# 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 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 tones (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 $m^2$ 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). Samples were preserved in individual sample bags at -80 ºC.

*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 $KH_2PO_4$, 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) polyvinylpyrrolidone (PVP), 153 mmol/L Mg $SO_4$, 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 4º C (Carregosa et al., 2014), and supernatants were preserved at -80 ºC 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 μM) 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

186 certified reference material (CRM; in this case TORT-2 and TORT-3 [lobster hepatopancreas,
187 National Research Council, Canada] for *E. superba*; DORM-4 [Fish protein, National
188 Research Council, Canada] for myctophids). The obtained values (mean ± standard deviation
189 for the whole of the CRM analyses (n=13) provided recoveries ranging from 84 to 96 % (TORT-
190 2: 88 ± 3 %; TORT-3: 89 ± 7 %; DORM-4: 91 ± 12%). The mass of CRM used for quality
191 control analyses was adjusted to be within the range of total Hg present in the samples. Blanks
192 were analysed at the beginning of each set of samples and the analyses were always
193 performed at least in duplicate, until coefficient of variation were below 10%.
194 Data obtained from toxicology, biochemical analyses and biological factor (size,
195 weight, sex and species) were submitted to permutational multivariate analysis of variance
196 with the PERMANOVA+add-on in PRIMER v6 (Anderson et al., 2009). The pseudo-F values
197 in the PERMANOVA main tests were evaluated in terms of significance. When the main test
198 revealed statistical significant differences ($p < 0.05$), pairwise comparisons were performed.
199 The null hypothesis tested for each parameter was: for each biochemical parameters and Hg
200 concentration, no significant differences existed among species.
201 Biochemical responses and Hg concentrations for the different species were used to
202 calculate the Euclidean distance similarity matrix. This matrix was simplified through the
203 calculation of the distance within the centroid matrix based on species, which was then
204 submitted to ordination analysis, performed by Principal Coordinates (PCO). Pearson
205 correlation vectors ($r > 0.85$) of physiological and biochemical descriptors and Hg
206 concentrations were provided as supplementary variables being superimposed on the top of
207 the PCO graph.
208 For all the studied species, both sexes were initially analysed separately and since no
209 significant differences were observed in terms of biochemical performance the total number
210 of organisms per species was pooled.
211
212 In terms of biochemical performance and Hg concentration in all the analysed
213 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* ≤ *G. braueri* ≤ *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* ≥ *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 μg/g and 0.08 μg/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

295 organism's tissues. The results obtained for GSTs activity were lower than the ones observed
296 by other authors for *E. superba* (Tremblay and Abele, 2015). Once again, these results may
297 indicate that due to the low Hg concentration in seawater, and consequently low accumulation
298 levels in the studied species, defence mechanisms were not responding to Hg tissues
299 concentrations. From the literature published on this topic it is possible to conclude that
300 antioxidant responses in *E. superba* vary between studies: SOD levels were lower in the
301 present study, but CAT activity was higher in samples collected in 2011, also around South
302 Georgia (Tremblay and Abele, 2015). GPx was also lower than in individuals captured in the
303 eastern Antarctic sector in 2006, when compared with the control individuals of the study
304 performed by Dawson 2017. Thus, the obtained results demonstrated that the activity of
305 antioxidant and biotransformation enzymes was similar among species, regardless the LPO
306 levels and Hg concentration. Such response may indicate low stress levels in the organisms
307 caused by low Hg concentrations accumulated by the organisms, with increased LPO levels
308 resulting from increased metabolic capacity rather than contamination levels.

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

319 The present study provides values for a number of metabolic parameters (PRO, GLY,
320 ETS, LPO, CAT, ETS, GPx, SOD, GSTs) for *E. superba* and three biomass dominant
321 myctophid species, all of which play key roles in Southern Ocean ecosystem function. At
322 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.

