



## When shape matters: Strategies of different Antarctic ascidians morphotypes to deal with sedimentation



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### ABSTRACT

Climate change leads to increased melting of tidewater glaciers in the Western Antarctic Peninsula region and sediment bearing glacial melt waters negatively affects filter feeding species as solitary ascidians. In previous work the erect-forms *Molgula pedunculata* and *Cnemidocarpa verrucosa* (Order Stolidobranchiata) appeared more sensitive than the flat form *Ascidia challengerii* (Order Phlebobranchiata). Sedimentation exposure is expected to induce up-regulation of anaerobic metabolism by obstructing the organs of gas exchange (environmental hypoxia) or causes enhanced squirting activity (functional hypoxia). In this study we evaluated the possible relationship between ascidian morphotype and their physiological response to sedimentation. Together with some behavioural observations, we analysed the response of anaerobic metabolic parameters (lactate formation and glycogen consumption) in different tissues of three Antarctic ascidians, exposed to high sediment concentrations (200 mgL<sup>-1</sup>). The results were compared to experimental hypoxia (10% pO<sub>2</sub>) and exercise (induced muscular contraction) effects, in order to discriminate the effect of sediment on each species and morpho-type (erect vs. flat forms). Our results suggest that the styled (erect) *C. verrucosa* increases muscular squirting activity in order to expulse excessive material, while the flat-form *A. challengerii* reacts more passively by down-regulating its aerobic metabolism under sediment exposure. Contrary, the erect ascidian *M. pedunculata* did not show any measurable response to the treatments, indicating that filtration and ingestion activities were not reduced or altered even under high sedimentation (low energetic material) which could be disadvantageous on the long-term and could explain why *M. pedunculata* densities decline in the study area.

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### 1. Introduction

It is well recognised that changes in community composition and structure can be related to the level of disturbance acting in an ecosystem (Brown et al., 2004; Gutt and Piepenburg, 2003; Momo et al., 1997; Smale, 2007). As a consequence of climate change, massive glacier melting in the South Shetland Islands in the past 50 years produced an increase of coastal sediment run-off (Monien et al., 2011; Rückamp et al., 2011; Schloss et al., 2012). Sedimentation has been described as an important stressor for marine systems, especially for filter-feeders (Thrush et al., 2004). Underwater surveys performed during the last 15 years documented

important changes in the benthic communities of Potter Cove (25 de Mayo/King George Island, South Shetlands, West Antarctic Peninsula) with respect to abundance and depth distribution of different filter-feeders groups. These changes included a population decrease of the solitary flat-form ascidians *Corella antarctica* (= *Corella eumyota*; Tatian et al., 1998) and *Ascidia challengerii* (Order Phlebobranchiata) and a more intense decline of the erect-forms *Cnemidocarpa verrucosa* and *Molgula pedunculata* (Order Stolidobranchiata). The last one was the most dramatically affected, from being dominant in 1994 to almost disappear in 2010, reducing more than six times their densities (Sahade et al., unpublished results).

As a first approach, to connect these environmental changes with ascidians population decline, the respiratory response of these species in response to increasing sediment concentrations was measured. *M. pedunculata* increased its oxygen consumption already at lower sediment concentrations than the other two

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species ( $15 \text{ mgL}^{-1}$ ) and kept it high up to the end of the experiment ( $400 \text{ mgL}^{-1}$ ), indicating a higher energetic cost under sediment exposure than the other species. Contrary *C. verrucosa* and *A. challengerii* increased oxygen consumption at higher sediment loads (50 and  $100 \text{ mgL}^{-1}$  respectively) up to a critical concentration after which oxygen consumption declined ( $200 \text{ mgL}^{-1}$ ) (Torre et al., 2012). While it has previously been shown that inorganic matter negatively affects ascidians survival (Robbins, 1985), in the present case the three species showed a distinct responses to sedimentation and also belonged to different morphotypes. Hence, we were interested in the possible relationship between morphological and physiological features of these ascidians species.

Ascidians are the largest and most diverse class of the sub-phylum Tunicata and can be found in all marine habitats around the world. They exhibit a wide variety of body forms, reproduction and larval dispersion strategies, colonizing polar to tropical latitudes from shallow waters to abyssal depths (Lambert, 2005; Shenkar and Swalla, 2011). Ascidian filtration and respiration are performed by the same structure, the branchial sac, through which water is pumped via the oral siphon and where suspended particles are trapped within a mucous net. The mucus is produced and transported by the endostyle (a ciliated groove coated with mucosal cells) and flows to the dorsal lamina, from where it enters the esophagus for digestion of filtered particles (Goodbody, 1975; Holley, 1986). Initially, the branchial sac was ascribed mostly nutritional function with a secondary role in gas exchange. Later findings indicated, however, that most of the gas exchange takes place in this organ and that oxygen partial pressure ( $pO_2$ ) in the branchial sac also regulates filtration and metabolic rates (Evans and Huntington, 1992; Fiala-Médioni, 1979; Modig and Ólafsson, 1998; Petersen, 2007; Robbins, 1984, 1985). Across ascidian species there is no consensus upon the response pattern to increasing suspended particles concentration. Experiments summarized by Petersen (2007) yielded contradictory results i.e. different species can exhibit increases or decreases in ciliary beat frequency, filtration rates, squirting behaviour and aerobic metabolism in response to augmenting suspended material concentrations. Furthermore, in many cases the response is not linear with respect to concentration and may also vary with exposure time (Evans and Huntington, 1992).

