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Effects of UV radiation on the RNA/DNA ratio of Copepods from Antarctica and Chile

Paulo F. Lagos, M. Jesús Valdés & Karen Manríquez*

Faculty of Ecology and Natural Resources, School of Marine Science, Marine Research Center, Quintay (CIMARQ), Valparaíso, Chile

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Abstract The effect of ultraviolet (UV) radiation on marine organisms has been an important focus of recent research, with depletion of the ozone layer resulting in increased UV radiation at high latitudes. Several studies have identified negative impacts of UV radiation on the biology of zooplanktonic organisms. This study used the RNA/DNA ratio as a measure of stress in copepod assemblages from Fildes Bay in Antarctica and Quintay Bay on the central coast of Chile, two areas with high UV radiation but different photobiologic histories. Controlled time-light experiments were performed with copepods from the two locations, exposing them to white light, UV light, or darkness. The results showed different responses to UV radiation. Copepods from Fildes Bay showed a slow metabolic response to UV radiation after 4 and 8 h of exposure. Copepods from Quintay Bay showed a fast metabolic response after 4 h of exposure (4 orders of magnitude higher than that for Fildes Bay copepods) followed by a rapid return toward baseline after 8 h of exposure. These different responses probably reflect the time the copepod assemblages have been exposed to increased UV radiation and the extent of adaptive stress responses to cope with that increased UV radiation. The results of this study show that the RNA/DNA ratio is a useful indicator of the physiologic status of marine organisms and is a useful tool to measure the effects of changing environmental conditions on marine ecosystems, such as those associated with global climate change.

Keywords copepods, RNA/DNA ratio, physiology, ultraviolet radiation

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1 Introduction

Zooplankton has a key role in marine ecosystems, and is responsible for the transfer of energy from phytoplankton to higher trophic levels[1-2]. Despite the importance of this role, little is known about the influence of environmental forcing factors on the life cycle dynamics of zooplankton, which limits accurate diagnostics and modeling programs[3].

The different life cycle strategies of copepods (holoplankton or mesozooplankton for part of the life cycle) are fundamental from an ecologic point of view because they allow the dispersal of copepod populations, and therefore favor their survival[4]. In both life stories, zooplankton is sensitive to disturbances in external environmental conditions such as wind, temperature, or ultraviolet (UV) radiation[5-9]. Therefore, zooplankton can be used as a sensitive indicator of environmental changes at diverse space-time scales, including those associated with global climate change[10].

Short- and long-term changes in ambient UV radiation are in part a consequence of changes in atmospheric ozone and climate. Stratospheric ozone depletion is a well-recognized global problem[11], and the Southern Hemisphere is the worst affected. Studies evidenced a 50% decrease in the ozone that covers Antarctica and a 14% per decade increase in the UV radiation (305 nm) in central Chile[12], suggesting

* Corresponding author (email: k.manriquezdiaz@uandresbello.edu)

that the ozone that covers this region is also decreasing. In marine systems, UV radiation is very relevant because approximately 95% penetrates the water surface and up to 50% reaches a depth of 3 m[13]. However, there have been few studies of marine zooplankton in terms of sensitivity to UV radiation[14], which is considered a stress factor related to climate change[7]. Aquatic organisms have developed several strategies to counteract the negative effects of UV radiation[15], sometimes remaining at greater depths in the water column, a strategy described for several zooplanktonic organisms such as copepods[16], cladoceran crustaceans[17], and fish larvae[18]. However, that strategy is not applicable in shallow waters with high transparency, or in locations with strong vertical mixing[9].

A second mechanism used to mitigate the effects of increased UV radiation exposure is associated with the synthesis or acquisition of UV protective or antioxidant compounds. This mechanism has been described for freshwater copepods[7-19] and in several studies of marine copepods[8-16]. Another defense strategy involves enzymatic mechanisms that repair DNA and cellular damage. For example, in copepods, UV radiation modifies the synthesis of enzymes that prevent cell apoptosis[20-21] and proteins that prevent denaturation[8]. Although most studies of UV effects on zooplankton have focused on these responses, others mention how UV radiation affects survival[22], reproduction[23], and damage at the DNA level[23-24].

Physiologic approximations to investigate the effects of UV radiation have been centered on the repair strategies of different zooplankton species. However, at an ecophysiologic level, one of the most commonly used indicators in marine ecology is the RNA/DNA ratio. The amount of DNA in organisms is stable, whereas the amount of RNA is variable and changes with different environmental conditions[25-26]. Studies that incorporate the RNA/DNA ratio have focused mainly on understanding how this index varies with the nutritional state and ontogenetic development of organisms such as mollusks[27], fish[28], phytoplankton[29], and copepods[30-32]. In recent years, the technique has been used to examine changes in the physiologic state of marine organisms in response to physical variables such as temperature[33] and solar radiation[31]. However, few studies have used the RNA/DNA ratio to measure physiologic changes caused by UV radiation in marine zooplankton in Antarctica.

Investigations into how UV radiation impacts on the Antarctic ecosystem began in the 1990s. At that time it became apparent that increasing UV radiation could affect Antarctic krill populations and increase the susceptibility of krill to DNA damage[34-36]. Subsequently, other studies have shown the effects of UV radiation on vertical migration patterns and mortality of copepods[37], but the majority of studies have focused on freshwater copepods or non-Antarctic zooplankton communities. This study is the first to investigate the effects of UV radiation on marine copepods at a community level in contrasting environments, from a physiologic perspective and in terms of photobiologic history. The RNA/DNA ratio was used to examine the effects of UV radiation, applied in a controlled laboratory situation, on copepods from Fildes Bay in Antarctica and from Quintay Bay on the central coast of Chile. The results suggest that the RNA/DNA ratio as a measure of the physiologic state of marine organisms can be a useful tool to investigate and more accurately predict some effects of changes in marine ecosystems that result from climate change and other factors.

2 Methods

2.1 Study locations

Samples were obtained from two locations with important differences in oceanographic and biologic variables. The first study location was Fildes Bay in Antarctica. At this location, three sampling stations were established between 1.47 and 9.6 km from the coast (Figure 1a). The second study location was Quintay Bay, on the central coast of Chile, in the vicinity of Valparaíso. At this location three sampling stations were established between 1 and 1.5 km from the coast (Figure 1b).

2.2 UV Radiation

The intensity of environmental UV radiation ($\mu W \cdot cm^{-2}$) was measured at both study locations using a broadband radiometer (Sper Scientific UVA/B Light Meter). At Fildes Bay, the daily radiation cycle was determined based on three daily measurements (at 0900, 1300, and 1800) during a period of 21 d. Additionally, the intensity of UV radiation was recorded over the 11 h between 0900 and 1900 under two different atmospheric conditions, a clear day (absence of clouds) and a cloudy day (presence of clouds). Similar recordings were made at Quintay Bay, under the same two atmospheric conditions. To correct the UV radiation values obtained for Antarctica and coastal Chile, the data were compared to atmospheric ozone time series obtained from the OMI/AURA L2G satellite of the National Aeronautics and Space Administration (NASA).