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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.

| *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 |

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 resul from the literature.

| | Hg | GLY | PROT | ETS | LPO | | SOD | | CAT | | GPx | | GSTs | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Species | μg $g^{-1}$ 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*** | | | | | | | | | | | | | | |
| *Euphausia superba* | 0.04 (± 0.01)[a] | 18.65 (±3.27)[a] | 41.36 (±2.48)[a] | 33.80 (±8.37)[a] | 24.82 (±3.90)[a] | 0.82 (±0.21) | 1.96 (±0.92)[a] | 0.02 (±0.005) | 10.79 (±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*** | | | | | | | | | | | | | | |
| *Electrona antarctica* | **0.22 (± 0.08)[b]** | 21.19 (±4.52)[a, b] | 64.81 (±9.17)[b] | 17.88 (±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) | 2.03 (±1.97)[a] | 0.07 (±0.07) | 0.05 (±0.01)[a] | 1.23 (±0.22) |
| *Gymnoscopelus braueri* | 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] | 0.01 (±0.009) | **22.60 (±7.96)[b]** | 0.13 (±0.02) | 3.48 (±2.27)[a] | 0.09 (±0.06) | 0.04 (±0.02)[a] | 0.77 (±0.53) |
| *Gymnoscopelus nicholsi* | 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] | 0.01 (±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) |

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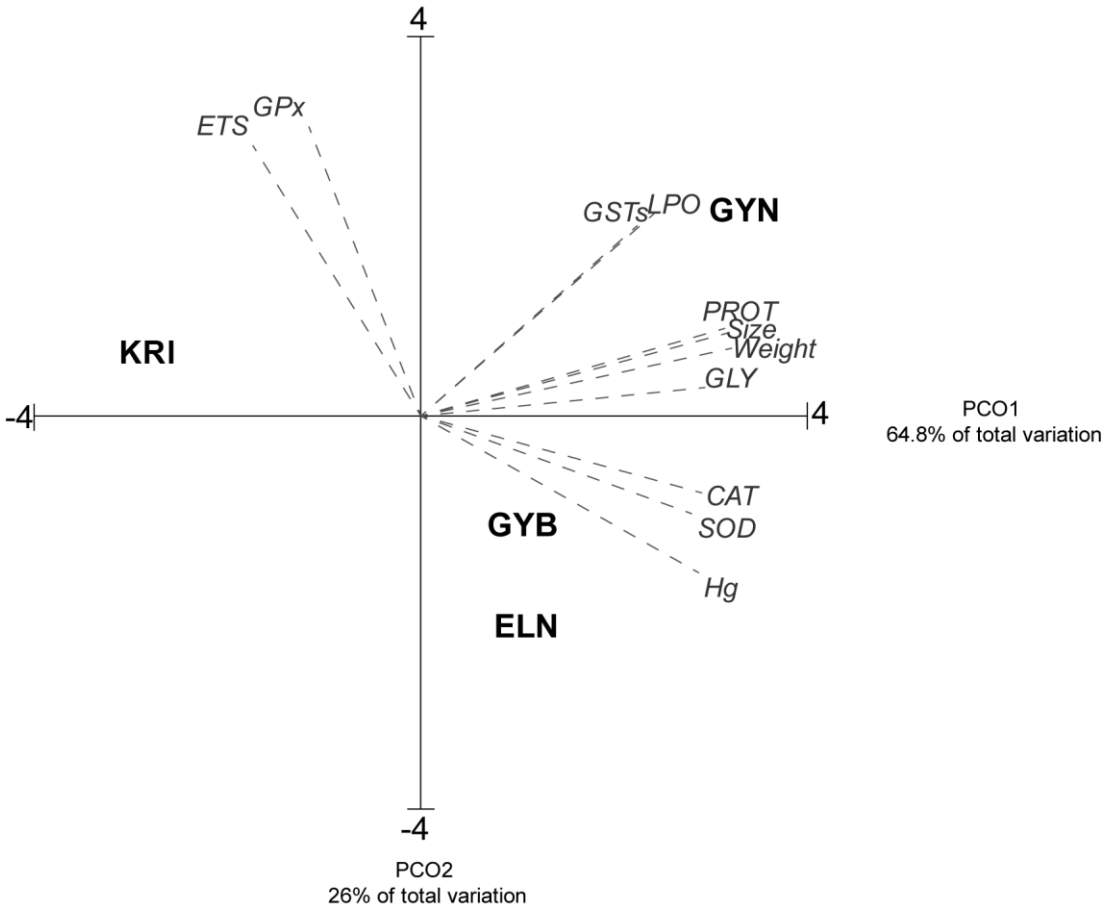


