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ORIGINAL PAPER

Comparison of *Artemia*–bacteria associations in brines, laboratory cultures and the gut environment: a study based on Chilean hypersaline environments

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Abstract The brine shrimp *Artemia* (*Crustacea*) and a diversity of halophilic microorganisms coexist in natural brines, salterns and laboratory cultures; part of such environmental microbial diversity is represented in the gut of *Artemia* individuals. Bacterial diversity in these environments was assessed by 16S rRNA gene denaturing gradient gel electrophoresis (DGGE) fingerprinting. Eight natural locations in Chile, where *A. franciscana* or *A. persimilis* occur, were sampled for analysis of free-living and gut-associated bacteria in water from nature and laboratory cultures. The highest ecological diversity (Shannon's index, H') was found in brines, it decreased in the gut of wild and laboratory animals, and in laboratory water. Significant differences in H' existed between brines and laboratory water, and between brines and gut of wild animals. The greatest similarity of bacterial community composition was between brines and the gut of field animals, suggesting a transient state of the gut microbiota. Sequences retrieved from DGGE patterns ($n = 83$) exhibited an average of 97.8 % identity with 41 bacterial genera from the phyla *Proteobacteria* (55.4 % of sequences match), *Bacteroidetes* (22.9 %), *Actinobacteria* (16.9 %) and *Firmicutes* (4.8 %). Environment-exclusive genera distribution was seen in *Sphingomonas* and *Paenibacillus* (gut of field animals), *Amaricoccus* and *Ornithinimicrobium* (gut of laboratory animals), and *Hydrogenophaga* (water of laboratory cultures). The reported ecological and physiological capabilities of such bacteria can help to understand *Artemia* adaptation to natural and laboratory conditions.

Keywords *Artemia* · Bacteria · 16S rRNA gene · Ecological diversity · Salty lakes · Salterns · Chile

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Introduction

Hypersaline lakes are inland-like ecosystems scattered over tropical and subtropical areas with high rates of evaporation (Van Stappen 2002). Their unique ecological characteristics, stemming from high salt concentration, anoxia and salinity fluctuation, high exposure to UV radiation, and wide variation in water temperature and ionic composition (Castro et al. 2006), make them extreme and unique ecosystems for the survival of aquatic species (Wharton 2007). Not surprisingly, the brine shrimp *Artemia* is the sole animal adapted to such conditions (Gajardo and Beardmore 2012), though the organism coexists with a diversity of viruses, bacteria, *Archaea* and fungi (Benlloch et al. 2002; Zafrilla et al. 2010; Ghai et al. 2011; Riddle et al. 2013). In the context of host-adapted bacteria relationships (Toft and Andersson 2010), the *Artemia*–bacteria historical coexistence presuppose some kind of functional relationship, for example bacteria being part of the *Artemia* food web,

somewhat expected for a non-selective filter feeder able to ingest particles up to 50 μm in size (adults). It is now known that besides microalgae and detritus (Savage and Knott 1998), *Artemia* also feed on bacteria as demonstrated by laboratory experiments under algae-limited and high bacteria biomass conditions (Marques et al. 2005; Toi et al. 2013). In spite of this functional relationship, studies on *Artemia* and halophilic bacteria have followed independent tracks, with some recent exceptions (Riddle et al. 2013). On one hand, *Artemia* as a model extremophile for studies of evolution and adaptation owing to the particular life cycle evolved to cope with harsh environmental conditions (Clegg and Trotman 2002; Gajardo et al. 2002; Gajardo and Beardmore 2012), which includes the ability of females to switch offspring between highly resistant cysts (diapause embryos) and free-swimming nauplii, in response to stressful or stable conditions, respectively. Cysts are highly demanded in the aquaculture market as upon hatching they release nauplii that are extremely valuable as live diet for the larviculture of marine fish (Dhont and Sorgeloos 2002). On the other hand, hypersaline lakes are reservoirs of high microbial genetic diversity (Casamayor et al. 2002; Demergasso et al. 2004; Dorador et al. 2009; Casamayor et al. 2013; Triadó-Margarit and Casamayor 2013) likely to be the source of novel variants of biotechnological importance, owing to the peculiarities of salty lakes. The recognition that microorganisms are ubiquitous to almost every environment, and able to establish different types of association, such as mutualism or parasitism (Fraune and Bosch 2010; Toft and Andersson 2010), led us to assume they should affect *Artemia* fitness (survival and reproduction) both in nature and the laboratory environment. The non-selective filter feeder condition of *Artemia* offers chances for microbial communities of salty lakes to be represented in the gut microbiota, thus helping *Artemia* to digest microalgae by providing important enzymes which also assist marine fish larvae feeding on *Artemia* nauplii, to take-up and digest nutrients (Gorospe et al. 1996; Orozco-Medina et al. 2002; Seoka et al. 2007; Nayak 2010; Tkavc et al. 2011). The microbiota also boosts immunological response and more complex reproductive phenotypes like courtship, as shown for other invertebrates (Sharon et al. 2010).

The purpose of this research was to assess bacterial diversity in the natural environment, hypersaline lakes (brines) and man-made coastal solar salterns, the laboratory environment—water of *Artemia* cultures—and in the *Artemia* gut environment (microbiota) of field and cultured animals. The study was based on water samples and individuals collected from salty lagoons scattered in salt flats of northern Chile (Atacama Desert) (Gajardo and Beardmore 1993; Gajardo et al. 2002; Demergasso et al. 2004; Dorador et al. 2009; Stivaletta et al. 2011), salterns in central Chile which are artificial *Artemia* environments, and salty lagoons in southern latitudes (Patagonia) (Clegg and Gajardo 2009). Specific *Artemia*–bacteria association is assumed depending on the environment evaluated owing to the differential selective pressures bacteria encounter. Knowing the functional abilities of bacterial types adapted to each condition should provide insights on the functionality of such association. The natural sites assessed contain the New World *Artemia* species, *A. franciscana* (distributed from 21 to 24° latitude South) and *A. persimilis* (50–54°S), which add the species dimension to the study (Gajardo et al. 2002). In solar salterns (34.5–35.0°S) located in between atypical *A. franciscana* individuals have been described (Mura and Gajardo 2011). This is the southern edge of the species distribution in the Americas.

Materials and methods

Origin of samples

Water samples and *Artemia* individuals were collected from eight natural locations in Chile, three salty lagoons in the north, two salterns in the center, and three lagoons in Patagonia, southern Chile (Table 1). These samples allowed comparisons between the following three types of environments: (a) field, water (brine) samples from natural locations (F_W); (b) laboratory, water samples (L_W) from laboratory-reared *Artemia* cultures; (c) gut, corresponds to the microbiota assessed in the digestive tube of wild (F_G) and laboratory-reared animals (L_G). Laboratory cultures were established from wild *Artemia* animals collected from each location, except for *Laguna de los Cisnes* (LAC) where only cysts were available at sampling, as explained below. For *Laguna de la sal* (LDS) only water samples (brine) were available for analysis since *Artemia* individuals did not survive under captivity. Adult and juveniles collected in nature were acclimatized to laboratory conditions in 5 L aquaria filled with water from the site which was then slowly replaced by artificial seawater (35 ppt), made according to Kalle (1971, p 687). Animals were fed either *Dunaliella tertiolecta* or *Isochrysis galbana* (1.2×10^6 cells mL^{-1} per individual) every 2 days, according to Gajardo and Beardmore (1993).

The LAC's laboratory population was the only one started from cysts hatched in the laboratory. These were washed to remove soil and debris and put into brine to discard empty or unviable ones. When needed, these were hatched without prior decapsulation to keep their bacterial load, following standards procedures (Lavens and Sorgeloos 1996).

