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Baseline

Oxidative stress, metabolic activity and mercury concentrations in Antarctic krill *Euphausia superba* and myctophid fish of the Southern Ocean

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ABSTRACT

Indicators of oxidative stress and metabolic capacity are key factors in understanding the fitness of wild populations. In the present study, these factors were evaluated in the pelagic Southern Ocean taxa Antarctic krill (*Euphausia superba*) and myctophid fish (*Electrona antarctica*, *Gymnoscopelus braueri* and *G. nicholsi*) to establish a baseline record for future studies. Mercury (Hg) concentrations were also analysed to evaluate its potential impacts on species biochemical performance. *E. superba* had higher metabolic activity than most of the myctophid species, which may explain the comparatively lower energy reserves found in the former. The activity of antioxidant enzymes showed, generally, a lower level in *E. superba* than in the myctophid species. The lack of any relationship between Hg concentrations and organisms' antioxidant and biotransformation defence mechanisms indicate that levels of Hg accumulated in the studied species were not high enough to affect their biochemical processes adversely.

The Southern Ocean ecosystem is characterised by its low temperatures, large levels of seasonal sea ice (Alberello et al., 2018), high nutrients concentrations (Brierley and Thomas, 2002) and the productive upwelling regions (Morrison et al., 2015). This region is also experiencing some of the highest levels of warming and ocean acidification globally (Freer et al., 2017; IPCC, 2019; Rintoul et al., 2018; Turner et al., 2013), and these changes may affect individuals at a subcellular level and, in turn, alter patterns of distribution and food webs' structure (Atkinson et al., 2004; Xavier and Peck, 2015). Warming will increase the release of freshwater into the Southern Ocean, particularly through accelerating the flow of glaciers, which liberates contaminants (such as mercury) that they store. These contaminants may have the potential to cause stress to Southern Ocean fauna, and it is important to describe and understand the scale of potential impacts, particularly to key biomass-dominant taxa.

Antarctic krill *Euphausia superba* is a key species in the Southern Ocean trophic web, being a major link between primary production and vertebrate predators (Everson, 2000). *E. superba* is also a very important commercial species, with 312 million tonnes harvested in 2018 (Nicol et al., 2000; Tou et al., 2007; CCAMLR, 2020). This species is predominantly herbivorous, feeding on phytoplankton and on some copepod crustaceans (Everson, 2000; Schmidt and Atkinson, 2016). Whales, seals, penguins and flying seabirds are among those species that consume high quantities of *E. superba* (Armstrong and Siegfried, 1991;

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Croxall et al., 1999; Xavier et al., 2003). However, given evidence of a 50–80% decline in *E. superba* over the last century (Atkinson et al., 2004), predators may have to switch to alternative prey groups. Myctophid fish, the most abundant group in the mesopelagic fish community worldwide (Gjøsaeter and Kawaguchi, 1980), can serve as a major alternative energy source to some predators in situations of low *E. superba* abundance (Murphy et al., 2007; Saunders et al., 2018). In the Southern Ocean, this group has an estimated biomass ranging between 70 and 200 million tonnes (Collins et al., 2008; Suzuki et al., 2005). Myctophid fish are therefore an important independent trophic link between primary consumers and a wide range of higher predators, including king penguins (Olsson and North, 1997), albatrosses (Xavier et al., 2003), Antarctic fur seals (Davis et al., 2006), squids (Kear, 1992) and Patagonian toothfish (Collins et al., 2007). Myctophid fish prey mainly on zooplankton (Saunders et al., 2019) and undertake diurnal vertical migration to feed and to avoid predators in surface waters during the day. Prey-selection differs between myctophid species: *Electrona antarctica* consumes the amphipod *Themisto gaudichaudii* during summer time; *Gymnoscopelus braueri* prey on different species, including *Themisto gaudichaudii*, *Metridia* spp., *E. superba*, *Pleuromamma robusta* and ostracods; and the diet of *G. nicholsi* is dominated by *Metridia* spp. and *E. superba* during the summer (Lourenço et al., 2017; Saunders et al., 2019, 2018). The vertical distribution of these myctophid species varies from 0 to 700 m in depth, with *E. antarctica* being the species with the widest spread of depths through the water column (Collins et al., 2008; Saunders et al., 2019, 2014). Because of their major trophic roles, it is important to understand stresses to, and responses by, myctophids and krill.