2.3 Sample collection

2.3.1 Fildes Bay, Antarctica

Zooplankton samples were collected from the three sampling stations between 15 December 2011 and 8 February 2012 (Figure 1a). Surface samples were collected using a floating epineustonic net with 200 μm mesh size and a 1.2×0.5 m rectangular opening[38]. Samples were also collected from a depth of 15 m using an oblique drag with a WP-2 net with 200 μm mesh size and a 60 cm diameter opening and 2 kg of ballast to sink the net. The two nets were dragged simultaneously for 1 800 s at a speed between 0.25 and 0.51 $m \cdot s^{-1}$, and the process was repeated three times at each sampling station. Both nets were equipped with a flowmeter (Hydrobios) to record the volume of filtered water.

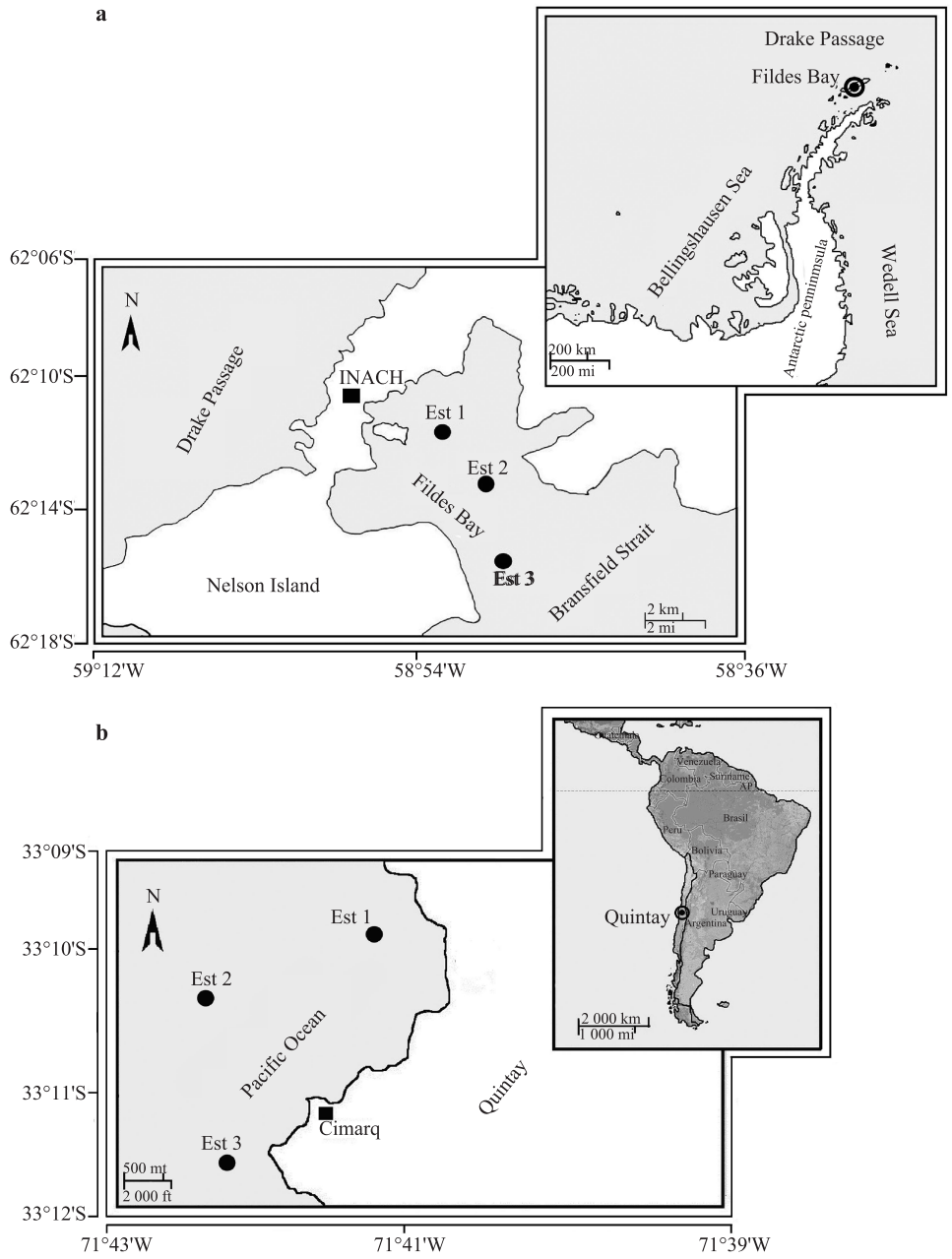

Figure 1 Study locations. **a**, Fildes Bay, Antarctica, (●)sampling stations, (■) location of Professor Julio Escudero Base (INACH), (⊙) South Shetland Islands; **b**, Quintay Bay, central coast of Chile, (●) sampling stations, (■) location of the marine research center (CIMARQ), (⊙) region of Valparaíso, central coast of Chile.

The samples were stored in 1 L plastic jars in a solution of absolute alcohol for later analysis of abundance. One quarter of each sample was kept *in vivo* and transported to the laboratories of the Chilean Antarctic Institute (INACH) for UV exposure experiments. In addition to sample collection, oceanographic data (temperature, salinity, and density) were obtained using a CTD profiler instrument (Seabird SBE-19) that was positioned using a GPS at each of the sampling points.

2.3.2 Quintay Bay, central coast of Chile

Samples at Quintay Bay were collected from the three sampling stations during March 2013 (Figure 1b). Surface samples were collected using a floating epineustonic net as previously described. Samples at 12 mt depth were collected using a WP-2 net dragged for a period of 1 200 s at a constant speed between 0.25 and 0.51 $m \cdot s^{-1}$. However, because of weather conditions during the sampling period, deep samples were not obtained from Station 2. The samples were stored in 1 L plastic jars in a solution of absolute alcohol for later analysis of abundance. One quarter of each sample was kept *in vivo* and transported to the Marine Research Center,

Quintay (CIMARQ), Andrés Bello University, for UV exposure experiments. Additionally, oceanographic data from the three stations were collected using a CTD profiler (3" Micro CTD; Falmouth Scientific Inc.) that provided temperature, salinity, and density data for the water column from 1 to 26 m (Figure 2) .

