **Sampling**

107 Abalone were collected by divers equipped with diving tanks in the Bay of Brest, in Brittany,
108 NW of France. The sampling site was situated on the east part of the "Ile des Morts" in the
109 Bay of Roscanvel located on the SW part of the Bay of Brest (48° 18,225' N, 4° 32,134' W,
110 France). Abalone were found under rocks at a depth between 3m and 10m depending on the
111 tide. They were carefully removed by hand, placed into a net until the end of the diving
112 session and subsequently placed within a closed transportable tank containing seawater.

113

114 **Laboratory conditions**

115 In the laboratory, abalone were placed into a flow through system and kept unhandled for at
116 least 24 hours before starting any measurements to minimize handling stress. Filtered
117 seawater was provided from the Bay of Brest by the nearby pumping station of the Institut
118 français pour l'exploitation de la mer (Ifremer) at Sainte-Anne du Portzic. Seawater
119 temperature was thus at the same temperature as *in situ*. A mix of algae from their natural
120 habitat was added for food.

121

122 **Methodology**

123 SEASON COMPARISON IN ALL PHYSIOLOGICAL PARAMETERS

124 Aquatic and aerial respiration rate, calcification rate and ammonium excretion rate were
125 calculated in adult abalone collected in the four seasons (Figure 1, n = 16 in summer, n = 15
126 in autumn, n = 17 in winter and in spring). These sampling periods were especially chosen to
127 test the effect of seawater temperature upon the measured parameters. For instance, seawater
128 temperature recorded in 2014-2015 was on average 17.1 ± 0.4 °C ($\bar{x} \pm SD$, mean ± standard
129 deviation) in summer (18.3°C in September experimental day), 15.1 ± 2.2 °C in autumn

(14.6°C in November experimental day), 10.5 ± 0.9 °C in winter (11°C in January experimental day) and 13.2 ± 1.8 °C in spring (14.1°C in May experimental day) (SOMLIT: Service d'Observation en Milieu Littoral, INSU-CNRS, St Anne du Portzic; Figure 1).

Abalone ranging from 70 mm to 80 mm in length were sexually mature *i.e.* size over 50 mm (Hayashi, 1980a). This size range, corresponding to adult individuals, is the most abundant in the sampling area and was selected to facilitate sampling. Aquatic incubations were performed around midday in each season. Aerial respiration rates were measured upon emersion as soon as individuals were removed from their aquatic incubation bottle. As animals were free to feed prior to experiments, measured metabolic rate may include postprandial metabolic demand.

Diurnal comparison in aquatic physiological parameters

In order to compare metabolic rates which can vary through time at a diurnal scale (Lorrain *et al.*, 2015), aquatic incubations were repeated overnight in autumn (November 2014) on the same individuals to compare aquatic respiration, calcification and excretion rates during day and night periods.

Individual size effect upon aquatic physiological parameters

Calcification, aquatic respiration and ammonium excretion rates were calculated in abalone (n = 45) from a wide range of size (length: 35 to 106 mm) in September 2014. Smaller individuals (< 50mm, n=15 abalone for a total of 107 abalone tested) were provided by the France Haliotis abalone farm (48°36′46N; 4°33′30W, Plouguerneau, France). These abalone were the third generation bred in the farm and resulted from systematic mating between wild and farmed broodstock (either males or females were wild broodstock) for each generation.

155 AERIAL RESPIRATION DURING 6H AIR EXPOSURE AND TEMPERATURE EFFECT

156 Abalone (n = 8) were gently detached from the laboratory tank and emersed for 6 hours.
157 Individual aerial respiration rates were measured each hour in spring (May 2015) at 14°C.
158 Incubations were also conducted at 18°C and 10°C on successive days. These air temperatures
159 were chosen as they are representative of the temperature that can be experienced by abalone
160 during either ambient temperature or cooled temperature transport. The spring season was
161 chosen because it corresponds to usual fishing period for *H. tuberculata* (fishing is forbidden
162 in summer in Europe and practically difficult for the fisherman in winter due to poor weather
163 conditions).

164

165 **Physiological measurements**

166 Aquatic incubations of 1.5 to 2 hours (dark conditions) were conducted to calculate abalone
167 aquatic respiration (dissolved inorganic carbon, DIC), calcification/decalcification ($CaCO_3$)
168 and ammonium excretion (NH_4^+ fluxes). Individuals were gently detached and placed into 2-L
169 watertight plastic bottles filled with natural filtered seawater (1 individual/bottle) immersed
170 into a 580 L tank filled with running seawater to maintain a constant temperature during
171 incubations. Bottles were gently rotated once in the middle of incubations to ensure
172 homogenization.

173

174 AQUATIC RESPIRATION RATE

175 Aquatic respiration within each bottle (R, µmol DIC h^{-1}) was calculated as the variation in the
176 dissolved inorganic carbon (*DIC)* concentration between the start and the end of the
177 incubation using the following equation: $R = (\Delta DIC \times v)/(\Delta t \times 10^3) - G$, where ΔDIC is
178 the variation in *DIC* during incubation (µmol DIC l^{-1}), Δt is the incubation time (h), v is the
179 bottle volume (l) and G is the calcification rate (µmol $CaCO_3$ h^{-1}). To determine the dissolved

inorganic carbon (DIC) concentration (µmol L^{-1}), seawater samples were taken from incubation bottles at the start and at the end of each incubation in order to measure total alkalinity (TA), pH and seawater temperature.

TA samples were obtained by filtering water through 0.7µm Whatman GF/F filters. Samples were then stored with 250ml plastic bottles in the dark. Laboratory analysis consisted in estimating TA (µmol L^{-1}) within 20ml subsamples (average of 6 subsamples per bottle) by automatic potentiometric titration (Radiometer, Titrilab TIM 865). Subsamples were titrated by adding small increments of 0.01 mol L^{-1} HCL with 0.7 mol kg^{-1} NaCl to approximate the ionic strength of sea water (Dickson & Goyet, 1994) until about pH 3. TA was determined using the modified Gran method by determining the second endpoint of the titration curve.

The pH (total scale) was measured using a pH probe (Radiometer pHC2401) which was standardized with buffer solutions in synthetic seawater of 35‰ (Tris-HCL: 2-amino-2 hydroxymethyl-1,3-propanediol hydrochloride; 2-amminopyridine/HCL). pH values were measured immediately after opening bottles in order to prevent effects of CO_2 exchange with the air.

DIC concentration was calculated from pH, TA, temperature, salinity, phosphate and silicate concentrations using the CO2SYS program (Pierrot *et al.*, 2006). The natural salinity, phosphate and silicate concentrations were obtained using data collected in the Bay of Brest by the French coastal observation service (SOMLIT: Service d'Observation en Milieu Littoral, INSU-CNRS, St Anne du Portzic). Dissociation constants for carbonic acid K1 and K2 were taken from Roy *et al.* (1993).

CALCIFICATION RATE

Calcification was determined using the alkalinity anomaly technique (Smith & Key, 1975) in each incubated bottle. Calcification rate (G in µmol $CaCO_3$ h^{-1}) was calculated as follows:

$G = (\Delta TA \times v)/(2 \times \Delta t)$. ΔTA, Δt and v corresponds to the variation in TA during incubation (µmol l^{-1}), incubation time (h) and bottle volume (L), respectively. This equation is based on the evidence that the precipitation of one mole of $CaCO_3$ implies the consumption of 2 moles of HCO_3^- which decreases TA by 2 equivalents (Frankignoulle *et al.*, 1994). $G > 0$ is indicative of calcification, whilst $G < 0$ indicates carbonate dissolution.