Water samples were collected in 1 L polyethylene brand new bottles previously washed three times with sterile

Table 1 Locations of *Artemia* sites used in this study, coordinates and some basic limnological characteristics of these sites

Map	Zone	Sampling site	Code	Coordinates		Salinity (ppt)	pH	T °C
				South	East			
	North	Cejar	CJR	23.063.410	68.214684	154	9.10	13.5
		Piedra	LAP	23.057742	68.217907	97	9.90	9.0
		Barro Negro	BNG	23.288228	68.177551	151	8.90	10.0
	Center	Cahuil	CAH	34.507177	71.981357	252	7.65	34.0
		Lo Valdivia	LOV	34.698113	72.012154	231	7.71	31.8
	South	Amarga	LAM	50.973499	72.732699	104	9.80	6.0
		De la Sal	LDS	53.284127	70.390975	112	8.30	12.5
		De los Cisnes[a]	LAC	53.240633	70.343.870	35	7.85	19.3

[a] *Artemia* samples from these locations were used to establish laboratory cultures, except for De los Cisnes Lagoon, the only site where only cysts were exclusively found. Sites in the north are inland athalassohaline lagoons (CJR, LAP and BNG), those in the center are coastal man-made solar salterns (CAH and LOV), while those in the south are athalassohaline (LAM and LAC) and thalassohaline (LDS). Map indicates the number of samples collected in northern, central and southern Chile

distillated water, and then filtered with Miracloth (Calbiochem) filter paper (typical pore size 22–25 μm) and 5 μm filter paper (Advantec MFS, No. 2), then between 30 and 500 mL of each sample was filtered with 0.2 μm acetate cellulose filters (Sartorius, 47 mm diameter). Samples were kept in 1.6 mL vials at −20 °C. *Artemia* individuals (adults) were collected with a plankton mesh and kept in 1.6 mL tubes filled with 1 mL of 70 % ethanol (Merck).

DNA extraction

For field and laboratory samples, *Artemia* adults, male or female, kept in ethanol (70 %) were washed tree times with sterile ultrapure water and dissected with sterile tools under a stereoscope microscope to first cut-off thoracopods, head, ovisac and furca to isolate the digestive tube (gut), ideally free of bacteria adhered to the external surface, which was processed in 1.6 mL tubes with 500 μL of lysis buffer (40 mM EDTA, Tris 50 mM, sucrose, pH 8.3). Filters with bacterial samples taken from brines and from laboratory cultures were cut-off into small pieces and covered with 500 μL of lysis buffer. Gut samples were homogenized with a pipette by blowing up and down until complete homogenization of the soft *Artemia* tissues was achieved, and incubated with lysozyme (Sigma) for 45 min. at 37 °C, with 5 μg of RNAse (Qiagen) during 45 min at 37 °C and, finally, with 100 μg of Proteinase K (Sigma) and 1 % SDS over 1 h at 55 °C. DNA was extracted with phenol–chloroform–isoamyl alcohol (25:24:1, vol/vol/vol) and with chloroform: isoamyl alcohol (24:1, vol/vol), and the aqueous phase was purified and concentrated with Amicon Ultra-4 tubes (100 K) as in Dumestre et al. (2002). Extracts were quantified with Quant IT system (Invitrogen) following manufacturer's instructions. PCRs were made in a total of 50 μL volume of reaction containing: 1.5 mM $MgCl_2$; 0.18 μg/μL BSA; 300 μM each dNTP's (Promega); 500 nM of each bacteria-specific primer and one unit of Taq polymerase (Biotaq, Bioline). Primers considered were specific to bacteria, BAC358F-GC: 5′-CCTACGGGAGGCAGCAG-3′ (Muyzer et al. 1993); BAC907RM: 5′-CGTCAATTCMTTTGAGTTT-3′ (Casamayor et al. 2000), while the CG-clamp utilized was: CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCG-3′ (Muyzer et al. 1993). PCR cycles considered 94 °C (2′), 94 °C (30″), 55 °C (1′), 72 °C (1′) 35 times and 72 °C (5′) one time. DGGE gels were loaded with 500–600 ng of PCR product and run at 100 V in a 7.5 % polyacrylamide gel with a 20–80 % urea–formamide gradient during 14 h at 60 °C, and stained with SYBR gold. Gels were observed under a BioRad UV system, and the relevant bands were excised and incubated overnight in Milli-Q sterile water. The extracts were re-amplified following the protocol mentioned above, but using primers without GC-clamp. Amplicons were sequenced by Macrogen, Korea, and electropherograms were manually screened.

Data analysis

The curated sequences were manually analyzed with BioEdit (Hall 1999) and Bellerophon (Huber et al. 2004) to discard chimeras. Alignment was performed with PyNAST (Caporaso et al. 2010), whereas matching to the closest

relative genera was done using "nucleotide blast" (blastn) in GenBank. The taxonomic identity was further refined comparing 16S rDNA sequences with the ARB phylogeny package (Ludwig et al. 2004; Database 2007). Partial sequences retrieved from DGGE gels were inserted in the tree keeping the overall topology using the Parsimony Interactive tool implemented in ARB.

Gel analyses (number of bands and intensity) were made using Image J (http://rsb.info.nih.gov/ij/) in comparable DGGE gels (Fig. 7). Diversity (Shannon diversity index, H') and statistical analyses were performed with PAST diversity software (Hammer et al. 2001). Shannon was calculated for every location and each environment assessed (F_W, F_G, L_W, L_G). Diversity analysis for each environment considered all locations averaged. Statistical significance was estimated with Student's t distribution (2–2), with $n-1$ degrees of freedom. Two types of comparisons were done, first, to evaluate how natural (field) bacterial communities were represented, or conserved, in the gut of field and *Artemia* laboratory-reared animals, as well as in the water of laboratory cultures. The analysis was extended to compare species. Such comparisons were made with R Project for Statistical Computing: http://www.r-project.org (R Core Team 2013), using the "vegan" package and the "vegdist" function. The Bray–Curtis quantitative dissimilarity index was used to get data from bands intensity, while the qualitative Sørensen (similarity index) for such binaries was calculated. To facilitate interpretation of Bray–Curtis index, data were expressed as 1-dissimilarity, and for this the following R functions were considered: aov for one-way ANOVA and Tukey HSD, a posteriori comparison, to detect significant differences.

Nucleotide sequence accession numbers

Sequences retrieved from bands were deposited in GenBank with Accession Numbers from LK391422 to LK391467 for DGGE bands 1–46 (corresponding to water samples), and from LK391468 to LK391504 for bands 47–83 (corresponding to *Artemia* gut samples).

Results

A total of 28 samples were analyzed: 14 water samples (7 natural sites and 7 laboratory cultures) and 14 *Artemia* gut samples (7 natural locations and 7 laboratory cultures). 192 DGGE sequenced bands were available for analysis, but after detailed inspection of electropherograms 83 sequences were finally considered (Fig. 1). Alignments revealed an average of 97.8 % identity and 99.8 % of coverage to 41 bacterial genera (Table 2) belonging to Phyla *Proteobacteria* (55.4 % of sequences match), *Bacteroidetes* (22.9 %), *Actinobacteria* (16.9 %) and *Firmicutes* (4.8 %). Figure 2 displays the most closely related genera found in GenBank, and their phylogenetic relationship to the sequences isolated from DGGE gels. The percentage of total band intensity in the DGGE fingerprints reported per Phyla, sample site, and environment (Field, Lab, and Gut) is provided in Fig. 3. Except for BNG (L_W), a site in the north with all bands sequenced, the non-sequenced bands were over 60 %. However, non-sequenced bands showed, on average, lower intensity in relation to the total band intensity than those sequenced hence most of the potentially predominant bacterial populations were targeted (see Fig. 4).