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Figure 1. Ordination diagram based on biomarkers, biological characteristic and mercury (Hg) concentration. ELN - *Electrona Antarctica*, GYB - *Gymnoscopelus braueri*; GYN - *Gymnoscopelus nicholsi*; KRI - *Euphausia superba*; PROT - protein content; 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.

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**REFENCES:**


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., Cherel, 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

421 Collins, M.A., Ross, K.A., Belchier, M., Reid, K., 2007. Distribution and diet of juvenile
422 Patagonian toothfish on the South Georgia and Shag Rocks shelves (Southern Ocean).
423 Mar Biol 152, 135–147.
424 Collins, M.A., Xavier, J.C., Johnston, N.M., North, A.W., 2008. Patterns in the distribution of
425 myctophid fish in the northern Scotia Sea ecosystem. Polar Biol. doi:10.1007/s00300-
426 008-0423-2
427 Coppola, F., Henriques, B., Soares, A.M.V.M., Figueira, E., Pereira, M.E., Freitas, R., 2018.
428 Influence of temperature rise on the recovery capacity of *Mytilus galloprovincialis*
429 exposed to mercury pollution. Ecological Indicators 93, 1060–1069.
430 doi:10.1016/j.ecolind.2018.05.077
431 Croxall, J.P., Reid, K., Prince, P.A., 1999. Diet, provisioning and productivity responses of
432 marine predators to differences in availability of Antarctic krill. Mar. Ecol. Prog. Ser. 177,
433 115–131. doi:10.3354/meps177115
434 Cruz, D., Almeida, Â., Calisto, V.N., Esteves, V.I., Schneider, R.J., Wrona, F.J., Soares,
435 A.M.V.M., Figueira, E., Freitas, R., 2016. Caffeine impacts in the clam Ruditapes
436 philippinarum: Alterations on energy reserves, metabolic activity and oxidative stress
437 biomarkers. Chemosphere 160, 95–103. doi:10.1016/j.chemosphere.2016.06.068
438 Davis, D., Staniland, I.J., Reid, K., 2006. Spatial and temporal variability in the fish diet of
439 Antarctic fur seal (*Arctocephalus gazella*) in the Atlantic sector of the Southern Ocean.
440 Can. J. Zool. 84, 1025–1037. doi:10.1139/z06-071
441 De Coen, W.M., Janssen, C.R., 1997. The use of biomarkers in *Daphnia magna* toxicity
442 testing. IV. Cellular Energy Allocation: a new methodology to assess the energy budget
443 of toxicant-stressed *Daphnia* populations. J. Aquat. Ecosyst. Stress. Recover 6, 43–55.