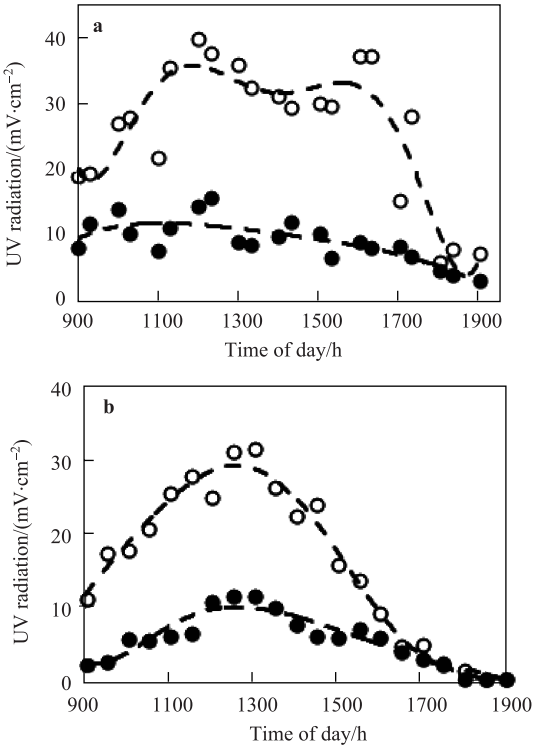

Figure 2 Graphs of field measurements of ultraviolet (UV) radiation during clear sky conditions (○) and cloudy conditions (●) for Fildes Bay, Antarctica (**a**); Quintay Bay, region of Valparaíso, central coast of Chile (**b**).

2.4 Experiments with UV radiation

The subsamples (one quarter of each sample) from Fildes Bay, kept *in vivo* with natural salt water, were rapidly transported to the laboratory at the INACH. They were placed in an experimental system that consisted of three 15 L plastic containers completely covered with tin foil to prevent contamination by outside light sources. Each plastic container received a different light treatment. The first container (UV) received only UV light for exposure periods of 4 and 8 h. The UV light was provided by two 40-W UV lamps (Phillips Actinic BL) that emitted a dose of UV radiation (316–400 nm) at 6 μW·cm^{-2}. The lamps were placed at the top of the container and their upper side was covered with tin foil. A second container, the positive control C(+), received only white light provided by a 38-W LED lamp. This system was refrigerated by air with a radiator (Uninov) to prevent the lamp from generating heat. The third container, the negative control C(-), was kept in darkness throughout the experiments. A system of three Petri plaques was placed inside each container, each containing samples of approximately <1 000 copepods of different species and developmental stages. Sample size variation was corrected by weighing and standardizing copepod samples prior to nucleic acid extraction. A second plaque containing ice was placed under each Petri plaque to maintain water temperature stability during the experiments.

In the first step of the experiment, the zooplankton samples were irradiated with either white light or UV light, or were kept in darkness for 4 h. At the end of that period, and within 5 min to minimize lag between irradiation periods, five 1 mL samples containing copepods were extracted from each Petri plaque using a 1 mL plastic pipette and placed in Eppendorf tubes. After the first extraction, the state of the copepods was analyzed visually to determine any mortality. Following this, the remaining copepods were treated for another period of 4 h, completing a total of 8 h of exposure to white light, UV light, or darkness. At the end of 8 h, copepods were again extracted as described for the 4 h sample. No food was provided to copepods during the experiments.

The same protocol was used for the subsamples collected at Quintay Bay during March 2013. These samples were immediately transported to the laboratories at the Marine Research Center, Quintay (CIMARQ), Andrés Bello University, where the experiments were performed.

2.5 Sample storage

The experimental samples of copepods from Fildes Bay were stored in liquid nitrogen and transported to the laboratories at Valtek S.A in Santiago, Chile, where they were kept at −80°C for later molecular analysis. The experimental samples of copepods from Quintay Bay were stored at −21°C and transported to the laboratories of Valtek S.A. where they were stored at −80°C for later molecular analysis.

2.6 Nucleic extraction procedures

The RNA and DNA extraction was performed with whole adult organisms (based on visual assessment of copepod sizes), but the accidental incorporation of juvenile copepods could not be ruled out. The extraction was performed on samples of a standardized total weight of 10 ± 0.01 mg measured using an analytic scale (Ohaus Pioneer). This weight was selected according to procedures described in previous studies[32-33].

The copepods were placed in 2 mL Eppendorf tubes and then macerated by suction with a 10 mL syringe and subsequently using a sonic tissue disruptor (Sonic Ruptor 250; Omni International) with guanidinium thiocyanate (GTC) lysis buffer plus 20 μL of 2-mercaptoethanol for each 1 mL of GTC lysis buffer. Extraction and isolation of DNA and RNA was performed simultaneously using a Total DNA/RNA Isolation Kit (Omega Bio-Tek), following the manufacturer's instructions.

2.6.1 Quantification of RNA and DNA

Each sample was diluted with 800 μl of Tris-EDTA buffer

and the absorbance of the samples was measured using a spectrophotometer (UV1800). Absorbance was measured at two different wavelengths, 260 nm and 280 nm. The purity, quantity (μg·mL^{-1}), and RNA/DNA ratio were quantified according to the methodology described in previous reports[25,39-40].

2.7 Statistical analysis

A linear correlation analysis was used to evaluate the relationship between atmospheric ozone and UV radiation values at Fíldes Bay. General linear models (GLMs), one-way analysis of variance (ANOVA), and Tukey post-hoc analysis were used evaluate significant differences between the RNA/DNA ratios of copepods from the two study locations, using the values of the RNA/DNA ratio as dependent variables. All analyses were performed using Statistica v7.0 software.

3 Results

3.1 UV radiation

Measurements at Fíldes Bay revealed considerable daily variability in the intensity of UV radiation that reached the Earth's surface. The maximum radiation occurred at 1300 on the clear sky day and cloudy day, respectively. The minimum radiation occurred at 0900 in the morning on the clear sky day and cloudy day, respectively and at 1900 in the evening on the clear sky day and cloudy day, respectively (Figure 2a). A similar situation was observed for Quintay Bay, with maximum radiation close to noon on the clear sky day and cloudy day, respectively and minimum radiation at 0900 in the morning on the clear sky day and cloudy day, respectively and at 1900 in the evening on the clear sky day and cloudy day (Figure 2b). The results of the time series of 21 days at Fíldes Bay indicated a broad synoptic variability in the values of UV radiation, with different maximum and minimum values of radiation at the same time on different days. Linear regression analysis of ozone and UV radiation at the Antarctic study location did not reveal a significant correlation, although there was an inverse relationship between the ozone concentration and the intensity of UV radiation ($R^2 = -0.16$, $P>0.05$).