Ecological diversity of microbial assemblages

Environmental (field) bacterioplankton (Fig. 5) turned out to be the most ecologically diverse (Shannon–Weaver index, H' 2.69 ± 0.30, $n = 7$), and such diversity was subsequently reduced in the gut of laboratory-reared (H' 2.35 ± 0.45, $n = 7$) and field individuals (H' 2.05 ± 0.28, $n = 7$), and in the laboratory (water) environment (H' 1.97 ± 0.56, $n = 7$), the less diverse of all the environments assessed. Significant differences occurred between FLD/WTR and LAB/WTR samples ($p < 0.01$), and these were highly significant for FLD/WTR and FLD/GUT ($p < 0.001$). There were no significant differences between ecological diversity of laboratory samples (water and gut) and gut samples (field and laboratory). With regard to the natural sites sampled, the higher to lower ecological diversity ranking showed the gut of Cejar (CJR) laboratory individuals in first place (H' 3.2) followed by De la Sal (LDS) field water samples, while water of Lo Valdivia *Artemia* cultures exhibited the lowest bacterial diversity (H' 1.39).

Similarity of bacterial communities to field water (brines) samples

Field water samples (F_W), were pairwise compared with the remaining samples (Fig. 6) and significant differences (One-way ANOVA, $p < 0.05$) were found. The closest similarity to the natural condition was found in the gut of field-collected individuals (F_G), whose diversity significantly differed to that observed in the gut of laboratory animals (L_G) (Tukey's Honest Significant Difference, p value <0.05). Such difference was greater using the Bray–Curtis index (15 % to Bray–Curtis and 19 % to Sorensen) that allowed to report inter-species differences by comparing presence/absence of DGGE bands.

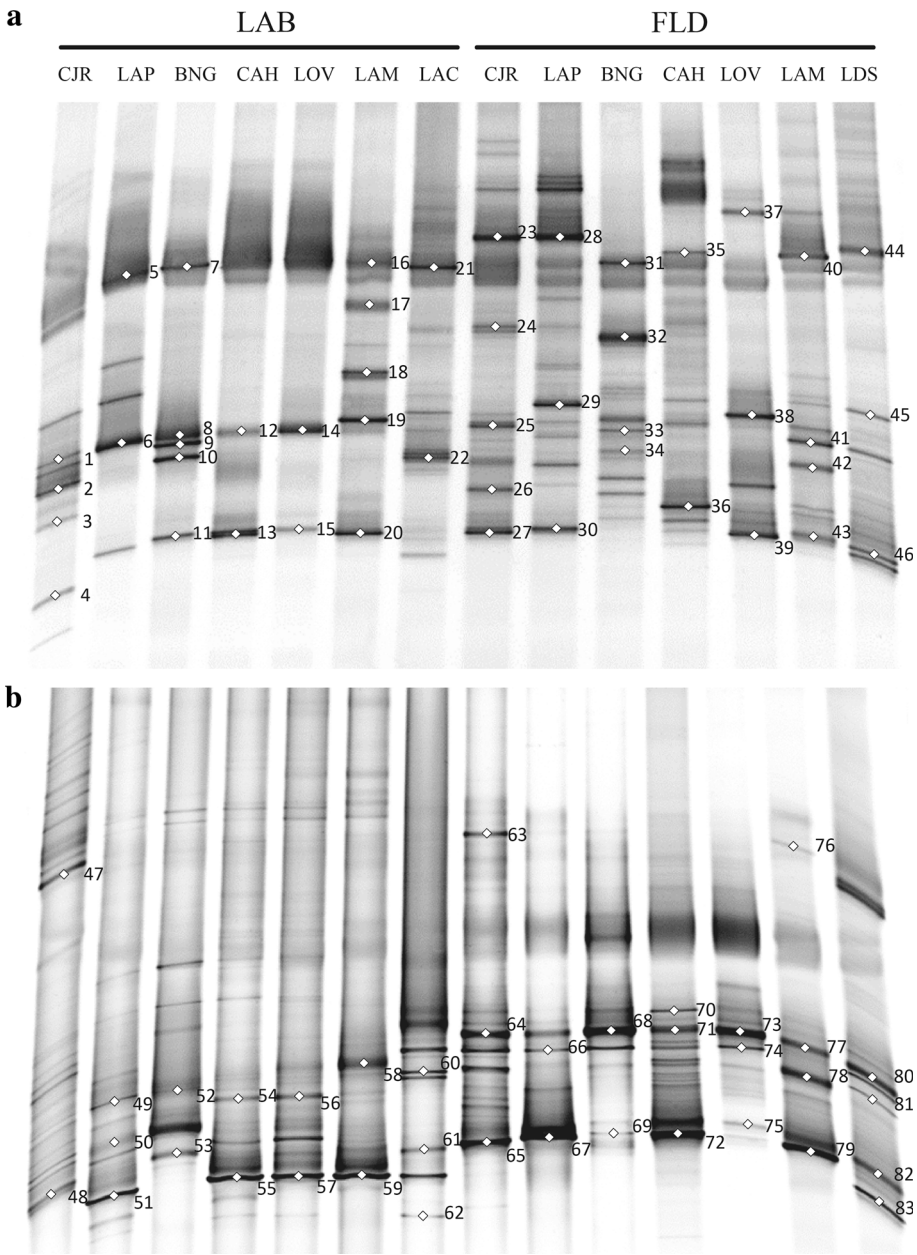

Fig. 1 Negative image of DGGE gels of: **a** water samples from laboratory cultures (LAB) and field (FLD); **b** *Artemia* gut of laboratory-reared (LAB) and animals collected in the field (FLD). *White points* in the gels indicate those bands excised and successfully sequenced (in correlative number)
728 × 970 mm (96 × 96 DPI)

Environment–bacteria relationship

None of the bacterial genera detected was present in all the environments assessed (field, lab and gut), except *Psychroflexus* that appeared in almost all field water samples and other environments assessed. Other common genera detected were *Chromohalobacter, Sphingomonas* and *Amaricoccus*. Three genera were solely detected in one specific environment such as *Sphingomonas* (F_G), *Amaricoccus* (L_G), *Ornithinimicrobium* (L_G) and *Firmicutes* (*Paenibacillus* and *Halolactibacillus*) (F_G). Only 24 bacterial genera were not repeated in other locations.

Discussion

From an ecosystem point of view, the *Artemia*–bacteria coexistence in hypersaline lakes should translate in some sort of functional relationship, a view in agreement with the fact that bacteria can interact in many ways (parasitic, mutualistic, symbiotic) with all kind of organisms, including aquatic invertebrates (Harris 1993). The non-selective filter feeder condition of *Artemia* provides opportunities for bacterial colonization of the gut. This study approached such coexistence at different levels: the field-natural realm, including salterns (semi-artificial

Table 2 BLAST searches of sequences recovered from excised DGGE bands. Bands are listed in correlation to Fig. 1

