TITLE

From behaviour to complex communities: Resilience to anthropogenic noise in a fish-induced trophic cascade

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SUMMARY

Sound emissions from human activities represent a pervasive environmental stressor. Individual responses in terms of behaviour, physiology or anatomy are well documented but whether they propagate through nested ecological interactions to alter complex communities needs to be better understood. This is even more relevant for freshwater ecosystems that harbour a disproportionate fraction of biodiversity but receive less attention than marine and terrestrial systems. We conducted a mesocosm investigation to study the effect of chronic exposure to motorboat noise on the dynamics of a freshwater community including phytoplankton, zooplankton, and roach as a planktivorous fish. As expected under the trophic

47 cascade hypothesis, roach predation induced structural changes in the planktonic
48 communities. Surprisingly, although roach changed their feeding behaviour in response to
49 noise, the dynamics of the roach-dominated planktonic communities did not differ between
50 noisy and noiseless mesocosms. This suggests that the top-down structuring influence of
51 roach on planktonic communities might be resilient to noise and reveals the difficulties on
52 extrapolating impacts form individual responses to complex communities.
53
54 **1. INTRODUCTION**
55
56 The trophic cascade, one of the most influential concepts in ecology, specifies the effects of
57 predators that propagate downward through food webs across multiple trophic levels [1,2].
58 Considering a series of nested consumer-resource interactions (i.e., a food chain), top
59 predators have a direct negative effect on mesopredators and indirect positive and negative
60 effects alternatively on lower trophic levels. Top-down cascade effects can result from
61 changes in predator density (density-mediated trophic cascade) or behaviour (trait-mediated
62 trophic cascade) and much attention has focused on identifying the intrinsic and extrinsic
63 determinants of their strength [3,4]. In particular, this helped to better understand the
64 structural impact of several anthropogenic stressors including warming, salinization,
65 chemical pollution or habitat degradation [5–7].
66 Noise emissions from transportation, cities, industry, military and recreational
67 activities represent another pervasive anthropogenic stressor [8,9]. They span all ecosystems
68 even in the most remote places [10] and have been shown to alter communication, social
69 interactions, use of space, activity patterns, foraging and reproduction in a wide range of taxa

70 [11–13]. First evidence of the cascading effects of noise pollution came from the long-term
71 investigations conducted in the natural gas fields of northwest New Mexico. Bird response to
72 gas-well-compressor noise was found species-specific with some key seed dispersers
73 (mountain bluebirds and Woodhouse's scrub-jays) avoiding noisy areas where pollinators
74 like hummingbirds had on the contrary higher reproductive success [14]. Long-term
75 consequences include alterations in plant communities that persist after removal of the noise
76 source [15]. The propensity of anthropogenic noise to indirectly affect species, and typically
77 primary producers, through a series of nested direct interactions had also been suggested
78 experimentally. Barton *et al*.[16] exposed a three-level terrestrial food chain to various
79 soundscapes for 14 days in plant growth chambers and found that urban sounds and rock
80 music made lady beetles less effective predators, reducing the strength of top-down control
81 on aphids, whose density increased. More aphids ultimately resulted in reduced soybean
82 biomass. Although freshwaters harbour a disproportionate fraction of earth's biodiversity
83 [17] and suffer a greater decline in species richness compared to terrestrial and marine
84 habitats [18,19], they often receive less attention, and research on the impacts of noise
85 pollution is no exception. For instance, we known that fish responses to noise include
86 changes in behaviour and abundances [20–23] but whether these effects spread along food
87 webs to alter planktonic communities through cascading effects remains to be investigated.
88 Similarly, the response of freshwater plankton to noise is largely overlooked. Available
89 evidence to date comes from water fleas, *Daphnia* spp., which are widespread pelagic
90 crustaceans (Cladocera) and an important source of food for upper trophic levels [24,25].
91 Surprisingly, knowing that marine invertebrates of similar size were found to adjust their
92 swimming activity in response to natural or artificial sounds [26], water fleas exposed to

93 band-pass filtered white noise either continuous or intermittent did not show any alteration in
94 swimming speed or depth [27,28]. However, long-term effects of chronic exposure on their
95 survival and reproductive success have yet to be explored, and overall, we lack knowledge on
96 the dynamics of plankton under anthropogenic noise. Here we conducted a mesocosm
97 investigation to study the temporal dynamics of a zooplankton – phytoplankton system under
98 the presence or absence of a planktivorous fish, with and without exposure to motorboat
99 noise. We used the roach *Rutilus rutilus* as top predator: a widespread Eurasian cyprinid fish
100 whose response to motorboat noise has been documented. Roach have specialized hearing
101 structures, the Weberian ossicles, that conduct sound from the swim-bladder to the inner ear
102 and provide high sensitivity to sound pressure [29–31]. They can detect sounds between 10
103 Hz and 5 kHz with a maximum sensitivity of 60 dB re. 1 µPa between 500 and 1000 Hz [32].
104 Roach have been found to respond to authentical motorboat sounds with fewer feeding
105 attempts, higher latency to enter the open area and longer time spent in the vegetation, and
106 these effects persisted after five days of exposure suggesting the absence of habituation [33].
107 After our mesocosm investigation, and in order to get insights into fish growth and behaviour,
108 the roach have been collected, weighted, and measured, and moved to aquaria to assess prey
109 consumption, mobility and group cohesion in the presence or absence of boat noise. Given
110 the lack of knowledge on how freshwater plankton respond to chronic noise exposure, we had
111 no clear prediction on the effect of boat noise on the dynamics of the zooplankton –
112 phytoplankton system without fish. Under the trophic cascade hypothesis, we expected roach
113 presence to reduce the top-down control of phytoplankton by zooplankton. Considering that
114 motorboat noise was found to negatively influence foraging in roach [33], we predicted a
115 decrease in the strength of top-down cascading effect. Concerning roach behaviour, we

