

Relationship between viral and prokaryotic abundance on the Bajo O'Higgins 1 Seamount (Humboldt Current System off Chile)

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SUMMARY: There is little known about the ecology of microbial communities living in the water column over seamounts. Here, for the first time, the spatial distribution and abundance of virus-like particles (VLP) are described over a seamount. The association between VLP distribution, prokaryotic abundance, and environmental variables is also analyzed. Sampling was conducted in December 2004 on the Bajo O'Higgins 1 seamount (32°54'S, 73°53'W) located in the Humboldt Current System off Chile. A oxygen minimum layer (OMZ) was clearly present between 130 and 280 m in the water column over the seamount. Water samples were taken with Niskin bottles at 10 oceanographic stations over the seamount at depths of 5, 20, 50, 75, 100, and 150 m and at the benthic boundary layer (BBL; 5-12 m over the sediments). Temperature, salinity, oxygen, chlorophyll *a*, and phaeopigments were measured at each station. Viral and prokaryotic abundances were determined with fluorochrome SYBR Green I. Viral abundance ranged from 1.53×10^9 VLP L⁻¹ - 16.48×10^9 VLP L⁻¹, whereas prokaryotic abundance ranged from 1.78×10^8 cell L⁻¹ - 14.91×10^8 cell L⁻¹. The virus-like particle/prokaryote ratio varied widely among the analyzed layers (i.e. surface, OMZ, and BBL), probably due to the presence of different prokaryotic and viral assemblages in each layer. Our results indicate that the environmental conditions, mainly the concentration of dissolved oxygen in the water column over Bajo O'Higgins 1 seamount, shape the association between viral and prokaryotic abundance.

Keywords: virus-like particles, prokaryotes, seamount, oxygen minimum zone, benthic boundary layer, Humboldt Current System.

RESUMEN: RELACIÓN ENTRE LA ABUNDANCIA VIRAL Y PROCARIÓTICA SOBRE EL MONTE SUBMARINO BAJO O'HIGGINS 1 (SISTEMA DE LA CORRIENTE DE HUMBOLDT FRENTE A CHILE). – La ecología de comunidades microbianas que habitan en la columna de agua sobre montes submarinos es escasamente conocida. Aquí, por primera vez, se describe la distribución y abundancia de partículas virales (VLP) sobre un monte submarino. La asociación entre la distribución de VLP, abundancia de procariotas y variables ambientales es también analizada. El muestreo fue realizado en Diciembre del 2004 sobre el monte submarino Bajo O'Higgins 1 (32°54'S, 73°53'W) localizado en el Sistema de la Corriente de Humboldt frente a Chile. Una zona de mínimo oxígeno se detectó entre 130 y 280 m en la columna de agua sobre el monte submarino. Las muestras de agua fueron tomadas con botellas Niskin desde 10 estaciones oceanográficas a 5, 20, 50, 75, 100, y 150 m de profundidad y en la capa de interfase bentónica (BBL; 5-12 m sobre el sedimento). Las variables temperatura, salinidad, oxígeno, clorofila *a* y feopigmentos fueron medidas en cada estación. La abundancia de virus y procariotas se determinó utilizando el fluorocromo SYBR Green I. La abundancia viral varió entre 1.53×10^9 VLP L⁻¹ - 16.48×10^9 VLP L⁻¹, mientras que la abundancia de células procariotas lo hizo entre 1.78×10^8 células L⁻¹ - 14.91×10^8 células L⁻¹. La razón virus/procariotas varió fuertemente entre los estratos analizados (i.e. superficial, zona de mínimo oxígeno, capa de interfase bentónica), probablemente debido a la presencia de diferentes ensamblajes procarióticos y virales en cada estrato. Nuestros resultados indican que las condiciones ambientales, principalmente la concentración de oxígeno disuelto en la columna de agua sobre el monte submarino Bajo O'Higgins 1, modula la asociación entre la abundancia viral y la abundancia de células procariotas.

Palabras claves: partículas virales, procariotas, monte submarino, capa de mínimo de oxígeno, capa de interfase bentónica, Sistema de Corriente de Humboldt.

TABLE 1. – Station location, pigment concentrations, and the abundance of virus-like particles and prokaryotes in the benthic-boundary layer (BBL) on the Bajo O'Higgins I seamount. n.a. = data not available.