3.2 Water column profiles

At Fíldes Bay, the water temperature ranged from 1.15°C to 1.43°C among stations, and was only slightly colder (about 0.3°C) in deeper layers of the water column (coldest at 15–18 m) with no pronounced thermocline (Figure 3a). At Quintay Bay, water temperature was about 3°C colder in deeper layers of the water column at stations E2 and E3,

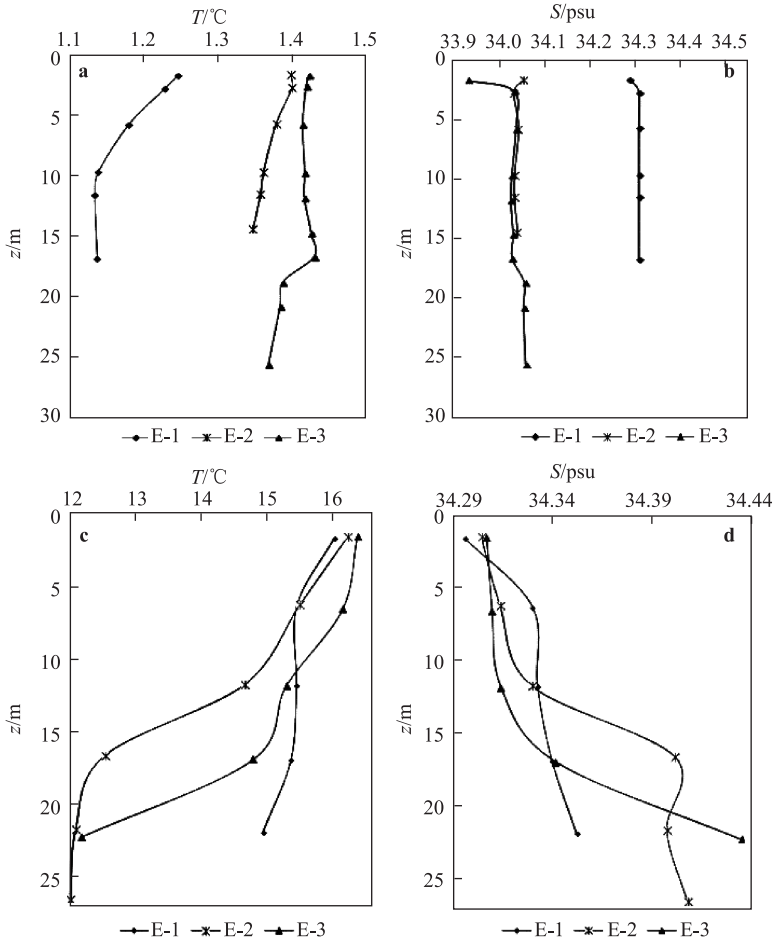

Figure 3 Temperature and salinity profiles for the three sampling stations at each study location. Fíldes Bay (**a**, **b**); Quintay Bay (**c**, **d**) .

with a clear thermocline at around 10–15 m (Figure 3c). Salinity at Fíldes Bay showed minimal variability from the surface to 15–25 m. There were differences in salinity among the three stations with a difference of almost one order of magnitude between station E1 and the other two stations, E2 and E3 (Figure 3b). At Quintay Bay the deeper layers of the water column were considerably more saline. There was a consistent change in salinity at 15–18 m, which was congruent with the existence of a thermocline at the same depth (Figure 3d).

3.3 Abundance of zooplankton at Fíldes Bay and Quintay Bay

The number of taxa at Fíldes Bay was less than half that at Quintay Bay. At both locations the dominant group was copepods but the abundance was up to 97.8% higher at Quintay Bay. At Fíldes Bay, copepods, mollusks and amphipods were the three most dominant groups and together accounted for 95% of the total abundance of organisms (Table 1). At Quintay Bay, copepods, ichthyoplankton, and amphipods were the three most dominant groups and together accounted for 99% of the total abundance of organisms (Table 2). Comparing the abundance of copepods caught in the different nets at both locations, at Fíldes Bay 57% of the copepods were caught near the surface with the epineustonic net, whereas at Quintay Bay 51% were caught with the WP-2 net at the deeper sampling depths used in this study.

3.4 RNA/DNA ratio of copepods from Fíldes Bay and Quintay Bay

Extractions using the methodology described in Section 2.6 produced large amounts of nucleic acids from small tissue samples. Average yields of 41.6 $\mu g \cdot mL^{-1}$ of RNA and 17.6 $\mu g \cdot mL^{-1}$ of DNA were achieved for Fíldes Bay samples, while for Quintay Bay samples the average yields were slightly lower at 41.26 $\mu g \cdot mL^{-1}$ of RNA and 13.496 $\mu g \cdot mL^{-1}$ of DNA (Table 3). A high level of purity of nucleic acids was achieved in the majority of extractions. For Fíldes Bay samples the purity of RNA/DNA was no less than 50%. For one sample from Quintay Bay pollutants dropped the purity level to lower than 10% but all other samples had purity of not less than 70% (Table 4).

After 4 h, the RNA/DNA ratio of UV-exposed copepods from Fíldes Bay was significantly higher compared with the negative control at 4 h and the positive control at 8 h, and significantly lower compared with the ratio of UV-exposed

Table 1 Relative abundance of major taxa of zooplankton found at Fíldes Bay, Antarctica

Abundance/(ind·1 000 m^{-3})									
	Sampling stations							Net types	
	EST1 EPI	EST1 WP2	EST2 EPI	EST2 WP2	EST3 EPI	EST3 WP2	Total	EPI	WP2
Mollusk	29	0	0	0	3	8	40	8	32
Copepods	1 270	204	173	664	593	648	3 552	2 036	1 516
Euphausids	21	0	0	0	3	8	32	24	8
Ictioplanckton	0	0	0	0	0	8	8	0	8
Amphipods	21	4	0	0	6	4	35	27	8
N/Id	14	11	11	13	15	24	88	40	48
Total	1 384	219	195	681	620	704	3 803	2 175	1 628

Table 2 Relative abundance of major taxa of zooplankton found at Quintay Bay, central coast of Chile

Abundance/(ind·1 000 m^{-3})									
	Sampling stations							Net type	
	EST1 EPI	EST1 WP2	EST2 EPI	EST2 WP2	EST3 EPI	EST3 WP2	Total	EPI	WP2
Molusk	0	37	7	0	0	0	44	7	37
Copepods	34 071	40 782	42 049	41 897	3 313	0	162 112	79 433	82 679
Ictioplanckton	262	58	331	79	0	0	730	593	137
Anphipods	0	0	3	0	329	0	332	332	0
Quetognatos	112	0	3	3	16	0	134	131	3
Crostacean	37	67	1	0	7	0	112	45	67
Yellifish	0	14	1	0	2	0	17	3	14
Ostracods	0	0	5	0	0	0	5	5	0
Siphonofora	0	0	3	3	120	0	126	123	3
Salpidae	0	0	0	0	2	0	2	2	0
Apendicularia	0	0	0	0	2	0	2	2	0
Total	34 482	40 958	42 396	41 982	3 791	0	163 616	80 676	82 940

copepods from Quintay Bay at 4 h (Tukey $P<0.05$; Figure 4). However, there was no significant difference between the RNA/DNA ratio of UV-exposed copepods from Fildes Bay at 4 h and the ratio of UV-exposed copepods from Quintay Bay at 8 h (Tukey $P>0.05$). There was no significant difference in ratios between the positive and negative controls at 4 or 8 h (Tukey $P>0.05$). After 8 h the RNA/DNA ratio of UV-exposed copepods from Fildes Bay was significantly higher compared with the ratio at 4 h, and significantly higher compared with positive and negative controls at 4 and 8 h (Tukey $P<0.05$). However, there was no significant difference between the RNA/DNA ratio of the UV-exposed group from Fildes Bay at 8 h and the ratio for UV-exposed copepods from Quintay Bay at 4 h (Tukey $P>0.05$).