AMMONIUM EXCRETION RATE

Ammonia is assumed to be the primary end product of catabolism of amino acids in molluscs (Bayne and Newell, 1983). Besides providing us information regarding *H. tuberculata* ammonium excretion, NH_4^+ concentrations were measured to correct TA since ammonium excretion is one of the processes that may affect TA in our experiments (Gazeau *et al.*, 2014, 2015). Ammonium excretion was obtained by collecting seawater samples at the beginning and end of each incubation in 10 ml vials and kept at -20°C until analysis. The phenol-hypochlorite method (Koroleff, 1969; Sororzano, 1969) was used to determine NH_4^+ concentration (µmol L^{-1}). After adding reagents, the coloration of each sample was measured at 630 nm using a spectrophotometer. Nitrification process was not assessed here since it can be considered to be negligible under short incubations (Tagliarolo *et al.*, 2013b; Lorrain *et al.*, 2015).

AERIAL RESPIRATION MEASUREMENTS

Each abalone were detached and placed into a 0.1-L airtight dark chamber connected to a closed circulation system with an integrated infrared CO_2 analyser (Li-Cor, Li-820) and a desiccation column filled with anhydrous calcium sulphate (Drierite, Xenia, USA) just after emersion. An adjustable pump maintained air flow at 0.8-0.9 L min^{-1}. Aerial respiration within each chamber was estimated by the linear slope of CO_2 concentration increase

measured every 5 seconds over 3 minutes using the Li-820 software (Clavier *et al.*, 2009). Fluxes of CO_2 (µmol CO_2 h^{-1}) were corrected for the net volume of the system and incubation time. The aerial temperature was maintained at that of the seawater during aquatic incubations.

Data analysis

SHELL COMMUNITY CONTRIBUTION AND INDIVIDUAL BIOMASS

Calcification, aquatic and aerial respiration measurements were repeated on empty shells of the same individuals. Shells were washed, cleared of any flesh and carefully dabbed inside with a paper towel damped with 70% ethanol. The measured parameters for the shell community were then subtracted to the total calcification and respiration rates. The parameter values presented in this study are the shell community corrected values. The contribution of the dissolution of the inner shell part was considered as negligible (maximum empty shell calcification rate of 0.17 µmol $CaCO_3.h^{-1}.g^{-1}$ in September, and a minimal flux of -0.03 µmol $CaCO_3.h^{-1}.g^{-1}$ in January).

All the studied parameters were further normalized to biomass as described below. Individual biomass was estimated as ash-free dry weight (AFDW) through loss on ignition (4 h combustion at 450°C of 60°C dried individuals until constant weight) and expressed in g $AFDW^{-1}$.

STATISTICAL ANALYSIS

The relationship between the biomass (AFDW) and the physiological aquatic parameters were assessed using the following allometric equation $Y = a \times W^b$ (Marsden *et al.*, 2012) where a corresponds to the physiological rate per gramme AFDW, and b shows the rate at which the physiological parameter evolves with the biomass.

255 Normality and homogeneity of variances of the data distributions of calcification, aquatic
256 and aerial respiration, and ammonium excretion were investigated using the Shapiro-Wilk and
257 Bartlett tests, respectively. An ANOVA was performed to study the seasonal variability.
258 When normal distribution of the data distributions were not verified, the Kruskal Wallis test
259 was used. When the assumption of homogeneity of variance was not verified, a Welch's
260 ANOVA test was performed as recommended by Day and Quinn (1989). Subsequent non
261 parametric post hoc analyses were conducted with the Tukey and Kramer (Nemenyi) test
262 (Pohlert, 2014) to distinguish differences between seasons. The Wilcoxon Signed-Ranks test
263 was run to compare aquatic and aerial respiration at each season.
264 The relationships between the aquatic respiration, calcification and excretion rates, and the
265 seawater temperature experienced at each season were investigated using the Arrhenius
266 equation after logarithmic transformation as a function of T^{-1} as follows:

$$\ln Flux = \ln a - \frac{E_A}{K} \times \frac{1}{T}$$

267 where *Flux* is respiration (ΔDIC aquatic or ΔCO_2 in the air, μmol g^{-1} h^{-1}), *a* is a normalization
268 constant, *E*a is the activation energy (J $mole^{-1}$), *K* is Boltzmann's constant (8.31 J K^{-1} mol^{-1}),
269 and *T* is the absolute temperature (°K).
270 Comparison of calcification, aquatic respiration and ammonium excretion between day-
271 and night-time were conducted with the Wilcoxon Signed-Rank test.
272 As the data were normally distributed ($p > 0.05$), hourly variations in aerial respiration
273 over 6 hours emersion at different ambient temperatures (10°C, 14°C and 18°C) were
274 assessed using repeated measures ANOVA with a Greenhouse-Geisser Correction when the
275 data violated the assumption of sphericity (Mauchly's test of sphericity). Post hoc tests
276 incorporating the Bonferroni correction were done to distinguish differences in aerial
277 respiration at different times of emersion. Also, ANOVA tests were conducted to compare
278 abalone respiration rates between different temperatures (10°C, 14°C and 18°C) at each hour

of emersion. When significant, a subsequent posthoc test (Tukey's HSD test) was run to distinguish which group(s) significantly differed.

RESULTS

Seasonal variations

A seasonal effect was observed on aquatic respiration (Welch test, $F_{3,32} = 19.25$, $P < 0.001$) and aerial respiration (Anova test, $F_{3,60} = 3.63$, $P < 0.05$). Aquatic respiration was significantly lower in autumn (8.41 ± 1.34 µmol DIC g $AFDW^{-1}$ h^{-1}) and winter (6.34 ± 1.93 µmol DIC g $AFDW^{-1}$ h^{-1}) than in summer (13.17 ± 3.66 µmol DIC g $AFDW^{-1}$ h^{-1}, Figure 2). Aerial respiration was significantly lower in winter (1.49 ± 0.66 µmol CO_2 g $AFDW^{-1}$ h^{-1}) than in summer (2.21 ± 0.83 µmol CO_2 g $AFDW^{-1}$ h^{-1}, Figure 2).

Aerial respiration was significantly lower than aquatic respiration over the 4 seasons with abalone respiring between 5 and 6 times more underwater than during emersion (Table 1; Figure 2).

Calcification rates of abalone (net CaCO3 fluxes) had a mean annual value of 0.55 ± 0.53 µmol $CaCO_3$ g $AFDW^{-1}$ h^{-1}. A seasonal effect was observed (Welch test, $F_{3,27.5} = 26.78$, $P < 0.001$) with $CaCO_3$ fluxes significantly lower in winter (Figure 2) but with still 53% of individuals exhibiting shell calcification ($G > 0$). $CaCO_3$ fluxes were positive in 88% of individuals in spring, and 100% of individuals in summer and autumn.

Ammonium fluxes were on average 0.29 ± 0.26 µmol NH^{4+} $AFDW^{-1}$ h^{-1} over the year. A seasonal effect was found (Welch test, $F_{3,27.4} = 5.88$, $P < 0.01$) with lower values measured in spring (0.17 ± 0.20 µmol NH^{4+} $AFDW^{-1}$ h^{-1}) and winter (0.19 ± 0.07 µmol NH^{4+} $AFDW^{-1}$ h^{-1}) than in summer (0.36 ± 0.22 µmol NH^{4+} $AFDW^{-1}$ h^{-1}) and/or autumn (0.47 ± 0.36 µmol NH^{4+} $AFDW^{-1}$ h^{-1}, Figure 2).