116 expected behavioural alterations consistent with the weakening of the trophic cascade in the
117 noisy mesocosms.
118
119 **2. MATERIALS AND METHOD**
120
121 The investigation has been conducted at the PLANAQUA experimental platform of the
122 CEREEP-Ecotron Île-de-France research station (48° 16'10.92 N, 2° 43'50.879 E, Seine et
123 Marne, France) and lasted six weeks (August 31 – October 14, 2020), which corresponds to a
124 prolonged exposure to motorboat noise following Johansson et al. (2016). We assigned three
125 mesocosms to one of four treatments (N = 3 replicates, 12 mesocosms in total): (1) no fish -
126 no noise, (2) no fish - noise, (3) fish – no noise, and (4) fish – noise.
127
128 2.1 Preparation of the mesocosms and animal collection
129
130 Each mesocosm (3.4 m diameter, 1.1 m depth) was filled with 9,079 L of water from the
131 storage lakes of the field station that naturally host zooplankton and phytoplankton
132 communities. An underwater loudspeaker (Electrovoice UW30) was submerged five cm
133 below the surface in the center of each mesocosm. It was connected to an amplifier (Dynavox
134 CS-PA 1MK) and then to an audio player (Handy's H4n zoom), both placed inside a
135 waterproof electric box next to the mesocosm. To promote the growth of phytoplankton, we
136 added 30 mL of Algoflash® (41.4 µg of phosphorus, nitrogen, and potassium per liter) at the
137 beginning of the investigation (August 20) and 50 additional mL at the middle of the
138 investigation (September 19) after we detected a drop in the amount of chlorophyll in the

mesocosms using a multiparameter probe (YSI ExO-2). From March to April 2020, we used fish traps to gradually collect roach from the storage lakes of the PLANAQUA platform, and we stored them in a pond containing the same planktonic communities as the mesocosms. These fish were the descendants of roach used in previous investigations conducted on the platform. They grew in quiet conditions and had never experienced motorboat noise before. At Day 0, 96 roach of similar size (8.54 ± 2.32 cm for standard length, SL) were randomly collected from the storage pond using a seine net, measured, and weighted to the nearest 0.01 cm and 1 g, and distributed in groups of 16 between the six mesocosms (fish – no noise and fish – noise treatments) so as to homogenize size distribution and total biomass between the mesocosms. We placed anti-bird nets on top of the mesocosms to avoid avian predation.

2.2 Plankton dynamics

To assess plankton dynamics, we sampled the mesocosms 13 times from Day 0 to Day 42 and every two or four days. The temporal variation of phytoplankton was assessed through the quantification of green algae, cyanobacteria, and diatom densities. We sampled eight liters of water per mesocosm using a 2-L sampling bottle (Uwitec) at four different positions. Analyses were made in the laboratory using a BBE FluoroProbe^{TM} spectrofluorometer (BBE Moldaenke GmbH, Schwentinental) on a 125-mL subsample previously kept in the dark for one hour. For detecting potential top-down effects on zooplankton, we choose to focus on mesoplankton organisms (Cladocera, copepodits and adults of Copepods, and *Chaoborus* larvae), which appeared as the most responsive organisms in previous mesocosm experiments realized in comparable conditions [34]. To assess their temporal variation in zooplankton, we

sampled 24 liters of water using a 2-L sampling bottle at twelve different positions and depths in each mesocosm. Water was filtered with a 50-µm nylon filter and zooplankton fixed in 15-mL of 90% ethanol. Taxa identification and counting was made on a 3-mL subsample following [35] for cladocerans and copepods (nauplii not counted), and [36] for aquatic insects.

2.3 Fish growth and behaviour

The behavioural tests took place in an experimental room of the PLANAQUA platform thermo-regulated at 17°C. We equipped four 110-L aquaria (80 cm length x 35 cm width x 40 cm height) with an underwater loudspeaker (Electrovoice UW30) in the middle of the left end surrounded by acoustic foam (1.5-cm thick) to attenuate vibrations, a neon light, and a camera (HD-TVI ABUS TVVR33418) above, and black plastic boards outside to avoid visual contacts with the experimenters that may provide stress. The speaker was connected to an amplifier (Dynavox CS-PA 1MK) and to an audio player (Handy's H4n zoom). The aquaria were filled with water from the control mesocosms (no fish – no noise treatment) filtered through a 50-µm nylon mesh filter to remove zooplankton. This experimental design allowed us to run four tests simultaneously with one treatment *per* aquarium depending on the noise condition in the mesocosm and later in the aquarium ("mesocosm – aquarium" noise conditions): (1) no noise – no noise, (2) no noise – noise, (3) noise – no noise and (4). Between two consecutive runs of tests, each aquarium was assigned another treatment to avoid an effect of the aquarium, while water was changed to remove chemical cues.

