Trophic conditions influence widespread distribution of Aster-Like nanoparticles within aquatic environments

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Title

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Abstract

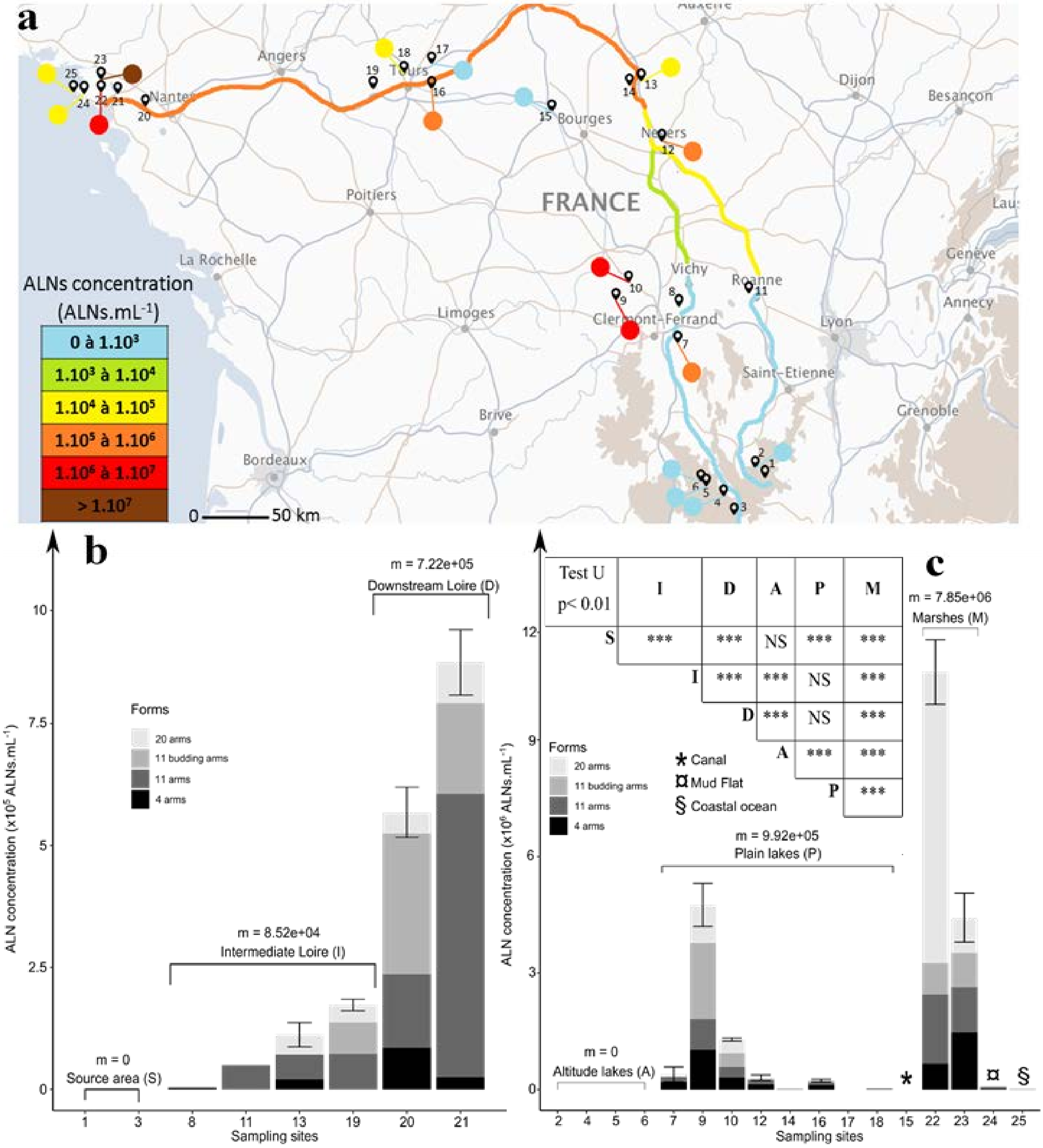
Aster Like Nanoparticles (ALNs) are newly described femto-entities. Their ecology (*e.g.* geographic distribution, spatial dynamic, preferences, forcing factors) is still unknown. Here we report that these entities, which have largely been ignored until now, can develop or maintain themselves in most aquatic environments in the Loire River catchment, France. We observed a significant influence of the trophic state on ALN ecological distributions. A positive relationship between prokaryotic abundance and ALN (r^2 =0.72, $p<0.01$) has been identified, but its exact nature remains to be clarified. Combined with their ubiquitous distribution and high abundances (up to 7.9 x 10^6 ALNs.mL^{-1}) recorded in our samples, this probably makes ALNs an overlooked functional component in aquatic ecosystems.