304

305 A good relationship was observed between aquatic respiration and calcification, and
306 temperature (respectively, $R^2 = 0.88$ and $R^2 = 0.70$, table 2). The relationship between aerial
307 respiration and temperature was rather high but lower than that of aquatic respiration ($R^2 =$
308 0.68, table 2). A poor relationship was observed between ammonium excretion and
309 temperature ($R^2 = 0.35$, table 2).

310

311 **Day-night variations (in autumn only)**

312 Day- and night-time aquatic respiration rates were 8.41 ± 1.34 and 9.10 ± 3.74 µmol DIC g
313 $AFDW^{-1}$ h^{-1}, respectively, and did not differ significantly (Wilcoxon signed rank test, P =
314 0.64).

315 $CaCO_3$ fluxes were significantly higher (Wilcoxon signed rank test, $P < 0.01$) during the
316 night than during the day (1.19 ± 0.72, and 0.71 ± 0.47 µmol $CaCO_3$ g $AFDW^{-1}$ h^{-1},
317 respectively).

318 Likewise, NH_4^+ fluxes were significantly greater (Wilcoxon signed rank test, $P < 0.05$)
319 during the night than during the day (0.50 ± 0.17, and 0.47 ± 0.36 µmol NH_4^+ $AFDW^{-1}h^{-1}$,
320 respectively).

321

322 **Individual size effect upon aquatic physiological parameters**

323 Both aquatic respiration and calcification rates per unit biomass decreased as the individual
324 biomass increased (aquatic respiration: $y = 28.066 \times x^{-0.484}$, $R^2 = 0.71$; calcification:
325 $y = 3.831 \times x^{-0.885}$, $R^2 = 0.76$; Figure 3). No distinguishable pattern was found between
326 the individual biomass and ammonium excretion rate ($R^2 = 0.004$).

327

328 **Hourly variations in aerial respiration rates**

329 At 10°C, aerial respiration rates were lower at one hour of emersion than at 3, 5 and 6 hours
330 of aerial exposure (Repeated measure Anova test, $F_{5,35} = 13.98$, $P < 0.001$, Figure 4). At 14°C,
331 abalone significantly respired less after one hour of emersion than at 4 and 6 hours of
332 emersion (repeated measures Anova test, $F_{3.1,21.6} = 5.43$, $P < 0.01$, Figure 4). No difference in
333 respiration rate was observed over the 6 hour emersion at 18°C ($F_{2.1,6.7} = 1.13$, Figure 4).
334 Significant differences in aerial respiration rates between temperatures were obtained at 1
335 hour (Anova test, $F_{2,21} = 5.46$, $P < 0.05$), 2 hours (Anova test, $F_{2,21} = 5.25$, $P < 0.05$), 4 hours
336 (Anova test, $F_{2,20} = 3.94$, $P < 0.05$) and 6 hours (Anova test, $F_{2,20} = 5.54$, $P < 0.05$) of emersion
337 : abalone significantly displayed lower respiration rates at 10°C than at 18°C after 1 hour of
338 emersion (Test HSD de Tukey, $P < 0.01$, Figure 4). At 2 and 4 hours of emersion, abalone
339 displayed lower rates at 10° than 14°C (Test HSD de Tukey, $P < 0.05$, Figure 4). At 6 hours
340 of emersion, abalone displayed lower rates at 18° than 14°C (Test HSD de Tukey, $P < 0.05$,
341 Figure 4).
342
343 DISCUSSION
344 **Effect of *Haliotis tuberculata* biomass upon its physiological rate**
345 Body size has been identified as a good proxy for metabolic rates of animals (Newell, 1973).
346 Abalone respiration rates increase as weight increases (Gaty & Wilson, 1986; Basuyaux *et al.*,
347 2001; Cunningham *et al.*, 2016). An allometric relationship between aquatic respiration and
348 calcification rates per unit biomass was observed with negative *b* values much less than unity
349 which indicates that respiration and calcification do not decrease in direct proportion to
350 biomass (Marsden *et al.*, 2012). Our results *i.e.* higher aquatic respiration and calcification
351 rates per unit biomass in smaller *H. tuberculata* individuals, corroborate the evidence that
352 metabolic demand and growth decrease with age (at least during the first years of growth) like
353 in others molluscs such as *Tectus niloticus* (Lorrain *et al.*, 2015), *Crepidula fornicata* (Martin

354 *et al.*, 2006), *Mytilus edulis* and *M. galloprovincialis* (Tagliarolo *et al.*, 2012). Previous
355 findings in *H. tuberculata* have also found that growth rate, underpinned by metabolism and
356 energy budgets, decreases progressively as its size increases (Hayashi, 1980b; Clavier &
357 Richard, 1986; Roussel *et al.*, 2011). However, because one part of the small abalone were
358 from a farmed origin, resulting from systematic mating between wild and farmed broodstock,
359 it cannot be excluded that acclimation to the farm may have slightly modified physiological
360 rates compared to the wild abalone. In addition, because respiration was tested in still water
361 conditions, both respiration and calcification values may be greater in animals in moving
362 water on farm or in the wild (Taylor and Ragg, 2005).
363
364 No relationship was observed between *H. tuberculata* ammonium excretion rate and
365 biomass as in the trochus *T. niloticus* (Lorrain *et al.*, 2015). This may indicate a similar
366 excretion rate per unit biomass for adults and juveniles. The rate of excretion per unit of
367 biomass diminishes in mussels as individuals grow larger (Vaughn & Hakenkamp, 2001;
368 Tagliarolo *et al.*, 2012). A positive correlation however has been reported between body size
369 and ammonium excretion rates per individual in *Haliotis discus discus*, *H. gigantea* and *H.*
370 *madaka* (Ahmed *et al.*, 2008).
371
372 **Seasonal variations in *Haliotis tuberculata* physiological parameters**
373 *H. tuberculata* aquatic respiration showed a 2-fold seasonal difference with minimal rates in
374 winter and maximal rates in summer. Seasonal variations in aquatic respiration have been
375 observed in many others benthic molluscs. From 1.6 up to five-fold differences in aquatic
376 respiration rates between winter and summer have been observed in *Patella vulgata*
377 (Tagliarolo *et al.*, 2013b), *C. fornicata* community (Martin *et al.*, 2007), *Crassostrea gigas*

(Lejart *et al.*, 2012), the trochus *T. niloticus* (Lorrain et al 2015) and the abalone *H. tuberculata* (Gaty & Wilson, 1986).

In our experiment, aerial respiration was minimal in winter (1.49 ± 0.66 µmol CO_2 g $AFDW^{-1}$ h^{-1}) and maximal in summer (2.21 ± 0.83 µmol CO_2 g $AFDW^{-1}$ h^{-1}) with an average 5-fold higher for aquatic respiration compared to aerial respiration. Seasonal aerial respiration has not been extensively studied in molluscs. However, this experiment showed that season has similar effects on underwater and aerial respiration. This can have direct consequences on live transport procedure. As respiration rates and energy demand are higher in summer, it might be more appropriate to transport live abalone during winter when energy needs are lower and when the recovery time post-air exposure may be less.

$CaCO_3$ accretion showed that 53% of *H. tuberculata* individuals did not stop their growth over winter in Britanny temperature conditions. The seasonal growth pattern reported in this study is consistent with previous observations in *H. tuberculata* : abalone growth rate was found to drop in winter whilst 70% of annual growth occurred between May and November (Clavier & Richard, 1986). Similarly, more recent studies using stable oxygen isotopes techniques have found that *H. tuberculata* does not stop its growth in winter (Roussel *et al.*, 2011; Jolivet *et al.*, 2015). $CaCO_3$ accretion in *H. tuberculata* was greater in warmer months when the temperature and the development of the gonad maturation are near its maximum (Hayashi, 1980a) and decreased over winter leading to a net calcification rate over the year (0.55 ± 0.53 µmol $CaCO_3$ g $AFDW^{-1}$ h^{-1}). In contrast, the abalone *Haliotis discus hannai* showed retardation in growth during gonad maturation and spawning (Sakai, 1960). Seasonal variation in $CaCO_3$ accretion is common in molluscs; higher $CaCO_3$ fluxes have been found in warmer months (Martin *et al.*, 2006; Lejart *et al.*, 2012) whilst $CaCO_3$ accretion decreases or stops in winter (Lejart *et al.*, 2012; Tagliarolo *et al.*, 2013b).

