



HAL
open science

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

José Seco, Rosa Freitas, José Xavier, Paco Bustamante, João Coelho, Francesca Coppola, Ryan Saunders, Ângela Almeida, Sophie Fielding, Miguel Pardal, et al.

► To cite this version:

José Seco, Rosa Freitas, José Xavier, Paco Bustamante, João Coelho, et al.. Oxidative stress, metabolic activity and mercury concentrations in Antarctic krill *Euphausia superba* and myctophid fish of the Southern Ocean. *Marine Pollution Bulletin*, 2021, 166, pp.112178. 10.1016/j.marpolbul.2021.112178 . hal-03489978

HAL Id: hal-03489978

<https://hal.science/hal-03489978v1>

Submitted on 13 Jan 2022

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

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

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

José Seco^{1,2}, Rosa Freitas^{3*}, José C. Xavier^{4,5}, Paco Bustamante^{6,7}, João P. Coelho³, Francesca Coppola³, Ryan A. Saunders⁴, Ângela Almeida³, Sophie Fielding⁴, Miguel A. Pardal⁸, Gabriele Stowasser⁴, Giulia Pompeo¹, Geraint A. Tarling⁴, Andrew S. Brierley², Eduarda Pereira¹

¹ Department of Chemistry and CESAM/REQUIMTE, University of Aveiro, 3810-193 Aveiro, Portugal

² Pelagic Ecology Research Group, Scottish Oceans Institute, Gatty Marine Laboratory, University of St Andrews, St Andrews KY16 8LB, Scotland, UK

³ CESAM – Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal

⁴ British Antarctic Survey, NERC, High Cross, Madingley Road, Cambridge CB3 0ET, UK

⁵ MARE—Marine and Environmental Sciences Centre, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

⁶ Littoral Environnement et Sociétés (LIENSs), UMR 7266 CNRS - La Rochelle Université, 2 rue Olympe de Gouges, 17000 La Rochelle, France

⁷ Institut Universitaire de France (IUF), 1 rue Descartes 75005 Paris, France

⁸ CFE - Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

⁹ Department of Chemistry and REQUIMTE, University of Aveiro, 3810-193 Aveiro, Portugal

*Corresponding author: Rosa Freitas

rosafreitas@ua.pt

CESAM – Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal

ABSTRACT

Indicators of oxidative stress and metabolic capacity are key factors in understanding the fitness of wild populations. In this 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 in tissues were also analysed to evaluate its potential impacts on species biochemical performance. *E. superba* had higher metabolic activity than 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 levels and organism's antioxidant and biotransformation defense mechanisms indicate that levels of Hg accumulated in the studied species were not high enough to affect their biochemical processes adversely.

Keywords: Toxicity, Biochemical performance, Antioxidant capacity, Base line.

The Southern Ocean ecosystem is distinctive for 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). Globally, it is also experiencing some of the highest levels of warming and ocean acidification (Freer et al., 2017; IPCC, 2019; Rintoul et al., 2018; Turner et al., 2013), which can affect individuals at a subcellular level and, in turn, alter patterns of distribution and food web structure (Atkinson et al., 2004; Xavier and Peck, 2015). Warming increases the release of freshwater into the Southern Ocean, particularly through accelerating the flow of glaciers, which liberates the contaminants that they store. These contaminants have the potential to cause stress to Southern Ocean fauna and it is important to describe and understand the impacts that they may have, 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 260000 tonnes harvested in 2016 (Nicol et al., 2000; Tou et al., 2007); CCAMLR, 2017). This species is predominantly herbivorous, feeding mainly on phytoplankton and rarely on some copepod crustaceans (Everson, 2000). Whales, seals, penguins and flying seabirds are amongst those species that consume high quantities of *E. superba* (Armstrong and Siegfried, 1991; Croxall et al., 1999; Xavier et al., 2003). However, given evidence of a 50-80 % decline in *E. superba* over long term (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 be considered a major alternative energy source to predators in 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), squid (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* preys on different species, including *Themisto gaudichaudii*, *Metridia* spp, *E. superba*, *Pleuromamma robusta* and ostracods; and *G. nicholsi* diet 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, 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).