444 doi:10.1023/A:1008228517955
445 DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric Method
446 for Determination of Sugars and Related Substances. Anal. Chem. 28, 350–356.
447 doi:10.1021/ac60111a017
448 Elia, A.C., Galarini, R., Taticchi, M.I., Dörr, A.J.M., Mantilacci, L., 2003. Antioxidant
449 responses and bioaccumulation in Ictalurus melas under mercury exposure. Ecotoxicol.
450 Environ. Saf. 55, 162–167. doi:10.1016/S0147-6513(02)00123-9
451 Everson, I., 2000. Krill: Biology, Ecology and Fisheries. Blackwell Science, London.
452 Freer, J.J., Partridge, J.C., Tarling, G.A., Collins, M.A., Genner, M.J., 2017. Predicting
453 ecological responses in a changing ocean: the effects of future climate uncertainty. Mar
454 Biol 165, 1–18. doi:10.1007/s00227-017-3239-1
455 Freitas, R., Cardoso, C.E.D., Costa, S., Morais, T., Moleiro, P., Lima, A.F.D., Soares, M.,
456 Figueiredo, S., Águeda, T.L., Rocha, P., Amador, G., Soares, A.M.V.M., Pereira, M.E.,
457 2020. New insights on the impacts of e-waste towards marine bivalves: The case of the
458 rare earth element Dysprosium. Environ. Pollut. 260, 113859.
459 doi:10.1016/j.envpol.2019.113859
460 Gjøsaeter, J., Kawaguchi, K., 1980. A review of the world resources of mesopelagic fish, in:.
461 Gon, O., Heemstra, P., 1990. Fishes of the Southern Ocean. JLB Smith Institute of
462 Ichthyology, Grahamstown, South Africa.
463 Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases the first enzymatic
464 step in mercapturic acid formation. J. Biol. Chem 249, 7130–7139.
465 Hulley, P.A., 1990. Myctophidae, in: Gon, O., Heemstra, P.C. (Eds.), Fishes of the Southern
466 Ocean. JLB Smith Institute of Ichthyology, pp. 146–178.
467 IPCC, 2019. Summary for Policymakers, in: Shukla, J. Skea, E. Calvo Buendia, V. Masson-
468 Delmotte, H.-O. Pörtner, D. C. Roberts, P. Zhai, R. Slade, S. Connors, R. van Diemen,
469 M. Ferrat, E. Haughey, S. Luz, S. Neogi, M. Pathak, J. Petzold, J. Portugal Pereira, P.
470 Vyas, E. Huntley, K. Kissick, M. Belkacemi, J. Malley, (Eds.), Global Warming of .C. an
471 IPCC Special Report on the Impacts of Global Warming of .C Above Pre-Industrial
472 Levels and Related Global Greenhouse Gas Emission Pathways, in the Context of
473 Strengthening the Global Response to the Threat of Climate Change, Sustainable
474 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

