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1 **Title**

2 Trophic conditions influence widespread distribution of Aster Like Nanoparticles
3 within aquatic environments

4

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14

15 **Abstract**

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

25

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

47

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

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

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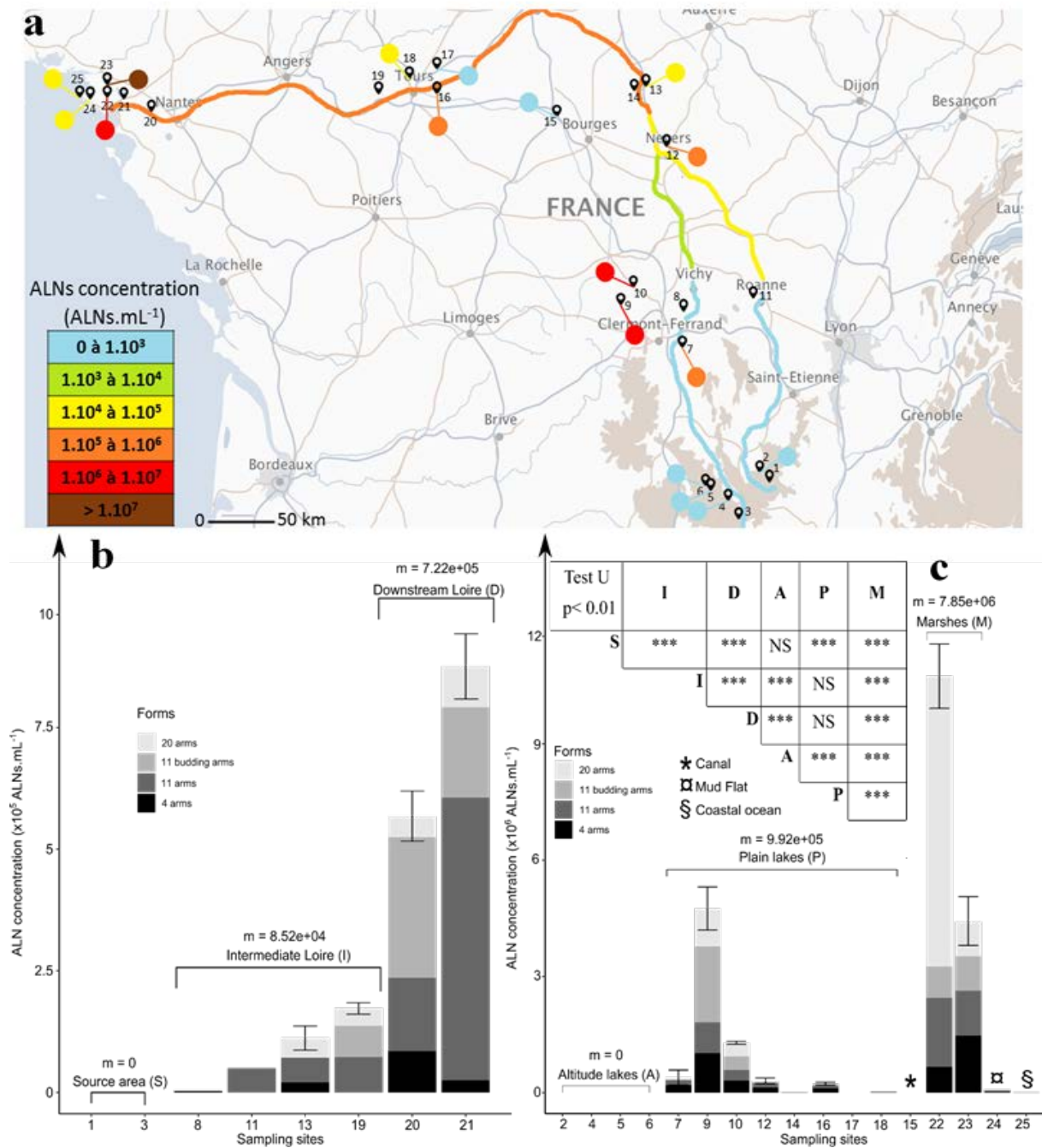
61 ALNs have the ability to colonize different aquatic environments: lakes, rivers, marshes, coastal
62 oceanic waters, except source areas and altitude lakes (Figure 1). They were found in
63 environments with contrasting physico-chemical (*e.g.* conductivity, oxygen, nutrients) and
64 biological (*e.g.* virus, prokaryotes, algae) characteristics (Supplementary Table S1). This
65 defines them as tolerant and ubiquitous entities capable of maintaining themselves in the
66 majority of continental and coastal aquatic environments. The abundances of ALNs recorded
67 ($1.1 \times 10^7 \pm 0.17 \times 10^7$ ALNs.mL⁻¹) indicate preferential development in plain lakes, marshes,
68 Loire estuary and in lesser extent within free-flowing river channels (Figure 1b-c). Detection
69 of ALNs has also been mentioned in the tropical estuarine system of Ha Long Bay (Vietnam)
70 and in the Saloum River in Senegal, thus broadening the habitat preferences and potential
71 distribution of these entities [6].