The discovery of the importance of extracellular vesicles [1], bacteria CPR (*Candidate Phyla Radiation*), archaea DPANN (*Diapherotrites, Parvarchaeota, Aenigmarchaeota, Nanoarchaeota, Nanohaloarchaea*) [2,3] and 'biomimetic mineralo-organic particles' [4] permitted to reconsider the diversity and ecological role of the femtoplankton, hitherto confined to the sole viruses, some <0.2µm filterable prokaryotes [5]. The recent discovery of "*Aster Like Nanoparticles*" (ALNs) in pelagic aquatic environments showed that the femtoplankton still hosts many unknown and undervalued entities [6]. In a previous, unprecedented study on these entities, the authors have shown that ALNs are amorphous star-shaped entities suspected to be organic in nature (composed mainly of carbon, oxygen, calcium and nitrogen with traces of potassium). Pleomorphic, these entities present three main morphotypes, with 4, 11 and 20 arms, and a reduced biovolume, lower than of the smallest known prokaryote. These original characteristics, combined with their sensitivity to biocidal treatments and their ability to grow in the absence of cells, raise questions about their exact nature and origin [6]. Their high seasonal abundances (up to $9.0 \pm 0.5 \times 10^{7}$ entities·mL^{-1}), in the range of virus-like particles, may exceed those of prokaryotes by up to about one order of magnitude [6]. Added to observations of phenotypic close contact with prokaryotes [6], such numerical abundance levels probably make ALNs a significant actor in the functioning of aquatic systems. For example, its calcium composition [6] could have a significant impact on calcium homeostasis of aquatic environments. Without yet knowing their full biological nature, we hypothesized that their distribution and dynamics in a wide range of aquatic environments will open a window on the understanding of their ecological significance.

Except for first findings obtained from lake samples [6], we still lack information on the ecological distribution and spatio-temporal dynamics of ALNs, in relation to the potential environmental forcing factors.

Results acquired in this second study demonstrate that ALNs are overlooked femto-entities, widely distributed and that their dynamics are controlled by trophic status, in particular by the biological environment. Identification and quantification of ALNs and of their morphological variants are described in Colombet and collaborators [6]. Twenty-five study sites distributed over the Loire River catchment (France) were selected to represent different aquatic systems (rivers, canal, marshes, lakes, mudflat, and coastal ocean) as well as longitudinal gradient from the source to the estuary (Figure 1a). Sampling sites were classified into three trophic states according to all abiotic and biotic parameters measured *in-situ*. Further details on the methodology used are provided in the supplementary text and data.

ALNs have the ability to colonize different aquatic environments: lakes, rivers, marshes, coastal oceanic waters, except source areas and altitude lakes (Figure 1). They were found in environments with contrasting physico-chemical (*e.g.* conductivity, oxygen, nutrients) and biological (*e.g.* virus, prokaryotes, algae) characteristics (Supplementary Table S1). This defines them as tolerant and ubiquitous entities capable of maintaining themselves in the majority of continental and coastal aquatic environments. The abundances of ALNs recorded ($1.1 \times 10^7 \pm 0.17 \times 10^7$ ALNs.mL^{-1}) indicate preferential development in plain lakes, marshes, Loire estuary and in lesser extent within free-flowing river channels (Figure 1b-c). Detection of ALNs has also been mentioned in the tropical estuarine system of Ha Long Bay (Vietnam) and in the Saloum River in Senegal, thus broadening the habitat preferences and potential distribution of these entities [6].