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 and non-enzymatic forms such as the enzymes superoxide dismutase, catalase, glutathione peroxidase, and reduced glutathione (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 on their metabolic capacity, in marine organisms (Coppola et al., 2018; Monteiro et al., 2019).

Despite the ecological importance of these two groups (*E. superba* 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 this aspect in *E. superba*, and virtually none in Southern Ocean myctophid, only two looking into ETS as a proxy for respiration rates (Belcher et al., 2020; 2019). The main

goal of the present study is to describe the general oxidative stress and metabolic status of *E. superba* and of other 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 reserves content were evaluated in all 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 m² 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 m² mouth-opening (RMT25; mesh size reducing from 8 mm to 4.5 mm in the cod end) (Roe and Shale, 1979). Samples 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 mercury (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, 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). 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 KH₂PO₄, 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) polyvinylpyrrolidone (PVP), 153 mmol/L Mg SO₄, 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 0–10 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/M/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/mM/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 μ M) 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/mM/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/mM/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

certified 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 \pm standard deviation for the whole of the CRM analyses (n=13) provided recoveries ranging from 84 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, until coefficient of variation were below 10%.

Data obtained from toxicology, biochemical analyses and biological factor (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 for each parameter was: for each biochemical parameters and Hg concentration, no significant differences existed among species.

Biochemical responses 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 biochemical performance 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 (Figure 1).

In term 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, 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*) 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 inhabitants 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 (Figure 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.

PROT and GLY contents were lower in *E. superba* compared to the three myctophid fish, which 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 inhabitants 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 inter-specifically ($G. nicholsi \geq E. antarctica > E. superba > G. braueri$). *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 *Dicentrarchus labrax*, demonstrated that in some cases higher contamination levels do not result in LPO increase (Hg 0.04 $\mu\text{g/g}$ and 0.08 $\mu\text{g/g}$) (Mieiro et al., 2011).

Regarding the activity of antioxidant enzymes (Table 2, Figure 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 tissues 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 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 2017. 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 in the organisms 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 inhabitants, 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 (PRO, GLY, ETS, LPO, CAT, ETS, 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 tissues. 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.

ACKNOWLEDGEMENTS: We thank the officers, crew and scientists aboard RSS *James Clark Ross* during cruises JR15004 for their assistance in collecting samples. We acknowledge the financial support of the Portuguese Foundation for the Science and Technology (FCT) through a PhD grant to José Seco (SRFH/PD/BD/113487), and the Portuguese Polar Program PROPOLAR. This study also benefited from the strategic program of MARE (Marine and Environmental Sciences Centre) financed by FCT (UIDB/04292/2020). Rosa Freitas was funded by national funds (OE), through FCT, in the scope of the framework contract foreseen in the numbers 4, 5 and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19. JP Coelho was funded by the Integrated Program of SR&TD 'Smart Valorization of Endogenous Marine Biological Resources Under a Changing Climate' (Centro-01-0145-FEDER-000018), co-funded by Centro 2020 program, Portugal 2020 and the European Regional Development Fund. Thanks are due for the financial support to CESAM (UIDB/50017/2020+UIDP/50017/2020). The Institut Universitaire de France (IUF) is acknowledged for its support to P. Bustamante as a Senior Member. RAS, SF, GS and GT were supported by the British Antarctic Surveys Ecosystem Programme, which is part of NERC and UKRI.

Table 1. Total number, 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.

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

Table 2. Biomarkers and mercury (Hg) concentration measured in each species. Biomarkers: PROT: protein content; LPO: lipid peroxidation levels; ETS: Electron transport system; GLY: Glycogen content; GPx: Glutathione peroxidase; GSTs: Glutathione S-transferases activity; SOD: superoxide dismutase activity; CAT: catalase activity. Units are presented per gram of fresh weight (FW) and per mg of protein (PROT) for comparison with results from the literature.