184 At the end of the mesocosm investigation (Day 44), roach were removed from the
185 mesocosms using a seine net and measured and weighted to the nearest 0.01 cm and 1 g. We
186 matched these values with the weights and lengths measured at the beginning of the
187 mesocosm investigation to recognize fish and calculate individual growth. Roach were then
188 randomly assigned to groups of three individuals and moved into one of the four
189 experimental aquaria. In total, we formed 28 groups (N = 7 replicates per treatment) with two
190 or three groups per mesocosm. Once in the aquarium, they first experienced ambient noise
191 during an acclimatization period of one hour, and then 40 minutes of either ambient noise or
192 ambient noise supplemented with motorboat sounds, depending on the treatment (see section
193 2.4 for further detail on the playback tracks). At the middle of the exposure period (i.e. after
194 20 min), we introduced 50 *Chaoborus* larvae (Diptera) and 50 *Daphnia* sp. (Crustacera:
195 Cladocera), previously collected from the control mesocosms using a 2-L sampling bottle and
196 50-µm nylon mesh filter. Both invertebrates are common prey of roach, differ in terms of size
197 and mobility, and were found in the mesocosms. Although *Chaoborus* larvae are natural
198 predators of *Daphnia* [37], we expected no predation events considering the short duration of
199 the experiment. At the end of the experiment, roach and invertebrates were removed from the
200 aquarium, counted, and returned to the storage pond. The videos were analyzed using
201 Kinovea v. 0.9.4 to get the xy coordinates of each fish each second and during each boat
202 sound (for a total of 691s and corresponding to ambient noise for the other treatment), and
203 then the following parameters were calculated: (1) the cumulative swimming distance (total
204 distance covered by the three individuals) as a proxy of mobility, (2) the distance between the
205 barycenter of each group and the center of the speaker as a proxy of aversion to noise, and (3)
206 the area occupied by the group as a proxy of group cohesion.

Group's barycenter was calculated using individual coordinates as follows:

$$X_{barycenter} = (X_{fish1} + X_{fish2} + X_{fish3}) / 3$$

$$Y_{barycenter} = (Y_{fish1} + Y_{fish2} + Y_{fish3}) / 3$$

The distance between the barycenter and the loudspeaker (D in cm) was calculated as follows:

$$D = \sqrt{((X_{barycenter} - X_{loudspeaker})^2 + (Y_{barycenter} - Y_{loudspeaker})^2)}$$

Group's area (A in cm^2) was calculated as follows:

$$A = \sqrt{((P*(P-d_{fish1\text{-}fish2})*(P-d_{fish1\text{-}fish3})*(P-d_{fish2\text{-}fish3})))}$$

Where d corresponds to the distance between two fish in cm and P to the perimeter of the group in cm with:

$$P = ½ (d_{fish1\text{-}fish2} + d_{fish1\text{-}fish3} + d_{fish2\text{-}fish3})$$

Group barycenter and area were calculated every second during the boat sounds and then averaged.

The behavioural tests took place from 8 am to 6 pm and needed two consecutive days with four mesocosms (two from the fish – no noise treatment and two from the fish – noise treatment) processed on Day 43 and the two others (one *per* treatment) on Day 44. We also conducted four additional tests (two *per* noise condition) without roach to control for *Chaoborus* predation on *Daphnia* and overall invertebrate mortality in the absence of predation.

2.4 Noise treatments

We used Audacity 2.2.1 to generate the audio tracks and an Aquarian Audio H2A-HLR hydrophone (frequency response from 10 to 100 kHz) connected to a ZOOM H4next Handy recorder for all the recordings. The level of background noise did not differ between the mesocosms and ranged from 90 to 95 dB re. µPa. In the mesocosms without boat noise, a 1-hr audio track of silence was looped continuously. In the mesocosms with boat noise, we used the audio tracks described in [38] where 150 sounds of small recreational boats have been distributed over the nine consecutive 1-hr audio tracks of silence going from 9 a.m. to 6 p.m. so as to mimic the daily activity of a small leisure base (Fig. 1A and Supp. Mat. 1, see Rojas et al. 2021 for more details on the audio tracks and the original recordings). We broadcasted silence the rest of the time. We applied a linear fading on both ends of the boat sounds to make them emerge from background noise and adjusted their levels with Audacity to obtain naturally occurring signal-to-noise ratios (SNR) ranging from 4.81 to 27 dB. We used the SNR function of the *seewave* R package [39]:

$$SNR = 20\log_{10}(RMS_{boat\ sound}/RMS_{background\ noise})$$

where RMS is the root-mean-square sound pressure of either the re-recordings of the boat sounds in the mesocosm or the recording of background noise.

In the aquaria without boat noise and to encompass the 1-h acclimatization period and the 40-min exposure period, we used a 100-min audio track of background noise previously recorded in the center of one mesocosm from the no noise – no fish treatment. We adjusted sound level to match that of the mesocosms. In the aquaria with boat noise, we used the 100-min audio track of background noise to which we added twelve boat sounds randomly selected from those broadcasted in the mesocosms. We randomly distributed the sounds over the 40-min exposure period and adjusted their level to match the range of SNR values we had

252 in the mesocosms (approx. 4.81 to 27 dB). In terms of boat traffic, this acoustic regime was
253 representative of the highest activity that roach experienced within a day in the mesocosms
254 (Fig. 1B and Supp. Mat. 1).
255
256 2.5 Data analysis
257
258 All the statistics were performed using R [40] with a significance level of 0.05. We used the
259 Principal Response Curve (PRC, *prc* function of the *vegan* R package [41]) to study how the
260 planktonic communities exposed to roach, boat noise, or both, have diverged over time
261 compared to control communities (i.e. from the no fish – no noise treatment). PRC is a
262 special case of redundancy analysis including time-series data particularly suited to the study
263 of community dynamics in mesocosms [42]. It typically results in a diagram with one curve
264 for each treatment, the time on the x-axis, the first major component of the community effects
265 on the left y-axis and the weights of the taxa on the right y-axis. The more the weight
266 deviates from zero the more the corresponding taxon contributes to the deviation from the
267 control. We used Hellinger-transformed taxa (square root of the relative abundance) to reduce
268 the influence of both rare (low abundances and/or many zeros) and abundant taxa [43].
269 Significance in the PRC was tested using a permutation test (*anova.cca* function of the *vegan*
270 R package) accounting for the non-independence of data due to repeated measurements on
271 the same mesocosm. Significance in the difference between each treatment and the control
272 was assessed with a multiple comparison test (*multiconstrained* function of the *BiodiversityR*
273 R package).
274 Roach' growth rate (G) was computed using the formulae:
275 $$G = (L_{final} - L_{initial}) / L_{initial} * 100$$
276 where L_{final} and $L_{initial}$ are the final and initial SL of roach, respectively.