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

86

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

93

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

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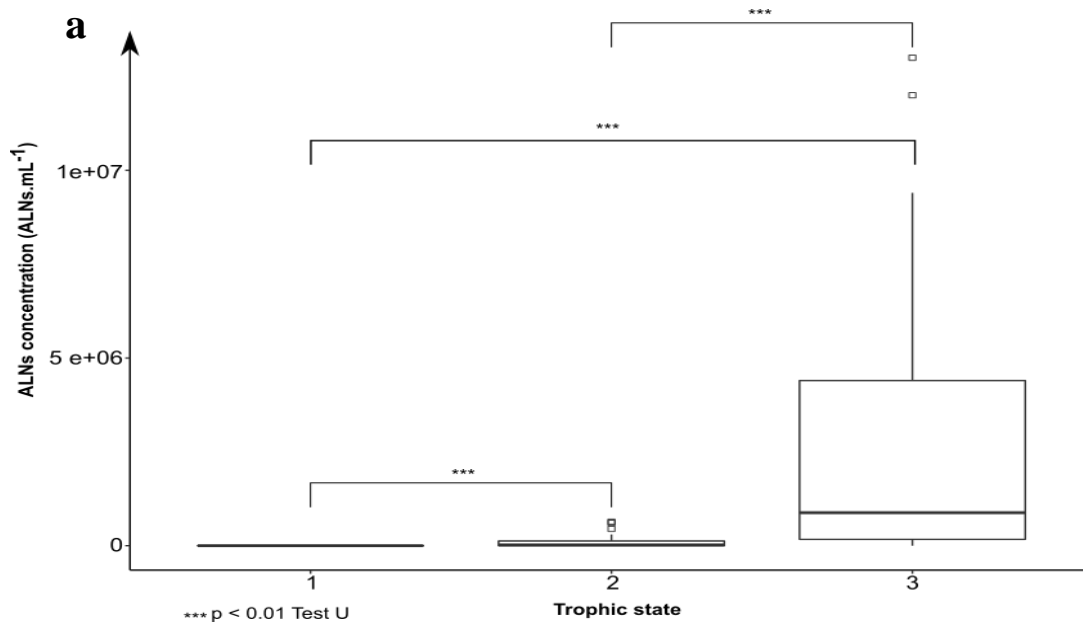
112 These observations also point to the hypothesis that ALNs could be present in all aquatic
113 environments but are selected by environmental factors (Baas Becking hypothesis) [10]. Such
114 an assumption is supported by the capacity of ALNs to develop in unrelated biomes, from
115 temperate regions separated by hundreds of kilometers as in our study, to tropical regions [6].
116 According to the parameters recorded, we reported a significant influence of the trophic state
117 on ecological distributions of ALNs(Fig. 2a) with a significant increase from undetectable
118 ALNs.mL⁻¹ in the environments classified in the first trophic state, to 2.2 x 10⁶ ALNs.mL⁻¹ in
119 those classified in the third trophic state [11]. In this study, the trophic state was estimated
120 according to the Trophic State Index, a reference method for the classification of aquatic
121 environments [12].

122

123 Therefore, we suggest that ALNs, in addition to certain microbial components such as
124 phytoplankton communities [13], may serve as a putative indicator of trophic conditions of
125 aquatic environments. Whatever the aquatic system considered, it seems that biological features
126 prevail over physico-chemical ones in the regulation of the distribution and abundances of
127 ALNs (Fig. 2b). The major correlative factor identified is the abundance of prokaryotes (r^2
128 =0.72, $p < 0.01$; Fig. 2b). A significant statistical correlation and the observation of direct
129 physical contact between prokaryotes and ALNs [6], suggest an interaction between these two
130 components. Previous study showed that ALNs can grow without a potential host [6], however,
131 the presence of prokaryotes could promote their development through mechanisms that remain
132 unknown [6]. Therefore, future investigations are needed to elucidate the nature of this mutual
133 relationship which, perhaps, could be related to either bottom-up resource-driven or to top-
134 down host-symbiont interactions.

135

136



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

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

137

138 **Fig 2.** Relationships between ALNs and physical-chemical and biological environment. **a.** Comparison
 139 of the average (test U, $p < 0.01$) of ALNs according to the low (state 1 = sites 1, 2, 3, 4), medium (state
 140 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,
 141 23) trophic levels of the different aquatic environments. **b.** Correlation analysis (Spearman test, $p < 0.01$)
 142 between the different physical chemical and biological parameters of the different sampling points. T°C
 143 = temperature, O2=dissolved oxygen, P= total organic phosphorus, N= total organic nitrogen, C=
 144 total organic carbon.

145

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

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187

188 **Conflict of Interest**

189 The authors declare no conflict of interest.

190

191 **Authors contribution**

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

194

195

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