When exposed to stressful conditions, including the presence of pollutants, organisms may resort to an overproduction of reactive oxygen species (ROS) in their cells, leading to a state known as oxidative stress (Regoli and Giuliani, 2014). To prevent the establishment of oxidative stress, cells possess an extensive antioxidant system, that includes enzymatic (such as the enzymes superoxide dismutase, catalase, glutathione peroxidase), and non-enzymatic (e.g. reduced glutathione) forms (Regoli et al., 2011). Depending on the stress level and organism's antioxidant capacity, cellular damage (namely through lipid peroxidation) and loss of redox balance (with increasing oxidation of reduced glutathione) may occur. To cope with oxidative stress, organisms may need to increase their metabolic capacity to ramp up their defence mechanisms, leading to increased electron transport system (ETS) activity and expenditure of energy reserves (e.g. glycogen) (Cruz et al., 2016; Freitas et al., 2020). Several pollutants, including mercury (Hg), have already been shown to cause oxidative stress, as well as alterations to metabolic capacity, in marine organisms (Coppola et al., 2018; Monteiro et al., 2019).

Despite the ecological importance of krill and myctophid fish in the pelagic realm, there is still a crucial knowledge gap regarding their ecophysiology (Atkinson et al., 2002; Meyer, 2012; Quetin and Ross, 1991), with only a few studies considering ecophysiology in *E. superba*, and virtually none in Southern Ocean myctophids, the exception being two studies looking into ETS as a proxy for respiration rates (Belcher et al., 2020, 2019). The main goal of the present study was to describe the general oxidative stress and metabolic status of *E. superba* and three species of Antarctic myctophid fish (*E. antarctica*, *G. braueri* and *G. nicholsi*), to evaluate Hg concentration in these species and possible impacts on their biochemical performance, and to establish a base record for future studies. For this, antioxidant capacity, cellular damage, redox balance, metabolic capacity and energy-reserve content were evaluated in the mentioned species.

Samples were collected on board of the British research vessel *RRS James Clark Ross* during the austral summers of 2015/2016 (December 2015 and January 2016) in the Scotia Sea (cruise JR15004). *Euphausia superba* specimens were collected from the water column using an 8 m^2 mouth-opening Rectangular Midwater Trawl (RMT8; mesh size reducing from 4.5 mm to 2.5 mm in the cod end) (Roe and Shale, 1979). The net was rigged with two nets that could be remotely opened and closed at different depths. Myctophid samples (*Electrona antarctica*, *Gymnoscopelus braueri* and *G. nicholsi*) were collected using a similar net design, with 25 m^2 mouth-opening (RMT25; mesh size reducing from 8 mm to 4.5 mm in the cod end) (Roe and Shale, 1979). After sampling, specimens were preserved in individual sample bags at −80 °C.

Euphausia superba in the catches were identified and total length (TL) of each individual was measured, from the anterior edge of the eye to the tip of the telson and rounded down (Morris et al., 1992). Sex and maturity stage were determined with reference to the presence of a petasma (males), thelycum (females) or absent (juveniles; individuals without visible external sexual characteristics; Ross and Quetin, 2000). Myctophids were identified using published guides (Gon and Heemstra, 1990; Hulley, 1990) and measured for the nearest mm using standard length (SL). Sex and maturity was determined whenever possible: in some myctophid species (e.g. *E. antarctica*) there is sexual dimorphism associated with the location of photophores, but when this was not possible, the gonads were examined following dissection (Yamamoto, 1969).

From each species (*E. superba* ($n = 20$); *E. antarctica* ($n = 5$); *G. braueri* (n = 5); *G. nicholsi* ($n = 3$)), samples were homogenized and used for biochemical markers measurements and for total Hg determination. For *E. superba*, whole individuals were used for biochemical and Hg analysis, while for the myctophids species only muscle was analysed to avoid the inclusion of any bone.