After 4 h, the RNA/DNA ratio of the UV-exposed copepods from Quintay Bay was significantly higher compared with both positive and negative controls at 4 h, and compared with UV-exposed copepods from Fildes Bay at 4 h (Tukey $P<0.05$), but not compared with copepods from Fildes Bay at 8 h (Tukey $P>0.05$). The ratio decreased significantly from 4 to 8 h, and at 8 h there was no significant difference between the ratio of the UV-exposed copepods and positive or negative controls (Tukey $P>0.05$). However, at 8 h the RNA/DNA ratio of UV-exposed copepods from Quintay Bay was significantly lower compared with the ratio of UV-exposed copepods from Fildes Bay (Tukey $P<0.05$).

Analysis of the effects of the independent variables on the RNA/DNA ratio of copepods from the Antarctica and the central coast of Chile showed that the interaction between the time of exposure to the different treatments was not a determining factor of the RNA/DNA ratio (ANOVA, $F(1,24)=0.01$; $P=0.91$). However, differences associated with the location were observed (ANOVA, $F(1,24)=4.61$; $P=0.041$). The analysis showed that in Antarctic copepods the RNA/DNA ratio was lower after 4 h of exposure to UV radiation and higher after 8 h of exposure to UV radiation. Conversely, copepods from the central coast of Chile showed a higher RNA/DNA ratio after 4 h of exposure to UV radiation and a lower ratio after 8 h of exposure, with responses that depended on the treatment, the time of exposure, and the location (ANOVA, $F(2,24)=24.102$, $P<0.05$; Figure 4).

Table 3 Average RNA and DNA concentrations of copepods from Fildes Bay and Quintay Bay exposed to 4 and 8 h of ultraviolet (UV) radiation

DNA(μg·mL^{-1})	Fildes Bay			Quintay Bay		
Treatments	**N**	**Average**	**±SD**	**N**	**Average**	**±SD**
C(-)/4 h	3	25.97	2.29	3	21.97	2.30
C(+)4 h	3	20.29	0.85	3	23.68	0.08
UV/4 h	3	9.31	0.77	3	12.49	0.26
Treatments	**N**	**Average**	**±SD**	**N**	**Average**	**±SD**
C(-)/8 h	3	19.00	1.22	3	2.11	0.70
C(+)/8 h	3	17.94	2.14	3	7.67	0.29
UV/8 h	3	13.19	1.13	3	13.02	1.66
RNA(μg·mL^{-1})	Fildes Bay			Quintay Bay		
Treatments	**N**	**Average**	**±SD**	**N**	**Average**	**±SD**
C(-)/4 h	3	37.10	3.53	3	42.07	0.56
C(+)4 h	3	36.56	1.23	3	48.61	10.08
UV/4 h	3	34.29	6.52	3	86.09	7.28
Treatments	**N**	**Average**	**±SD**	**N**	**Average**	**±SD**
C(-)/8 h	3	35.60	0.64	3	4.23	0.10
C(+)/8 h	3	23.71	14.64	3	23.71	14.64
UV/8 h	3	82.68	10.67	3	42.80	5.71

Notes: Positive control C(+), negative control C(-),UV-exposed group (UV).

Table 4 Average purity obtained from RNA and DNA extractions of copepods from Fildes Bay and Quintay Bay exposed to 4 and 8 h of ultraviolet (UV) radiation

A260/A280RNA	Fildes Bay			Quintay Bay		
Treatments	**N**	**Average**	**±SD**	**N**	**Average**	**±SD**
C(-)/4 h	3	2.0	0.181	3	1.9	0.043
C(+)4 h	3	1.9	0.172	3	2.2	0.375
UV/4 h	3	1.8	0.089	3	2.3	0.146
Treatments	**N**	**Average**	**±SD**	**N**	**Average**	**±SD**
C(-)/8 h	3	1.9	0.130	3	1.7	0.671
C(+)/8 h	3	2.3	0.474	3	2.3	0.474
UV/8 h	3	1.8	0.064	3	2.1	0.041
A260/A280DNA	Fildes Bay			Quintay Bay		
Treatments	**N**	**Average**	**±SD**	**N**	**Average**	**±SD**
C(-)/4 h	3	2.4	0.439	3	1.8	0.205
C(+)4 h	3	1.5	0.285	3	1.7	0.330
UV/4 h	3	2.7	0.493	3	2.3	0.083
Treatments	**N**	**Average**	**±SD**	**N**	**Average**	**±SD**
C(-)/8 h	3	2.0	0.071	3	0.7	0.194
C(+)/8 h	3	2.0	0.239	3	2.4	1.023
UV/8 h	3	1.9	0.237	3	1.7	0.285

Notes: Positive control C(+), negative control C(-), and UV-exposed group (UV).

4 Discussion

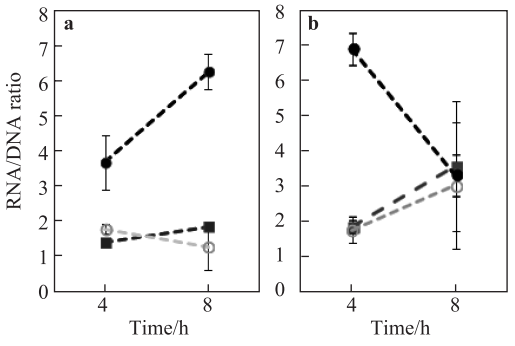

Figure 4 General linear model (ANOVA) for RNA/DNA ratio of copepods from the two study locations exposed to 4 and 8 h of ultraviolet (UV) radiation. **a**, copepods from Fildes Bay, Antarctica; **b**, copepods from Quintay Bay, central coast of Chile. (■) Negative control, (O) positive control, (●) UV-exposed. Vertical lines show interaction $F(2, 24)=24.102$, $P=0.000$. Data ± standard deviation.

4.1 UV radiation measurements

The measurements of UV radiation at both study locations showed a daily cycle with, as expected, high intensities close to noon, and low intensities during the morning and evening. The level of UV radiation was higher at Quintay Bay on the central coast of Chile than at Fildes Bay in Antarctica. This may be a result of the relatively short measurement cycle and short periods of cloudy weather during sunny days during the measurements in Antarctica. Longer measurement periods are warranted in future studies.

There is a lack of information on UV radiation at the Antarctic study location. Indexes of spectral UV radiation obtained in the present study were similar to those in a previous study[41] that reported a range of 1–90 $\mu W \cdot cm^{-2}$ for a time series of 360 d at an Antarctic study location closer to the south pole than the present study location. Another study reported UV radiation intensity of 1.8–8 $W \cdot m^{-2} \cdot nm^{-1}$ for a time series of 2 a between 2008 and 2009[42]. Considering the geographic and physical variation of the available information, it is essential to find new alternatives to validate atmospheric data.