Station	Latitude (S)	Longitude (W)	Station depth (m)	VLP ($\times 10^9$ VLP L ⁻¹)	Prokaryotes ($\times 10^8$ cell L ⁻¹)	VP-ratio	Chl <i>a</i> (μ g Chl <i>a</i> L ⁻¹)	Phaeop. (μ g Ph L ⁻¹)
1	32°53'27''	73°52'23''	513	1.26 ± 0.18	n.a.	n.a.	0.008	0.039
2	32°54'47''	73°52'97''	376	1.41 ± 0.24	1.72 ± 0.34	8.18	0.019	0.063
3	32°55'76''	73°52'08''	506	1.29 ± 0.18	1.34 ± 0.29	9.64	0.020	0.247
4	32°56'62''	73°53'80''	565	0.68 ± 0.16	1.19 ± 0.25	5.71	0.009	0.045
5	32°54'40''	73°51'11''	562	0.76 ± 0.17	1.14 ± 0.31	6.62	0.006	0.047
6	32°52'34''	73°53'67''	732	1.21 ± 0.15	1.31 ± 0.32	9.27	0.002	0.022
7	32°53'41''	73°55'18''	437	0.83 ± 0.11	1.13 ± 0.25	7.35	0.013	0.063
8	32°54'59''	73°54'64''	448	0.72 ± 0.11	1.27 ± 0.32	5.63	0.011	0.048
9	32°55'80''	73°55'20''	567	0.86 ± 0.14	0.92 ± 0.17	9.34	0.006	0.030
10	32°54'54''	73°56'34''	841	0.83 ± 0.08	0.91 ± 0.21	9.13	0.008	0.023

841 m; see Table 1) over the seamount (Niskin bottles, 2.5 L) at the following sampling depths: 5, 20, 50, 75, 100, and 150 m. Samples from 5 to 12 m over the sediments were also taken at all stations and, for practical purposes, will be referred to here as benthic-boundary layer (BBL) samples. Hydrographic data (temperature, salinity, and oxygen) were collected with a CTD Sea Bird Electronics SBE 9.

Chlorophyll *a* and phaeopigments

For each depth, 500 ml of water was filtered through a Whatman GF/F filter and then immediately frozen until processing. Pigments were extracted in acetone (90%) for 24 h, in the dark, at -20°C, and then determined using a fluorometer (TD-700 Turner Designs) according to Parson *et al.* (1984).

Virus-like particles (VLP) and prokaryotic abundance

Water samples for counting VLP and prokaryote cells were fixed immediately on board with glutaraldehyde (2% final concentration) and stored in the dark at 4°C until analyzed. The samples were processed within 10 days. As described by Noble and Fuhrman (1998), 3 ml were filtered (0.02 μ m pore diameter; Anodisc) and stained with fluorochrome SYBR Green I (stock 10%). The membranes were incubated in the dark for 10 minutes and then mounted on slides with a drop of buffer solution (50% glycerol, 50% sodium phosphate buffer, 0.1% *p*-phenylenediamine). VLP and prokaryote cell abundances were determined by counting between 10 and 20 randomly selected fields with a Zeiss epifluorescence microscope (model Axioskop 2 plus) at a magnification of 1600x.

Data analysis

Pearson's correlation and partial correlation analysis (surface stratum: 5-50 m; all data) were used to look for possible associations between variables. Lineal regression analysis was also performed between selected variables. An analysis of variance (ANOVA) was done to detect whether the analyzed variables (i.e. VLP abundance, prokaryotic abundance, temperature, oxygen, salinity, chlorophyll *a*, phaeopigments) varied according to geographic location on the seamount; different stations as well as vertical layers (i.e. 5-20 m, 5-50 m, and 50-150 m) were compared. When significant differences were found, the Tukey test was used to identify the stations. All variables were transformed logarithmically (\log_{10}) before carrying out any statistical analyses.

RESULTS

Environmental parameters

The water column was stratified on the seamount, and had a mixed layer around 35 m depth. Figure 2 shows the hydrographic parameters for each station. The temperature ranged from 17.7°C (surface) to 5.6°C (bottom) and salinity ranged from 34.31 (surface) to 34.58 (200 m). The oxygen levels varied widely along the water column, averaging 5.2 mL O₂ L⁻¹ at the surface and decaying to <0.5 mL O₂ L⁻¹ between 130 and 280 m depth. These low oxygen conditions reflect the strong influence of the Equatorial Subsurface Waters (ESSW) even at this distance from the shore (Schneider *et al.*, 2003). Below this, the oxygen level increased to a maximum of almost 4 mL O₂ L⁻¹ around 550 m depth.