Species	Hg	GLY	PROT	ETS	LPO		SOD		CAT		GPx		GSTs	
	$\mu\text{g g}^{-1}$ DW	mg/g FW	mg/g FW	nmol/min/g FW	nmol MDA/g FW	nmol.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														
<i>Euphausia superba</i>	<u>0.04</u> (± 0.01) ^a	<u>18.65</u> (± 3.27) ^a	<u>41.36</u> (± 2.48) ^a	33.80 (± 8.37) ^a	24.82 (± 3.90) ^a	0.82 (± 0.21)	<u>1.96</u> (± 0.92) ^a	0.02 (± 0.005)	<u>10.79</u> (± 3.83) ^a	0.13 (± 0.05)	5.15 (± 3.22) ^a	0.25 (± 0.16)	0.05 (± 0.02) ^a	0.78 (± 0.57)
Myctophids														
<i>Electrona antarctica</i>	0.22 (± 0.08) ^b	21.19 (± 4.52) ^{a, b}	64.81 (± 9.17) ^b	<u>17.88</u> (± 5.77) ^b	36.19 (± 8.41) ^{a, c}	0.28 (± 0.11)	4.19 (± 0.90) ^b	0.03 (± 0.01)	17.45 (± 6.80) ^{a, b}	0.13 (± 0.05)	<u>2.03</u> (± 1.97) ^a	0.07 (± 0.07)	0.05 (± 0.01) ^a	1.23 (± 0.22)
<i>Gymnoscopelus braueri</i>	0.17 (± 0.03) ^b	24.03 (± 4.38) ^{a, b}	85.34 (± 22.57) ^{b, c}	22.36 (± 8.82) ^{a, b}	15.77 (± 9.10) ^b	0.26 (± 0.05)	2.74 (± 0.81) ^{a, b}	<u>0.01</u> (± 0.009)	22.60 (± 7.96) ^b	0.13 (± 0.02)	3.48 (± 2.27) ^a	0.09 (± 0.06)	<u>0.04</u> (± 0.02) ^a	0.77 (± 0.53)
<i>Gymnoscopelus nicholsi</i>	0.17 (± 0.04) ^b	25.54 (± 1.41) ^b	118.99 (± 38.51) ^c	42.45 (± 10.71) ^{a, b}	76.86 (± 7.99) ^c	0.34 (± 0.17)	3.73 (± 0.84) ^b	<u>0.01</u> (± 0.005)	20.11 (± 10.20) ^{a, b}	0.09 (± 0.05)	5.07 (± 1.92) ^a	0.11 (± 0.04)	0.13 (± 0.04) ^a	1.79 (± 1.66)