277 Because growth data met the normality and homoscedasticity assumptions (Shapiro-Wilk and
278 Bartlett tests, all p values > 0.05), we used a linear mixed-effect model (*lme4* R package [44])
279 with the noise condition as fixed factor and the mesocosm as random factor to test for
280 significance of the difference in growth rate between the treatments.

281 The effects of noise and pre-exposure to noise on roach predation were assessed in
282 two ways. First, we used a generalized linear mixed-effect model assuming a Poisson
283 distribution to explain the total number of prey eaten as a function of the noise condition in
284 the mesocosm, the noise condition in the aquarium and their interaction as fixed factors, and
285 the mesocosm ID as random factor. Second, we estimated the preference of roach for
286 *Daphnia* over *Chaoborus* larvae using the Manly's alpha (α) preference index [45,46]:

287 $$\alpha_{daphnia} = \ln(50 - N_{\text{daphnia}}) / (\ln(50 - N_{\text{daphnia}}) + \ln(50 - N_{\text{chaoborus}}))$$

288 where 50 is the initial number of each prey, and N_{daphnia} and $N_{\text{chaoborus}}$ the numbers of daphnia
289 and *Chaoborus* larvae eaten. The Manly's alpha accounts for prey depletion during the
290 predation test and ranges from zero when only the alternative prey (here *Chaoborus* larvae) is
291 eaten to one when only the focal prey (here *Daphnia*) is eaten. The value of 0.5 indicates a
292 lack of preference. As recommended by [47], we compared obtained values to the theoretical
293 value of 0.5 using t tests except for the "no fish – noise" treatment where we used a Wilcoxon
294 test to deal with the non-normality of data.

295 Roach behaviour was analyzed with model averaging and an information-theory
296 approach. We used linear mixed-effect models to model each of the three response variables
297 (cumulative swimming distance, distance to the speaker and area of the group) as a function
298 of the noise condition in the mesocosm, the noise condition in the aquarium, the time, and
299 taking the mesocosm ID as random factor as several groups came from the same mesocosm.
300 Because the noise condition that roach have experienced in the mesocosm might have
301 changed their response to boat noise in the aquarium and because the effect of time may vary

with the noise condition, we also included the interactions between the two noise treatments and between each noise treatment and time in the predictors. All predictors were centered and scaled using the *standardization* function of the *arm* R package. We ranked all the submodels based on small sampled-corrected AIC values (AIC_c, *dredge* and *get.models* functions of the *MuMIn* Rpackage [48]) and performed model averaging on a confidence set of models using a cut-off of 10 AIC_c (*model.avg* function of the *AICcmodavg* R package). The predictors whose parameter estimate had a 95% confidence interval (CI) that included the value of zero were considered as having no significant effect.

3. RESULTS

The pelagic zooplankton communities of the mesocosms included four cladoceran families (Bosminidae, Daphniidae, Sididae and Chydoridae), cyclopoid and calanoid copepods, dipteran larvae of the genus *Chaoborus*, and some ostracods captured in the pelagic zone although being mainly benthic organisms. The diagram of the PRC analysis illustrates how adding roach, boat noise or both make the planktonic communities gradually deviate over time from those of the no fish – no noise treatment (i.e., control) considered as the baseline (Fig. 2). Adding boat noise made the communities deviate from the control and adding roach induced a larger deviation without variation between the two noise conditions (i.e., similar trajectories, Fig. 2). Table 1A shows that 30% of total variance was attributed to time and 34% to the treatment regime, including its interaction with time. On the basis of the permutation tests, the treatment regime as well as time and their interaction had a significant influence on the community dynamics (Table 1B). The pairwise comparisons revealed that the difference between the two treatments with roach (with or without noise) was the only to

be not significant (Table 1C). Bosminidae and in a lesser extent Chydoridae, both copepod taxa and green algae are indicated with a positive taxon weight in the PRC, suggesting they were expected to increase in abundance with the treatments relative to the control and in proportion to their weight. On the other hand, Daphniidae and, to a lesser extent ostracod, Sididae and *Chaoborus* larvae exhibited negative species weights and were expected to decrease in abundance with the treatments. The taxa weights of diatoms and cyanobacteria were the smallest and close to zero (Fig. 2).

Individual fish growth did not significantly differ between the noise conditions ($\mathcal{X}_1^2$ = 1.4813 and p = 0.2236, Fig. 3). In the absence of roach, prey survival in the aquaria was 100% for both prey in the absence of boat noise, and 100% for *Chaoborus* larvae and 98% for *Daphnia* in the presence of boat noise. We therefore considered prey mortality during the predation tests to be the result of fish predation only. The effect of noise on the total number of consumed prey depended on the noise condition in the mesocosms, with significantly less prey consumed only for roach coming from the noiseless mesocosms ($\mathcal{X}_1^2$ = 18.36 and p < 0.001 for the interaction between the two noise conditions, Fig. 4A). Whatever the noise condition in the mesocosm, the Manly's alpha index did not differ from the theoretical value of 0.5 in noiseless aquaria (t = 1.36 and p = 0.22 for ambient noise, t = -0.32 and p = 0.76 for boat noise) but was significantly higher with boat noise (V = 27 and p = 0.03 for ambient noise, t = 3.10 and p = 0.02 for boat noise, Fig. 4B). Concerning the cumulative swimming distance and the distance to the speaker, the 95% CI of the parameter estimate included the value of zero for all the predictors. Concerning the area occupied by the group, boat noise in the aquarium and time were the only predictors whose parameter estimate 95% CI did not include the value of zero, with significantly positive values (Fig. 5).