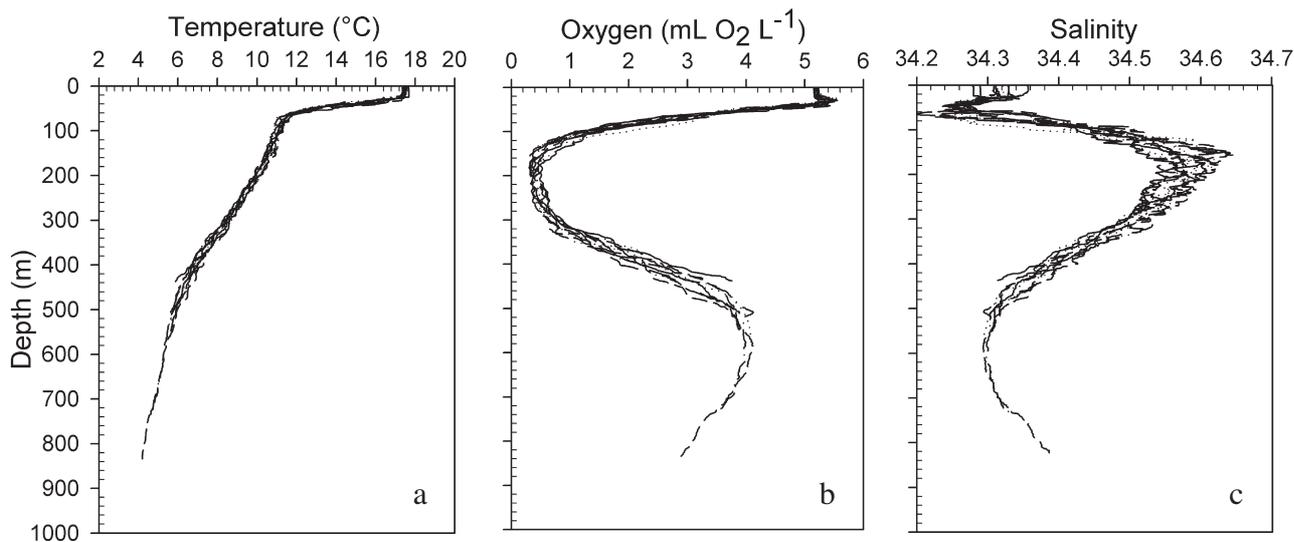


FIG. 2. – Vertical profiles of (a) temperature, (b) oxygen, and (c) salinity at the stations on Bajo O'Higgins 1 seamount.

A subsurface chlorophyll *a* and phaeopigment maximum was observed at 50 m depth in most stations (Fig. 3); the respective concentrations ranged from 0.21 $\mu\text{g L}^{-1}$ to 0.55 $\mu\text{g L}^{-1}$ (average $0.41 \pm 0.09 \mu\text{g L}^{-1}$) and from 0.15 $\mu\text{g L}^{-1}$ to 0.26 $\mu\text{g L}^{-1}$ (average $0.22 \pm 0.03 \mu\text{g L}^{-1}$). In the BBL samples, the highest concentrations were found in the shallowest bathymetric isolines (400 and 500 m; see Table 1).

Spatial distribution and abundance

VLP concentrations in the water column decreased with depth at all the stations sampled (Fig. 4a). VLP abundance varied between $10.93 \times 10^9 \text{ VLP L}^{-1}$ and $16.48 \times 10^9 \text{ VLP L}^{-1}$ (average $13.98 \pm 1.94 \times 10^9 \text{ VLP L}^{-1}$) at the surface and decreased to

$1.98 \pm 3.12 \times 10^9 \text{ VLP L}^{-1}$ at 150 m. Likewise, prokaryotic abundance decreased with depth (Fig. 4b), reaching $11.25 \pm 1.99 \times 10^8 \text{ cell L}^{-1}$ in surface waters and $2.92 \pm 0.5 \times 10^8 \text{ cell L}^{-1}$ at 150 m.