402 Finally, ammonium excretion rates in *H. tuberculata* also varied seasonally; lower average
403 values were measured in spring and winter (0.17-0.19 µmol NH_4^+ $AFDW^{-1}h^{-1}$, respectively)
404 whilst maximal average values were found in summer and autumn (0.37-0.47 µmol NH_4^+
405 $AFDW^{-1}h^{-1}$, respectively). These results are consistent with the variations in excretion rates
406 reported for the limpet *P. vulgata*; low shore individuals had fluxes ranging from 0.5 to 0.7
407 µmol NH_4^+ $AFDW^{-1}h^{-1}$ in winter and in summer, respectively (Tagliarolo *et al.*, 2013b). In
408 contrast, other mollusc species showed maximum excretion rates in spring (Martin *et al.*,
409 2006) and minimum rates in winter (Bayne & Scullard, 1977; Martin *et al.*, 2006).
410 This seasonal pattern in *H. tuberculata* physiological rates can be explained by both
411 environmental stressors (*e.g.* temperature), and biogenic factors (*e.g.* reproductive needs,
412 resource abundance, distribution and availability).
413 Seasonal variation in temperature was most likely the primary driver of the observed
414 seasonal variations in carbon fluxes in this study. This hypothesis is consistent with the strong
415 relationships observed between temperature and the studied physiological rates (except
416 ammonium rates); lower rates occurred in winter when the temperature was 11°C and
417 maximal rates were recorded in summer when the temperature reached 18.3°C. Temperature
418 is known to impact all physiological rates in ectotherms (Somero, 2002) and it has been
419 showed to influence the aquatic respiration of many mollusc species (Lejart *et al.*, 2012;
420 Tagliarolo *et al.*, 2013a, 2013b). As temperature increases, the oxygen demand increases as
421 more energy is required to fulfill physiological requirements. This has already been reported
422 in *H. tuberculata,* which exhibited lower oxygen consumption rates at 8°C compared to 16°C
423 or 18°C (Gaty & Wilson, 1986).
424 Biotic factors such as food abundance can contribute to respiration rates. Abalone are
425 herbivorous (Stephenson, 1924) and mostly sedentary (Clavier & Richard, 1982). Abalone
426 increase their feeding activity in summer (Allen *et al.*, 2006) when their resources *i.e.* drifting

seaweed (Clavier & Chardy, 1989) are more abundant following summer macroalgal blooms on the Atlantic coast (Dion & Le Bozec, 1996). Fresh algae were provided *ad libitum* before the experiment in order to reduce variability due to starvation since at the same temperature, oxygen consumption rate is 30% lower in 2-weeks starved abalone compared to fed *H. tuberculata* (Gaty & Wilson, 1986). Because *H. tuberculata* are more inclined to forage in summer (Roussel, pers. comm.), higher aquatic and aerial respiration rates in summer may be related to higher metabolic demands as more energy is required for digestion, absorption and assimilation processes (Widdows & Shick, 1985). Higher respiration rates may also be related to the production of large gonad. Indeed, the spawning period has been associated with increased metabolic demand as energy is required to produce gametes (Hayashi, 1983). Finally, the low growth and the decrease in excretion rates may be associated with less abundant resources over winter (Allen *et al.*, 2006).

Day-night variations in *H. tuberculata* physiological parameters

H. tuberculata is characterised like other abalone species (Momma & Sato, 1970; Barkai & Griffiths, 1987) by nocturnal behaviour with movements that are initiated an hour after sunset (Werner *et al.*, 1995). However, unlike other gastropods such as the abalone *H. discus hannai* (Uki & Kikuchi, 1975), and the trochus *T. niloticus* (Lorrain *et al.*, 2015) which display higher metabolic rates whilst being active overnight, no circadian rhythm in *H. tuberculata* aquatic respiration was observed. Movement of the animal could not be controlled in most of the experiment design. The apparatus used in this experiment allowed movement of the abalone inside the bottle, and no movement restraint was used. However, no abalone movements were observed during visual observation of the bottle, even if punctual movement cannot be completely excluded. This result probably indicates that aquatic respiration is

451 similar during night and day periods. However, we cannot exclude that aquatic respiration
452 would be in average higher at night when abalone is crawling to get food.

453 Intrinsic higher metabolic rates overnight may also explain the counterintuitive greater
454 ammonium fluxes at night than during the day, even though the difference was small (0.03
455 µmol NH_4^+ $AFDW^{-1}h^{-1}$). Indeed since *H. tuberculata* has a nocturnal feeding habit like the
456 abalone *H. midae*, ammonium excretion was expected to be higher during the diurnal
457 elimination phase (Barkai & Griffiths, 1987). Further research is required to determine the
458 factors driving day-night patterns of ammonium fluxes in *H. tuberculata*.

459 *H. tuberculata* calcifies 1.7 fold more at night than during the day. Diurnal growth ridge
460 formation in other gastropods like the limpet *Acmaea antillarum* have been related to the
461 light-dark cycle (Kenny, 1977). Since incubations at both day- and night-times were
462 conducted in dark conditions, the calcification process in *H. tuberculata* may be intrinsic and
463 be related to its nocturnal behaviour and related higher metabolic activity in the same way as
464 the trochus *T. niloticus* (Lorrain *et al.*, 2015). More research is required to examine the
465 day/night pattern in calcification rates in shelled gastropods, which has to date been poorly
466 documented.

467

468 **_H. tuberculata_ aerial respiration during 6-h exposure**

469 *H. tuberculata* aerial carbon respiration represents ca. 20% of aquatic carbon respiration,
470 which is in the range of what has been observed in intertidal mussels (19%-23%, Tagliarolo *et*
471 *al.*, 2012) but lower than others intertidal gastropod species (40%-230%) (Tagliarolo *et al.*,
472 2013a). The low emersion/immersion ratio *i.e.* 0.2, may be an energy saving strategy for
473 emerged *H. tuberculata* individuals which potentially implies a switch to anaerobic processes
474 (Widdows & Shick, 1985). Another explanation of this lower aerial respiration would be the
475 collapse of the gill in the air, so that the respiration would be limited. This indicates that *H.*

476 *tuberculata* can physiologically adapt to emersion like others intertidal gastropods. However,
477 regardless carefully handling of abalone, it cannot be excluded that acute stress response due
478 to detachment modified, at least partly, aerial respiration at the beginning of the measurement
479 period. Further research is however required to better understand aerobic and anaerobic
480 pathways during emersion in *H. tuberculata*.