Test U p< 0.01	I	D	A	P	M
S	***	***	NS	***	***
I		***	***	NS	***
D			***	NS	***
A				***	***
P					***

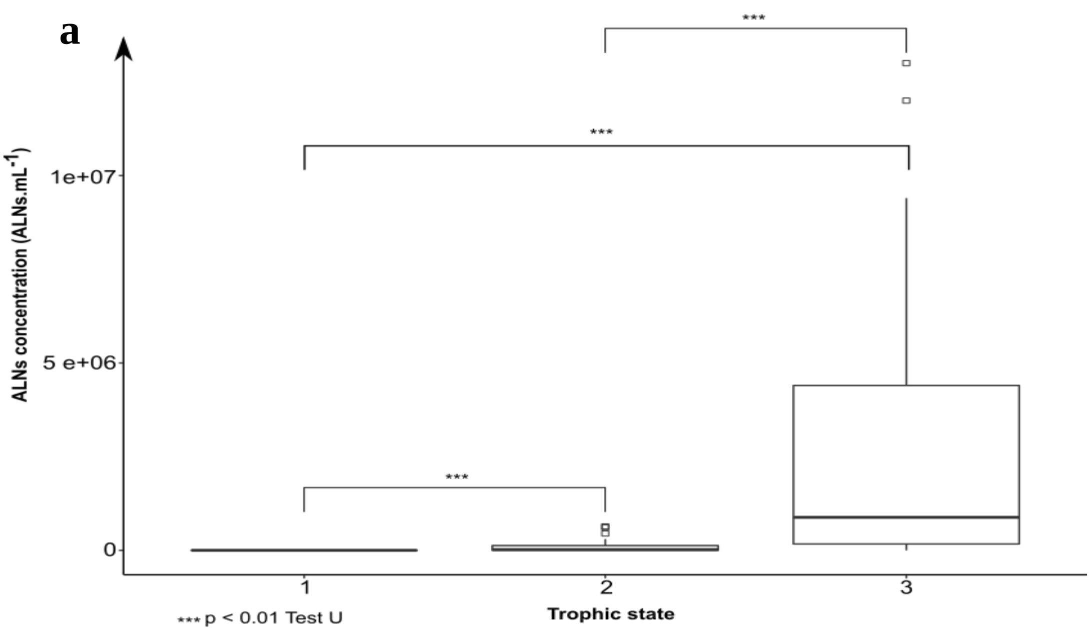
Fig 1. *Sampling site locations (1-25) and distribution of ALNs in different aquatic environments along the watershed of Loire River (France).* ***a.*** *Site locations are plotted on the map of the Loire watershed. Each site or river continuum is characterized by ALN concentration range.* ***b.*** *Distribution of the ALN concentrations and their different forms along the river continuum.* ***c.*** *Comparisons of the ALN concentrations and their different forms between the different aquatic environments sampled. Statistical differences between aquatic environments (test U, *** = p<0.01) are provided in the inserted table (S= Source area, I= Intermediate Loire, D= Downstream Loire, A= Altitude lakes, P=Plain lakes, M=Marshes, NS=Not significant). m= mean.*

However, sampling of these studies was sporadic, and these conclusions must be weighted by the high seasonal or even daily fluctuations of ALNs recorded previously [6]. The sampling period, *i.e*. spring, was based on the results obtained from the unique study on seasonal monitoring of ALNs in a eutrophic lake [6]. We recognize the need of additional data on the seasonal variability of ALNs in different aquatic environments in order to optimize sampling periods.

The presence of ALNs in the coastal ocean samples raises questions about their potential for development in the marine environment and their possible transfer from continental waters to the oceans. We suggest that the gradual and significant increase of ALN abundance (undetectable to 8.8 x 10^5 ALNs.mL^{-1}) from the source to the estuary (Figure 1b) can be explained through the ALN downstream transfer along the river continuum, as observed for other entities or pollutants [7-9]. The occurrence of 11-budding-arm forms in the downstream area suggests that ALNs could be transported from the upstream catchment to more favorable conditions for their development downstream. The occurrence of the different forms of ALN indicates dominance of 20-arm forms (69 % of total ALNs) in marshes, while 11-arm forms dominate in lakes and rivers (38 % and 47 % of total ALNs, respectively; Figure 1c). Environmental conditions probably select and/or favor the development of one type in respect to another, through a process that remains unexplained and which could either reflect the gradual development from one form to another or the occurrence and development of rather independent forms. In order to better understand spatial distributions of ALNs, future investigations will have to demonstrate if a form of resistance distinct from forms of propagation able to develop over time (i.e. assuming distinct growth stages of the same organism) [6], exist.