In the BBL samples, VLP abundance varied between $0.68 \times 10^9 \text{ VLP L}^{-1}$ and $1.41 \times 10^9 \text{ VLP L}^{-1}$ and prokaryotic abundance varied between $0.91 \times 10^8 \text{ cell L}^{-1}$ and $1.72 \times 10^8 \text{ cell L}^{-1}$ (see Table 1). In both cases, the highest concentrations were found over the meseta that constitutes the seamount's peak (~ 400 m).

VLP to prokaryote ratio (VP-ratio)

The VP-ratio in the water column decreased with depth at all stations ranging from an average of 12.56 (surface) to 4.3 (100 m depth). This trend of

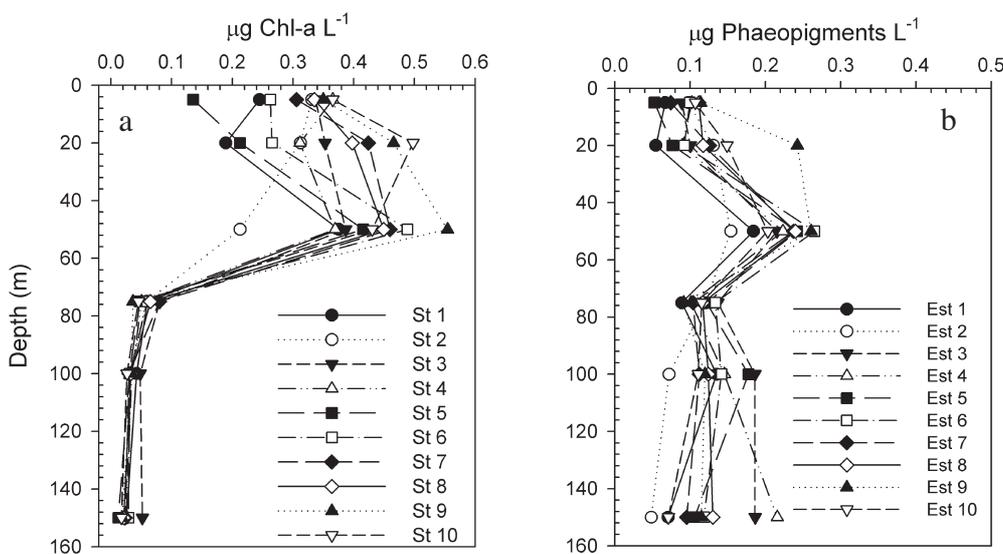


FIG. 3. – Vertical profiles of (a) chlorophyll *a* and (b) phaeopigments at the stations on Bajo O'Higgins 1 seamount.

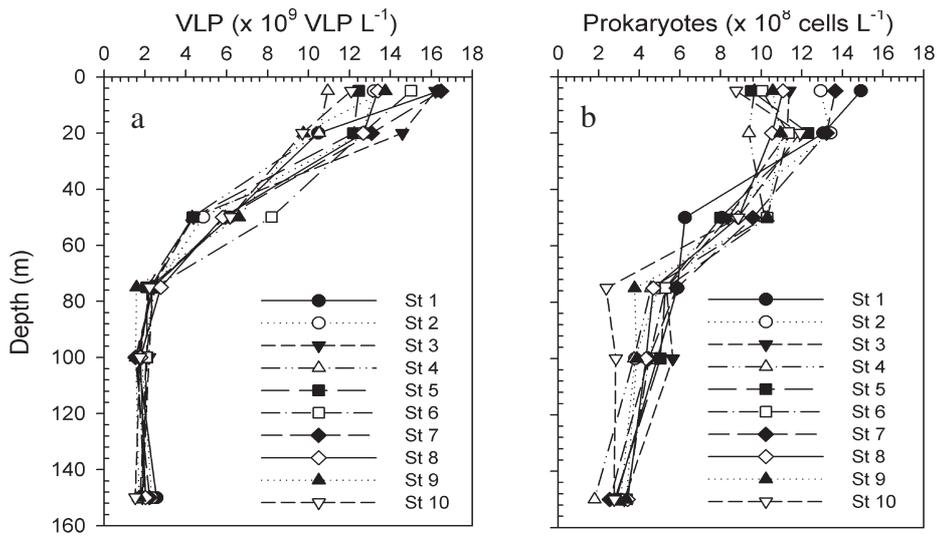


FIG. 4. – Vertical profiles of (a) virus-like particles and (b) prokaryotes at the stations on Bajo O'Higgins 1 seamount.

decreasing with depth reversed upon reaching the OMZ, where the average VP-ratio increased to 7.05 at 150 m (OMZ; Fig. 5a).