The relationship between the ozone concentration and UV radiation intensity has been studied extensively in both Antarctica and continental Chile[43-46]. Studies have used the relationship between the ozone concentration and solar radiation as an indicator of data quality[47]. In our study, the relationship between ozone and UV radiation at the Antarctic study location was negative as expected, although the relationship was not statistically significant, again probably because of the relatively short measurement period.

4.2 Distribution and abundance of copepods

There were clear differences in the distribution and abundance of copepods in Fildes Bay compared with assemblages described in other reports for the Antarctic zone. Previous studies at Almirantazgo Bay, Tierra Del Fuego[48-50] reported that representatives of Oithonidae were the dominant group and members of Calanidae were the second most abundant group. At the copepod family level, a total of 14 families were found in Fildes Bay, including Cyclopinidae, Oncaeidae, Oithonidae, Candaciidae, Metrinidae, Augaptilidae, Scolectrichidae, Phaennidae, Aetideidae, Clausocalanidae, Spinocalanidae, Eucalanidae, Calanidae, and Megacalanidae, with the dominant families being Oithonidae (38%), Calanidae (26%), and Augaptilidae (13%). These three families accounted for 77% of the total biomass of copepods found in Fildes Bay, while the rest of the taxa contributed about 1% to 3% each (Figure 5).

There were also clear differences between the distribution and abundance of copepods in Antarctica and Quintay Bay, and between previously published data for the central Chile region. Several studies have identified members of the families Calanidae, Paracalanidae, and Oithonidae as the most abundant copepods in the central coastal region of Chile[51-52], but in specific zones within the central coastal region changes in the community structure may occur. Members of the family Paracalanidae have been reported as the most abundant in the northern/central coastal region of Chile while members of the families Oithonidae and Calanidae are more abundant in the southern/central coastal region[52-53]. These differences are to be expected, considering the different oceanographic conditions between zones, and indicate that assemblages are comparable between study locations in terms of the families present but not necessarily in terms of their relative contribution. It has been reported that 83% of species from the Antarctic zone are cosmopolitan, so overlapping distribution between study locations, at least on a family level, was expected. This was the case for the Oithonidae and Calanidae families that were strongly represented at both study locations[49,50-54].

4.3 RNA/DNA ratios

Changes in climate may alter the geographic and vertical distribution of organisms in aquatic and terrestrial

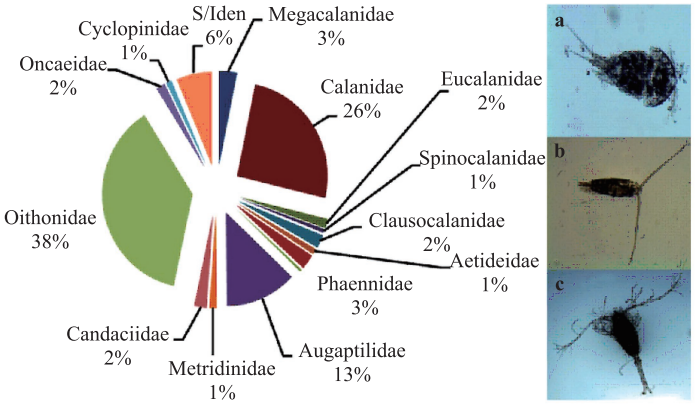

Figure 5 Percent composition of the copepod families found at Fildes Bay, Antarctica (Left). Images of representatives of the three families of copepods that accounted for 77% of the total abundance (Right); **a**, Augaptilidae, **b**, Calanidae, and **c**, Oithonidae.

ecosystems, exposing them to different levels of UV radiation with positive and negative effects. Responses to UV radiation are integral to how organisms function. In a changing climate some of these responses will be modified, resulting in benefits to some organisms and ecosystems and negative effects on others[55]. The RNA/DNA ratio was measured in this study to determine the physiologic status of zooplankton communities from two different locations in terms of photobiologic history and response to UV radiation. Increased transcription rates cause an increase in the RNA/DNA ratio[56]. The results showed that basal RNA/DNA ratios, as measured in the control treatments at the first time point, were similar between copepods from the two study locations. It is unclear why basal metabolic rates from two different locations with different temperatures and trophic states were similar. However, there are complex interactions among temperature and other environmental variables that may affect nucleic acid content[57] and act as additional homogenizing factors, as evidenced by the similarity in the families of copepods represented and the fact that no mortality was registered during the experiments.

The present study investigated the response to changing UV radiation at a community level, using changes in the RNA/DNA ratio. Whole copepod communities from both locations were included in the samples. Therefore, the samples included a large number of individuals with different metabolic basal states. In that sense, the basal metabolic rates found in this study could be a true representation of basal metabolic rates, but there are still questions to be addressed. The very different responses between the UV-exposed copepods from the two locations cannot be definitively explained, because the RNA/DNA ratios reflect a global level of metabolic activity and do not indicate which genes are responsible for the increased transcription levels. However, our interpretation of the results is as follows. The UV-exposed copepod samples from Fíldes Bay showed an increased RNA/DNA ratio compared with controls after 4 h of UV exposure, because stress-related genes were up-regulated. This continued on to even higher ratios after 8 h, as stress continued. In contrast, UV-exposed samples from Quintay Bay showed a rapid increase in RNA/DNA ratios after 4 h as stress response genes were up-regulated but the effect of this up-regulation was that stress was reduced and the ratios returned to levels comparable with the control groups. This suggests that copepods from the central coast of Chile have a more effective response to UV exposure that enables them to cope with UV exposure to some extent.

Specific adaptations such as pigmentation can delay the negative effects of UV radiation[20]. Some species of copepods accumulate carotenoids in response to high UV exposure[7], but most previous research has focused on freshwater copepods. In the present study, copepods from the central coast of Chile responded faster with immediate changes in metabolism to cope with the exposure to UV radiation. Future research should investigate the type of UV response and should determine whether the response is related to pigmentation or to other mechanisms that help copepods to deal with UV radiation. We believe that this response is probably related to the time scale of the photobiologic history. The copepods from the central coast of Chile have been exposed to high doses of UV radiation in the past few years, but those from Antarctica have been exposed to high UV radiation for the past few decades[46,58-59].