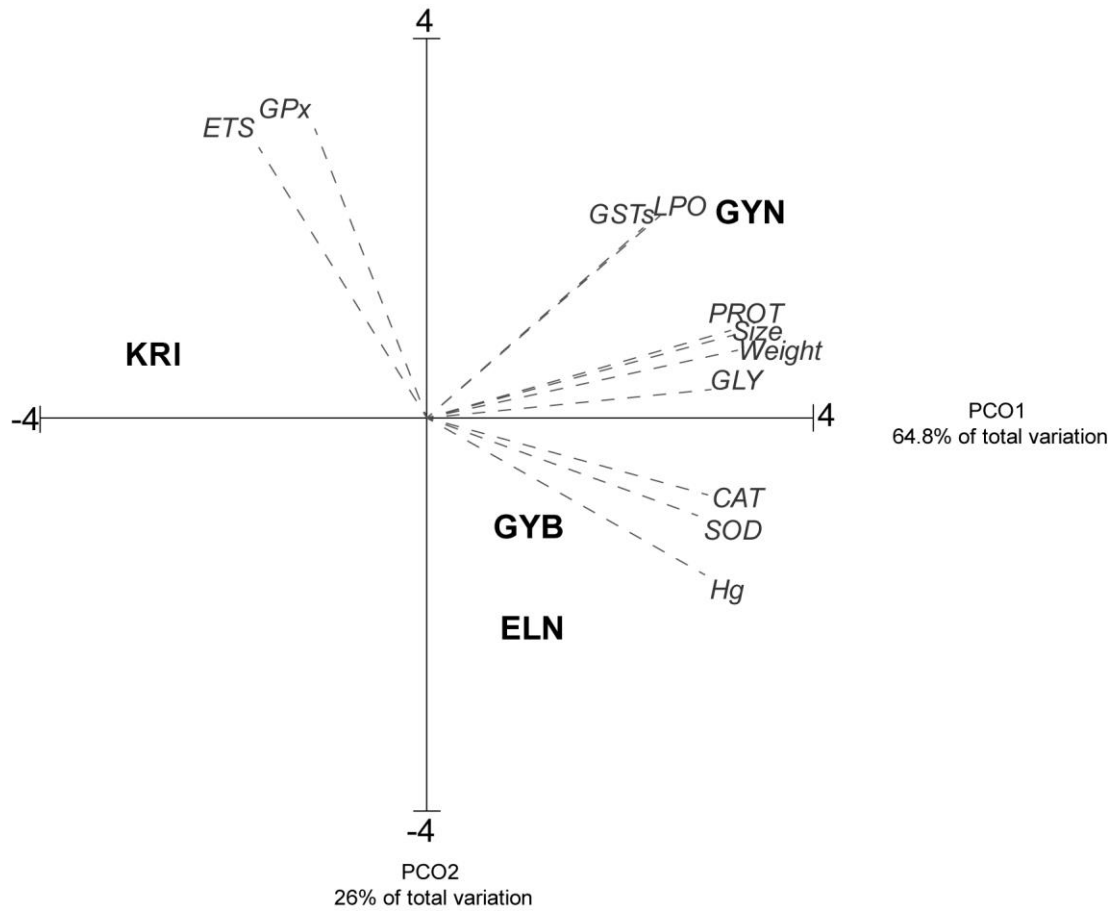


Figure 1. Ordination diagram based on biomarkers, biological characteristic and mercury (Hg) concentration. ELN - *Electrona Antarctica*, GYB - *Gymnoscopelus braueri*; GYN - *Gymnoscopelus nicholsi*; KRI - *Euphausia superba*; PROT - protein content; LPO - lipid peroxidation levels; ETS - Electron transport system activity; GLY - Glycogen content; GPx - Glutathione peroxidase; GSTs - Glutathione S-transferases activity; SOD - superoxide dismutase activity; CAT - catalase activity; Hg - Mercury concentration.

REFERENCES

- Abele, D., Puntarulo, S., 2004. Formation of reactive species and induction of antioxidant defence systems in polar and temperate marine invertebrates and fish. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 138, 405–415. doi:10.1016/j.cbpb.2004.05.013
- Alberello, A., Onorato, M., Bennetts, L., Vichi, M., Eayrs, C., MacHutchon, K., Toffoli, A., 2018. Brief communication: Pancake ice floe size distribution during the winter expansion of the Antarctic marginal ice zone. *The Cryosphere Discuss.* 1–9. doi:10.5194/tc-2018-155
- Almeida, Â., Calisto, V., Esteves, V.I., Schneider, R.J., Soares, A.M.V.M., Figueira, E., Freitas, R., 2014. Presence of the pharmaceutical drug carbamazepine in coastal systems: Effects on bivalves. *Aquat. Toxicol.* 156, 74–87. doi:10.1016/j.aquatox.2014.08.002
- Almeida, Â., Freitas, R., Calisto, V., Esteves, V.I., Schneider, R.J., Soares, A.M.V.M., Figueira, E., 2015. Chronic toxicity of the antiepileptic carbamazepine on the clam *Ruditapes philippinarum*. *Comparative Biochemistry and Physiology, Part C* 172-173, 26–35. doi:10.1016/j.cbpc.2015.04.004
- Anderson, M.J., Gorley, R.N., Clarke, K.R., 2009 PERMANOVA for PRIMER: Guide to Software and Statistical Methods. University of Auckland and PRIMER-E.
- Armstrong, A.J., Siegfried, W.R., 1991. Consumption of Antarctic krill by Minke whales. *Antarct. Sci.* 3, 13–18. doi:10.1017/S0954102091000044
- Atkinson, A., Meyer, B., Stuübing, D., Hagen, W., Schmidt, K., Bathmann, U.V., 2002. Feeding and energy budgets of Antarctic krill *Euphausia superba* at the onset of winter—II. Juveniles and adults. *Limnol Oceanogr* 47, 953–966. doi:10.4319/lo.2002.47.4.0953