Biochemical parameters were analysed in each species. For this, the whole tissue of 20 individuals of *E. superba* (10 females, 10 males), and muscle tissue from 5 *G. braueri* (2 males, 3 unknow), 3 *G. nicholsi* (2 females, 1 male) and 5 *E. antarctica* (3 females, 2 males) (Table 1) were homogenized using a mortar and pestle with liquid nitrogen and sonicated for 15 s at 4 °C, after buffer addition (1:2) (Carregosa et al., 2014). Specimens were homogenized individually and divided into different aliquots (for different buffers use). To determine the activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferases (GSTs) enzymes and the content of glycogen (GLY) and protein (PROT), supernatants were extracted with a potassium phosphate buffer (50 mmol/L KH_2PO_4, 1 mmol/L ethylenediamine tetraacetic acid disodium salt hydrate (EDTA), 1% (*v*/v) Triton X-100, 1 mmol/L dithiothreitol (DTT), pH 7.0). For lipid peroxidation (LPO) assessment, supernatants were obtained using 20% (*w/v*) trichloroacetic (TCA). For electron transport system (ETS) activity evaluation the homogenizing buffer (0.1 mol/L Tris-HCl, 15% (w/v)

Table 1
Total number (N), mean length and weight values for each collected species and location (latitude and longitude) of sampling areas on the Scotia sea, surveyed during the austral summer of 2015/2016. Values represent mean ± standard deviation.

Species	N	Total length (mm)	Weight (g)	Latitude	Longitude
Euphausia superba	20	5.1 (± 0.3)	1.2 (±0.3)	−60.3131	−46.8488
Electrona antarctica	5	82 (± 14)	6.3 (±5.4)	−59.9861	−47.22192
Gymnoscopelus braueri	5	104 (± 11)	9.1 (±2.9)	−59.9861	−47.22192
Gymnoscopelus nicholsi	3	148 (± 8)	14.7 (±5.9)	−60.33097	−46.67431

polyvinylpyrrolidone (PVP), 153 μmol/L $MgSO_4$, and 0.2% (*v*/v) Triton-X 100, pH 8.5) was used. Samples were centrifuged for 20 min at 10,000 *g* (3,000 *g* for ETS) and 4 °C (Carregosa et al., 2014), and supernatants were preserved at −80 °C or analysed immediately.

The GLY content was determined according to the sulfuric acid method (DuBois et al., 1956), using glucose standards (between 0 and 10 mg/mL) to obtain a calibration curve. Absorbance was measured at 492 nm after 30 min incubation at room temperature and results were expressed in mg per g of fresh weight (FW). The PROT content was determined according to Robinson and Hogden (1940), following the Biuret method that uses bovine serum albumin (BSA) as standard (0 to 40 mg/mL) to obtain a calibration curve. After 10 min incubation at 30 °C, the absorbance was read at 540 nm. The results were expressed in mg per g of FW.

Metabolic capacity was assessed by measuring the ETS activity, following the method of King and Packard (1975) and modifications by De Coen and Janssen (1997). Absorbance was measured during 10 min at 490 nm in 25 s intervals and the extinction coefficient (ε) 15,900/(mol/L)/cm was used to calculate the amount of formazan formed per unit time. Results were expressed in nmol min per g of FW.

Cellular damage was measured by the quantification of LPO levels following the method described in Ohkawa et al. (1979) with modifications referred by Carregosa et al. (2014). Absorbance was measured at 535 nm and LPO was determined using the extinction coefficient (ε) 156/(mmol/L)/cm and results expressed in nmol of MDA equivalents formed per g of FW.

The activity of SOD was quantified based on the method of Beaucham and Fridovic (1971). SOD standards (0.25–60 U/mL) were used to generate a calibration curve. After 20 min incubation at room temperature, absorbance was measured at 560 nm. Results were expressed in U per g FW and by U per mg of PROT. The activity of CAT was quantified following Johansson and Borg (1988). Formaldehyde standards (0–150 μmol/L) were used to produce a calibration curve. Absorbance was measured at 540 nm and results expressed in U per g FW and per mg of PROT. The activity of GPx was determined following the method of Paglia and Valentine (1967). Absorbance was measured at 340 nm during 5 min in 10 s intervals. Enzyme activity was calculated using the extinction coefficient (ε) 6.22/(mmol/L)/cm and the results were expressed in U per g FW and per mg of PROT. The activity of GSTs was determined at room temperature using 1- chloro-2,4-dinitrobenzene (CDNB) as substrate according to the method described by Habig et al. (1974) with modifications described in Carregosa et al. (2014). The activity was determined spectrophotometrically at 340 nm using the extinction coefficient (ε) 9.6/(mmol/L)/cm and absorbance was measured in intervals of 10 s during 5 min. The GSTs activity expressed in U per g FW and by U per mg of PROT.