4. DISCUSSION

Exposure to anthropogenic noise is known to elicit physiological or behavioural responses in individual organisms [8,11–13,22]. But to what extent these alterations spread across ecological interactions to alter community dynamics and ecosystem functions is not clear. We conducted a mesocosm investigation to study the impact of chronic exposure to motorboat noise on the dynamics of a zooplankton – phytoplankton system either alone or dominated by a planktivorous fish. Although we detected alterations in fish feeding and behaviour, the strength of top-down control and its consequences on the structure of the planktonic communities were resilient to motorboat noise. This suggests that individual responses to noise do not necessarily result in ecological effects at the level of communities.

The pelagic mesoplankton communities of our mesocosms were dominated by cladocerans and copepods, two major groups of herbivorous microcrustaceans widespread in freshwater bodies. In smaller proportions, they also included ostracods (coming from the benthic areas of the mesocosms), and predatory larvae of the *Chaoborus* genus, known to feed on small zooplankton [49]. In fishless mesocosms, daphnid cladocerans gradually became the most abundant taxa. Compared to copepods, cladocerans have higher reproduction rates. Moreover, because daphnids are the largest cladocerans, they suffer smaller predation risk by *Chaoborus* larvae than the other cladocerans [50]. The presence of roach made the planktonic communities gradually deviate from those of the fishless communities with a shift in the dominant taxon of zooplankton from daphnids to bosminids whose abundance greatly increased. Visual-feeding fish like roach tend to prefer large

372 zooplankton [51] and it might be that selective predation on the two largest taxa: *Chaoborus*
373 larvae and daphnids, has released bosminids from predation and competition. Alteration in
374 the daphnids – bosminids balance is symptomatic of fish presence [52]. To a lesser extent,
375 copepods and Chydoridae have also benefited from the roach-induced decrease in daphnids.
376 This might be explained by the greater availability of food resources like green algae, which
377 slightly increased in the presence of roach, but also rotifers that represent another important
378 taxon of freshwater zooplankton. Due to their small size, we did not count the number of
379 rotifers but they are known to increase in the presence of fish because of the removal of large
380 cladocerans [53]. The slight increase in green algae in the presence of roach is consistent with
381 the trophic cascade hypothesis: fish have a negative direct effect on zooplankton (here
382 daphnids) and indirectly benefit phytoplankton that is released from grazing [34]. Another
383 way roach can influence the planktonic communities is through the modulation of diel
384 migration patterns. Indeed, some taxa migrate to the bottom under chemical cues by predators
385 and become less frequent in the pelagic realm [54].

386 Motorboat noise did not alter the top-down structuring effect of roach on the
387 planktonic communities and particularly the shift from daphnids to bosminids. This suggests
388 that chronic exposure to noise had no effect on the feeding behaviour of roach, which is
389 consistent with the absence of difference in growth rate between the two noise conditions.
390 This is also consistent with the total number of prey eaten recorded during the predation tests,
391 which was significantly reduced by motorboat noise for the roach that never experienced boat
392 noise before but not for those pre-exposed to boat noise in the mesocosms. Weakening of the
393 response to noise after repeated exposure has been reported in other fish species [38,55,56],
394 and might reflect habituation through associative learning: naïve animals first allocate

attention to noise at the expense of other activities like feeding, and then resume normal behaviour as they learn that it is not associated with any threat. However, when looking closer to what has been eaten, we found motorboat noise to elicit selective preference for daphnids over *Chaoborus* larvae even for roach pre-exposed to boat noise in the mesocosm. This persistent response could find its origin in behaviour. Concerning invertebrates, although we did not record their behaviour, we know from past investigations that motorboat noise did not alter the mobility of daphnids [27] but triggers body rotations in *Chaoborus* larvae [38], interpreted as an anti-predatory response [57,58] that could have driven the choice of roach towards daphnids. Concerning roach behaviour, we found no alteration in mobility and no evidence for any avoidance of the sound source, but the area occupied by the three individuals was larger under motorboat noise. This effect seems to be persistent as it was also observed with the roach pre-exposed to boat noise in the mesocosms. Similarly, playback of pile driving was found to make juveniles of seabass les cohesive [59]. Noise could mask the perception of nearest neighbours' movements through the lateral line or impair the ability to process sensory information as a consequence of stress and/or distraction [59]. Compared to stress or distraction, masking does not weaken with repeated exposure. This could explain why the reduced group cohesion was also observed in the roach that experienced motorboat noise in the mesocosms. Disruption of group cohesion could ultimately compromise the benefits of grouping associated with the dilution and confusion effects [60]. Regarding feeding, we can expect the strength of intra-specific competition to decrease with the distance between individuals. Together with the lesser catchability of moving *Chaoborus* larvae, this could explain why the roach showed selective preference for daphnids under motorboat noise. At the level of communities, a selective preference for

418 daphnids, which were the main grazers, should have strengthened the trophic cascade, what
419 we did not observe. The change in feeding we found in the aquaria may not have been strong
420 enough to be detected in the mesocosms or maybe does not occur in a larger and more
421 complex environment where other prey items are available.