481 Some intertidal gastropods have been shown to either decrease aerial respiration over
482 emersion or to maintain stable rates (Tagliarolo *et al.*, 2013a, 2013b). Here, abalone increased
483 their aerial respiration over time at 10°C and 14°C. At 18°C, aerial respiration was already
484 high in the first hour of aerial exposition and no further increase was observed over time.
485 Similarly, mussels (*M. edulis*) showed an increase in aerial energy expenditure when exposed
486 to the air for longer than under natural conditions (Widdows & Shick, 1985). Abalone are
487 mostly found in subtidal areas or at the low shore level of intertidal rocky shores, therefore
488 intertidal abalone are emerged for short periods of time in nature *i.e.* less than 3 hours, rather
489 than the imposed 6 hours of emersion of this study. Respiration rates in *H. tuberculata* started
490 to increase after 3 hours of emersion at the anticipated time of re-immersion. This result
491 showed that *H. tuberculata* can handle emersion during 3 hours with probably very limited
492 energetic cost. However, after 3 hours of emersion, abalone increased their respiration rate (at
493 10ºC and 14ºC) which suggests higher metabolic cost of longer emersion period at these
494 temperatures (up to 6 hours in this study) and potential physiological impacts on the recovery
495 time (and higher post-emersion mortality). Respiration rates at 10°C were lower than at
496 greater temperatures after 1, 2 and 4 hours of emersion. This suggests that transporting live
497 ormers at low temperature (10 ºC) using ice packs for short time period may minimize the
498 metabolic cost of emersion and hence may improve survival rates during the post-emersion
499 recovery period (Buen-Ursua & Ludevese, 2011).
500

CONCLUSION

This study has underscored that (i) aquatic carbon respiration and calcification rates per unit biomass are higher in smaller ormers, (ii) rates of physiological parameters are lower in winter than in summer, in particular, ormers do not stop calcifying in winter, (iii) calcification and excretion rates are higher at night than during the day, (iv) aerial carbon respiration corresponds to 20% of aquatic respiration which indicates that *H. tuberculata* is adapted to periods of emersion. The clear patterns of variations in *H. tuberculata* physiological rates reported in the present study may constitute important information to understand the relationship between growth, survival and metabolism during farming procedures.

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REFERENCES

Ahmed F., Segawa S., Yokota M. and Watanabe S. (2008) Effect of light on oxygen consumption and ammonia excretion in *Haliotis discus discus*, *H. gigantea*, *H. madaka* and

526 their hybrids. *Aquaculture* 279, 160–165.

527 **Allen V.J., Marsden I.D., Ragg N.L.C. and Gieseg S.** (2006) The effects of tactile stimulants
528 on feeding, growth, behaviour, and meat quality of cultured Blackfoot abalone, *Haliotis iris*.
529 *Aquaculture* 257: 294–308.

530 **Baldwin J., Wells R.M.G., Low M. and Ryder J.M.** (1992) Tauropine and D-lactate as
531 metabolic stress indicators during transport and storage of live paua, (New Zealand abalone)
532 (*Haliotis iris*). *Journal of Food Science* 57: 280–282.

533 **Barkai R. and Griffiths C.L.** (1987) Consumption, absorption efficiency, respiration and
534 excretion in the South African abalone *Haliotis midae*. *South African Journal of Marine*
535 *Science* 5: 523–529.

536 **Basuyaux O., Mathieu M. and Richard O.** (2001) Influence de Ia taille, de la température et du
537 régime alimentaire sur la consommation d'oxygène de l'oursin. *Marine Life* 11: 57–67.

538 **Bayne B.L .and Scullard C.** (1977) Rates of nitrogen excretion by species of Mytilus (Bivalvia:
539 Mollusca). *Journal of the Marine Biological Association of the United Kingdom* 57: 355–369.

540 **Bayne B.L. and Newell R.C.** (1983). Physiological energetics of marine molluscs. *The*
541 *Mollusca* 4: 407–515.

542 **Buen-Ursua S.M.A. and Ludevese G.** (2011) Temperature and size range for the transport of
543 juvenile donkey's ear abalone *Haliotis asinina* Linne. *Aquaculture Research* 42: 1206–1213.

544 **Carefoot T.H., Taylor B.E. and Donovan D.A.** (1998) Seasonality in digestive-gland size and
545 metabolism in relation to reproduction in *Haliotis Kamtschatkana*. *Journal of Shellfish*
546 *Research* 17: 713–716.

547 **Chiou T.K., Lai M.M. and Shiau C.Y.** (2001) Seasonal variations of chemical constituents in
548 the muscle and viscera of small abalone fed different diets. *Fisheries Science* 67: 146–156.

549 **Clavier J., Castets M.-D., Bastian T., Hily C., Boucher G.and Chauvaud L.** (2009) An
550 amphibious mode of life in the intertidal zone: aerial and underwater contribution of
551 *Chthamalus montagui* to CO_2 fluxes. *Marine Ecology Progress Series* 375: 185–194.

552 **Clavier J. and Chardy P.** (1989) Investigation into the ecology of the ormer (*Haliotis*
553 *tuberculata* L.), factors influencing spatial distribution. *Aquatic living resources* 2: 191–197.

554 **Clavier J. and Richard O.** (1982) Etude expérimentale du déplacement de l'ormeau (*Haliotis*
555 *tuberculata*) dans le milieu naturel. *Revue des Travaux de l'Institut des Pêches Maritimes* 46:
556 315–326.

557 **Clavier J. and Richard O.** (1986) Growth of juvenile *Haliotis tuberculata* (Mollusca:
558 Gastropoda) in their natural environment. *Journal of the Marine Biological Association of the*
559 *United Kingdom* 66: 497–503.

560 **Cook P.A.** (2014) The worldwide abalone industry. *Modern Economy* 5: 1181–1186.

561 **Cooke S.J., Blumstein D.T., Buchholz R., Caro T., Fernández-Juricic E., Franklin C.E.,**
562 **Metcalfe J., O'Connor C.M., Clair C.C.S., Sutherland W.J. and Wikelski M.** (2014)
563 Physiology, Behavior, and Conservation. *Physiological and Biochemical Zoology* 87: 1–14.

564 **Crofts D.R.** (1929) *Haliotis* (No. 29). The University Press of Liverpool.

565 **Cunningham S.C., Smith A.M. and Lamare M.D.** (2016) The effects of elevated pCO_2 on
566 growth, shell production and metabolism of cultured juvenile abalone, *Haliotis iris*.
567 *Aquaculture Research* 47: 2375–2392.

568 **Day R. W. and Quinn G. P.** (1989) Comparisons of treatments after an analysis of variance in
569 ecology. *Ecological Monographs* 59: 433-463.

570 **Dickson A.G. and Goyet C.** (1994) *Handbook of methods for the analysis of the various*
571 *parameters of the carbon dioxide system in sea water*. Version 2. Oak Ridge National Lab.,

572 TN (United States).

573 **Dion P. and Le Bozec S.** (1996) *The French atlantic coasts.* In Marine Benthic Vegetation.
574 Springer Berlin Heidelberg. 251–264.

575 **Forster G.R.** (1967) The growth of *Haliotis tuberculata*: results of tagging experiments in
576 Guernsey 1963-65. *Journal of the Marine Biological Association of the UK* 47: 287–300.

577 **Frankignoulle M., Canon C. and Gattuso J.P.** (1994) Marine calcification as a source of
578 carbon dioxide: positive feedback of increasing atmospheric CO_2. *Limnology and*
579 *Oceanography* 39: 458–462.

580 **Gao X., Xian L., Meijie L., Changbin S. and Ying. L** (2016) Effects of light intensity on
581 metabolism and antioxidant defense in *Haliotis discus hannai* Ino. *Aquaculture* 465: 78–87.

582 **Gaty G. and Wilson J.H.** (1986) Effect of body size, starvation, temperature and oxygen
583 tension on the oxygen consumption of hatchery-reared ormers *Haliotis tuberculata* L.
584 *Aquaculture* 56: 229–237

585 **Gazeau F., Alliouane S., Bock C., Bramanti L., López Correa M., Gentile M., Hirse T.,**
586 **Pörtner H.-O. and Ziveri P.** (2014) Impact of ocean acidification and warming on the
587 Mediterranean mussel (*Mytilus galloprovincialis*). *Frontiers in Marine Science* 1: 1–12.