Phylum	Closest relative	Accession	Bands	Identity	Coverage
Proteobacteria	*Ahrensia* sp.	AB758568.1	2, 58	100.0	100
	Amaricoccus sp.	JF957137.1	49, 52, 54, 56, 61	93.7–95.4	100
	Bordetella sp.	AM990925.1	3, 27	97.1–98.1	99–100
	Buttiauxella agrestis	HF585059.1	75	100.0	100
	Chromohalobacter salexigens	KF952437.1	43, 48, 65, 67, 72, 82	99.0–99.5	100
	Halomonas alkaliphila	KF985241.1	79	100.0	100
	Halomonas meridiana	KC842224.1	46	99.4	100
	Hydrogenophaga caeni	NR_043769.1	12, 14	96.5–96.7	99–100
	Hydrogenophaga flava	AB681848.1	8	97.6	98
	Loktanella salsilacus	KF740556.1	42	98.8	100
	Marinobacter salsuginis	JN202614.1	19	98.5	100
	Maritimibacter sp.	GQ131651.1	70	98.0	100
	Oceanicella actignis	JQ864436.1	53	96.6	100
	Pseudoalteromonas sp.	GQ849227.1	45	99.1	100
	Pseudoruegeria sp.	KC196069.1	25	98.5	99
	Rhodobacter sp.	KC702687.1	34	99.5	100
	Roseovarius litoreus	NR_109594.1	1	99.4	100
	Roseovarius sp.	JF417975.1	10	100.0	100
	Roseovarius sp.	KF289476.1	38	99.8	100
	Roseovarius sp.	HE576999.1	83	99.3	100
	Salinivibrio sp.	KF418154.1	41	97.5	100
	Seohaeicola saemankumensis	NR_044437.1	33	99.3	100
	Simplicispira sp.	JQ229604.1	6	95.9	99
	Sphingomonas aquatilis	KF542913.1	64, 68, 71, 77, 80	99.0	99–100
	Sphingomonas aquatilis	EU423085.1	73	100.0	100
	Spiribacter sp.	CP005990.1	36	99.4	100
	Sulfitobacter sp.	KC247329.1	29	99.2	100
	Thiohalomonas denitrificans	EF117913.1	18	90.7	100
	Tropicibacter sp.	KC534265.2	22, 60	98.1	100
Bacteroidetes	*Algoriphagus halophilus*	KJ009558.1	26	97.0	100
	Aureimarina marisflavi	EF108215.1	5, 16	95.9–100.0	100
	Belliell sp.	KC762321.1	32	99.8	100
	Brumimicrobium sp.	EU195945.1	24	95.7	100
	Crocinitomix catalasitica	AB681013.1	31	96.5	92
	Muricauda ruestringensis	NR_074562.1	9	99.8	100
	Owenweeksia hongkongensis	CP003156.1	7, 17	86.6–99.8	100
	Psychroflexus lacisalsi	AB381940.1	23, 28, 40, 44, 47, 63, 76	90.0–99.8	99–100
	Psychroflexus sp.	JQ390292.1	37	96.7	100
	Psychroflexus torquis	NR_074147.1	21	98.4	100
	Psychroflexus tropicus	NR_028854.1	35	99.2	99
Actinobacteria	*Candidatus Rhodoluna*	AJ565415.1	11, 39	97.1–97.3	100
	Dietzia cercidiphylli	KF876893.1	4	100.0	100
	Ilumatobacter nonamiensis	AB360345.1	50, 81	98.8–99.2	100
	Ornithinimicrobium murale	NR_108532.1	51, 55, 57, 59	98.2	100
	Paraoerskovia marina	AB695379.1	62	96.2	100
	Pontimonas salivibrio	NR_109611.1	20	99.8	100
	Rhodoluna sp.	AB607304.1	15, 30	95.5–95.6	99–100
	Schumannella sp.	KF881974.1	13	95.5	100
Firmicutes	*Halolactibacillus miurensis*	AB681280.1	78	97.6	100
	Paenibacillus darwinianus	KF264457.1	66	93.4	100
	Paenibacillus tarimensis	NR_044102.1	69, 74	97.1–97.3	100

environments), the laboratory and the *Artemia* gut environments (microbiota). We also considered the species level represented by the two American species, *A. franciscana* and *A. persimilis*, both present in Chile, the former widely distributed in the Americas (North, Center and South), central Chile being the southern edge of its distribution, while *A. persimilis* is restricted to southern latitudes in Chile and Argentina (Gajardo et al. 2002; Gajardo and Beardmore 2012).

This study confirmed the large bacterial diversity existing in natural salty lakes, but at the same time showed how such natural diversity is greatly reduced in the gut environment of field and laboratory-reared animals and in the laboratory environment (water) as well, which turned out to be the least diverse in bacterial communities. Such bacteria stratification can be consequence of their differential survival to the selective pressures imposed by each environment, though further temporal monitoring along with more specific experimental studies would be required to confirm this. Such heterogeneous distribution of bacterial types is likely to affect, directly or indirectly, different aspects of the *Artemia* life cycle, the food web the animal depends on and, perhaps, its tolerance to abiotic conditions. Particularly sensitive should be the female reproductive mode (ovoviviparity, oviparity) which is synchronized by unknown environmental cues. The gut microbiota might also influence *Artemia* survival in nature and laboratory cultures by providing specific functional capabilities such as food processing and uptake (Intriago and Jones 1993; Gorospe et al. 1996), immunological competence (Orozco-Medina et al. 2002; Villamil et al. 2003) and other phenotypes alike, as shown for other invertebrates (Sharon et al. 2010).

Bacterial diversity

High average bacterial diversity has been reported in natural lakes of northern Chile (Demergasso et al. 2004, 2008; Dorador et al. 2009; Stivaletta et al. 2011) and in salterns elsewhere (Benlloch et al. 2002; Casamayor et al. 2002; Tkavc et al. 2011). Such high diversity is confirmed in this study, but the heterogeneities observed among the comparisons made, i.e., natural sites (brines), gut, and laboratory environment (water of *Artemia* cultures) can be attributed, or correlated to salinity differences as it has been previously reported in evaporation ponds of salterns, where bacteria community composition and ecological interactions strongly varied among the salinity gradients (Benlloch et al. 2002; Casamayor et al. 2002; Estrada et al. 2004; Gasol et al. 2004; Tkavc et al. 2011). The substantially different conditions under which *Artemia* cultures were maintained in the laboratory (for convenience, artificial seawater at 35 ppt) explain also why bacterial diversity in the natural and the laboratory realms (water) differs significantly. At the moment of sampling, salinity in natural salty lakes was about 97 ppt, and increased up to 252 ppt in salterns (Table 1). Also weather conditions affect the comparison between *Artemia* sites in northern, central and southern sites, and hence the physico-chemical and ionic water conditions. The latter is also explained by the fact that inland and coastal locations were sampled, which differ in the water ionic composition (Castro et al. 2006). This study is, therefore, considered a snapshot of the existing bacterial diversity at sampling, which was done along different periods owing to the remoteness of sites and the distance between them. For this reason, differences among sites are not emphasized, either comparisons to other studies for similar locations in Chile (Demergasso et al. 2004) or elsewhere (Riddle et al. 2013).

In addition, methodological biases could affect the Shannon diversity index which relates the total number of bands per sample and their relative abundance (intensity), overestimating diversity due to the possible presence of heteroduplex (Casamayor et al. 2002), though the refinement process based on manual analysis of sequences minimized this factor. Yet, the method is particularly good for monitoring changes in bacterial communities in the different environment assessed, according to Benlloch et al. (2002). Finally, the total number of sequenced bands was unevenly distributed over the environments compared. It is worth to highlight the natural environment as a bacterial diversity reservoir, and how such diversity is subsequently reduced in the gut of field and laboratory-reared animals, the water of laboratory cultures being the less variable, a finding consistent with the stringent selective pressures of laboratory conditions. In spite of having reduced variability, the gut of field animals maintains higher similarity in bacterial communities to natural brines (Fig. 6). Albeit partially, this means wild *Artemia* individuals tend to reflect the bacterial diversity of the environment they inhabit, be it nature or laboratory, a finding consistent with the fact that bacterial colonization of the gut is facilitated by the non-selective filter feeder nature of *Artemia*. It is known that brine shrimps contain gut resident bacteria (Niu et al. 2012), but what bacterial types become resident, or gut-adapted, depends on their functional capabilities such as ability for biofilm formation, or to synthesize adhesive molecules to allow attachment to the intestine membrane. It is worth noting in this regard that *Vibrio* species appear to be common colonizers of *Artemia* intestine in some saline systems (Tkavc et al. 2011), and such adhesive properties are importantly associated to its pathogenicity for *Artemia* and other crustaceans (Orozco-Medina et al. 2009). We found one band matching *Salinivibrio* in natural brines of Amarga lagoon in Patagonia.