422 In the fishless mesocosms, adding motorboat noise induced a small but detectable
423 deviation from the control communities. This is interesting but also tricky to interpret since
424 very little is known on the response of freshwater plankton to chronic anthropogenic noise.
425 *Chaoborus* larvae occupied the highest trophic level of the fishless communities and we
426 know that they are sensitive to motorboat sounds with more body rotations [38], interpreted
427 as an anti-predatory response [58]. If noise also interferes with prey processing and reduces
428 the capture efficiency of *Chaoborus* larvae, then it could be beneficial to small zooplankton.
429 Noise could also trigger vertical migration to the bottom, as chemical cues from predators do
430 [54], making some taxa like ostracods less detectable in the pelagic realm. Additional long-
431 term investigations in mesocosms but also *in situ* are needed to better understand the response
432 of planktonic communities to chronic anthropogenic noise.

433 Our investigation illustrates how extrapolating the impact of anthropogenic noise
434 from individual responses to complex communities if far from obvious. Although we
435 observed persistent alterations in roach behaviour with less group cohesion and altered
436 feeding preference, these effects did not propagate downward along the food chain through
437 trait-mediated cascading effects. A valuable perspective would be to study the dynamics of
438 roach under chronic anthropogenic noise to test whether the behavioural responses we
439 observed ultimately decrease survival and/or reproductive success [61], and result in density-
440 mediated cascading effects.

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ETHIC STATEMENTS

All the procedures were conducted in accordance with appropriate European (*Directive* 2010/63/EU) and French national guidelines, permits, and regulations regarding animal care and experimental use (Approval no. C42-218-0901).

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY

The data that support the findings of this study are open available in Zenodo at https://doi.org/10.5281/zenodo.6760791.

FIGURE LEGENDS

Figure 1: Sound spectra of the two noise conditions (control: dashed lines, boat noise: one solid lines for each hour from 9 am to 6 pm) broadcasted in A) the mesocosms and B) the aquaria. Spectra were made from 1-hour recordings in the mesocosms and 20-min recordings in the aquaria.

Figure 2: Principal Response Curve (PRC) showing the effects of adding fish (squares), motorboat noise (dots) or both (triangles) on freshwater plankton communities compared to control communities (no fish – no noise, horizontal line, see text for further detail). Species weights are on the left axis (bos: Bosminidae, cyclo: cyclopoid copepods, cala: calanoid copepods, chydo: Chydoridae, green: green algae, diat: diatoms, cyano: cyanobacteria, sidi: Sididae, chao: *Chaoborus* larvae, ostra: ostracods, daph: Daphniidae). See Table 1 for the percentages of variance accounted for and the significance levels.

Figure 3: Fish growth rates (medians and interquartile ranges) in the mesocosms depending on the noise condition (white box: ambient, grey box: boat noise, $n = 48$ *per* condition).

485 **Figure 4**: Results of the predation tests in aquaria with A) the total number of prey eaten *per*
486 group of three fish (n=7 groups per acoustic mesocosm-aquarium treatment) presented to 50
487 *Daphnia* and 50 *Chaoborus* larvae and B) the Manly's alpha preference index for *Daphnia*
488 over *Chaoborus* larvae (medians and interquartile ranges) as a function of the noise condition
489 previously experienced in the mesocosms and the noise condition in the aquaria (white box:
490 ambient, grey box: boat noise).
491
492 **Figure 5:** Model-averaged coefficient estimates and 95% confidence intervals for the
493 predictors included in the confidence set of models explaining the behaviour of roach *Rutilus*
494 *rutilus* in groups of three individuals when feeding on *Daphnia* and *Chaoborus* larvae during
495 the predation tests in aquaria. We used as response variables A) the area of the group, B) the
496 cumulative swimming distance and C) the distance to the speaker. Predictors correspond to
497 the noise condition in the mesocosms (boat noise or ambient noise as control), the noise
498 condition in the aquaria of the predation tests (boat noise or ambient noise as control), the
499 time, and the two-way interactions between time and the noise condition in the two
500 experimental units and between the noise conditions of the two experimental units.
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507

TABLE

Table 1: Results of the Principal Response Curve (PRC) for the effect of motorboat noise (absence / presence) and fish (absence / presence, for a total of four treatments) on freshwater planktonic communities. A) Proportion of the total variance explained by the constraints: time, treatment, and their interaction, captured by the canonical 1st axis of the PRC. B) Significance of the PRC diagram on the basis on the permutation test for Constrained Correspondence Analysis (CCA, 999 permutations). C) Pairwise comparisons for all the possible treatment combinations following a CCA analysis.

A)

	Inertia	Proportion	Rank
Total	0.236	1	
Conditional (% of the total variance explained by time)	0.071	0.30	12
Constrained (% of the total variance explained by time*treatment)	0.085	0.36	11
Unconstrained	0.080	0.34	11

(% of the total variance not explained by predictors)			

526

527 B)

	Df	Variance	F	Pr(>F)
Treatment	3	0.04	19.28	0.001 ***
Time	1	0.06	77.32	0.001 ***
Treatment*Time	3	0.02	8.98	0.001 ***

528

529 C)

Pairwise comparison	Df	Sum of Sqs	F	Pr(>F)
no fish - no noise fish - no noise	1	0.054	23.856	0.001 ***
no fish - no noise no fish - noise	1	0.011	4.801	0.003 **
no fish - no noise fish - noise	1	0.059	22.593	0.001 ***
fish - no noise no fish - noise	1	0.022	8.902	0.001 ***
fish - no noise fish - noise	1	0.005	1.624	0.177
no fish - noise fish - noise	1	0.027	9.523	0.001 ***