588 **Gazeau F., Urbini L., Cox T.E., Alliouane S. and Gattuso J.P.** (2015) Comparison of the
589 alkalinity and calcium anomaly techniques to estimate rates of net calcification. *Marine*
590 *Ecology Progress Series* 527: 1–12.

591 **González R.C., Brokordt K. and Lohrmann K. B.** (2012) Physiological performance of
592 juvenile *Haliotis rufescens* and *Haliotis discus hannai* abalone exposed to the withering
593 syndrome agent. *Journal of Invertebrate Pathology* 111: 20–26.

594 **Hatae K., Nakai H., Shimada A., Murakami T., Takada K., Shirojo Y. and Watabe S.**

595 (1995) Abalone (*Hariltis discus*): Seasonal variations in chemical composition and textural
596 properties. *Journal of Food Science* 60: 32–35.

597 **Hayashi I.** (1980a) The reproductive biology of the ormer, *Haliotis tuberculata*. *Journal of the*
598 *Marine Biological Association of the United Kingdom* 60: 415–430.

599 **Hayashi I.** (1980b) Structure and growth of a shore population of the ormer, *Haliotis*
600 *tuberculata*. *Journal of the Marine Biological Association of the United Kingdom* 60: 431–
601 437.

602 **Hayashi I.** (1983) Seasonal changes in condition factors and in the C: N ratio of the foot of the
603 ormer, *Haliotis tuberculata*. *Journal of the Marine Biological Association of the United*
604 *Kingdom* 63: 85–95.

605 **Jolivet A., Chauvaud L., Huchette S., Legoff C., Thébault J., Nasreddine K., Schöne B.R.**
606 **and Clavier J.** (2015) The ormer (*Haliotis tuberculata*): A new, promising paleoclimatic tool.
607 *Palaeogeography, Palaeoclimatology, Palaeoecology* 427: 32–40.

608 **Kenny R.** (1977) Growth studies of the tropical intertidal limpet *Acmaea antillarum*. *Marine*
609 *Biology* 39: 161–170.

610 **Koroleff F.** (1969) Direct determination of ammonia in natural waters as indophenol blue.
611 *International Council for the Exploration of the Sea* 9: 1–6.

612 **Lachambre S., Huchette S., Day R., Boudry P., Rio-Cabello A., Fustec T. and Roussel S.**
613 (2017) Relationships between growth, survival, physiology and behaviour - a multi-criteria
614 approach to *Haliotis tuberculata* phenotypic traits. *Aquaculture* 467: 190–197.

615 **Lejart M., Clavier J., Chauvaud L. and Hily C.** (2012) Respiration and Calcification of
616 *Crassostrea gigas* : contribution of an Intertidal Invasive Species to Coastal Ecosystem CO_2
617 Fluxes. *Estuaries and Coasts* 35: 622–632.

618 **Lorrain A., Clavier J., Thébault J., Tremblay-Boyer L., Houlbrèque F., Amice E., Le Goff**
619 **M. and Chauvaud L.** (2015) Variability in diel and seasonal *in situ* metabolism of the
620 tropical gastropod *Tectus niloticus*. *Aquatic Biology* 23: 167–182.

621 **Lu J., Shi Y., Cai S. and Feng J.** (2017) Metabolic responses of *Haliotis diversicolor* to *Vibrio*
622 *parahaemolyticus* infection. *Fish & Shellfish Immunology* 60: 265–274.

623 **Marsden I.D., Shumway S.E. and Padilla D.K.** (2012) Does size matter? The effects of body
624 size and declining oxygen tension on oxygen uptake in gastropods. *Journal of the Marine*
625 *Biological Association of the United Kingdom* 92: 1603–1617.

626 **Martin S., Thouzeau G., Chauvaud L., Jean F., Guérin L.and Clavier J.** (2006) Respiration,
627 calcification, and excretion of the invasive slipper limpet, *Crepidula fornicata* L.:
628 implications for carbon, carbonate, and nitrogen fluxes in affected areas. *Limnology and*
629 *Oceanography* 51: 1996–2007.

630 **Martin S., Thouzeau G., Richard M., Chauvaud L., Jean F. and Clavier J.** (2007) Benthic
631 community respiration in areas impacted by the invasive mollusk *Crepidula fornicata*. *Marine*
632 *Ecology Progress Series* 347: 51–60.

633 **Mateos H.T., Lewandowski P.A. and Su X.Q.** (2010) Seasonal variations of total lipid and
634 fatty acid contents in muscle, gonad and digestive glands of farmed Jade Tiger hybrid abalone
635 in Australia. *Food Chemistry* 123: 436–441.

636 **Momma H. and Sato R.** (1970) The locomotion behavior of the disc abalone, *Haliotis discus*
637 *hannai* Ino, in a tank. *Tohoku journal of agricultural research* 21: 20–25.

638 **Morash A.J.and Alter K.** (2015) Effects of environmental and farm stress on abalone
639 physiology: perspectives for abalone aquaculture in the face of global climate change.
640 *Reviews in Aquaculture* 8: 342–368.

641 **Newell R.C.** (1973) Factors affecting the respiration of intertidal invertebrates. *Integrative and*

642 *Comparative Biology* 13: 513–528.

643 **Pierrot D., Lewis E. and. Wallace D.W.R**. (2006). MS Excel program developed for CO_2
644 system calculations. ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center, Oak
645 Ridge National Laboratory, US Department of Energy, Oak Ridge, Tennessee.

646 **Pohlert T.** (2014) The pairwise multiple comparison of mean ranks package (PMCMR). R
647 package.

648 **Ragg, N.L.C. and Watts E.** (2015) Physiological indicators of stress and morbidity in
649 commercially handled abalone *Haliotis iris*. *Journal of Shellfish Research* 34: 455–467.

650 **Roussel S., Huchette S., Clavier J. and Chauvaud L.** (2011) Growth of the European abalone
651 (*Haliotis tuberculata* L.) *in* situ: seasonality and ageing using stable oxygen isotopes. *Journal*
652 *of sea research* 65: 213–218.

653 **Roussel V., Charreyron J., Labarre S., Van Wormhoudt A. and Huchette S.** (2013) First
654 steps on technological and genetic improvement of European abalone (*Haliotis tuberculata*)
655 based on investigations in full-sib families. *Open Journal of Genetics* 3: 224–233.

656 **Roy R.N., Roy L.N., Vogel K.M., Porter-Moore C., Pearson T., Good C.E., Millero F.J. and**
657 **Campbell D.M.** (1993) The dissociation constants of carbonic acid in seawater at salinities 5
658 to 45 and temperatures 0 to 45 ºC. *Marine Chemistry* 44: 249–267.

659 **Sakai S.** (1960) On the formation of the annual ring on the shell of the abalone *Haliotis discus*
660 *var Hannai* ino. *Tohoku journal of agricultural research* 11: 239–244.

661 **Shepherd S.A.** (1973) Studies on southern Australian abalone (genus *Haliotis*). I. Ecology of
662 five sympatric species. *Australian Journal of Marine and Freshwater Research* 24: 217–258.

663 **Smith S.V and Key G.S.** (1975) Carbon dioxide and metabolism in marine environments.
664 *Limnology and Oceanography* 20: 493–495.

665 **Somero G.N.** (2002) Thermal physiology and vertical zonation of intertidal animals: optima,
666 limits, and costs of living. *Integrative and comparative biology* 42: 780–789,

667 **Sororzano L.** (1969) Determination of ammonia in natural waters by the phenolhypochlorite
668 method. *Limnology Oceanography* 14: 799–801.