The three Antarctic studied species showed an increase in oxygen consumption in response to sedimentation, (Torre et al., 2012) that could be related to an increase in mucus production and ciliary activity to cope with excessive amounts of particles, or an increase of squirting behaviour frequency attempting to expel the unwanted material (Armsworthy et al., 2001). Squirting requires energy demanding muscular contractions (Carlisle, 1966; Hoyle, 1953). *C. verrucosa* and *A. challengerii* showed an increase of respiration at lower concentrations followed by a respiratory decline from a certain sediment concentration onwards. This decline was attributed to reduced energetic spending for filtration (Petersen et al., 1999), or muscle fatigue after excessive squirting contraction. On the other hand, the reduction in oxygen intake could also be the consequence of respiratory structures being clogged by sediment cover (Robbins, 1985; Thrush et al., 2004; Torre et al., 2012).

Both, increased muscle contraction and sediment coverage that blocks oxygen diffusion over the branchial sac may stimulate anaerobic metabolism which leads to the accumulation of (L)-lactate from anaerobic glycolysis (Kreutzer et al., 1989). Ascidians store glycogen in the pyloric glands, a system of tubules and ampullae in the intestinal wall (Ermak, 1977; Gaill, 1980). It can be expected that increased squirting activity would cause increased lactate levels in the muscular body wall and not so much in other tissues, which could cause acidification and “muscle fatigue”. To the contrary, under environmental hypoxia, when respiration was

impaired by sediments clogging of the branchial sac, we expect to see lactate accumulation in all tissues.

The aim of this study was to understand whether and to what extent, sedimentation induces up-regulation of anaerobic metabolism, either due to depriving the animals of oxygen (environmental hypoxia), or due to inducing enhanced squirting activity (functional hypoxia). In order to accomplish this goal, we analysed body wall and branchial sac lactate concentration and body wall and intestinal glycogen amounts in three Antarctic ascidians species exposed to high sediment concentrations ( $200 \text{ mgL}^{-1}$ ) during acute (1 day) and chronic (5 days) experimental conditions. The results are compared to experimental hypoxia (de-oxygenated water) and exercise treatments (induced muscular contraction), in order to discriminate the effect of sediment on each species and analyse the morpho-specific strategy (flat vs. erect forms) to deal with high inorganic sedimentation.

## 2. Materials and methods

### 2.1. Study area and sampling

This work was carried at Potter Cove, 25 de Mayo/King George Island, South Shetland Archipelago (S  $62^{\circ}14'$ , W  $58^{\circ}40'$ ) during January 2010 using the facilities of Dallmann laboratory in the Argentinean Carlini station (Fig 1).

Three species were chosen representing different ascidian morphotypes. *A. challengerii* was chosen as flat form, while *C. verrucosa* and *M. pedunculata* were representatives of erect morphotype. *A. challengerii* is characterised to have an oblong, elongate and depressed body. *M. pedunculata* presents ovoid or rectangular shape, longer than wide and it lives attached to the bottom with a stem stalk of variable length (between 10 and 20 cm). This species have a translucent tunic and a poorly developed muscular body wall. *C. verrucosa* is a cylindrical or “barrel-shaped” ascidian with a contractile tunic and a strong muscular body wall (Kott, 1969) (Fig. 2). Animals were collected by scuba diving and carefully cleaned from debris and attached organisms under running seawater. Then they were placed in a 140-L aquarium with aerated unfiltered seawater at  $0 \pm 1^{\circ}\text{C}$  for a recovery period (adjustment to the aquarium conditions) of 1 week prior experimentation. The animals were then placed into 1.5-L chambers, situated in 200-L aquarium tanks and connected to a recirculation system with aerated unfiltered sea water at stable temperature ( $0 \pm 1^{\circ}\text{C}$ ) and under dim light for 5 days to get use to experimental conditions. The water in the tanks was exchanged daily during recovery and experimentation periods.

### 2.2. Experimental design

All experiments and recovery process were performed on natural unfiltered sea-water at  $0 \pm 1^{\circ}\text{C}$ .

Each species was handled separately in the experiments. After one week of recovery in the aquarium system, specimens were randomly distributed into five groups of 8 animals each. Metabolic response to high sediment concentration were studied in two groups: Acute Sedimentation Exposure (ASE) and Chronic Sedimentation Exposure (CSE) to simulate the effects of a prolonged sediment exposure (e.g. during a prolonged storm). In the ASE group animals were exposed to  $200 \text{ mg L}^{-1}$  of suspended sediments for 1 day, whereas treatment time in the CSE experiment lasted 5 days. Sediment concentrations were chosen according to quantifications conducted in the natural environment and previous experiments (Pakhomov et al., 2003; Philipp et al., 2011; Torre et al., 2012). A water pump softly pumped water from the tanks into the chambers, maintaining water circulation into each animal chamber