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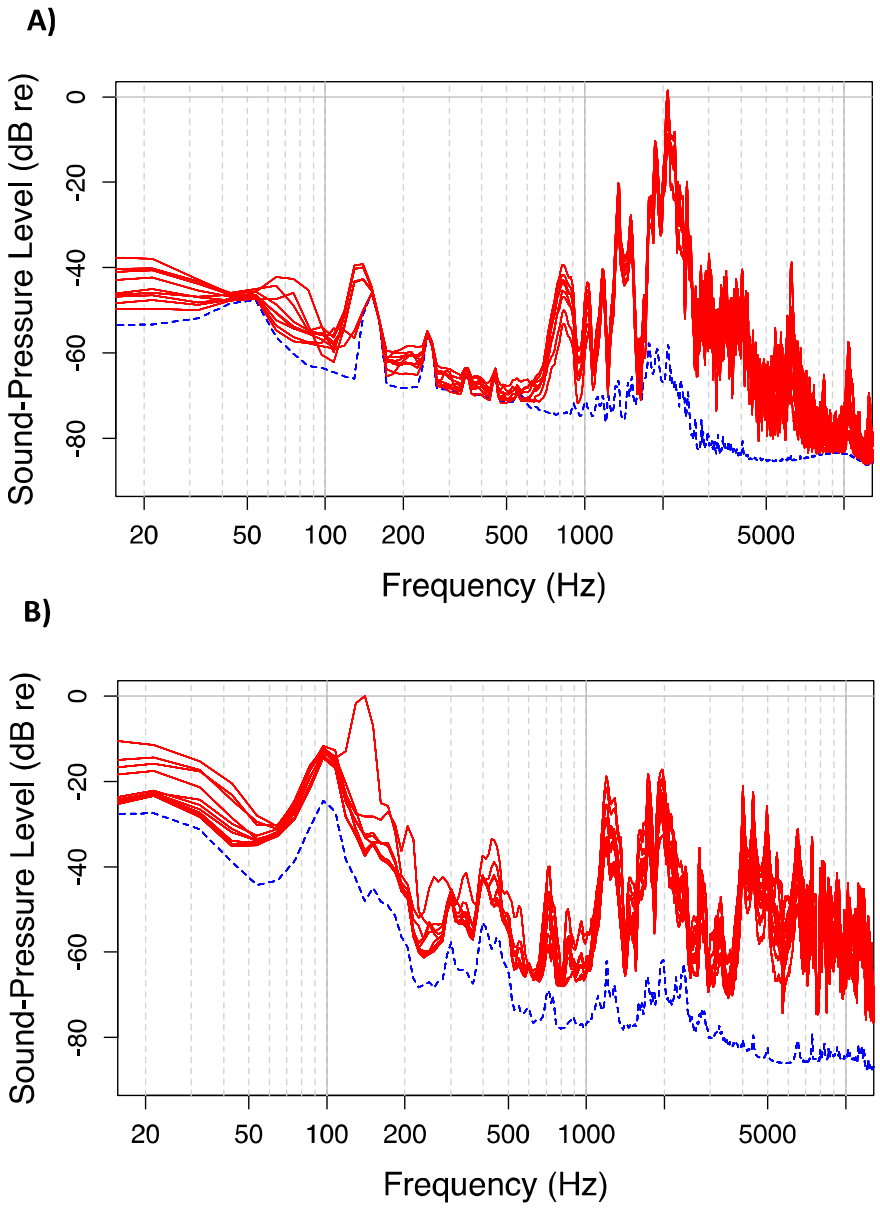

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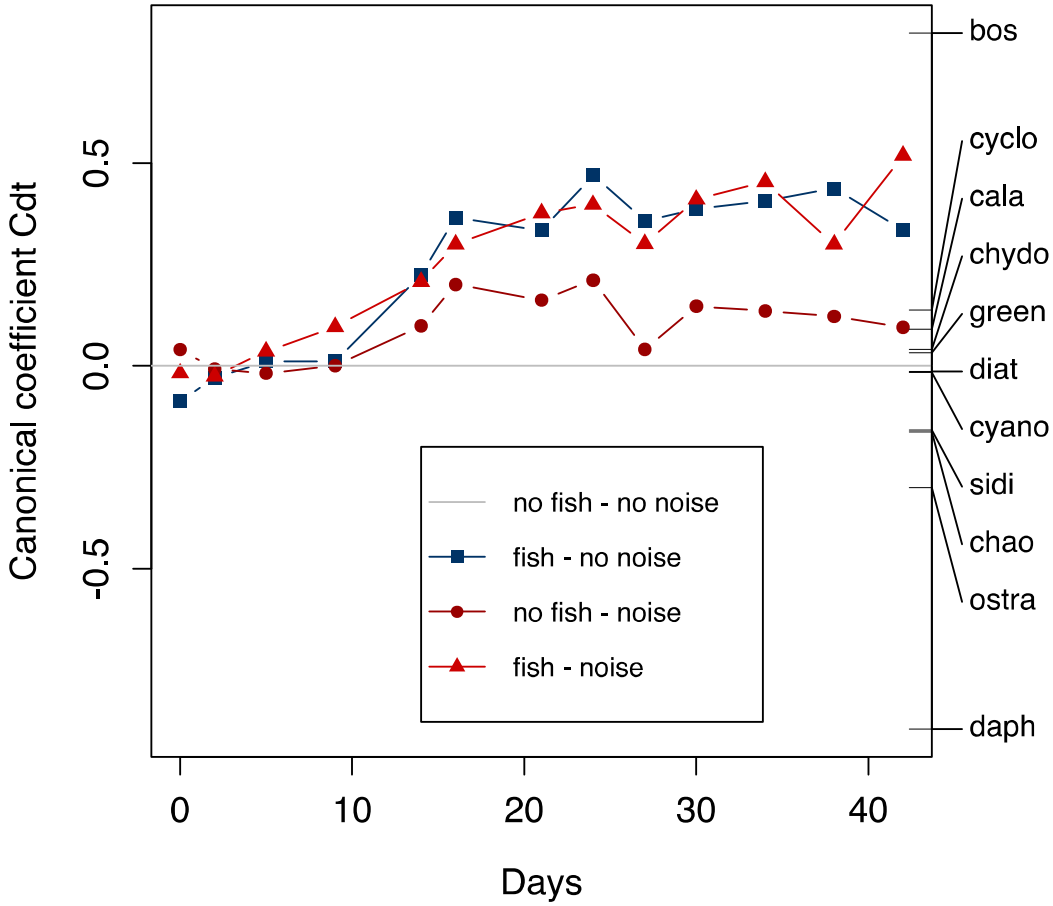