529 Rintoul, S.R., Chown, S.L., DeConto, R.M., England, M.H., Fricker, H.A., Masson-Delmotte,
530 V., Naish, T.R., Siegert, M.J., Xavier, J.C., 2018. Choosing the future of Antarctica.
531 Nature 558, 233–241. doi:10.1038/s41586-018-0173-4
532 Robinson, H.W., Hogden, C.G., 1940. The biuret reaction in the determination of serum
533 proteins I. A study of the conditions necessary for the production of a stable color which
534 bears a quantitative relationship to the protein concentration. J. Biol. Chem 135, 707–
535 725.
536 Roe, H.S.J., Shale, D.M., 1979. A new multiple rectangular midwater trawl (RMT 1+8M) and
537 some modifications to the institute of oceanographic sciences' RMT 1+8. Mar Biol 50,
538 283–288. doi:10.1007/BF00394210
539 Rosa, R., Nunes, M.L., 2003. Biochemical composition of deep-sea decapod crustaceans
540 with two different benthic life strategies off the Portuguese south coast. Deep Sea Res.
541 Part I Oceanogr. Res. Pap. 50, 119–130. doi:10.1016/S0967-0637(02)00147-4
542 Ross, R., Quetin, L.B., 2000. Reproduction in Euphausiacea, in: Krill. Blackwell Science Ltd,
543 Oxford, UK, pp. 150–181. doi:10.1002/9780470999493.ch6
544 Saunders, R.A., Collins, M.A., Shreeve, R., Ward, P., Stowasser, G., Hill, S.L., Tarling, G.A.,
545 2018. Seasonal variation in the predatory impact of myctophids on zooplankton in the
546 Scotia Sea (Southern Ocean). Prog. Oceanogr 168, 123–144.
547 doi:10.1016/j.pocean.2018.09.017
548 Saunders, R.A., Collins, M.A., Ward, P., Stowasser, G., Shreeve, R., Tarling, G.A., 2014.
549 Distribution, population structure and trophodynamics of Southern Ocean
550 *Gymnoscopelus* (Myctophidae) in the Scotia Sea. Polar Biol. 38, 287–308.
551 doi:10.1007/s00300-014-1584-9
552 Saunders, R.A., Hill, S.L., Tarling, G.A., Murphy, E.J., 2019. Myctophid Fish (Family
553 Myctophidae) Are Central Consumers in the Food Web of the Scotia Sea (Southern
554 Ocean). Front. Mar. Sci. 6, 142. doi:10.3389/fmars.2019.00530
555 Seco, J., Xavier, J.C., Bustamante, P., Coelho, J.P., Saunders, R.A., Ferreira, N., Fielding,
556 S., Pardal, M.A., Stowasser, G., Viana, T., Tarling, G.A., Pereira, M.E., Brierley, A.S.,
557 2020. Main drivers of mercury levels in Southern Ocean lantern fish Myctophidae.
558 Environ. Pollut. 264, 114711–10. doi:10.1016/j.envpol.2020.114711
559 Suzuki, N., Uchikawa, K., Yamada, H., Chow, S., 2005. Genetic Divergence and
560 Identification of Two Controversial Lanternfishes Myctophidae: *Diaphus*) Based
561 (Actinopterygii: on Mitochondrial Cytochrome *b* Sequences and PCR-RFLP Analysis.
562 Species Diversity 10, 289–299. doi:10.12782/specdiv.10.289
563 Tou, J.C., Jaczynski, J., Chen, Y.-C., 2007. Krill for human consumption: nutritional value
564 and potential health benefits. Nutr. Rev. 65, 63–77. doi:10.1301/nr.2007.feb.63–77
565 Tremblay, N., Abele, D., 2015. Response of three krill species to hypoxia and warming: an
566 experimental approach to oxygen minimum zones expansion in coastal ecosystems.
567 Mar Ecol Prog Ser 37, 179–199. doi:10.1111/maec.12258
568 Turner, J., Barrand, N.E., Bracegirdle, T.J., Convey, P., Hodgson, D.A., Jarvis, M., Jenkins,
569 A., Marshall, G., Meredith, M.P., Roscoe, H., Shanklin, J., French, J., Goosse, H.,
570 Guglielmin, M., Gutt, J., Jacobs, S., Kennicutt, M.C., Masson-Delmotte, V., Mayewski,
571 P., Navarro, F., Robinson, S., Scambos, T., Sparrow, M., Summerhayes, C., Speer, K.,
572 Klepikov, A., 2013. Antarctic climate change and the environment: an update. Polar
573 Record 50, 237–259. doi:10.1017/S0032247413000296
574 Xavier, J.C., Croxall, J.P., Trathan, P.N., Wood, A.G., 2003. Feeding strategies and diets of
575 breeding grey-headed and wandering albatrosses at South Georgia. Mar Biol 143, 221–
576 232. doi:10.1007/s00227-003-1049-0
577 Xavier, J.C., Peck, L.S., 2015. Life Beyond the Ice, in: Exploring the Last Continent: an
578 Introduction to Antarctica, Marine Ecosystems in the Southern Ocean. Springer
579 International Publishing, Cham, pp. 229–252. doi:10.1007/978-3-319-18947-5_12
580 Yamamoto, T.-O., 1969. Sex Differentiation, in: Hoar, W., Randall, D. (Eds.), Fish
581 Physiology. Deep-Sea Research I, pp. 117–175. doi:10.1016/S1546-5098(08)60113-2
582