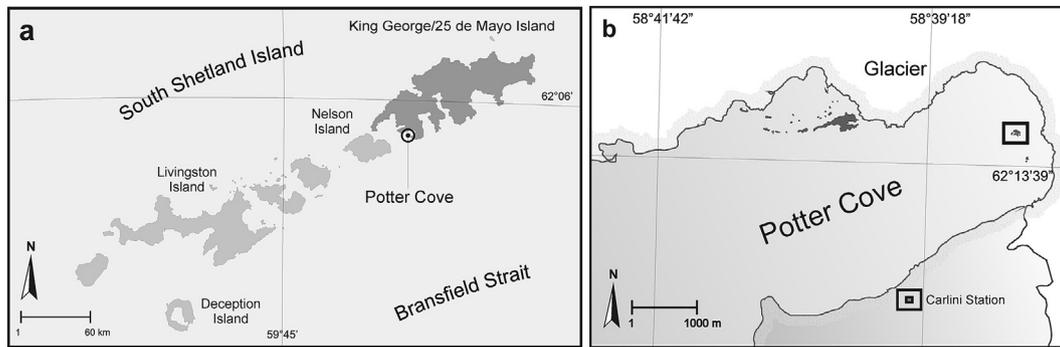


Fig. 1. a) The location of Potter Cove in the South Shetland Islands, Antarctic Peninsula. b) Map of Potter Cove, squares show positions of Carlini Station and sampling site.

while keeping the lowest possible disturbance. The animals were kept in this condition for several days before the experiments started, allowing them to get adjusted to this condition. Aerator and stronger water pumps kept particles suspended through the exposure in the tank supplying the chambers then assuring sediment exposure and without strong jet disturbances.

Additionally, exercise and hypoxic treatments were conducted. In the “hypoxic experimental group” a state severe under-oxygenation (hypoxia) was simulated, whereas the forced activity treatment (exercise) mimicked a situation in which the animals try to get rid from the sediments by squirting contractions. Hypoxia treatment was performed following [Kreutzer et al. \(1989\)](#) and consisted in 15 h exposure to approximately 10%  $pO_2$  by bubbling the chambers with nitrogen until the desired  $pO_2$  was reached (approx. 15 min) and chambers were hermetically closed per 15 h. Oxygen concentrations were determined using the spectrophotometric Winkler method following [Labasque et al. \(2004\)](#). For the exercise treatment, individual animals were stimulated to perform body contractions by touching the body wall repeatedly with a glass stick until exhaustion, (when animals were no longer able to contract their body or siphons). Exhaustion was in general reached in less than an hour. The control group was kept for 5 days in the

experimental chamber without addition of sediment or other disturbance. All treatments were run in parallel per species.

Summarizing, three tanks of 200 L to supply forty 1.5 L chambers were used in the set-up. One tank with sediment for CSE group ( $n = 8$ ) and the two other tanks with 16 chambers each one, where control, hypoxia, exercise and ASE groups were randomly distributed, until the last day when ASE group was transferred to the sediment tank and the rest of the treatments were performed as explained above. At the end of the experiment (day 5), animals were rapidly dissected and tissue samples (branchial sac, body wall and intestine without stomach) were snap frozen in liquid nitrogen. Samples were stored at  $-80^\circ\text{C}$  for later measurement of lactate and glycogen content (explained in details in section 2.4).

### 2.3. Behavioural observations of squirting frequency in *C. verrucosa*

As *C. verrucosa* is the only one of the studied species capable of noticeable squirting behaviour ([Torre et al., 2012](#)), its contraction response to sedimentation was visually monitored in the CSE group ( $n = 8$ ) and compared to the behaviour of control group ( $n = 8$ ).

During squirting, *C. verrucosa* individuals closed their atrial siphon and contracted the body wall, ejecting the major part of the water from their branchial cavity through the oral siphon while deflating their bodies. Immediately after squirting, both siphons are closed for some minutes after which the oral siphon slowly opens and start to inflate the atrial cavity with water. Once the maximum volume is reached again, both siphons open and the filtration process continues (*L. Torre pers. observation*). Behavioural recordings were conducted during 8 h per day starting on day 0 (where all specimens were in control conditions) and ending after 5 days of sediment exposure (to a total of 6 days). As simultaneous recording of every specimen was not possible, one observation per hour per individual during 8 h per day was made. In the observations the state of the siphon opening was recorded using the following behavioural categories (1) “both siphons open”, (2) “one siphon closed”, (3) “both siphons closed” and (4) squirting contraction. Squirting behaviour was frequent, but rarely occurred exactly during recording moments. However, considering the whole process as described above, categories 2 and 3 can be considered as good indicator of the occurrence of squirting behaviour.

### 2.4. Metabolite content

(L)-Lactate was measured by enzymatic determination following [Kunst et al. \(1984\)](#). Body wall and branchial sac were analysed separately. Each tissue sample (200–400 mg) was ground in liquid nitrogen and weighed into 2 mL Eppendorf cups. Perchloric acid

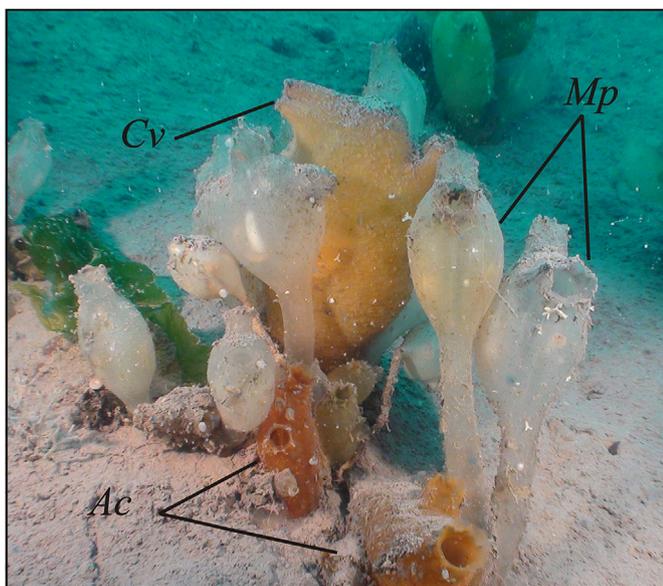


Fig. 2. The three Ascidians studied *Molgula pedunculata* (Mp), *Cnemidocarpa verrucosa* (Cv) and *Ascidia challengeri* (Ac). It is clear the upright disposition of *C. verrucosa* and *M. pedunculata* while *A. challengeri* is lying on the bottom.