F_G_LOV3_75
Maritimibacter sp. (GQ131651)
Marinobacter sp. (JN202614)
Buttiauxella agrestis (HF585059)
Simplicispira sp. (JQ229604)
Bordetella sp. (AM990925)
Salinivibrio sp. (KF418154)
F_W_LAM2_41
F_W_LDS2_45
Pseudoalteromonas sp. (GQ849227)
F_W_LAM4_43
L_G_CJR2_48
F_G_CJR3_65
F_G_LAP2_67
F_G_CAH3_72
F_W_LDS3_46
F_G_LDS3_82
F_G_LAM4_79
Chromohalobacter salexigens (KF952437)
Halomonas alkaliphila (KF985241)
Halomonas meridiana (KC842224)
Beta & Gammaproteobacteria
L_W_LAM4_19
L_W_LAM3_18
F_W_CJR5_27
L_W_CJR3_3
Hydrogenophaga flava (AB681848)
Hydrogenophaga caeni (NR_043769)
L_W_LOV1_14
L_W_LAP2_6
L_W_CAH1_12
L_W_BNG2_8
F_W_CAH2_36
Aherensia sp. (AB758568)
L_G_LAM1_58
L_W_CJR2_2
Roseovarius sp. (JF417975)
Roseovarius sp. (KF289476)
Roseovarius sp. (HE576999)
Pseudoruegeria sp. (KC196069)
F_W_CJR3_25
L_W_LAC2_22
L_G_LAC1_60
F_W_LOV2_38
F_G_LDS4_83
L_W_BNG4_10
L_W_CJR1_1
Seohaeicola saemankumensis (NR_044437)
F_W_BNG3_33
Sulfitobacter sp. (KC247329)
Tropicibacter sp. (KC534265)
F_W_LAP2_29
Loktanella salsilacus (KF740556)
F_W_LAM3_42
F_G_CAH1_70
Amaricoccus sp. (JF957137)
L_G_BNG1_52
L_G_LAP1_49
L_G_BNG2_53
L_G_LAC2_61
Oceanicella actignis (JQ864436)
Rhodobacter sp. (KC702687)
F_W_BNG4_34
L_G_LOV1_56
L_G_CAH1_54
F_G_BNG1_68
F_G_LOV1_73
F_G_CAH2_71
F_G_CJR2_64
Sphingomonas aquatilis (EU423085)
Sphingomonas aquatilis (KF542913)
F_G_LAM2_77
F_G_LDS1_80
Alphaproteobacteria
Thiohalomonas denitrificans (EFI17913)
Psychroflexus sp. (JQ390292)
Psychroflexus tropicus (NR_028854)
Psychroflexus lacisalsi (AB381940)
Psychroflexus torquis (NR_074147)
L_W_LAC1_21
F_W_CAH1_35
F_G_LAM1_76
F_W_LAM1_40
F_G_CJR1_63
L_G_CJR1_47
F_W_CJR1_23
F_W_LAP1_28
F_W_LDS1_44
F_W_LOV1_37
Muricauda ruestringensis (NR_074562)
L_W_BNG3_9
L_W_LAP1_5
L_W_LAM1_16
Aureimarina marisflavi (EF108215)
Brumimicrobium sp. (EU195945)
F_W_CJR2_24
F_W_BNG1_31
Crocinitomix catalasitica (AB681013)
L_W_BNG1_7
Owenweeksia hongkongensis
L_W_LAM2_17
F_W_CJR4_26
Belliella sp. (KC762321)
Algoriphagus halophilus (KJ009558)
F_W_BNG232
Bacteroidetes
L_W_BNG5_11
L_W_LOV2_15
L_W_CAH2_13
F_W_LOV3_39
F_W_LAP3_30
Rhodoluna sp. (AB607304)
Candidatus Rhodoluna (AJ565415)
Pontimonas salivibrio (NR_109611)
L_W_LAM5_20
Paraoerskovia marina (AB695379)
L_G_LAC3_62
L_G_LOV2_57
L_G_LAM2_59
L_G_CAH2_55
L_G_LAP3_51
Ornithinimicrobium murale (NR_108532)
L_W_CJR4_4
Dietzia cercidiphilli (KF876893)
Ilumatobacter nonamiensis (AB360345)
F_G_LDS2_81
L_G_LAP2_50
Actinobacteria
F_G_LAM3_78
Halolactibacillus miurensis (AB681280)
F_G_BNG2_69
F_G_LAP1_66
F_G_LOV2_74
Paenibacillus tarimensis (NR_044102)
Paenibacillus darwinianus (KF264457)
Firmicutes
0.10

◀**Fig. 2** Maximum parsimony tree showing the phylogenetic position of bacterial 16S rRNA sequences recovered from DGGE bands. Tree shows the closest relative matching each of the sequences included. Each sequence is scored as environment/site/band number, respectively, 273 × 372 mm (300 × 300 DPI)

This study cannot confirm bacterial types found in the gut as residents, since a different technical approach would be required to exclude the interference of transient (luminal) and body-surface bacterial types (Niu et al. 2012). It is interesting though to highlight that gut of wild-collected animals maintains a fraction of the natural diversity and, in some cases, bacteria not found in the water. How does happen remains uncertain, but it is useful to mention the salt crusts surrounding natural lakes as probable bacteria reservoirs, according to Stivaletta et al. (2011) who studied Piedra Lagoon (LAP), one of the locations sampled in this study. A seasonal wash-out of bacteria from salt crushes occurs during the rainy season, when water level increases. Hence, salt crushes act as a sort of bacterial shelter when ponds tend to dry-out. Such bacteria reservoirs have been also reported in Tunisian salty lakes (Baati et al. 2010).

Environment-type specific bacteria

The differential bacteria survival in each of the assayed environments raises the question on how such bacteria could affect *Artemia* fitness, once incorporated into the gut. Based on a previous study showing bacteria help *Artemia* to degrade microalgae (Intriago and Jones 1993), this section discusses the reported functional capabilities for the principal bacterial types observed in this work. We reckon such information provides preliminary insights on how such bacteria could contribute to *Artemia* survival and reproduction. For example, *Sphingomonas* (*Alphaproteobacteria*) was exclusively found in the gut of wild *Artemia* individuals, a genus reported to have the ability to degrade polycyclic aromatic hydrocarbons (PAHs),

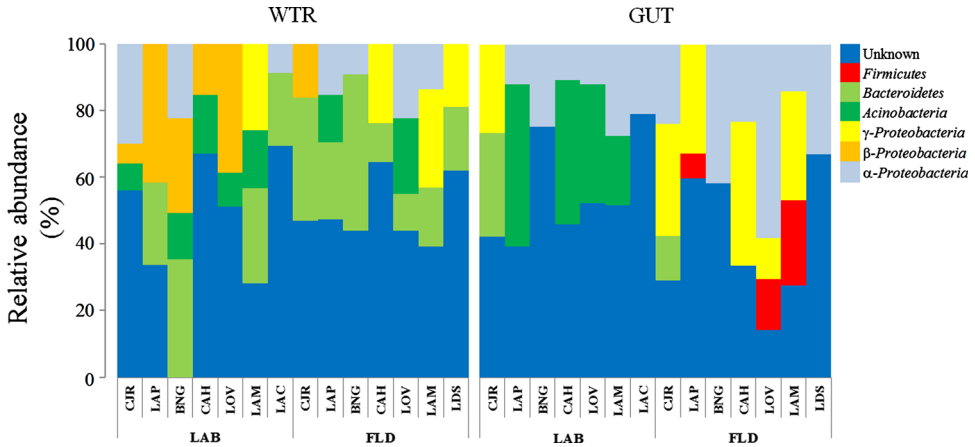

Fig. 3 Relative abundance of known and unknown DGGE bands distributed by Phylum, environment (WTR, GUT, FLD, LAB) and sample site (*CJR* Cejar, *BNG* Barro Negro, *LAP* Piedra, *CAH* Cahuil, *LOV* Lo Valdivia, *LAM* Amarga, *LAC* De los Cisnes, and *LDS* De la Sal). Chart considers bands over 5 % of relative abundance 359 × 167 mm (96 × 96 DPI)