- Atkinson, A., Siegel, V., Pakhomov, E., Rothery, P., 2004. Long-term decline in krill stock and increase in salps within the Southern Ocean. *Nature* 432, 100–103. doi:10.1038/nature02996
- Beaucham, C., Fridovic, I., 1971. Superoxide Dismutase - Improved Assays and an Assay Applicable to Acrylamide Gels. *Anal. Biochem.* 44, 276–287. doi:10.1016/0003-2697(71)90370-8
- Belcher, A., Cook, K., Bondyale-Juez, D., Stowasser, G., Fielding, S., Saunders, R.A., Mayor, D.J., Tarling, G.A., 2020. Respiration of mesopelagic fish: a comparison of respiratory electron transport system (ETS) measurements and allometrically calculated rates in the Southern Ocean and Benguela Current. *ICES J Mar Sci* 76, 690–13. doi:10.1093/icesjms/fsaa031
- Belcher, A., Saunders, R.A., Tarling, G.A., 2019. Respiration rates and active carbon flux of mesopelagic fishes (Family Myctophidae) in the Scotia Sea, Southern Ocean. *Mar. Ecol. Prog. Ser.* 610, 149–162. doi:10.3354/meps12861
- Brierley, A.S., Thomas, D.N., 2002. Ecology of Southern Ocean pack ice. *Adv. Mar. Biol.* 43, 171–276. doi:10.1016/S0065-2881(02)43005-2
- Carregosa, V., Velez, C., Pires, A., Soares, A.M.V.M., Figueira, E., Freitas, R., 2014. Physiological and biochemical responses of the *Polychaete Diopatra neapolitana* to organic matter enrichment. *Aquat. Toxicol.* 155, 32–42. doi:10.1016/j.aquatox.2014.05.029
- Cipro, C.V.Z., Chernel, Y., Bocher, P., Caurant, F., Miramand, P., Bustamante, P., 2018. Trace elements in invertebrates and fish from Kerguelen waters, southern Indian Ocean. *Polar Biol.* 41, 175–191. doi:10.1007/s00300-017-2180-6
- Coelho, J.P., Reis, A.T., Ventura, S., Pereira, M.E., Duarte, A.C., Pardal, M.A., 2008. Pattern and pathways for mercury lifespan bioaccumulation in *Carcinus maenas*. *Mar. Pollut. Bull.* 56, 1104–1110. doi:10.1016/j.marpolbul.2008.03.020
- Collins, M.A., Ross, K.A., Belchier, M., Reid, K., 2007. Distribution and diet of juvenile Patagonian toothfish on the South Georgia and Shag Rocks shelves (Southern Ocean). *Mar Biol* 152, 135–147.