(0.6 M) was added at a 1:1 (w/v) ratio to precipitate the proteins in the sample. The mixture was incubated for 10 min on ice and centrifuged for 15 min at 3000 g at 2 °C. The clear supernatant was transferred to 1.5 mL Eppendorf cups and 10 µL of methylene orange solution (0.05%, w/v) were added. Subsequently, 200 µL of the coloured supernatant were neutralized with 500 µL KOH (3 M) and after another 15 min of incubation on ice, the precipitated potassium perchlorate was removed by centrifugation. An aliquot of between 300 and 600 µL of the supernatant was used for the measurement and mixed with 250 µL glutamate buffer (glutamate 0.52 M; pH = 8.9), 30 µL NAD solution (NAD 24 mM), 20 µL L-alanine: 2-oxoglutarate aminotransferase (ALT) suspension water (80 kU L<sup>-1</sup>) and between 380 and 80 µL (depending on the sample volume used) of milli-Q water in order to obtain the final assay volume of 1000 µL. This mix was incubated in the cuvette for 10 min at 25 °C, and the first absorbance A<sub>1</sub> was read at 334 nm. Subsequently 20 µL of lactate dehydrogenase suspension (550 kU L<sup>-1</sup>) were added and thoroughly mixed using a plastic spatula. Absorbance was read again at 334 nm until constancy reached after approx. 30 min (A<sub>2</sub>). All samples were measured with a parallel blank, which consisted in the same mix of solutions, except that sample volume was replaced by milli-Q water, and was processed as the samples. The slope between A<sub>1</sub> and A<sub>2</sub> of each sample, minus its respectively blank, was used for the calculation of the concentration following Kunst et al. (1984). Lactate content is expressed as mg of lactate per g of fresh mass (mg gfm<sup>-1</sup>).

Glycogen concentration was determined after Kunst et al. (1984) and Keppler and Decker (1984). Body wall and intestine were analysed separately. Each tissue (100–200 mg) was grounded in liquid nitrogen and 0.5 mL of ice cold milli-Q water was added and the sample homogenized manually using a small glass homogenizer for 30 s on ice. The homogenate was heated to 95 °C for 10 min for protein denaturation. To hydrolyse glycogen to glucose, 250 µL of the homogenate were mixed with 500 µL acetate buffer (0.1 mol, pH 4.8) and 20 µL amyloglucosidase (Roche, Mannheim, Germany), and incubated for 2 h at 40 °C. The rest of the homogenate was kept on ice for later determination of the free glucose concentration. After incubation, both samples were centrifuged at 15,000 g for 10 min at 4 °C in a 5403 Eppendorf centrifuge (Germany). The supernatant was saved for glucose determination and measured using the glucose determination kit (D-glucose UV test, R-Biopharm, Darmstadt, Germany) following the manufacturer's instructions. The glycogen content was finally calculated as the difference between the hydrolysed and the non-hydrolysed subsamples. Glycogen content is expressed as µg of glycogen per g of fresh mass (µg gfm<sup>-1</sup>).

## 2.5. Data analyses

There were 8 behavioural observations per day per individual of *C. verrucosa*. To compare behaviour between control and CSE groups, the proportion of each behaviour was calculated its occurrence per day, assuming the total number of observations as 100%.

Because of the lack of homogeneity of variances, differences between groups in lactate and glycogen content were assessed by randomized analysis of variance (Randomized ANOVA) for each species. Tukey post-hoc test was applied to determine significant differences between groups at a significance level of at least  $p < 0.05$ . Because, as expected, greater amounts of lactate accumulated in the hypoxia treatment, this treatment was excluded from the analysis when necessary, and data reanalysed in order to find differences between the other treatments that were superposed by the effect of it. These statistical analyses were performed with R 2.12.2 (Gentleman and Ihaka, 2011). Outliers were identified

and excluded using the ESD method (GraphPad, 2012). All data are presented as mean values ± standard errors.

## 3. Results

### 3.1. Behavioural observations

During the experiments, *C. verrucosa* squirting behaviour was occasional and randomly observed with no clear internal squirting rhythm or response pattern becoming evident. Nevertheless, the behavioural analysis showed that closure of either one or both siphons occurred more frequently in sediment exposed animals than control ones until day 5 when, probably due to exhaustion, the ascidians are not anymore able to contract their body wall (Fig 3).