Mercury concentrations were determined by thermal decomposition atomic absorption spectrometry with gold amalgamation, using a LECO AMA-254 (Advanced mercury analyser) following Coelho et al. (2008) methodology. Analytical quality control was performed using certiffed reference material (CRM; in this case TORT-2 and TORT-3 [lobster hepatopancreas, National Research Council, Canada] for *E. superba*; DORM-4 [Fish protein, National Research Council, Canada] for myctophids). The obtained values (mean ± standard deviation) for the whole of the CRM analyses ($n = 13$) provided recoveries ranging from 82 to 96% (TORT-2: 88 ± 3%; TORT-3: 89 ± 7%; DORM-4: 91 ± 12%). The mass of CRM used for quality control analyses was adjusted to be within the range of total Hg present in the samples. Blanks were analysed at the beginning of each set of samples and the analyses were always performed at least in duplicate or until coefficient of variation were below 10%.

Data obtained from biochemical analyses and biological factors (size, weight, sex and species) were submitted to permutational multivariate analysis of variance with the PERMANOVA+add-on in PRIMER v6 (Anderson et al., 2009). The pseudo-F values in the PERMANOVA main tests were evaluated in terms of significance. When the main test revealed statistical significant differences ($p < 0.05$), pairwise comparisons were performed. The null hypothesis tested was: for each parameter, no significant differences existed among species.

Biochemical responses, size, weight and Hg concentrations for the different species were used to calculate the Euclidean distance similarity matrix. This matrix was simplified through the calculation of the distance within the centroid matrix based on species, which was then submitted to ordination analysis, performed by Principal Coordinates (PCO). Pearson correlation vectors ($r > 0.85$) of physiological and biochemical descriptors and Hg concentrations were provided as supplementary variables being superimposed on the top of the PCO graph.

For all the studied species, both sexes were initially analysed separately and since no significant differences were observed in terms of biochemical performance the total number of organisms per species was pooled.

In terms of physiological characteristics (size and weight), biochemical performance (metabolism and oxidative stress related biomarkers) and Hg concentration in all the analysed organisms, the PCO analysis clearly highlights differences between species, with PCO1 explaining 64.8% of the total variation and distinguishing between *E. superba* (KRI) on the negative side from the myctophids species in the positive side. PCO2 explains 26.0% of the total variation, separating *E. antarctica* (ELN) and *G. braueri* (GYB) in the negative side from *G. nicholsi* (GYN) and *E. superba* (KRI) in the positive side (Fig. 1).

In terms of Hg levels, most probably due to lower trophic level and lifespan, *E. superba* had significantly lower Hg concentrations than all the myctophid fish species analysed (Table 2), with Hg concentrations in *E. superba* within the range of values observed in previous studies with the same species (0.008 to 0.077 μg/g dry weight, DW, Seco et al., 2019). The results obtained also showed that Hg concentrations did not significantly differ among myctophid species. To our knowledge, there is only two previous studies that reports Hg concentration in Southern Ocean myctophids, with reported concentrations within the same range as the ones obtained in the current study (Cipro et al., 2018; Seco et al., 2020).