On the other hand, no significant spatial variations were found in the VP-ratio of the BBL samples (Fig. 5b). The average VP-ratio of the BBL was 7.87, slightly higher than that of the OMZ.

Associations between VLP, prokaryotes, and environmental variables

When analyzing all the data (i.e. water column and BBL samples; n = 70), a high correlation ($r^2 = 0.821$, $p < 0.05$) was found between viral and prokaryotic abundance. Likewise, VLP were highly associated with chlorophyll *a*, temperature and, to a lesser degree, oxygen (Table 2). Prokaryotic abundance, however, was highly associated with chlorophyll *a* and temperature and, to a much lesser extent, with oxygen and phaeopigments (Table 2).

An analysis of the surface stratum (between 5 and 50 m depth) revealed that VLP abundance is moderately correlated with prokaryotic abundance, phaeopigments, and salinity but strongly correlated with temperature and oxygen (Table 2). The environmental homogeneity observed during the cruise in the OMZ (below 150 m) and at the BBL does not allow a proper correlation analysis within each of these strata.

When determining the association between VLP and prokaryotic abundance, the co-variance of both variables with environmental variables such as oxygen and temperature must be considered. Furthermore, the environmental variables could be associated with each other. Due to this, a partial correlation analysis was done pooling all the data together (i.e. surface layer, OMZ, and BBL; n = 70), which revealed a significant relationship between VLP and chlorophyll *a*, phaeopigments, temperature, oxygen, and salinity (Table 3). The partial correlation analysis also showed a significant relation-

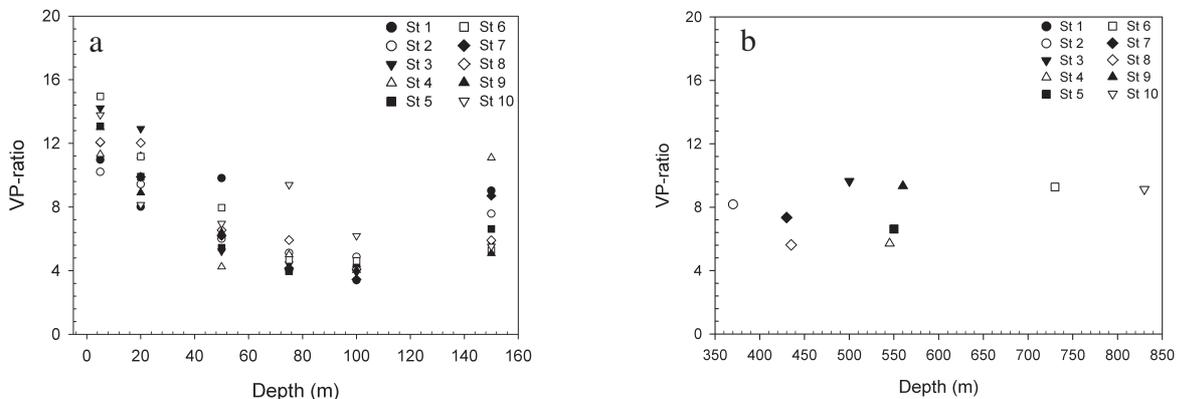


FIG. 5. Virus-like particles/prokaryote ratio (VP-ratio) in the (a) water column (5 - 150 m) and (b) benthic boundary layer on Bajo O'Higgins 1 seamount.

TABLE 2. – Correlation coefficients between biotic and abiotic variables in the water column of the Bajo O'Higgins 1 seamount. Sample size for VLP and prokaryotes: all data (n = 70), surface layer (i.e. 5-50 m; n = 30). All correlation coefficients are significant at $p \leq 0.05$. (-) No significant correlation ($p > 0.05$).

	VLP	Prokaryotes	Chl <i>a</i>	Phaeopigments	Oxygen	Temperature	Salinity
All data							
VLP		0.90	0.89	0.28	0.55	0.79	- 0.39
Prokaryotes	0.90		0.92	0.48	0.44	0.93	- 0.35
Surface layer							
VLP		0.68	-	- 0.76	0.91	0.92	0.74
Prokaryotes	0.68		-	- 0.50	0.61	0.58	0.75

TABLE 3. – Partial correlation analysis between biotic and abiotic variables in the water column of the Bajo O'Higgins 1 seamount. Sample size for VLP and prokaryotes: all data (n = 70), surface layer (i.e. 5 - 50 m; n = 30). All correlation coefficients are significant at $p \leq 0.05$. (-) No significant correlation ($p > 0.05$).