### 3.2. Metabolic parameters

Coincidentally with behavioural analyses, *C. verrucosa* was the species that behaved more responsive during the exercise

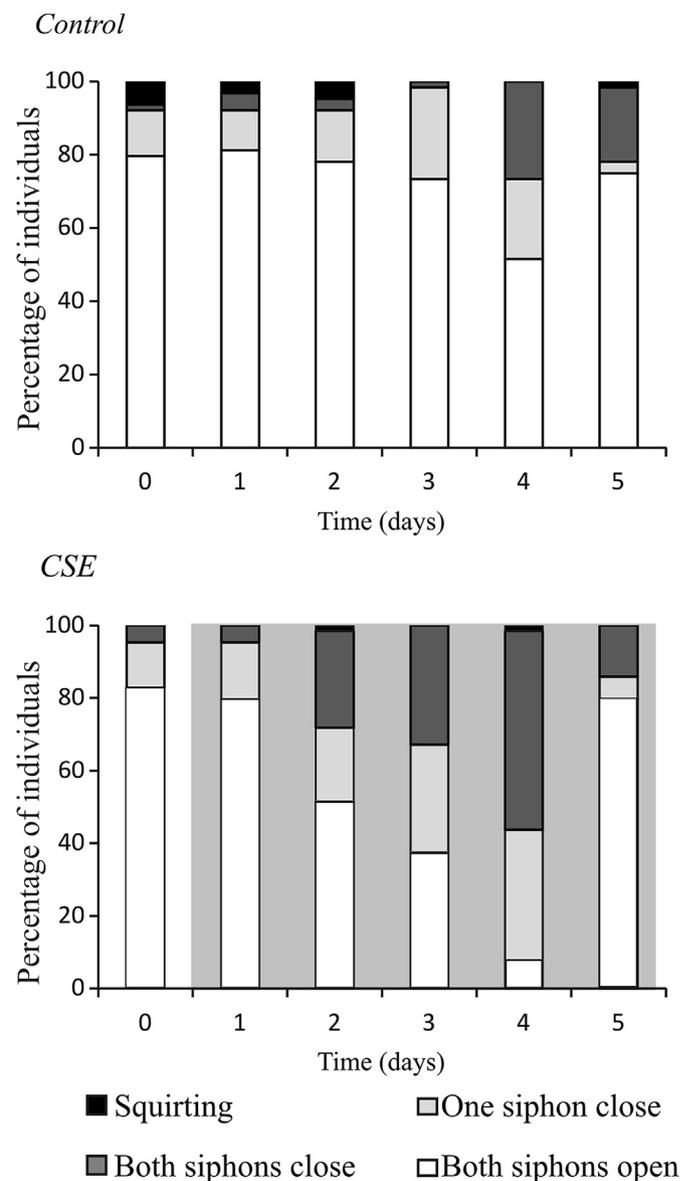
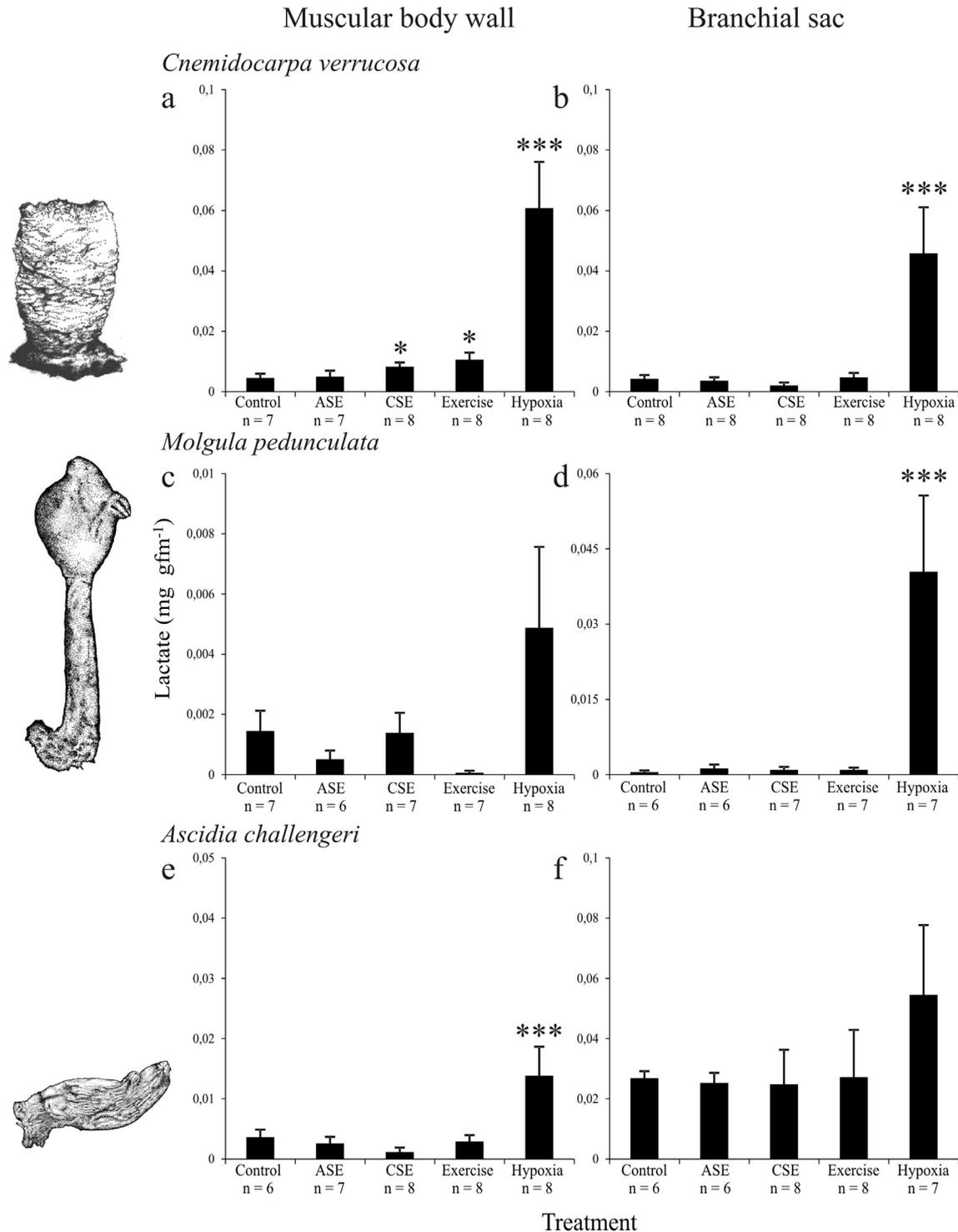