The oceanography of the Fíldes Bay location was more stable and the waters were more transparent compared with the Quintay Bay location[60]. These differences facilitated the penetration of UV light at Fíldes Bay compared to Quintay Bay, which had more dynamic oceanographic features and upwelling events that resulted in a greater amount of particles in suspension, masking the effects of UV radiation. It was not unexpected that copepods from Quintay Bay showed a higher RNA/DNA ratio than those from Fíldes Bay, independent of experimental exposure to UV radiation, because of the shorter photobiologic history associated with oceanographic factors that mask the effects of UV radiation. Although higher RNA/DNA ratios are observed after short-term exposure to UV radiation, this pattern is known to be a long-term response wherein a decrease in the basal levels of the RNA/DNA ratio could be related to compensatory mechanisms (such as the heat shock protein Hsp70) that decrease the stress produced in the initial stages of increased UV radiation. These mechanisms could be less visible for copepods from Fíldes Bay, although it remains unclear whether, with longer exposure to UV radiation, the copepods from Fíldes Bay would show a return to baseline RNA/DNA ratios, which would indicate that the responses of the copepods from the two study locations were asynchronous.

The RNA/DNA ratios found in the present study corresponded to the ranges for copepods and other organisms reported in previous studies[29,32-33,46-61]. However, there is a need for better understanding of how the RNA/DNA ratio changes in different ontogenic stages and in different copepod species, to ensure that the methods used in this study have the necessary validity to explain physiologic responses related to UV radiation.

References

1 Banse K. Zooplankton: Pivotal role in the control of ocean production. ICES J Mar Sci, 1995, 52(3–4): 265–277

2 Williams R, Conway D V P, Hunt H G. The role of copepods in the planktonic ecosystems of mixed and stratified waters of the Europeans shelf seas. Hydrobiologia, 1994, 292–293: 521–530

3 Zagarese H E, Williamson C E. Modeling the impacts of UV-B radiation on ecological interactions in freshwater and marine ecosystems// Biggs R H, Joyner M E B. Stratospheric ozone depletion/UV-B radiation in the biosphere. Berlin, Heidelberg: Springer, 1994: 315–328

4 Lalli C M, Parson T R. Biological oceanography: an introduction, 2nd edn. Butterworth Heinemann: Elsevier, 1997

5 Harris R P, Irigoien X, Head R N, et al. Feeding, growth, and reproduction in the genus *Calanus*. ICES J Mar Sci, 2000, 57(6):

1708–1726
6 Irigoien X, Harris R P, Head R N. Does turbulence play a role in feeding and reproduction of *Calanus finmarchicus*?. J Plankton Res, 2000, 22(2): 399–407
7 Rautio M, Tartarotti B. UV radiation and freshwater zooplankton : damage, protection and recovery. Freshw Rev, 2010, 3(2): 105–131
8 Tartarotti B, Torres J J. Sublethal stress: impact of solar UV radiation on protein synthesis in the copepod *Acartia tonsa*. J Exp Mar Biol Ecol, 2009, 375(1–2): 106–113
9 Zagarese H E, Cravero W, Gonzales P, et al. Copepod mortality induced by fluctuating levels of natural ultraviolet radiation simulating vertical water mixing. Limnol Oceanogr, 1998, 43(1): 169–174
10 Beaugrand G, Reid P C, Ibañez F, et al. Reorganization of North Atlantic Marine Copepod Biodiversity and Climate. Science, 2002, 296(5573): 1692–1694
11 Erlykin A D, Sloan T, Wolfendale A W. Clouds, solar irradiance and mean surface temperature over the last century. J Atmos Sol-Terr Phy, 2010, 72(5–6): 425–434
12 Cabrera S, Moreno G, Fuenzalida H. Radiación ultravioleta en Chile: variaciones con la altura y la latitud. Bol Cient Asoc Chil Segur, 2000, 2(4): 66–71
13 Delgado L. Índice ultravioleta. Departamento de Física, Universidad de Antofagasta. 2003
14 Häder D P, Kumar H D, Smith R C, et al. Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. Photochem Photobiol, 2007, 6(3): 267–285
15 Zengling M A, Wei L I, Kunshan G A O. Horizontal migration of *Acartia pacifica* Steuer (copepoda) in response to UV-radiation. J Photochem Photobiol B: Biol, 2010, 101(3): 233–237
16 Yu J, Yang G P, Tian J Y. Effects of UV-B radiation on ingestion, fecundity, population dynamics and antioxidant enzyme activities of *Schmackeria inopinus* (Copepoda Calanoida). J Exp Mar Biol Ecol, 2009, 381(2): 74–81
17 Leech D M, Padeletti A, Williamson C E. Zooplankton behavioral responses to solar UV radiation vary within and among lakes. J Plankton Res, 2005, 27(5): 461–471
18 Speekmann C L, Bollens S M, Avent S R. The effect of ultraviolet radiation on the vertical distribution and mortality of estuarine zooplankton. J Plankton Res, 2000, 22(12): 2325–2350
19 Rocco V E, Oppezzo O, Pizarro R, et al. Ultraviolet damage and counteracting mechanisms in the freshwater copepod *Boeckella poppei* from the Antarctic Peninsula. Limnol Oceanogr, 2002, 47(3): 829–836
20 Souza M S, Modenutti B E, Balseiro E G. Antioxidant defenses in planktonic crustaceans exposed to different underwater light irradiances and Andean lakes. Water Air Soil Pollut, 2007, 183(1–4): 49–57
21 Souza M S, Hansson L A, Hylander S, et al. Rapid enzymatic response to compensate UV radiation in copepods. PLoS One, 2012, 7(2): e32046
22 Mangel M, Richerson K, Cresswell K A, et al. Modelling the effects of UV radiation on the survival of Antarctic krill (*Euphausia superba Dana*) in the face of limited data. Ecol Model, 2010, 221(17): 2095–2101
23 Browman H I, Vetter R D, Rodríguez C A, et al. Ultraviolet (280–400 nm) induced DNA damage in the eggs and larvae of *Calanus finmarchicus* G. (Copepoda) and Atlantic Cod (*Gadus morhua*). Photochem Photobiol, 2003, 77(4): 397–404
24 Browman H I, Rodríguez C A, Béland F, et al. Impact of ultraviolet radiation on marine crustacean zooplankton and ichthyoplankton: a synthesis of results from the estuary and gulf of St. Laurence, Canada. Mar Ecol Prog Ser, 2000, 199: 293–311
25 Chícharo A M, Chícharo L. RNA: DNA ratio and other nucleic acid derived indices in marine ecology. Int J Mol Sci, 2008, 9(8): 1453–1471
26 Laflamme S, Côté C, Gagnaire P, et al. RNA/DNA ratios in American glass eels (*Anguila rostrata*): evidence for latitudinal variation in physiological status and constrains to oceanic migration?. Ecol Evol, 2012, 2(5): 875–884
27 Jamius W Y, Leung K M. Effect of animal size and nutritional status on the RNA/DNA ratio in different tissues of the green-lipped mussel (*Perna viridis*). J Mar Biol Assoc UK, 2011, 93(1): 217–225
28 Pepin P, Evans G T, Shears T H. Patterns of RNA/DNA ratios in larval fish and their relationship to survival in the field. ICES J Mar Sci, 1999, 56: 697–706
29 Dortch Q, Roberts T L, Clayton J R, et al. RNA/DNA ratios and DNA concentrations as indicators of growth rate and biomass in planktonic marine organisms. Mar Ecol Prog Ser, 1983, 13: 61–71
30 Pommier J, Frenette J J, Glémet H. Relating RNA: DNA ratio in *Eurytemora affinis* to ceston fatty acids in a highly dynamic environment. Mar Ecol Prog Ser, 2010, 400: 143–154
31 Pommier J, Frenette J J, Massicotte P, et al. Seston fatty acid composition and copepod RNA : DNA ratio with respect to the underwater light climate in fluvial Lac Saint-Pierre. Aquat Sci, 2012, 74(3): 539–553
32 Wagner M, Durbin E, Buckley L. RNA: DNA ratios as indicators of nutritional condition in the copepod *Calanus finmarchicus*. Mar Ecol Prog Ser, 1998, 162: 173–181
33 Speekmann C L, Nunez B S, Buskey E J. Measuring RNA: DNA ratios in individual Acartia tonsa (Copepoda). Mar Biol, 2006, 151(2): 759–766
34 Jarman S, Elliot N, Nicol S, et al. The base composition of the krill genome and its potential susceptibility to damage by UVB. Antarct Sci, 1999, 11(1): 23–26
35 Naganobu M, Kutsuwada K, Sasai Y, et al. Relationship between Antarctic Krill (*Euphausia superba*) variability and westerly fluctuations and ozone depletion in the Antarctic Peninsula area. J Geophys Res, 1999, 104(C9): 20561–20665
36 Newman S J, Nicol S, Ritz D, et al. Susceptibility of Antarctic krill (*Euphausia superba Dana*) to ultraviolet radiation. Polar Biol, 1999, 22(1): 50–55
37 Ban S, Ohi N, Leong S C Y, et al. Effects of solar ultraviolet radiation on survival of Krill larvae and copepods in Antarctic Ocean. Polar Biol, 2007, 30(10): 1295–1302
38 DiSalvo L H. Observations on the larval and post-metamorphic life of *Concholepas concholepas* (Bruguiére, 1789) in laboratory culture. Veliger, 1988, 30: 358–368
39 Barbas C F, Burton D R, Scott J K, et al. Quantification of DNA and RNA. Cold Spring Harb Protoc, 2007
40 Berdalet E, Roldán C, Olivar P M. Quantifying RNA and DNA in planktonic organisms with SYBR Green II and nucleases. Part B. Quantification in natural samples. Sci Mar, 2005, 69(1): 17–30
41 Lubin D, Frederick J E. The ultraviolet radiation environment of the Antarctic Peninsula: The roles of Ozone and Cloud Cover. J Appl Meteor, 1991, 30(4): 478–493
42 Vitale V, Petkov B, Goutail F, et al. Variations of UV irradiance at Antarctic station Concordia during the springs of 2008 and 2009. Antarct Sci, 2009, 23(4): 389–398
43 Casiccia C, Kirchhoff V W J H, Torres A. Simultaneous measurements of ozone and ultraviolet radiation: spring 2000, Punta Arenas, Chile. Atmos Environ, 2003, 37(3): 383–389
44 Junk J, Feister U, Helbig A, et al. The benefit of modeled ozone