	Prokaryotes	Chl <i>a</i>	Phaeopigments	Oxygen	Temperature	Salinity
VLP (all data)	-	0.51	- 0.45	0.41	0.38	0.40
VLP (5-50 m)	0.45	-	-	-	-	-

ship between viral abundance and prokaryotic abundance at the surface (5-50 m) (Table 3).

Between-station comparisons

Significant differences ($p < 0.05$) were detected for the surface layer (5-20 m depth; n = 20) for prokaryotic abundance, chlorophyll *a*, temperature, oxygen, and salinity. The Tukey test among stations only indicated significant differences for chlorophyll *a*, temperature, and salinity between some stations (data not shown). No significant differences ($p < 0.05$) were found for the other analyzed layers (i.e. all the data and 50-150 m.).

DISCUSSION

This study describes, for the first time, the distribution and abundance of VLP on a seamount and the relationship of this distribution with prokaryotic abundance as well as with environmental factors.

The spatial distribution of chlorophyll *a* on the seamount did not vary much between sampling stations (Fig. 3a). Nevertheless, this observed homogeneity should be taken with caution considering the short time scale involved in our sampling. The chlorophyll *a* and phaeopigment levels measured on the seamount are low (Fig 3), but within the ranges reported for adjacent areas (Pizarro *et al.*, 2001). Unfortunately, no information is available on primary production from this area, making it impossible to

determine the level of biological productivity for Bajo O'Higgins 1. However, increased levels of phytoplanktonic biomass and productivity were found in areas influenced by seamounts (e.g. Comeau *et al.*, 1995) and therefore it is possible that primary productivity could be enhanced on the Bajo O'Higgins 1 seamount in some periods of the year. In fact, the high fishery activity on species such as Orange Roughy (*Hoplostethus atlanticus*) and Alfonsino (*Beryx splendens*), which concentrate on this seamount (Young *et al.*, 2000), suggests that the seamount's topography may influence biological and oceanographic processes, therefore enhancing biological productivity. Seamounts can generate physical disturbances that cause water masses to rise from the sea bottom to the photic zone, increasing primary production (Boehlert and Genin, 1987; Sime-Ngando *et al.*, 1992; Pusch *et al.*, 2004) and creating conditions capable of sustaining a high diversity and abundance of organisms (Rowden and Clark, 2004).

With the increase in research in the field of marine virology, it is becoming more evident that virioplankton play a significant role in aquatic communities (e.g. Suttle *et al.*, 1990; Eissler and Quiñones, 2003; Winter *et al.*, 2004). Accordingly, the precision and accuracy of the methods for estimating viral abundance are crucial. However, the discussion regarding the best methodology to be used is on-going. The methodology utilized in this research (i.e. fixation with glutaraldehyde and storing at 4°C) is the most common method used to preserve and store viruses for determining their abundance in aquatic ecosys-

tems (e.g. Noble and Fuhrman, 1998; Chen *et al.*, 2001; Hewson *et al.*, 2001; Culley and Welschmeyer, 2002; Taylor *et al.*, 2003; Corinaldesi *et al.*, 2003; Glud and Middelboe, 2004; Lunau *et al.*, 2005; Vanucci *et al.*, 2005). Nevertheless, this methodology is becoming controversial because there is evidence showing that it can underestimate viral abundance (Brussaard, 2004; Wen *et al.*, 2004). It is highly likely that there has been a certain level of underestimation with the methodology we used here. We recognize the importance of the role of fixing and storing in estimating viral abundance and, for that matter, bacterial abundance. In fact, Wen *et al.* (2004) suggest that it is much more reliable to store the samples at -80°C . Wen's conclusions are coherent with those of Brussaard (2004), who uses flow cytometry combined with liquid nitrogen, deep freezing (-80°C), and glutaraldehyde. In contrast, other authors have reached the conclusion that storing at -80°C is more effective only when the objective is to keep the samples for long periods of time (more than 40 days) but that for shorter periods storing at 4°C is better (Olson *et al.*, 2004). It is important to note that methodological controversies are frequent in the fields of marine microbiology and biological oceanography. For instance, the controversies concerning the real efficiency of filters of different materials and pore sizes (e.g. Stockner *et al.*, 1990; Lee *et al.*, 1995), the most appropriate methodology for measuring DOC (e.g. Sugimura and Suzuki, 1988; Suzuki *et al.*, 1992; Wangersky, 1993), or even the most adequate methodology for obtaining an optimum measurement of chlorophyll in seawater (e.g. Holm-Hansen and Riemann, 1978; Baker *et al.*, 1983; Stramski, 1990). The history of these controversies has shown that it is necessary to accumulate a large body of evidence