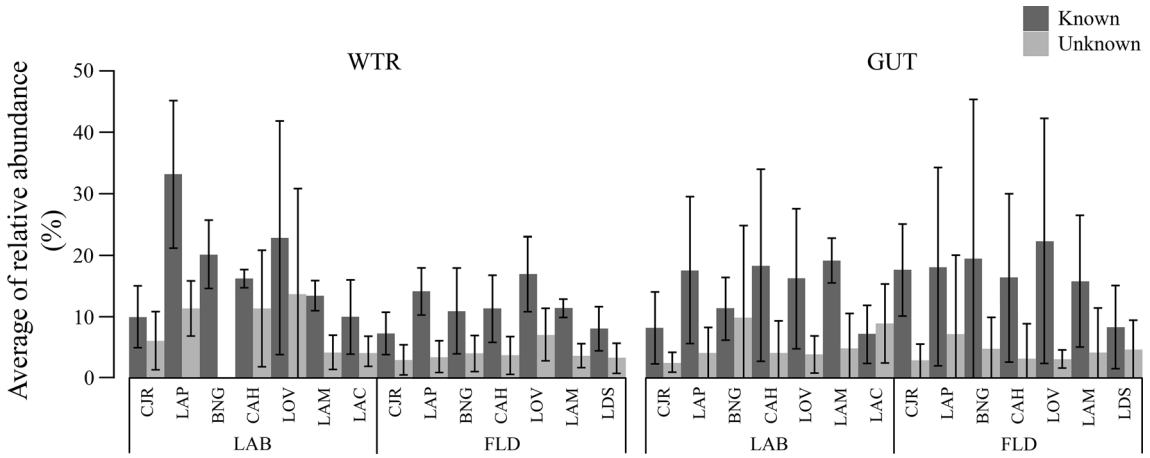

Fig. 4 Average relative abundance in DGGE fingerprints for known (*dark gray*) and unknown (*light gray*) bands in wáter samples (WTR) from laboratory cultures (LAB) and field sites (FLD); *Artemia* gut (GUT) from field and laboratory individuals. For site abbreviations see Table 1 83 × 27 mm (300 × 300 DPI)

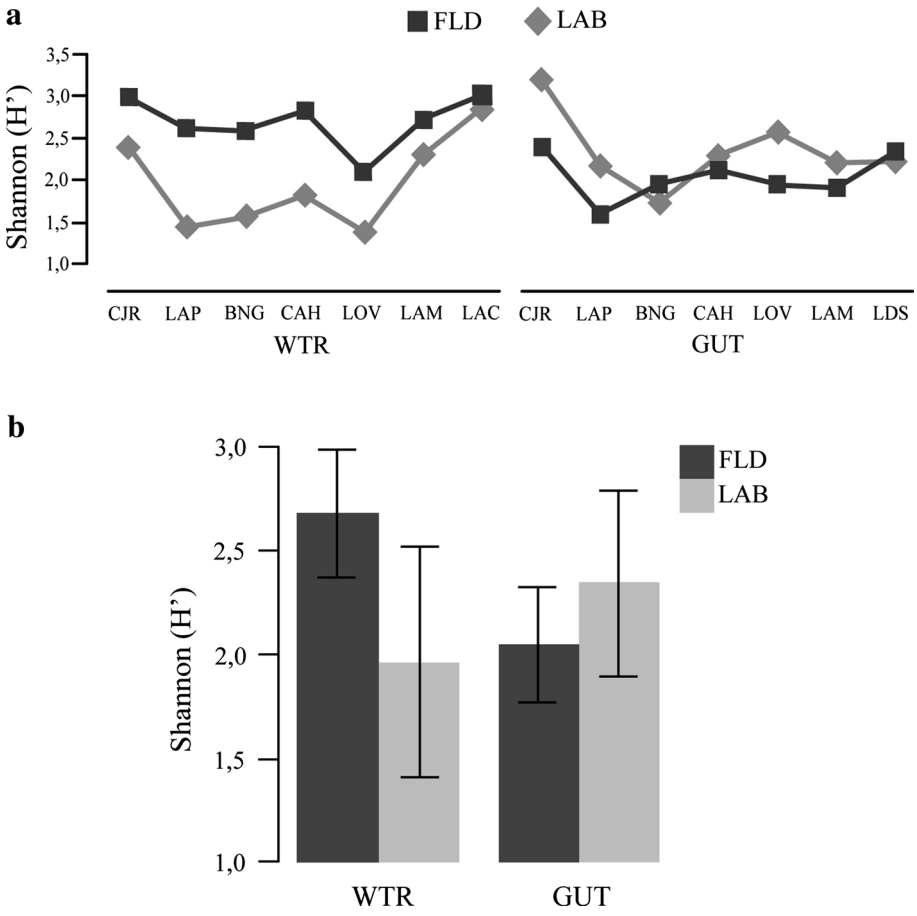

Fig. 5 Ecological diversity (Shannon diversity index, H') in water (WTR) and *Artemia* gut (GUT) from field (FLD) and laboratory-reared cultures individuals (LAB). **a** *lines* represent diversity (H') for all locations sampled; **b** *bars* correspond to an average H' considering all sites ($n = 7$) and environments assessed. Standard deviation is indicated

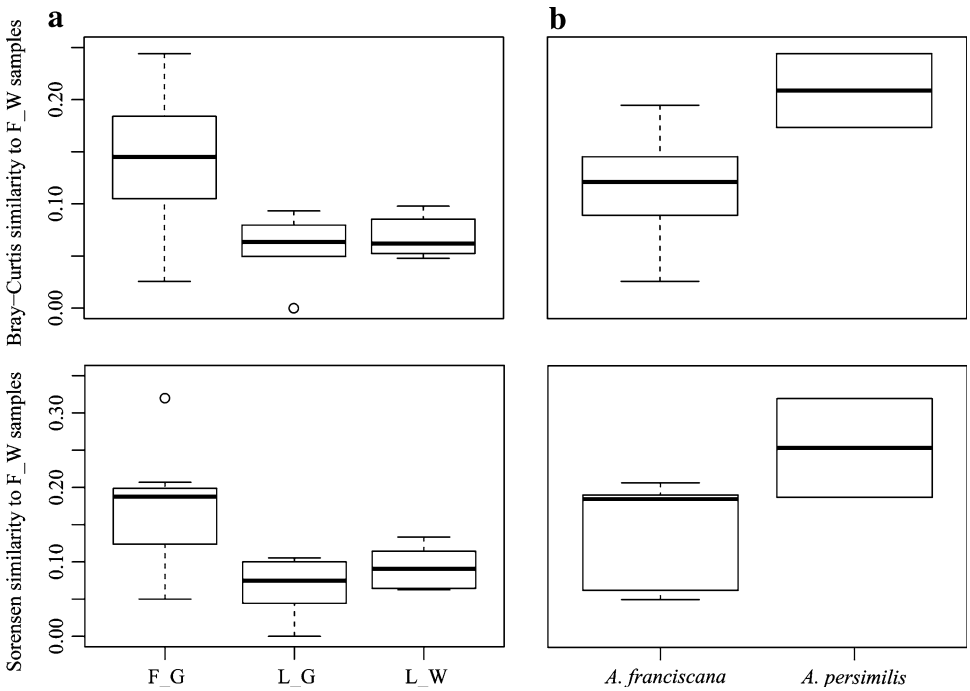

Fig. 6 Similarity to F_W samples of bacterial communities in different environments: **a** Gut of field (F_G) and laboratory-reared animals (L_G), water from laboratory cultures (L_W); **b** *Artemia* species
200 × 150 mm (300 × 300 DPI)

known as persistent pollutants accumulating in the food chain (Yabuuchi et al. 2001; Jones et al. 2001). *Chromohalobacter* (*Gammaproteobacteria*) was mainly identified in the gut of wild *Artemia* collected in north, center and south Chile, but also in some cultured animals. Members of this genus (*C. salexigens*) produce ectoine (or hydroxyectoine) a compound protecting proteins from degradation, and from other environmental stressors such as salinity changes, oxidative stress and high UV radiation (Cánovas et al. 1998; Vargas et al. 2008). Ectoine and other compatible solutes also act as osmoprotectants facilitating bacteria establishment in saline environments (Oren 2008). *Psychroflexus* (*Bacteroidetes*) was the most ubiquitous genus, previously described in the Atacama Desert (Demergasso et al. 2004, 2008, 2010; Dorador et al. 2009), as well as in inland salt lakes in Spain (Casamayor et al. 2013). It was found in water and some gut samples of animals collected in Cejar (north) and Amarga (south), two quite contrasting natural hypersaline lagoons (3,600 km apart). Psychrophilic bacteria are known to produce anti-freeze proteins, and some bacteria found in salty lakes of the Atacama Desert are phylogenetically closer to some types found in the Antarctic. Such similarity tells about convergent environmental conditions in spite of the contrasting latitudes (Dorador et al. 2008). The species *P. salinarum* has been reported in salterns elsewhere (Yoon et al. 2009), an indication of the ecological and physiological abilities of psychrophilic bacteria.