669 **Stephenson T.A.** (1924) Notes on *Haliotis tuberculata*. I. *Journal of the Marine Biological*
670 *Association of the United Kingdom* 13: 480–495.

671 **Su X.Q., Antonas K., Li D.and Nichols P.** (2006) Seasonal variations of total lipid and fatty
672 acid contents in the muscle of two Australian farmed abalone species. *Journal of Food Lipids*
673 13: 411–423.

674 **Tagliarolo M., Clavier J., Chauvaud L. and Grall J.** (2013a) Carbon emission associated with
675 respiration and calcification of nine gastropod species from the intertidal rocky shore of
676 Western Europe. *Marine Biology* 160: 2891–2901.

677 **Tagliarolo M., Clavier J., Chauvaud L., Koken M. and Grall J.** (2012) Metabolism in blue
678 mussel: intertidal and subtidal beds compared. *Aquatic Biology* 17: 167–180.

679 **Tagliarolo M., Grall J., Chauvaud L. and Clavier J.** (2013b) Aerial and underwater
680 metabolism of *Patella vulgata* L.: comparison of three intertidal levels. *Hydrobiologia* 702:
681 241–253.

682 **Taylor, H. H. and Ragg, N. L. C.** (2005) The role of body surfaces and ventilation in gas
683 exchange of the abalone, *Haliotis iris*. *Journal of Comparative Physiology B-Biochemical*
684 *Systemic and Environmental Physiology*, 175: 463-478.

685 **Travers M.A., Le Goic N., Huchette S., Koken M. and Paillard C.** (2008) Summer immune
686 depression associated with increased susceptibility of the European abalone, *Haliotis*
687 *tuberculata* to *Vibrio harveyi* infection. *Fish & Shellfish Immunology* 25: 800–808.

688 **Uki N. and Kikuchi S.** (1975) Oxygen consumption of the abalone, *Haliotis discus hannai* in
689 relation to body size and temperature. *Bulletin of Tohoku Regional Fisheries Research*
690 *Laboratory* 35: 73-84.

691 **van Wormhoudt A., Gaume B., Le Bras Y., Roussel V. and Huchette S.** (2011) Two different
692 and functional nuclear rDNA genes in the abalone *Haliotis tuberculata*: Tissue differential
693 expression. *Genetica* 139: 1217–1227.

694 **Vaughn C.C. and Hakenkamp C.C.** (2001) The functional role of burrowing bivalves in
695 freshwater ecosystems. *Freshwater Biology* 46: 1431–1446.

696 **Venter L., Loots D.T., Vosloo A., van Rensburg P.J. and Lindeque J.Z.** (in press) Abalone
697 growth and associated aspects: now from a metabolic perspective. *Reviews in Aquaculture*.

698 **Wells R.M.G.and Baldwin J.** (1995) A comparison of metabolic stress during air exposure in
699 two species of New Zealand abalone, *Haliotis iris* and *Haliotis australis*: Implications for the
700 handling and shipping of live animals. *Aquaculture* 134: 3–4.

701 **Werner I., Flothmann S. and Burnell G.** (1995) Behaviour studies on the mobility of two
702 species of abalone (*Haliotis tuberculata* and *H. discus hannai*) on sand: implications for
703 reseeding programmes. *Marine and Freshwater Research* 46: 681–688.

704 **Widdows J. and Shick J.M.** (1985) Physiological responses of *Mytilus edulis* and *Cardium*
705 *edule* to aerial exposure. *Marine Biology* 85: 217–232.

706 **Young J.L., Bornik Z.B., Marcotte M.L., Charlie K.N., Wagner G.N., Hinch S.G. and**
707 **Cooke S.J.** (2006) Integrating physiology and life history to improve fisheries management
708 and conservation. *Fish and Fisheries* 7: 262–283.

709

TABLE CAPTIONS

Table 1. Comparison of aquatic (UW) and aerial (A) respiration in *Haliotis tuberculata* over 4 seasons (Wilcoxon Signed Ranks Test).

Table 2. Relationships between metabolic parameters and temperature described by Arrhenius equation $\ln Flux = \ln a - \frac{E_A}{K} \times \frac{1}{T}$. Standards errors are in brackets.

FIGURE CAPTIONS

Fig. 1. Sea water temperature variations recorded at the SOMLIT station (St Anne du Portzic site) over 12 months in 2014-2015. Grey squares indicate the field seawater temperature at each sampling period (Sum: summer; Aut: autumn; Win: winter; Spr: spring).

Fig. 2. *Haliotis tuberculata* aquatic and aerial respiration, calcification and ammonium excretion over 4 seasons in regards to seawater temperature (white dots: summer; dark grey dots: autumn; black dots: winter; light grey dots: spring.) in 2014-2015. Values are means and error bars are standard deviations. n = 16 in summer, n = 15 in autumn, n = 17 in winter and in spring. Significant differences ($p < 0.05$) between seasons from Tukey and Kramer post hoc tests are indicated by letters.

Fig. 3. Allometric relation ($aAFDW^b$) between individual abalone biomass (AFDW) and aquatic respiration (top graph), and calcification (bottom graph) in summer 2014.

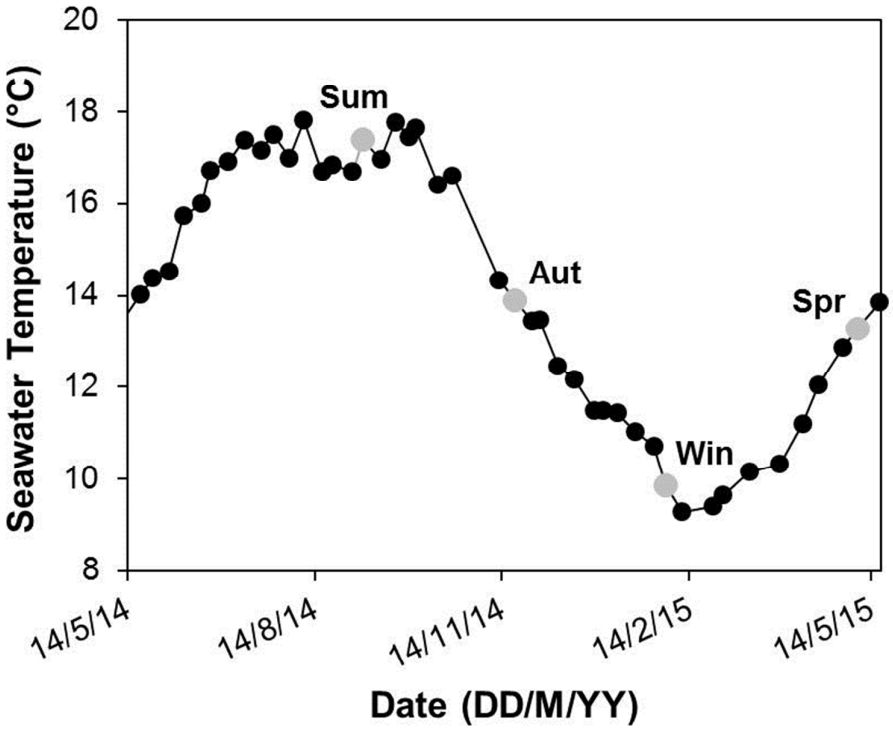
Fig. 4. *Haliotis tuberculata* aerial respiration during emersion at 10°C, 14°C and 18°C. Values are means and error bars are standard deviations (n = 8 at 10°C and 14°C; $6 < n < 8$ at 18°C).