Fig. 3. Siphon closure and squirting frequency in *C. verrucosa* over time under sediment exposure conditions compared with control group. Results are expressed in % of individuals. Grey background in CSE graph indicates sediment exposure period.

treatment, contracting the whole body together with the siphons. As the time of perturbations advanced, the contractions became weaker and shorter and, after an hour of induced activity, the muscle fibres were exhausted and unable to respond at all. Contrary, the contractions of *M. pedunculata* and *A. challengeri* only involved their siphons and were never as strong and fast as the response of *C. verrucosa*. After 30 min of triggered activity, no

individual was capable of siphon closing. No particular behaviour like contraction or siphons closing was observed during hypoxia treatment in any of the species.

The analysis of lactate levels between treatments in all three species demonstrated the highest levels of the anaerobic metabolite build up under hypoxia treatment compared to the rest of the treatments (Fig. 4). The effect was statistically significant in



**Fig. 4.** Lactate content of body wall and branchial tissue of *C. verrucosa* (a–b), *M. pedunculata* (c–d) and *A. challengeri* (e–f) exposed to ASE (acute sediment exposure), CSE (chronic sediment exposure), exercise and hypoxia treatment and control conditions. Bars indicate standard error (SEM). Stars indicate significant difference to control value with \*:  $p < 0.05$  and \*\*\*:  $p < 0.001$  in the Tukey HSD.

*C. verrucosa* and *M. pedunculata* branchial sac ( $F = 6.71$ ,  $p < 0.001$  and  $F = 5.46$ ,  $p = 0.002$  respectively) (Fig. 4b, d) and also in *A. challengeri* and *C. verrucosa* body wall tissue ( $F = 4.43$ ,  $p < 0.001$  and  $F = 18.97$ ,  $p < 0.001$ , Fig. 4a, e). When hypoxia treatment groups were excluded from the analysis, only the *C. verrucosa* CSE and exercise treated animals had significantly elevated lactate content compared with control and ASE levels (Fig. 4a) (ANOVA  $F = 7.30$ ,  $p = 0.002$ ). Although no statistical difference between treatments was found for respiratory tissue, lactate content in *A. challengeri* branchial sac appeared astonishingly high even in the control group. This may indicate some extent of constitutive anaerobic energy production in the tissue, also known from bivalves and other hypoxia tolerant species.

There were no statistical differences in body wall glycogen levels between treatments for any of the analysed species. Nevertheless, the amount of glycogen was smaller in all treatments compared with the control group, particularly in CSE, exercise and hypoxia. This tendency was more evident in *C. verrucosa* and *M. pedunculata* (Fig. 5 a, c).

Only in the hypoxia treatment of *M. pedunculata* glycogen was almost depleted in the intestinal tissue, (ANOVA  $F = 3.64$ ,  $p = 0.016$ ). As the sediment used in the experiment was almost completely inorganic, a diminishment of intestinal glycogen content was also expected for the chronic sediment treatment. However chronic exposure to sedimentation did not affect tissue glycogen levels in any of the species.

## 4. Discussion

### 4.1. Ascidian physiological responses

The three species exhibited different strategies to cope with sedimentation, with *C. verrucosa* responding more actively compared with the other two species. *C. verrucosa* was also the only species that evidently performed squirting, with increasing incidence of this behaviour when exposed to inorganic suspended material. Although an increase in spontaneous squirting in ascidians has been previously described under low oxygen concentrations (Evans and Huntington, 1992), our results showed that the response to the experimental sedimentation exposure was clearly different to the hypoxic treatment response. Under sediment exposure squirting supports ejection of inorganic material, and indeed analysis of *C. verrucosa* stomach contents showed almost no inorganic material even during summer periods when inorganic particle load in the water column is higher in Potter Cove (Schloss et al., 2012; Tatián et al., 2002). Clearly the extra energetic cost under sediment exposure described for this species (Torre et al., 2012) is related to the enhanced muscular activity in the presence of high amounts of suspended particles. The later decline in oxygen consumption during chronic stress exposure is probably related to a cessation of muscle contractions as a consequence of the high accumulation of lactate in the body wall, which was nearly as high as the lactate concentration in the body wall of animals from the exhaustive exercise treatment.