An exclusive and recurrent bacteria detected in *Artemia* gut of the laboratory environment was *Amaricoccus* sp. (*Alphaproteobacteria*), and particularly *A. kaplicensis,* exhibits high capacity to store poly β-hydroxybutyrate (PHB) (Falvo et al. 2001), an effective inhibitor of pathogenic *Vibrio campbelli* in *Artemia franciscana* (Defoirdt et al. 2007; Halet et al. 2007). This molecule (PHB) could be considered a cost-effective method for bacterial control in aquaculture if, for example, it is encapsulated in *Artemia* to feed fish larvae. The finding of *Amaricoccus* in the gut of cultured *Artemia* individuals could be related to the high pathogen exposure under laboratory conditions.

Another aspect worth to mention is the fact that a significant part of bacteria found in the gut of field animals were not detected in the gut of laboratory-reared animals, for example *Paenibacillus* sp. and *Sphingomonas* sp, *Betaproteobacteria* appeared mainly in laboratory water samples (Fig. 3).

Some final words on the relevance of bacteria associated with the *Artemia* species found in Chile. *A. franciscana* is the most widely distributed species, a feature attributed to its plasticity, genetic variability at coding and not-coding regions, and many other virtues (Gajardo et al. 2002). Instead, *A. persimilis* is restricted to southern latitudes and differs genetically and phenotypically from *A. franciscana,* but the species seems to preserve higher environmental bacteria than *A. franciscana* (Fig. 6b). The meaning of this requires further investigation, and both species are nice model systems to go any further on this topic. Finally, the study of *Artemia*–bacteria association has practical consequences for the use of *Artemia* as live diet for the larviculture of marine fish, as *Artemia* cysts are bacterial vectors to the laboratory environment (Riddle et al. 2013).

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References

Baati H, Guermazi S, Gharsallah N, Sghir A, Ammar E (2010) Microbial community of salt crystals processed from Mediterranean seawater based on 16S rRNA analysis. Can J Microbiol 56:44–51

Benlloch S, López-López A, Casamayor EO et al (2002) Prokaryotic genetic diversity throughout the salinity gradient of a coastal solar saltern. Environ Microbiol 4:349–360

Cánovas D, Vargas C, Calderón MI, Ventosa A, Nieto JJ (1998) Characterization of the genes for the biosynthesis of the compatible solute ectoine in the moderately halophilic bacterium *Halomonas elongata* DSM 3043. Syst Appl Microbiol 21:487–497

Caporaso JG, Bittinger K, Bushman FD, De Santis TZ, Andersen GL, Knight R (2010) PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics 26:266–267

Casamayor EO, Schäfer H, Bañeras H, Pedrós-Alió C, Muyzer G (2000) Identification of spatio-temporal differences between microbial assemblages from two neighboring sulfurous lakes: comparison by microscopy and denaturing gradient gel electrophoresis. Appl Environ Microbiol 66:499–508

Casamayor EO, Massana R, Benlloch S, Overeas L, Diez B, Goddard V et al (2002) Changes in archaeal, bacterial, and eukaryal assemblages along a salinity gradient by comparison of genetic fingerprinting methods in a multipond solar saltern. Environ Microbiol 4:338–348

Casamayor EO, Triadó-Margarit XC, Castañeda C (2013) Microbial biodiversity in saline shallow lakes of the Monegros Desert, Spain. FEMS Microbiol Ecol 85:503–518

Castro TB, Gajardo G, Castro M, Castro GM (2006) A biometric and ecologic comparison between *Artemia* from México and Chile. Saline Syst. doi:10.1186/1746-1448-2-13

Clegg JS, Trotman C (2002) Physiological and biochemical aspects of *Artemia* ecology. In: Abatzopoulos Th, Beardmore JA, Clegg JS, Sorgeloos P (eds) *Artemia*: basic and applied biology. Kluwer Academic Publishers, Dordrecht, pp 129–159

Clegg JS, Gajardo G (2009) Two highly diverged New World *Artemia* species *A. franciscana* and *A. persimilis*, from contrasting hypersaline habitats express a conserved stress protein complement. Comp Biochem Physiol A 153:451–456

Core Team R (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Defoirdt T, Halet D, Vervaeren H, Boon N, Van de Wiele T, Sorgeloos P et al (2007) The bacterial storage compound poly-β-hydroxybutyrate protects *Artemia franciscana* from pathogenic Vibrio campbellii. Environ Microbiol 9:445–452

Demergasso C, Casamayor EO, Chong G, Galleguillos P, Escudero L, Pedrós-Alió C (2004) Distribution of prokaryotic genetic diversity in athalassohaline lakes of the Atacama Desert, Northern Chile. FEMS Microbiol Ecol 48:57–69

Demergasso C, Escudero L, Casamayor EO, Chong G, Balagué V, Pedrós-Alió C (2008) Novelty and spatio-temporal heterogeneity in the bacterial diversity of hypersaline Lake Tebenquiche (Salar de Atacama). Extremophiles 12:491–504

Demergasso C, Dorador C, Meneses D, Blamey J, Cabrol N, Escudero L, Chong G (2010) Prokaryotic diversity pattern in high-altitude ecosystems of the Chilean Altiplano. J Geophys Res. doi:10.1029/2008JG000836

Dhont J, Sorgeloos P (2002) Applications of *Artemia*. In: Abatzopoulos Th, Beardmore JA, Clegg JS, Sorgeloos P (eds) *Artemia*: basic and applied biology. Kluwer Academic Publishers, Dordrecht, pp 251–277

Dorador C, Vila I, Imhoff JF, Witzel KP (2008) Cyanobacterial diversity in Salar de Huasco, a high altitude saline wetland in northern Chile: an example of geographical dispersion. FEMS Microbiol Ecol 64:419–432

Dorador C, Meneses D, Urtuvia V, Demergasso C, Vila I, Witzel KP, Imhoff JF (2009) Diversity of *Bacteroidetes* in high-altitude saline evaporitic basins in northern Chile. J Geophys Res. doi:10.1029/2008JG000837

Dumestre JF, Casamayor EO, Massana R, Pedrós-Alió C (2002) Changes in bacterial and archaeal assemblages in an equatorial river induced by the water eutrophication of Petit Saut dam reservoir (French Guiana). Aquat Microb Ecol 26:209–221

Estrada M, Hendriksen P, Gasol JM, Casamayor EO, Pedrós-Alió C (2004) Diversity of planktonic photoautotrophic microorganisms along a salinity gradient as depicted by microscopy, flow cytometry, pigment analysis and DNA-based methods. FEMS Microbiol Ecol 49:281–293

Falvo A, Levantesi C, Rossetti S, Seviour RJ, Tandoi V (2001) Synthesis of intracellular storage polymers by *Amaricoccus kaplicensis*, a tetrad forming bacterium present in activated sludge. J Appl Microbiol 91:299–305

Fraune S, Bosch TC (2010) Why bacteria matter in animal development and evolution. BioEssays 32:571–580

Gajardo GM, Beardmore JA (1993) Electrophoretic evidence suggests that the *Artemia* found in the Salar de Atacama, Chile, is *A. franciscana Kellogg*. Hydrobiologia 257:65–71