data for the reconstruction of a 99-year UV radiation time series. J Geophys Res, 2012, 117(D16), doi: 10.1029/2012JD017659
45 Karentz D. Considerations for evaluating ultraviolet radiation-induced genetic damage relative to Antarctic ozone depletion. Environ Health Persp, 1994, 102(3): 61–63
46 Purich A, Son S W. Impact of Antarctic ozone depletion and recovery on southern hemisphere precipitation, evaporation and extreme changes. J Climate, 2012, 25(9): 3145–3154
47 Xiong F S, Day T A. Effect of solar ultraviolet-B radiation during springtime ozone depletion on photosynthesis and biomass production of Antarctic vascular plants. Plant Physiol, 2001, 125(2): 738–751
48 Razouls S, Razouls C, de Bovée F. Biodiversity and biogeography of Antarctic copepods. Antarct Sci, 2000, 12(3): 343–362
49 Walkusz W, Kwaśniewski S, Dmoch K, et al. Characteristics of the Arctic and Antarctic mesozooplankton in the neritic zone during summer. Pol Polar Res, 2004, 25(3–4): 275–291
50 Zmijewska M. *Copepoda* in the southern part of Drake Passage and in Bransfield Strait during early summer. Polar Res, 1985, 6(1–2): 79–93
51 Hidalgo P, Escribano R, Vergara O, et al. Patterns of copepods diversity in the Chilean coastal upwelling system. Deep-Sea Res, 2010, 57(24–26): 2089–2097
52 Manríquez K, Escribano R, Riquelme-Bugueño R. Spatial structure of the zooplankton community in the coastal upwelling system off central-southern Chile in spring 2004 as assessed by automated image analysis. Prog Oceanogr, 2012, 92–95: 121–133
53 Castro L R, Troncoso V A, Figueroa D R. Fine-scale vertical distribution of coastal and offshore copepods in the Golfo de Arauco, central Chile, during the upwelling season. Prog Oceanogr, 2007, 75(3): 486–500
54 Takahashi K T, Kawaguchi S, Hosie G W, et al. Surface zooplankton distribution in the Drake Passage recorded by Continuous Plankton Recorder (CPR) in late austral summer of 2000. Polar Sci, 2010, 3(4): 235–245
55 Williamson C E, Zeep R G, Lucas R M, et al. Solar ultraviolet radiation in changing climate. Nat Clim Change, 2014, 4(6): 434–441
56 Spicer J I, Gaston K J. Physiological diversity: ecological implications. Oxford, London: Blackwell Science Ltda, 1999
57 Ning J, Li C L, Yang G, et al. Use of RNA: DNA ratios to evaluate the condition and growth of the copepod *Calanus sinicus* in the Southern Yellow Sea. Deep-Sea Res Part II: Top Stud Oceanogr, 2013, 97: 109–116
58 Gies P, Watzl R, Javorniczky J, et al. Measurement of the UVR exposures of expeditioners on Antarctic resupply voyages. Photochem Photobiol, 2009, 85(6): 1485–1490
59 de Laat A T J, van der A R J, Allaart M A F, et al. Extreme sunbathing : Three weeks of small total O_3 columns and high UV radiation over the southern tip of South America during the 2009 Antarctic O_3 hole season. Geophys Res Lett, 2010, 37(14), doi: 10.1029/2010GL043699
60 Chang K I, Jun H K, Park G T, et al. Oceanographic conditions of Maxwell Bay, King George Island, Antarctica (Austral summer 1989). Korean J Pol Res, 1990, 1(1): 27–46
61 McNamara P T, Caldarone E M, Buckley L J. RNA/DNA ratio and expression of 18S ribosomal RNA, actin and myosin heavy chain messenger RNAs in starved and fed larval Atlantic cod (*Gadus morhua*). Mar Biol, 1999, 135(1): 123–132