before discarding one method and the knowledge obtained from it.

The high VLP abundance observed on the Bajo O'Higgins 1 seamount is consistent with previously published values for other oceanic areas (see Table 4), although it is slightly lower than that observed in the water column over the continental shelf off central Chile (average 2.05×10^{10} VLP L^{-1} ; unpublished results). Viral abundance decreases with depth on the seamount (Fig. 4a), which is in accordance with trends previously reported in other oceanic ecosystems (e.g. Hara *et al.*, 1996; Steward *et al.*, 1996). Similarly, the prokaryotic abundance is within the ranges registered off central Chile, especially in oceanic zones of the HCS (e.g. Cuevas *et al.*, 2004). The decreasing prokaryotic vertical distribution observed on Bajo O'Higgins 1 (Fig. 4b) is also consistent with the pattern described for other areas, such as the Adriatic Sea (Corinaldesi *et al.*, 2003) and the North Pacific Ocean (Culley and Welschmeyer, 2002).

The significant correlation found between VLP abundance and prokaryote cells (Table 2) suggests that the members of the latter group are the main hosts for viruses, which is a typical trend in marine systems (e.g. Hara *et al.*, 1996; Weinbauer and Suttle, 1997; Culley and Welschmeyer, 2002). Nevertheless the correlation found between VLP and chlorophyll *a* (Table 2) suggests that phytoplankton may also play a relevant role as hosts.

Viral distribution and abundance in the ocean can be controlled or modified by environmental factors such as light, temperature, oxygen (e.g. Gantzer *et al.*, 1998; Weinbauer and Höfle, 1998; Bettarel *et al.*, 2003; Corinaldesi *et al.*, 2003), and turbidity (Winter *et al.*, 2005). Likewise, abiotic factors like nutrient availability and temperature affect prokary-

TABLE 4. – Comparison of viral abundance in the water column from oceanic areas. EFM = epifluorescence microscopy; FC = flow cytometry; TEM = transmission electron microscopy. Table extracted and modified from Wommack and Colwell (2000).

Area	Method	VLP ($\times 10^6$ mL^{-1})	VBR	Reference
North Pacific (subartic)	EFM	0.06-0.38	1.1 – 4.5	Hara <i>et al.</i> (1996)
North Pacific (subtropical)	EFM	0.4-1.9	1.0 – 8.7	Hara <i>et al.</i> (1996)
Equatorial Pacific	FC	5.3	-	Marie <i>et al.</i> (1999)
Sargassum Sea	TEM	0.003 ± 0.0015	-	Proctor and Fuhrman (1990)
East Caribbean Sea	TEM	1.9 ± 1.3	-	Proctor and Fuhrman (1990)
West Caribbean Sea	TEM	4.8 ± 3	-	Proctor and Fuhrman (1990)
North Atlantic	TEM	14.9	50	Bergh <i>et al.</i> (1989)
Barents Sea	TEM	0.06	3	Bergh <i>et al.</i> (1989)
Mediterranean Sea	FC	2.3 – 6.5	-	Marie <i>et al.</i> (1999)
Alboran Sea	EFM- TEM	1.8 – 0.014	1.4-20	Alonso <i>et al.</i> (2001)
Baltic Sea (BY 15)	EFM	23.4	-	Weinbauer <i>et al.</i> (2002)
Baltic Sea (Teili 1)	EFM	32.9	-	Weinbauer <i>et al.</i> (2002)
North Sea (Stn 112)	EFM	27.1	-	Weinbauer <i>et al.</i> (2002)
Eastern South Pacific	EFM	0.68-16.48	4-15	Present study

ote distribution and abundance (e.g. Brett *et al.*, 1999; Heidelberg *et al.*, 2002). Thus, environmental gradients in the ocean that are able to modify viral or prokaryotic abundance can potentially influence the VLP/prokaryote relationship. Similarly, biotic factors could also modulate the relationship between VLP and prokaryotes such as grazing by protists (i.e. ciliates and flagellates) over prokaryotes (e.g. Fuhrman and Noble, 1995; Cuevas *et al.*, 2004).