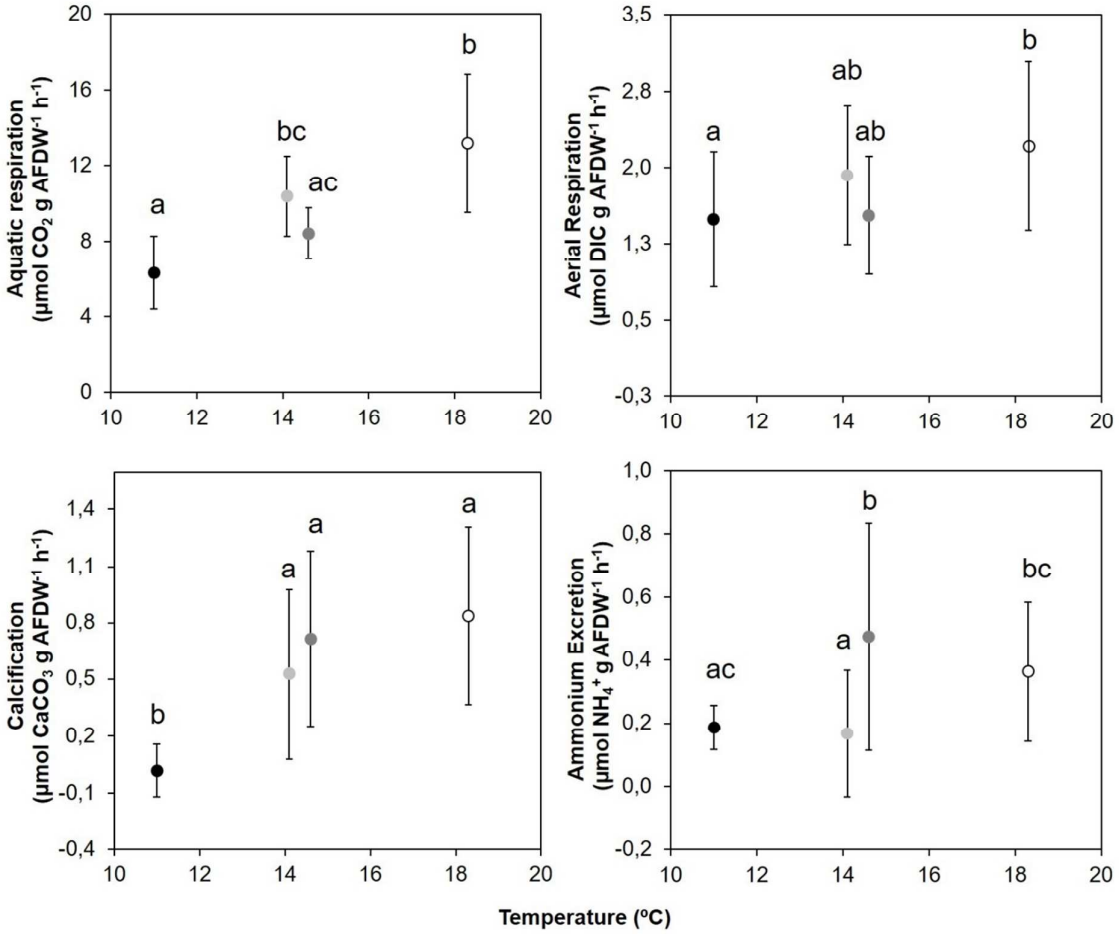
Season	*Z*	*P*	Results
Summer	-3.516	< 0.001	UW > A
Autumn	-3.408	0.001	UW > A
Winter	-3.574	< 0.001	UW > A
Spring	-3.516	< 0.001	UW > A

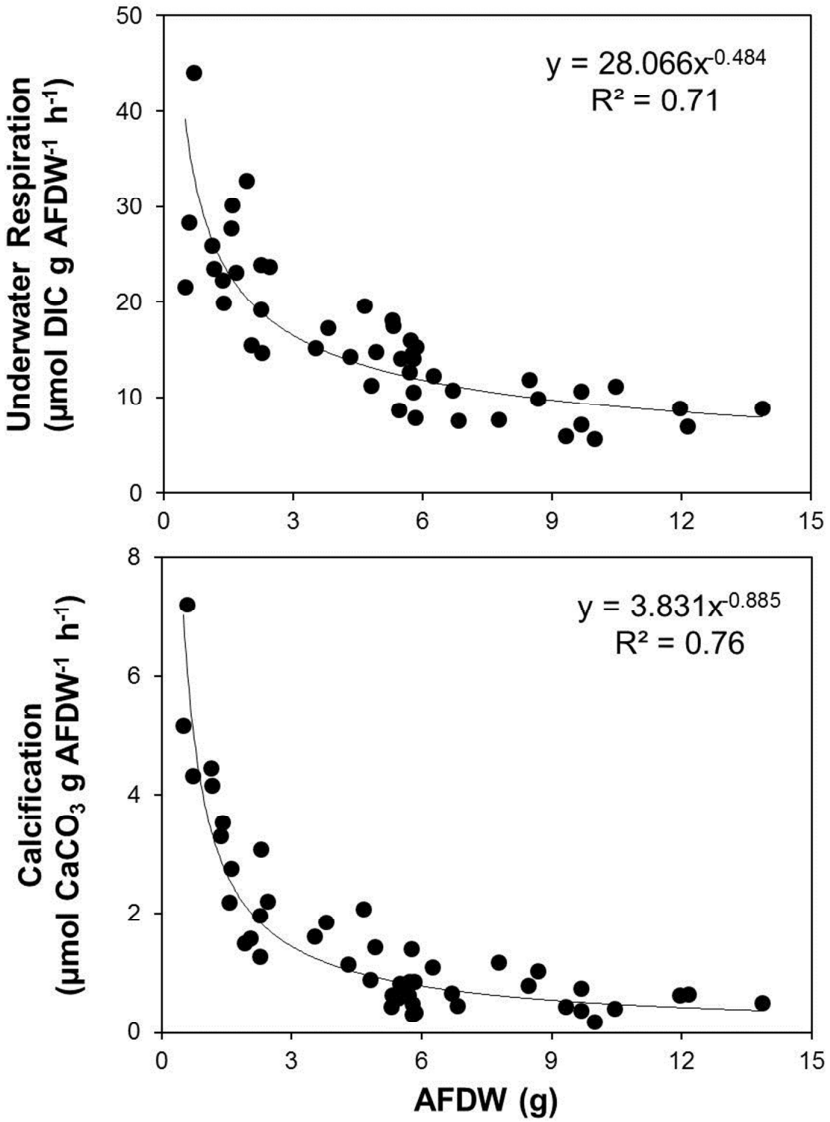
1

Parameter	$ln\ a$	Ea/K	R^2
Aquatic respiration	30.23 (7.46)	8.06 (2.15)	0.88 (0.13)
Calcification	143.42 (67.31)	41.62 (19.36)	0.70 (1.22)
Ammonium excretion	27.47 (27.96)	8.28 (8.04)	0.35 (0.50)
Aerial respiration	15.65 (7.27)	4.34 (2.10)	0.68 (0.13)

2




Aquatic respiration (µmol CO_2 g AFDW^{-1} h^{-1})
a
bc
ac
b
Aerial Respiration (µmol DIC g AFDW^{-1} h^{-1})
a
ab
ab
b
Calcification (µmol $CaCO_3$ g AFDW^{-1} h^{-1})
b
a
a
a
Ammonium Excretion (µmol NH_4^+ g AFDW^{-1} h^{-1})
ac
a
b
bc
Temperature (ºC)


Underwater Respiration
(µmol DIC g AFDW-1 h-1)
y = 28.066x-0.484
R² = 0.71
Calcification
(µmol CaCO3 g AFDW-1 h-1)
y = 3.831x-0.885
R² = 0.76
AFDW (g)