In terms of energy reserves, individuals of *E. superba* had a relatively low PROT content (Table 2) when compared with other crustaceans (*Aristeus antennatus*, *Parapenaeus longirostris* and *Nephrops norvegicus*)

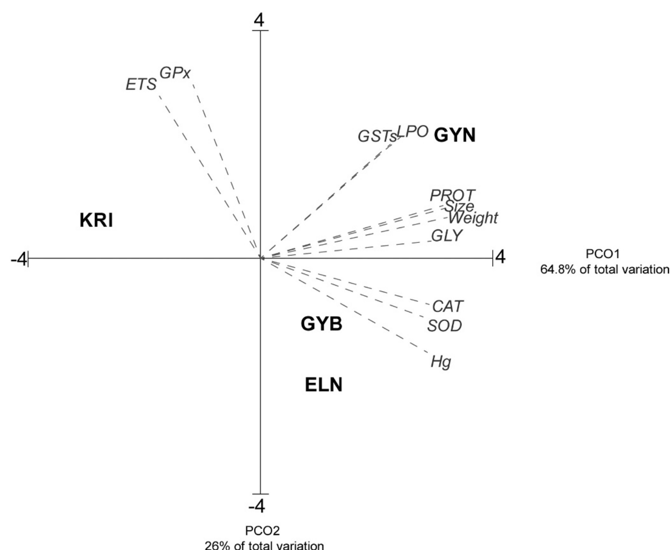

Fig. 1. Ordination diagram based on biomarkers, physiological characteristic (size and weight) and mercury (Hg) concentration. ELN - *Electrona Antarctica*, GYB - *Gymnoscopelus braueri*; GYN - *Gymnoscopelus nicholsi*; KRI - *Euphausia superba*; PROT - protein content; LPO - lipid peroxidation levels; ETS - electron transport system activity; GLY - glycogen content; GPx - glutathione peroxidase; GSTs - glutathione S-transferases activity; SOD - superoxide dismutase activity; CAT - catalase activity.

Table 2
Mercury (Hg) concentration (dry weight, DW) and biomarkers measured in each species. Biomarkers: GLY: glycogen content; PROT: protein content; ETS: electron transport system activity; LPO: lipid peroxidation levels; SOD: superoxide dismutase activity; CAT: catalase activity; GPx: glutathione peroxidase activity; GSTs: glutathione S-transferases activity. Units are presented per g of fresh weight (FW) and per mg of protein (PROT) for comparison with results from the literature. Values represent mean ± standard deviation. For each parameter, the highest value is highlighted in bold while the lowest value is underlined.

Species	Hg	GLY	PROT	ETS	LPO		SOD		CAT		GPx		GSTs	
	μg/g DW	mg/g FW	mg/g FW	nmol/min/g FW	nmol MDA/g FW	nmol MDA/mg PROT	U/g FW	U/mg PROT	U/g FW	U/mg PROT	U/g FW	U/mg PROT	U/g FW	U/mg PROT
Crustaceans														
Euphausia superba	0.04 (± 0.01)[a]	18.7 (±3.3)[a]	41(±3)[a]	34 (±8)[a]	25 (±4)[a]	0.82 (±0.21)	1.9 (±0.9)[a]	0.02 (±0.005)	10.9 (±3.8) [a]	0.13 (±0.05)	**5.2 (±3.2)[a]**	0.25 (±0.16)	0.05 (±0.02)[a]	0.78 (±0.57)
Myctophids														
Electrona antarctica	**0.22 (± 0.08)[b]**	21.2 (±4.5)[a,b]	65 (±9)[b]	18 (±6)[b]	36 (±8)[a, c]	0.28 (±0.11)	**4.2 (±0.9)[b]**	0.03 (±0.01)	17.5 (±6.8)[a, b]	0.13 (±0.05)	2.0 (±2.0)[a]	0.07 (±0.07)	0.05 (±0.01)[a]	1.2 (±0.2)
Gymnoscopelus braueri	0.17 (± 0.03)[b]	24.0 (±4.4)[a, b]	85 (±23)[b, c]	22 (±9)[a, b]	16 (± 9)[b]	0.26 (±0.05)	2.7 (±0.8)[a, b]	0.01 (±0.009)	**22.6 (±8.0)[b]**	0.13 (±0.02)	3.5 (±2.3)[a]	0.09 (±0.06)	0.04 (±0.02)[a]	0.77 (±0.53)
Gymnoscopelus nicholsi	0.17 (± 0.04)[b]	**25.5 (±1.4)[b]**	**118 (±38)[c]**	**42 (±11)[a,b]**	77 (±8)[c]	0.34 (±0.17)	3.7 (±0.8)[b]	0.01 (±0.005)	20.1 (±10.2)[a, b]	0.09 (±0.05)	5.1 (±1.9)[a]	0.11 (±0.04)	**0.13 (±0.04)[a]**	1.8 (±1.7)