The HCS is characterized by sharp vertical gradients, especially of oxygen, generated by the presence of several water masses, namely SubAntarctic Waters (SAAW), ESSW, and Antarctic Intermediate Waters (AAIW) (Strub *et al.*, 1998). The ESSW brought by the Gunther Current is particularly relevant to the present study, since these waters are very poor in dissolved oxygen (Brandhorst, 1971; Sievers and Silva, 1982; Silva, 1983). The environment generated by the OMZ associated with the ESSW represents an important biological barrier for organisms relying on aerobic metabolism (Eissler and Quiñones, 1999). Based on the oxygen vertical distribution (Fig. 2b), three systems were identified in the water column on the Bajo O'Higgins 1 seamount. The surface system (i.e. 0 to 100 m) is characterized by high oxygen concentrations ($>2 \text{ mL O}_2 \text{ L}^{-1}$) and influenced by light. The second system is below 100 m and corresponds largely to ESSW, with extremely low oxygen conditions ($<0.5 \text{ mL O}_2 \text{ L}^{-1}$). Finally, at the BBL, oxygen concentrations are higher than at the OMZ due to the influence of the more oxygenated waters of the AAIW.

Although the microbial assemblages inhabiting the OMZ of the HCS remain largely unidentified, some evidence indicates that they are very different from those dwelling in oxic waters. For example, the abundance of archaea (Levipan, 2006) and assemblages of denitrifying microorganisms (Molina, 2004; Castro-González *et al.*, 2005) are important in the OMZ of the HCS. Consequently, the prokaryote community of the OMZ is expected to be very different from the microbial communities that live at the surface and bottom of the ocean, which is reflected in the different VP-ratios obtained (Fig. 5). A similar phenomenon was described by Taylor *et al.* (2003) for variations of viral particles and prokaryotic abundance between three layers (oxic, transitional, and hypoxic) of the water column in Cariaco Basin. These authors found viral distribution to be marked by an environmental oxygen gradient, which was clearly reflected in VP-ratios; the higher proportions corresponded to the hypoxic zone. Similarly, Weinbauer *et al.* (2003) showed an incre-

ment in the abundances of both VLP and prokaryotes in anoxic waters of the Baltic Sea. In fact, the same trend was observed in the present study, where the VP-ratios decreased with depth in the oxic zone and then drastically increased under hypoxic conditions.

The differential VLP/prokaryote association with respect to environmental gradients on the Bajo O'Higgins 1 seamount could be due to the fact that the microbial assemblage compositions, particularly for prokaryotes, are very different in these layers (i.e. surface, OMZ, BBL). A low VP-ratio may indicate that the observed relationship between VLP and host prokaryotic cells could be explained by the presence of a low infecting rate, a low number of viruses per available host cell, or a high virus decay rate (Alonso *et al.*, 2001). In addition, the difference in the VP-ratios could be caused by virus-host specificity (Middelboe *et al.*, 2000) or the different viral assemblages found in the oxic and hypoxic layer. Another alternative hypothesis for the lack of correlation between VLP and prokaryotes and the high VP-ratio in the OMZ is a relative increase in virioplankton not able to infect the existing prokaryotes, as Bettarel *et al.* (2003) observed in some lakes.

In contrast to the rest of the water column, no change in the VP-ratio associated with the dissolved oxygen content was observed in the BBL, probably due to the spatial variability of the community living in the sediment. It is known that the BBL is strongly influenced by the resuspension of organic matter (Schallenberg and Burns, 2004), sinking of POM from surface layers (Turley, 2000), and degradation processes acting on the organic matter found at the sea bottom (Suess, 1980; Peterson *et al.*, 1988; Graco *et al.*, 2001).

Given these results, we conclude that the association between viral and prokaryotic abundance is significantly shaped by the environmental conditions, mainly dissolved oxygen concentration in the water column associated with the Bajo O'Higgins 1 seamount.

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