These observations also point to the hypothesis that ALNs could be present in all aquatic environments but are selected by environmental factors (Baas Becking hypothesis) [10]. Such an assumption is supported by the capacity of ALNs to develop in unrelated biomes, from temperate regions separated by hundreds of kilometers as in our study, to tropical regions [6]. According to the parameters recorded, we reported a significant influence of the trophic state on ecological distributions of ALNs(Fig. 2a) with a significant increase from undetectable ALNs.mL^{-1} in the environments classified in the first trophic state, to 2.2 x 10^6 ALNs.mL^{-1} in those classified in the third trophic state [11]. In this study, the trophic state was estimated according to the Trophic State Index, a reference method for the classification of aquatic environments [12].

Therefore, we suggest that ALNs, in addition to certain microbial components such as phytoplankton communities [13], may serve as a putative indicator of trophic conditions of aquatic environments. Whatever the aquatic system considered, it seems that biological features prevail over physico-chemical ones in the regulation of the distribution and abundances of ALNs (Fig. 2b). The major correlative factor identified is the abundance of prokaryotes (r^2 =0.72, $p< 0.01$; Fig. 2b). A significant statistical correlation and the observation of direct physical contact between prokaryotes and ALNs [6], suggest an interaction between these two components. Previous study showed that ALNs can grow without a potential host [6], however, the presence of prokaryotes could promote their development through mechanisms that remain unknown [6]. Therefore, future investigations are needed to elucidate the nature of this mutual relationship which, perhaps, could be related to either bottom-up resource-driven or to top-down host-symbiont interactions.



b

	Prokaryotes	Viruses	Microphytoplankton	Picocyanobacteria	Picophytoplankton	T°C	pH	O2	Conductivity	P	N	C
ALNs	0.72	0.55	0.53	0.66	0.5	0.47	0.46	-0.3	0.45	0.31	0.19	0.36
Prokaryotes		0.71	0.68	0.67	0.69	0.44	0.5	-0.18	0.45	0.32	0.14	0.61
Viruses			0.38	0.46	0.37	0.36	0.42	-0.02	0.28	0.12	-0.12	0.59
Microphytoplankton				0.5	0.57	0.1	0.16	-0.16	0.15	0.32	0.23	0.56
Picocyanobacteria					0.6	0.6	0.46	-0.12	0.6	0.15	0.3	0.23
Picophytoplankton						0.46	0.35	-0.19	0.36	0.2	0.15	0.44
T°C							0.51	-0.41	0.79	-0.09	0.13	0.08
pH								0.33	0.63	0.27	0.13	0.17
O2									-0.1	0.1	-0.08	-0.13
Conductivity										-0.04	0.17	0.09
P											0.38	0.4
N												-0.09

Spearman correlation coefficient > 0.60 / < -0.60 p-value < 0.01

Fig 2. *Relationships between ALNs and physical-chemical and biological environment.* ***a.*** *Comparison of the average (test U, p<0.01) of ALNs according to the low (state 1 = sites 1, 2, 3, 4), medium (state 2 = sites 5, 6, 8, 11, 14, 15, 16, 18, 19, 20, 24, 25) and high (state 3 = sites 7, 9, 10, 12, 13, 17, 21, 22, 23) trophic levels of the different aquatic environments.* ***b.*** *Correlation analysis (Spearman test, p<0.01) between the different physical chemical and biological parameters of the different sampling points. T°C = temperature, O2=dissolved oxygen, P= total organic phosphorus, N= total organic nitrogen, C= total organic carbon.*

Our findings reinforced the hypothesis of the biological origin of ALNs [6] and the hypothesis that ALNs are ubiquitous and widespread within continental freshwaters and coastal brackish waters. The estimation of the ecological importance of femtoplanktonic entities depends closely on their abundance and functions. This is demonstrated for viruses [14,15], extracellular vesicles, ultramicro-prokaryotes [2,3], and suspected for non-biological entities such as 'biomimetic mineralo-organic particles' [4]. The average abundances of 9.9×10^5 and 7.9×10^6 we recorded respectively in plain lakes and marshes area (see fig.1), are in the range of those of prokaryotes or viruses. The strong empirical relationship with prokaryotes and the regulation by their environment implies that ALNs are additional and so far, ignored actors in aquatic ecosystems. Furthermore, to fully understand the ecological significance of ALNs in plankton dynamics, it is crucial to validate their biological nature.

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Conflict of Interest

The authors declare no conflict of interest.

Authors contribution

MF, HB, MM, JC designed the study and analyzed data. All authors wrote the manuscript, contributed critically and gave final approval for publication.

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