but the content of GLY was higher than in other crustacean species (Rosa and Nunes, 2003). These findings can be related to the fact that the current study was performed during the austral summer, when Southern Ocean organisms tend to build up energy reserves for winter time. For the 3 myctophid species, significant differences of the quantity of PROT (*E. antarctica* $\leq$ *G. braueri* $\leq$ *G. nicholsi*) were observed between *E. antarctica* and *G. nicholsi*, which may be related to the difference in size between both species' individuals (*G. nicholsi* individuals were 2 times the length of *E. Antarctica* individuals; Table 1). This result is corroborated by the PCO analysis, where a close relationship between PROT content and size and weight were found (Fig. 1). No significant differences on the PROT content were observed between *G. braueri* and the other 2 myctophid species. In terms of GLY content, no significant differences were observed among the 3 myctophid species, while *E. superba* had lower GLY values than *G. nicholsi* but there were no significant differences between *E. superba* and the other two myctophid species. Lower PROT and GLY contents in *E. superba* compared to the three myctophid fish may indicate higher energetic requirements by the crustacean compared with the fish species. However, in the present study, *E. superba* was not the species with the highest metabolic activity and, therefore, lower GLY and PROT concentrations did not result from their expenditure in response to increased metabolism. In fact, in terms of metabolic capacity, no significant differences were observed between *E. superba* and both *Gymnoscopelus* species (Table 2). Also, the highest ETS activity observed in *G. nicholsi* was not accompanied by higher energy expenditure as this species presented the highest GLY and PROT contents. Low energy reserves content in *E. superba* can be related with lower production and/or accumulation capacity in the crustacean compared with the fish species, however there is still a lack of knowledge regarding the energy cycle and reverses in this species. We can also hypothesize that differences in the energy reserves between *E. superba* and myctophid fish may be related with the dietary differences between the groups as *E. superba* feeds on phytoplankton (Everson, 2000) whereas myctophid fish feed mainly in zooplankton (e.g.: *E. superba*, *Metridia* spp. and on *Rhincalanus gigas* (Saunders et al., 2018)).

As demonstrated previously, organisms tend to reduce their metabolism as a strategy to avoid accumulation of toxic substances, reducing for example their filtration rate and, consequently, ingestion of contaminants, as reported in estuarine bivalves (Almeida et al., 2014, 2015; Pinto et al., 2019). Nevertheless, in the present study, the lowest ETS activity (*E. antarctica*) was not associated with the lowest Hg concentrations and, in the same way, the highest ETS activity observed in *G. nicholsi* did not correspond to higher Hg concentrations. The ETS activity in myctophids showed to be positively correlated with size, as demonstrated in a previous study looking into ETS as a respiration rate, larger myctophids had higher ETS (Belcher et al., 2020). These preliminary data suggest that both groups of organisms may not decrease their metabolism as a strategy to avoid accumulation of pollutants or, most probably, Hg concentration in the environment was not the factor that conditioned species metabolism, since accumulated levels were very low.

Many Southern Ocean cold waters organisms have generally slow activity and low metabolic rates (Abele and Puntarulo, 2004). In theory, this should result in lower rates of reactive oxygen species (ROS) formation in ectotherm species. It is well known that mitochondria respiration system is responsible for the generation of ROS which are responsible for cellular damage (including LPO). In the present study although lower metabolic rate (identified by lower ETS values) observed in *E. antarctica* was not accompanied by lower LPO values, higher LPO levels observed in *G. nicholsi* may result from higher ETS activity recorded in this species. In particular, LPO levels varied interspecifically (*G. nicholsi* $\geq$ *E. antarctica* $>$ *E. superba* $>$ *G. braueri*). *Euphausia superba* presented significantly higher LPO levels than *G. braueri*, but lower than *G. nicholsi*. In the fish group, *E. antarctica* and *G. nicholsi* had higher levels of LPO than *G. braueri* (Table 2). It is well described that LPO may occur as a consequence of pollutants exposure

due to overproduction of ROS and inefficiency of antioxidant mechanisms (among others, Regoli and Giuliani, 2014). In the present study, the highest LPO levels identified in *G. nicholsi* did not correspond to higher Hg tissue concentrations, which, once again, may corroborate the hypothesis that Hg concentrations observed in organisms were not high enough to induce cellular alterations. Also, a study on oxidative stress profiles on seabass, *Dicentrarchus labrax*, demonstrated that in some cases higher contamination levels do not result in LPO increase (Hg 0.04 μg/g and 0.08 μg/g DW) (Mieiro et al., 2011).