- Collins, M.A., Xavier, J.C., Johnston, N.M., North, A.W., 2008. Patterns in the distribution of myctophid fish in the northern Scotia Sea ecosystem. *Polar Biol.* doi:10.1007/s00300-008-0423-2
- Coppola, F., Henriques, B., Soares, A.M.V.M., Figueira, E., Pereira, M.E., Freitas, R., 2018. Influence of temperature rise on the recovery capacity of *Mytilus galloprovincialis* exposed to mercury pollution. *Ecological Indicators* 93, 1060–1069. doi:10.1016/j.ecolind.2018.05.077
- Croxall, J.P., Reid, K., Prince, P.A., 1999. Diet, provisioning and productivity responses of marine predators to differences in availability of Antarctic krill. *Mar. Ecol. Prog. Ser.* 177, 115–131. doi:10.3354/meps177115
- Cruz, D., Almeida, Â., Calisto, V.N., Esteves, V.I., Schneider, R.J., Wrona, F.J., Soares, A.M.V.M., Figueira, E., Freitas, R., 2016. Caffeine impacts in the clam *Ruditapes philippinarum*: Alterations on energy reserves, metabolic activity and oxidative stress biomarkers. *Chemosphere* 160, 95–103. doi:10.1016/j.chemosphere.2016.06.068
- Davis, D., Staniland, I.J., Reid, K., 2006. Spatial and temporal variability in the fish diet of Antarctic fur seal (*Arctocephalus gazella*) in the Atlantic sector of the Southern Ocean. *Can. J. Zool.* 84, 1025–1037. doi:10.1139/z06-071
- De Coen, W.M., Janssen, C.R., 1997. The use of biomarkers in *Daphnia magna* toxicity testing. IV. Cellular Energy Allocation: a new methodology to assess the energy budget of toxicant-stressed *Daphnia* populations. *J. Aquat. Ecosyst. Stress. Recover* 6, 43–55. doi:10.1023/A:1008228517955
- DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric Method for Determination of Sugars and Related Substances. *Anal. Chem.* 28, 350–356. doi:10.1021/ac60111a017
- Elia, A.C., Galarini, R., Taticchi, M.I., Dörr, A.J.M., Mantilacci, L., 2003. Antioxidant responses and bioaccumulation in *Ictalurus melas* under mercury exposure. *Ecotoxicol. Environ. Saf.* 55, 162–167. doi:10.1016/S0147-6513(02)00123-9
- Everson, I., 2000. *Krill: Biology, Ecology and Fisheries*. Blackwell Science, London.
- Freer, J.J., Partridge, J.C., Tarling, G.A., Collins, M.A., Genner, M.J., 2017. Predicting ecological responses in a changing ocean: the effects of future climate uncertainty. *Mar Biol* 165, 1–18. doi:10.1007/s00227-017-3239-1
- Freitas, R., Cardoso, C.E.D., Costa, S., Morais, T., Moleiro, P., Lima, A.F.D., Soares, M., Figueiredo, S., Águeda, T.L., Rocha, P., Amador, G., Soares, A.M.V.M., Pereira, M.E., 2020. New insights on the impacts of e-waste towards marine bivalves: The case of the rare earth element Dysprosium. *Environ. Pollut.* 260, 113859. doi:10.1016/j.envpol.2019.113859
- Gjøsaeter, J., Kawaguchi, K., 1980. A review of the world resources of mesopelagic fish, in: Gon, O., Heemstra, P., 1990. *Fishes of the Southern Ocean*. JLB Smith Institute of Ichthyology, Grahamstown, South Africa.
- Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases the first enzymatic step in mercapturic acid formation. *J. Biol. Chem* 249, 7130–7139.
- Hulley, P.A., 1990. Myctophidae, in: Gon, O., Heemstra, P.C. (Eds.), *Fishes of the Southern Ocean*. JLB Smith Institute of Ichthyology, pp. 146–178.
- IPCC, 2019. Summary for Policymakers, in: Shukla, J. Skea, E. Calvo Buendia, V. Masson-Delmotte, H.-O. Pörtner, D. C. Roberts, P. Zhai, R. Slade, S. Connors, R. van Diemen, M. Ferrat, E. Haughey, S. Luz, S. Neogi, M. Pathak, J. Petzold, J. Portugal Pereira, P. Vyas, E. Huntley, K. Kissick, M. Belkacemi, J. Malley, (Eds.), *Global Warming of .C. an IPCC Special Report on the Impacts of Global Warming of .C Above Pre-Industrial Levels and Related Global Greenhouse Gas Emission Pathways, in the Context of Strengthening the Global Response to the Threat of Climate Change, Sustainable Development, and Efforts to Eradicate Poverty*. Geneva, Switzerland, p. 32.
- Kear, A.J., 1992. The Diet of Antarctic Squid - Comparison of Conventional and Serological Gut Contents Analyses. *J Exp Mar Biol Ecol* 156, 161–178. doi:10.1016/0022-0981(92)90243-4