In a previous study, *M. pedunculata* responded to sedimentation with increased oxygen consumption indicating enhanced metabolic turnover (Torre et al., 2012). Unable to distinguish the quality of the suspended material and also unable to perform squirting, this species may keep-up filtration, digestion, and excretion rates as suspended particulate matter rises or even increases the activities in order to enhance ventilation. In fact, increased activity together with the low energetic value of the ingested particles could be the cause of glycogen mobilization under prolonged exposure to sediment (CSE), exercise and hypoxia that in the long term could be affecting species fitness. Inter-individual glycogen concentrations

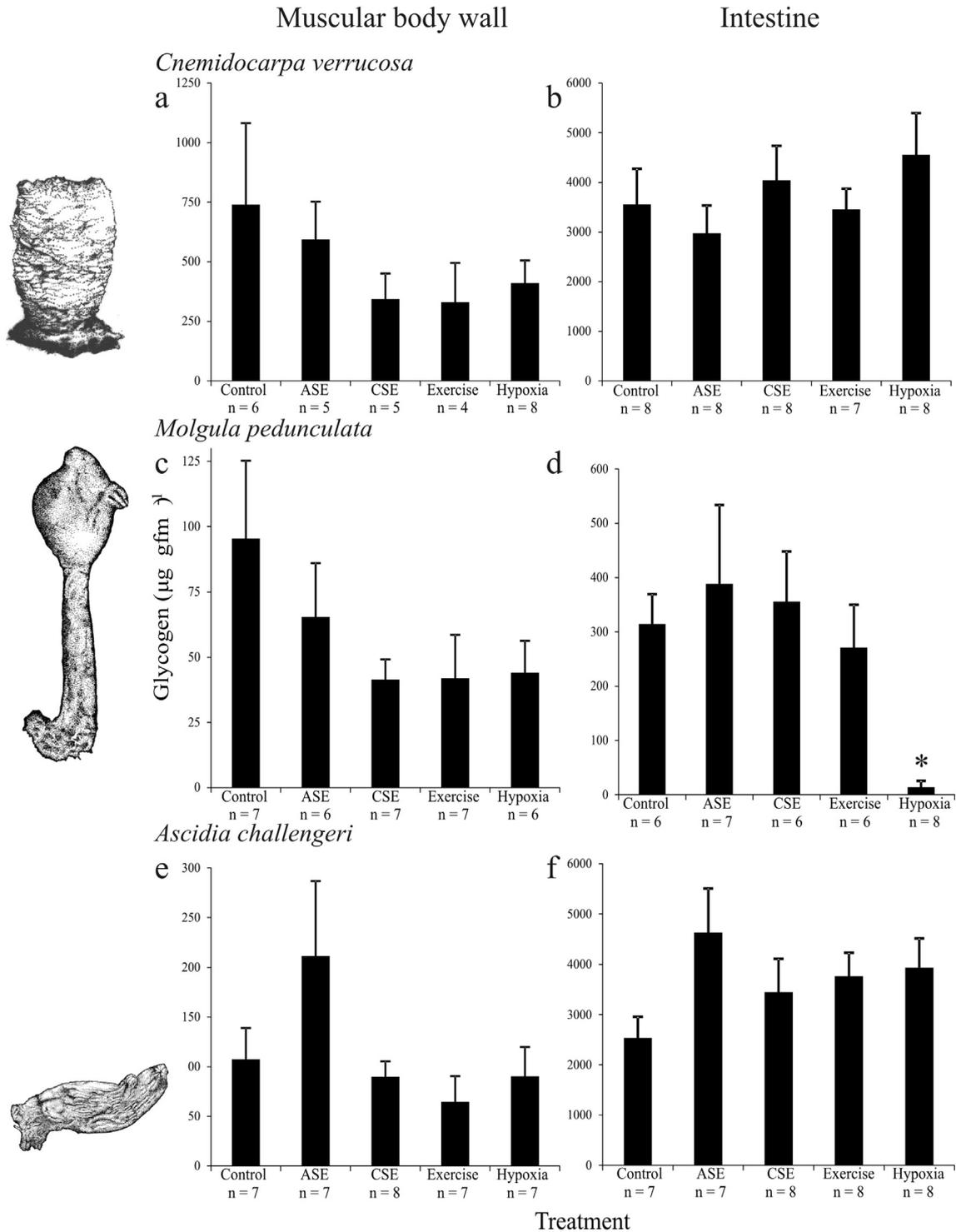
in this species are highly variable (see large SEM in control group), and an experimental period of 5 days may simply not have been long enough to obtain significant results. Gaill (1980) recorded significantly diminished glycogen stores after 15 days of starvation in the temperate species *Dendrodoa grossularia*. Additionally, this is the only species that showed a significant decrease of its small intestinal glycogen store under hypoxia suggesting that *M. pedunculata* is much more susceptible than the other ascidians to environmental stressor as hypoxia.

When facing intense sedimentation, *A. challengeri* showed a slight increase in respiration as *M. pedunculata* but, instead of keeping respiration up like *M. pedunculata* does, it decrease the oxygen uptake above a certain sediment concentration ( $200 \text{ mgL}^{-1}$ ), like *C. verrucosa* (Torre et al., 2012). Nevertheless, in the case of *A. challengeri* no lactate accumulates in the body wall indicating absence of exhaustive muscle contraction and squirting. Instead, diminished respiration indicates down-regulation of aerobic metabolism to wait for better environmental conditions. It has been observed that ascidians filtration rates depend mostly of branchial sac area (Petersen and Svane, 2002), so species with unfolded branchial sacs (Phlebobranchiata), and therefore a lower branchial surface area, are assumed to invest less energy into filtration than species with a folded branchial sac (Stolidobranchiata). Therefore flat-form Antarctic ascidians, like *A. challengeri* and *C. antarctica*, both belonging to the order Phlebobranchiata have the lowest respiration and filtration rates (Kowalke, 1999), and seem to be better adapted to deal with low oxygen availability. *A. challengeri* even appears to have constitutive anaerobic activity according to the high basal lactate levels in the branchial sac under control conditions. This metabolic strategy, which aligns with the low metabolic rate and scope for activity in this species (Torre et al., 2012), may be an adaptation to the special niche in the sediment water interface, to avoid competition with the erect-forms for food, space and oxygen higher up in the water column. Its low energy demand also explains the high tolerance of sedimentation in this species.