Gajardo G, Abatzopoulos T, Kappas I, Beardmore J (2002) Evolution and speciation. In: Abatzopoulos Th, Beardmore JA, Clegg JS, Sorgeloos P (eds) *Artemia*: basic and applied biology. Kluwer Academic Publishers, Dordrecht, pp 225–250

Gajardo G, Beardmore J (2012) The brine shrimp *Artemia*: adapted to critical life conditions. Front Physiol. doi:10.3389/fphys.2012.00185

Gasol JM, Casamayor EO, Join I, Garde K, Gustavson K, Benlloch S, Díez B, Schauer M, Massana R, Pedrós-Alió C (2004) Control of heterotrophic prokaryotic abundance and growth rate in hypersaline planktonic environments. Aquat Microb Ecol 34:193–206

Ghai R, Pašić L, Fernández AB, Martin-Cuadrado AB et al (2011) New abundant microbial groups in aquatic hypersaline environments. Sci Rep. doi:10.1038/srep00135

Gorospe JN, Nakamura K, Abe M, Higashi S (1996) Nutritional contribution of *Pseudomonas sp*. in *Artemia* culture. Fisheries Sci 62:914–918

Halet D, Defoirdt T, Van Damme P, Vervaeren H et al (2007) Poly-β-hydroxybutyrate-accumulating bacteria protect gnotobiotic *Artemia franciscana* from pathogenic Vibrio campbellii. FEMS Microbiol Ecol 60:363–369

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser 41:95–98

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: paleontological statistics software package for education and data analysis. Palaeontol Electron 4:9

Harris JM (1993) The presence, nature, and role of gut microflora in aquatic invertebrates: a synthesis. Microbial Ecol 25:195–231

Huber T, Faulkner G, Hugenholtz P (2004) Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. Bioinformatics 20:2317–2319

Intriago P, Jones DA (1993) Bacteria as food for *Artemia*. Aquaculture 113:115–127

Jones P, Kannan K, Newsted JL, Tillitt DE, Williams LL, Giesy JP (2001) Accumulation of 2,3,7,8-tetrachlorodibenzo-p-dioxin by rainbow trout (*Oncorhynchus mykiss*) at environmentally relevant dietary concentrations. Environ Toxicol Chem 20:344–350

Kalle K (1971) Salinity marine ecology. In: Kinne O (ed) A comprehensive, integrated treatise on life in ocean and coastal waters. Wiley, New York, pp 683–688

Lavens P, Sorgeloos P (1996) Manual on the production and use of live food for aquaculture. Food and Agriculture Organization (FAO), Germany, p 361

Ludwig W et al (2004) ARB: a software environment for sequence data. Nucleic Acids Res 32:1363–1371

Marques A, Dinh T, Ioakeimidis C, Huys G et al (2005) Effects of bacteria on *Artemia franciscana* cultured in different gnotobiotic environments. Appl Environ Microb 71:4307–4317

Mura G, Gajardo G (2011) The highly divergent New World *Artemia* species (*Branchiopoda*, *Anostraca*), *A. franciscana* and *A. persimilis*, show subtle differences in morphological traits involved in mating. Zootaxa 2912:37–48

Muyzer G, de Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microb 59:695–700

Nayak SK (2010) Probiotics and immunity: a fish perspective. Fish Shellfish Immunol 29:2–14

Niu Y, Defoirdt T, Rekecki A, De Schryver P, Van den Broeck W, Dong S, Sorgeloos P, Boon N, Bossier P (2012) A method for the specific detection of resident bacteria in brine shrimp larvae. J Microbiol Meth 89:33–37

Oren A (2008) Microbial life at high salt concentrations: phylogenetic and metabolic diversity. Saline Syst. doi:10.1186/1746-1448-4-2

Orozco-Medina C, Maeda-Martìnez AM, López-Cortés A (2002) Effect of aerobic Gram-positive heterotrophic bacteria associated with *Artemia franciscana* cysts on the survival and development of its larvae. Aquaculture 213:15–29

Orozco-Medina C, Lopez-Cortes A, Maeda-Martinez AM (2009) Aerobic gram-positive heterotrophic bacteria *Exiguobacterium mexicanum* and *Microbacterium sp*. in the gut lumen of *Artemia franciscana* larvae under gnotobiotic conditions. Curr Sci India 96:120–129

Riddle MR, Baxter BK, Avery BJ (2013) Molecular identification of microorganisms associated with the brine shrimp *Artemia franciscana*. Aquat Biosyst. doi:10.1186/2046-9063-9-7

Savage A, Knott B (1998) *Artemia parthenogenetica* in Lake Hayward, Western Australia. II. Feeding biology in a shallow, seasonally stratified, hypersaline lake. Int J Salt Lake Res 7:13–24

Seoka M, Kurata M, Kumai H (2007) Effect of docosahexaenoic acid enrichment in *Artemia* on growth of Pacific bluefin tuna *Thunnus orientalis* larvae. Aquaculture 270:193–199

Sharon G, Segal D, Ringo JM, Hefetz A, Zilber-Rosenberg I, Rosenberg E (2010) Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. P Natl Acad Sci USA 107:20051–20056

Stivaletta N, Barbieri R, Cevenini F, Lopez-Garcia P (2011) Physicochemical conditions and microbial diversity associated with the evaporite deposits in the Laguna de la Piedra (Salar de Atacama, Chile). Geomicrobiol J 28:83–95

Tkavc R, Ausec L, Oren A, Gunde-Cimerman N (2011) Bacteria associated with *Artemia spp.* along the salinity gradient of the solar salterns at Eilat (Israel). FEMS Microbiol Ecol 77:310–321

Toft C, Andersson SG (2010) Evolutionary microbial genomics: insights into bacterial host adaptation. Nat Rev Genet 11:465–475

Toi HT, Boeckx P, Sorgeloos P, Bossier P, Van Stappen G (2013) Bacteria contribute to *Artemia* nutrition in algae-limited conditions: a laboratory study. Aquaculture. doi:10.1016/j.aquaculture.2013.01.005

Triadó-Margarit X, Casamayor EO (2013) High genetic diversity and high novelty in planktonic protists inhabiting inland and coastal high salinity water bodies. FEMS Microbiol Ecol 85:27–36

Van Stappen G (2002) Zoogeography. In: Abatzopoulos Th, Beardmore JA, Clegg JS, Sorgeloos P (eds) *Artemia*: basic and applied biology. Kluwer Academic Publishers, Dordrecht, pp 171–215

Vargas C, Argandoña M, Reina-Bueno M, Rodríguez-Moya J et al (2008) Unravelling the adaptation responses to osmotic and temperature stress in *Chromohalobacter salexigens*, a bacterium with broad salinity tolerance. Saline Syst. doi:10.1186/1746-1448-4-14

Villamil L, Figueras A, Planas M, Novoa B (2003) Control of *Vibrio alginolyticus* in *Artemia* culture by treatment with bacterial probiotics. Aquaculture 219:43–56

Wharton D (2007) Life at the Limits. Organisms in extreme environments. Cambridge University Press, Cambridge. doi:10.1017/CBO9780511541568

Yabuuchi E, Yamamoto H, Terakubo S, Okamura N, Naka T, Fujiwara N, Kobayashi K, Kosako Y, Hiraishi A (2001) Proposal of *Sphingomonas wittichii* sp. nov. for strain RW1(T), known as a dibenzo-*p*-dioxin metabolizer. Int J Syst Evol Microbiol 51:281–292

Yoon JH, Kang SJ, Jung YT, Oh TK (2009) *Psychroflexus salinarum sp*. nov., isolated from a marine solar saltern. Int J Syst Evol Micr 59:2404–2407

Zafrilla B, Martínez-Espinosa RM, Alonso MA, Bonete MJ (2010) Biodiversity of Archaea and floral of two inland saltern ecosystems in the Alto Vinalopó Valley. Saline Syst, Spain. doi:10.1186/1746-1448-6-10