- King, F.D., Packard, T.T., 1975. Respiration and the activity of the respiratory electron transport system in marine zooplankton. *Limnol Oceanogr* 20, 849–854.
doi:10.4319/lo.1975.20.5.0849
- Lourenço, S., Saunders, R.A., Collins, M.A., Shreeve, R., Assis, C.A., Belchier, M., Watkins, J.L., Xavier, J.C., 2017. Life cycle, distribution and trophodynamics of the lanternfish *Krefftichthys anderssoni* (Lonnberg, 1905) in the Scotia Sea. *Polar Biol.* 40, 1229–1245.
doi:10.1007/s00300-016-2046-3
- Meyer, B., 2012. The overwintering of Antarctic krill, *Euphausia superba*, from an ecophysiological perspective. *Polar Biol.* 35, 15–37. doi:10.1007/s00300-011-1120-0
- Mieiro, C.L., Pereira, M.E., Duarte, A.C., Pacheco, M., 2011. Brain as a critical target of mercury in environmentally exposed fish (*Dicentrarchus labrax*) -Bioaccumulation and oxidative stress profiles. *Aquat. Toxicol.* 103, 233–240.
doi:10.1016/j.aquatox.2011.03.006
- Monteiro, R., Costa, S., Coppola, F., Freitas, R., Vale, C., Pereira, M.E., 2019. Evidences of metabolic alterations and cellular damage in mussels after short pulses of Ti contamination. *Sci. Total Environ.* 650, 987–995. doi:10.1016/j.scitotenv.2018.08.314
- Morris, D.J., Ricketts, C., Watkins, J.L., Buchholz, F., Priddle, J., 1992. An assessment of the merits of length and weight measurements of Antarctic krill *Euphausia superba*. *Deep-Sea Res. II* 39, 359–371. doi:10.1016/0198-0149(92)90113-8
- Morrison, A.K., Frölicher, T.L., Sarmiento, J.L., 2015. Upwelling in the Southern Ocean. *Physics Today* 68, 27–32. doi:10.1063/PT.3.2654
- Murphy, E.J., Watkins, J.L., Trathan, P.N., Reid, K., Meredith, M.P., Thorpe, S.E., Johnston, N.M., Clarke, A., Tarling, G.A., Collins, M.A., Forcada, J., Shreeve, R.S., Atkinson, A., Korb, R., Whitehouse, M.J., Ward, P., Rodhouse, P.G., Enderlein, P., Hirst, A.G., Martin, A.R., Hill, S.L., Staniland, I.J., Pond, D.W., Briggs, D.R., Cunningham, N.J., Fleming, A.H., 2007. Spatial and temporal operation of the Scotia Sea ecosystem: a review of large-scale links in a krill centred food web. *Phil. Trans. R. Soc. B* 362, 113–148.
doi:10.1098/rstb.2006.1957
- Nicol, S., Forster, I., Spence, J., 2000. Products derived from krill, in: *Krill: Biology, Ecology and Fisheries*. Blackwell Science, London, pp. 262–283.
- Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* 95, 351–358. doi:10.1021/bi971641i
- Olsson, O., North, A.W., 1997. Diet of the king penguin *Aptenodytes patagonicus* during three summers at South Georgia. *Ibis* 504–512. doi:10.1111/j.1474-919X.1997.tb04666.x
- Paglia, D.E., Valentine, W.N., 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* 70, 158–169.
- Pinto, J., Costa, M., Leite, C., Borges, C., Coppola, F., Henriques, B., Monteiro, R., Russo, T., Di Cosmo, A., Soares, A.M.V.M., Polese, G., Pereira, M.E., Freitas, R., 2019. Ecotoxicological effects of lanthanum in *Mytilus galloprovincialis*: Biochemical and histopathological impacts. *Aquat. Toxicol.* 211, 181–192.
doi:10.1016/j.aquatox.2019.03.017
- Quetin, L.B., Ross, R.M., 1991. Behavioral and Physiological Characteristics of the Antarctic Krill, *Euphausia superba*. *Amer. Zool.* 31, 49–63.
- Regoli, F., Giuliani, M.E., 2014. Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms. *Mar. Environ. Res.* 93, 106–117.
doi:10.1016/j.marenvres.2013.07.006
- Regoli, F., Giuliani, M.E., Benedetti, M., Arukwe, A., 2011. Molecular and biochemical biomarkers in environmental monitoring: A comparison of biotransformation and antioxidant defense systems in multiple tissues. *Aquat. Toxicol.* 105, 56–66.
doi:10.1016/j.aquatox.2011.06.014
- Rintoul, S.R., Chown, S.L., DeConto, R.M., England, M.H., Fricker, H.A., Masson-Delmotte, V., Naish, T.R., Siegert, M.J., Xavier, J.C., 2018. Choosing the future of Antarctica. *Nature* 558, 233–241. doi:10.1038/s41586-018-0173-4