Regarding the activity of antioxidant enzymes (Table 2, Fig. 1), significantly lower values were observed in *E. superba* than in some of the myctophid group (*E. antarctica* and *G. nicholsi* for SOD and *G. braueri* for CAT), while no significant differences were found among the three myctophid species. GPx activity presented no changes among all the analysed species whereby it may not be influenced by environmental conditions, with similar response in all studied species. Detoxification enzymes, like GSTs, are related to elimination routes of contaminants (e.g., Hg) (Elia et al., 2003). In the present study GSTs levels showed no significant differences among species, with no relationship with the Hg concentrations in organism's tissues. The results obtained for GSTs activity were lower than the ones observed by other authors for *E. superba* (Tremblay and Abele, 2015). Once again, these results may indicate that due to the low Hg concentration in seawater, and consequently low accumulation levels in the studied species, defence mechanisms were not responding to Hg accumulated concentrations. From the literature published on this topic it is possible to conclude that antioxidant responses in *E. superba* vary between studies: SOD levels were lower in the present study, but CAT activity was higher in samples collected in 2011, also around South Georgia (Tremblay and Abele, 2015). GPx activity was also lower than in individuals captured in the eastern Antarctic sector in 2006, when compared with the control individuals of the study performed by Dawson et al. (2018). Thus, the obtained results demonstrated that the activity of antioxidant and biotransformation enzymes was similar among species, regardless the LPO levels and Hg concentration. Such response may indicate low stress levels caused by low Hg concentrations accumulated by the organisms, with increased LPO levels resulting from increased metabolic capacity rather than contamination levels.

Overall, the present findings highlighted that *G. nicholsi* presented higher levels in almost all the analysed biochemical parameters. This performance may be due to a difference in size, compared with the other analysed species. To the best of our knowledge, this is the first study reporting biochemical parameters in Southern Ocean myctophid fish. The unique environmental features of the Southern Ocean, and its highly specialized organisms, are likely to make it very sensitive to environmental change. With ocean warming, increased levels of glacial melt and higher amounts of freshwater input, levels of contaminants (like Hg) may increase further into the future. Oxidative stress and metabolic capacity will be among the first biological responses of resident species to contaminants. So, it is important to describe levels of natural variation in these parameters for future comparisons.

The present study provides values for a number of metabolic parameters (PROT, GLY, ETS, LPO, CAT, GPx, SOD, GSTs) for *E. superba* and three biomass dominant myctophid species, all of which play key roles in Southern Ocean ecosystem function. At present, none of the studied biochemical parameters shows any positive or negative relationship with levels of Hg found within species. Nevertheless, these values provide an important baseline to establish whether any future increases in contamination levels are having a notifiable effect on species metabolic capacity and biochemical performance.

CRediT authorship contribution statement

José Seco: Conceptualization, Formal analysis, Writing - Original Draft.
Rosa Freitas: Conceptualization, Resources, Writing - Original Draft.
José C. Xavier: Writing - Review & Editing.
Paco Bustamante: Writing - Review & Editing.
Francesca Coppola: Formal analysis.
João P. Coelho: Writing - Review & Editing.
Ryan A. Saunders: Writing - Review & Editing.
Ângela Almeida: Formal analysis.
Sophie Fielding: Writing - Review & Editing.
Miguel A. Pardal: Writing - Review & Editing.
Gabriele Stowasser: Writing - Review & Editing.
Giulia Pompeo: Formal analysis.
Geraint A. Tarling: Resources, Writing - Review & Editing.
Andrew S. Brierley: Writing - Review & Editing.
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Declaration of competing interest

The Authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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