In accordance with Ermak (1977) and Gaill (1980), our results confirm that intestinal glycogen can be an important energy reserve for ascidians and is rapidly depleted as observed in *M. pedunculata* under hypoxia exposure. In this context it is important to note the importance of studying energy allocation separately in each ascidian tissue, as tissue specific resource utilization or reallocations can be overlooked or underestimated when only whole body content is considered (i.e. Kreutzer et al., 1989).

### 4.2. Ecological implications

From an ecological point of view, the physiological analysis related to different morphotypes helps to understand some of the changes recorded in the benthic community of Potter Cove during the last 15 years (Sahade et al., 2008; Sahade et al., unpublished results). In benthic systems, space has been considered as one of the most important resources species have to compete for (Paine, 1984). In this context, the erect solitary organisms have an advantage as they can develop high biomasses with minimal space utilization (Barnes, 1995; Bell and Barnes, 2000b; Hart and Marshall, 2012). Nevertheless coexistence of different growth forms within habitats indicates that other environmental factor also favour the flat forms (Gutt, 2006; Jackson, 1977). In Potter Cove the erect forms, in particular *M. pedunculata*, were dominant below 25 m depth (Sahade et al., 1998). Presumably because of their higher filtration and growth rates (Kowalke et al., 2001) and the presence of the stalk which allows them to reach higher quality food (higher organic fraction) in 25 cm above the sediment–water interface (Schloss et al., 1999). Moreover, it has also been described



**Fig. 5.** Glycogen content of body wall and intestinal tissue of *C. verrucosa* (a–b), *M. pedunculata* (c–d) and *A. challengeri* (e–f) exposed under ASE (acute sediment exposure), CSE (chronic sediment exposure), exercise and hypoxia treatment and control conditions. Bars indicate standard error (SEM). Stars indicate significant difference to control value with  $p < 0.05$  in the Tukey HSD.

that the length of the stalk varies, and that stalks are growing higher in response to competitive pressure (Monniot et al., 2011).

Contrary *C. verrucosa* also has an erect body shape but has been less successful in colonizing these particular soft bottoms (Sahade et al., 1998). This may be linked to the slower growth of the more muscular and compact body mass of *M. pedunculata* (Kott, 1969; Kowalke et al., 2001). Nevertheless under the current

environmental conditions the ability to perform squirting and the fact that this species reproduces during winter (Sahade et al., 2004) may favour *C. verrucosa*. Indeed, its population density in the cove showed just slight changes in the last 15 years (from 1.84 to 1.63 ind  $\text{m}^{-2}$ ) compared to the practical disappearance of *M. pedunculata* (from 16.32 to 2.5 ind  $\text{m}^{-2}$ ) (Sahade et al., 2008). Finally, flat forms like *A. challengeri*, which are limited by space and

food availability, may be favoured under high sedimentation scenarios. This might explain the relative success of flat forms like *C. antarctica* and *A. challengerii* under a marked increment of sedimentation in Potter Cove (from 8.64 ind m<sup>-2</sup> to 13.12 ind m<sup>-2</sup>), while erect ascidians were drastically reduced (*M. pedunculata*) or showed no variations at all (*C. verrucosa*) (Sahade et al., 2008).

Several studies have already showed adaptations of “body-plan” or specific morphological structures that support adaptations to specific niches or microhabitats and explain distribution patterns of species with respect to environmental conditions (Barnes, 1995; Bell and Barnes, 2000a; Teixidó, 2003; Weihe and Abele, 2008). The approach in the present paper was to relate the response of different ascidian morphotypes to sedimentation and their specific physiological capacities to counteract or endure the stress. We found that flat form ascidians, assumed to be inferior in competition for colonization space, growth rate and food intake, will be better in dealing with increasing sediment loads and oxygen deficit.

## 5. Conclusions

Each ascidian morphotype has its own strategy to deal with sedimentation, which explains the differential sensitivity to this type of stress and the observed differences in population density change Potter Cove. Our results indicate that erect-forms up-regulate metabolism: *C. verrucosa* increasing squirting in order to get rid of excessive inorganic material until exhaustion and *M. pedunculata* increasing filtration and ingestion activities. On the contrary, the flat-form ascidian *A. challengerii* reduces its aerobic metabolism under stressful condition. Whether this relationship between morphology and function can be extended to other ascidian species or is just a feature of the examined species is still an open question that deserves future research.

This kind of comparative studies helps to elucidate the discrepancies summarized by Petersen (2007) about ascidian responses to inorganic matter exposure, which do not only relate to experimental setups. These findings may relate to the variability with respect to behaviour physiology and morphology across ascidian species. This variability may explain the coexistence and also the recent community changes based not only on the competition for food and space, but also based on their particular adaptation to stressful conditions produced by glacier melting.

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