- Robinson, H.W., Hogden, C.G., 1940. The biuret reaction in the determination of serum proteins I. A study of the conditions necessary for the production of a stable color which bears a quantitative relationship to the protein concentration. *J. Biol. Chem* 135, 707–725.
- Roe, H.S.J., Shale, D.M., 1979. A new multiple rectangular midwater trawl (RMT 1+8M) and some modifications to the institute of oceanographic sciences' RMT 1+8. *Mar Biol* 50, 283–288. doi:10.1007/BF00394210
- Rosa, R., Nunes, M.L., 2003. Biochemical composition of deep-sea decapod crustaceans with two different benthic life strategies off the Portuguese south coast. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 50, 119–130. doi:10.1016/S0967-0637(02)00147-4
- Ross, R., Quetin, L.B., 2000. Reproduction in Euphausiacea, in: *Krill*. Blackwell Science Ltd, Oxford, UK, pp. 150–181. doi:10.1002/9780470999493.ch6
- Saunders, R.A., Collins, M.A., Shreeve, R., Ward, P., Stowasser, G., Hill, S.L., Tarling, G.A., 2018. Seasonal variation in the predatory impact of myctophids on zooplankton in the Scotia Sea (Southern Ocean). *Prog. Oceanogr* 168, 123–144. doi:10.1016/j.pocean.2018.09.017
- Saunders, R.A., Collins, M.A., Ward, P., Stowasser, G., Shreeve, R., Tarling, G.A., 2014. Distribution, population structure and trophodynamics of Southern Ocean *Gymnoscopelus* (Myctophidae) in the Scotia Sea. *Polar Biol.* 38, 287–308. doi:10.1007/s00300-014-1584-9
- Saunders, R.A., Hill, S.L., Tarling, G.A., Murphy, E.J., 2019. Myctophid Fish (Family Myctophidae) Are Central Consumers in the Food Web of the Scotia Sea (Southern Ocean). *Front. Mar. Sci.* 6, 142. doi:10.3389/fmars.2019.00530
- Seco, J., Xavier, J.C., Bustamante, P., Coelho, J.P., Saunders, R.A., Ferreira, N., Fielding, S., Pardal, M.A., Stowasser, G., Viana, T., Tarling, G.A., Pereira, M.E., Brierley, A.S., 2020. Main drivers of mercury levels in Southern Ocean lantern fish Myctophidae. *Environ. Pollut.* 264, 114711–10. doi:10.1016/j.envpol.2020.114711
- Suzuki, N., Uchikawa, K., Yamada, H., Chow, S., 2005. Genetic Divergence and Identification of Two Controversial Lanternfishes Myctophidae: *Diaphus*) Based (Actinopterygii: on Mitochondrial Cytochrome *b* Sequences and PCR-RFLP Analysis. *Species Diversity* 10, 289–299. doi:10.12782/specdiv.10.289
- Tou, J.C., Jaczynski, J., Chen, Y.-C., 2007. Krill for human consumption: nutritional value and potential health benefits. *Nutr. Rev.* 65, 63–77. doi:10.1301/nr.2007.feb.63–77
- Tremblay, N., Abele, D., 2015. Response of three krill species to hypoxia and warming: an experimental approach to oxygen minimum zones expansion in coastal ecosystems. *Mar Ecol Prog Ser* 37, 179–199. doi:10.1111/maec.12258
- Turner, J., Barrand, N.E., Bracegirdle, T.J., Convey, P., Hodgson, D.A., Jarvis, M., Jenkins, A., Marshall, G., Meredith, M.P., Roscoe, H., Shanklin, J., French, J., Goosse, H., Guglielmin, M., Gutt, J., Jacobs, S., Kennicutt, M.C., Masson-Delmotte, V., Mayewski, P., Navarro, F., Robinson, S., Scambos, T., Sparrow, M., Summerhayes, C., Speer, K., Klepikov, A., 2013. Antarctic climate change and the environment: an update. *Polar Record* 50, 237–259. doi:10.1017/S0032247413000296
- Xavier, J.C., Croxall, J.P., Trathan, P.N., Wood, A.G., 2003. Feeding strategies and diets of breeding grey-headed and wandering albatrosses at South Georgia. *Mar Biol* 143, 221–232. doi:10.1007/s00227-003-1049-0
- Xavier, J.C., Peck, L.S., 2015. Life Beyond the Ice, in: *Exploring the Last Continent: an Introduction to Antarctica, Marine Ecosystems in the Southern Ocean*. Springer International Publishing, Cham, pp. 229–252. doi:10.1007/978-3-319-18947-5_12
- Yamamoto, T.-O., 1969. Sex Differentiation, in: Hoar, W., Randall, D. (Eds.), *Fish Physiology*. Deep-Sea Research I, pp. 117–175. doi:10.1016/S1546-5098(08)60113-2