Fig. 2

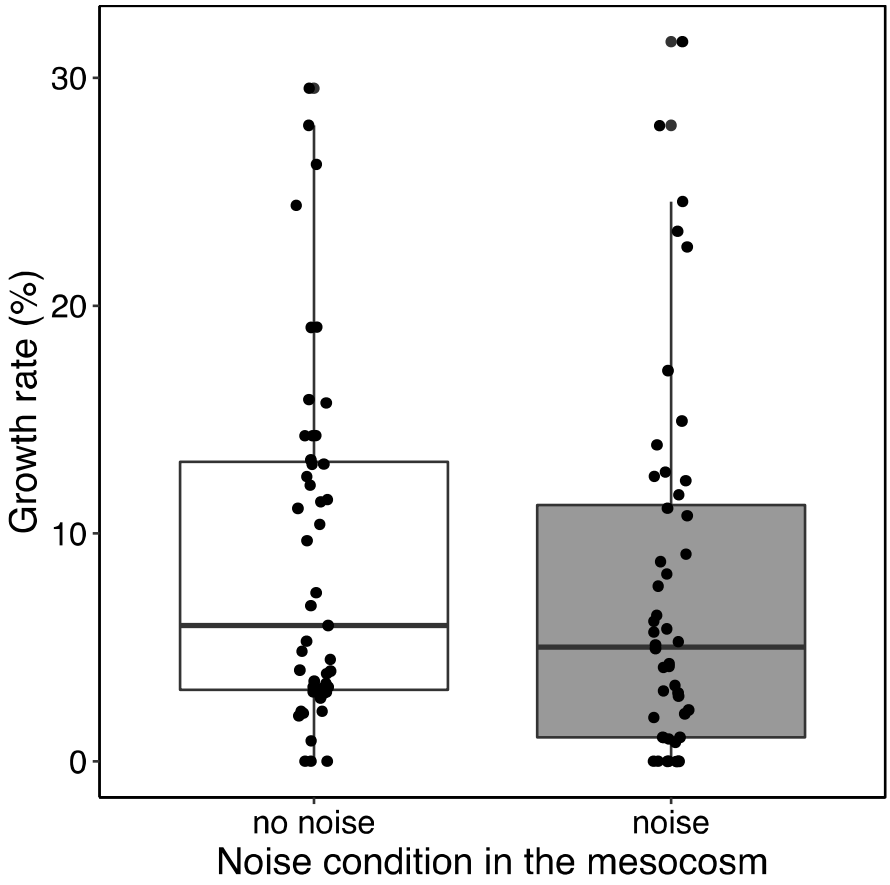

Fig. 3

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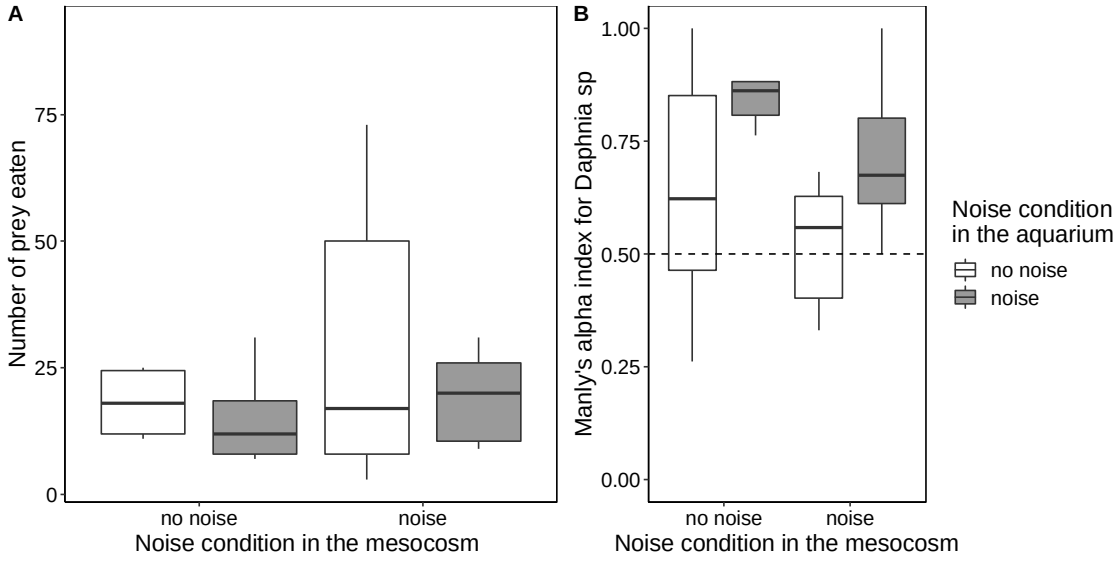
735 **Fig. 4**



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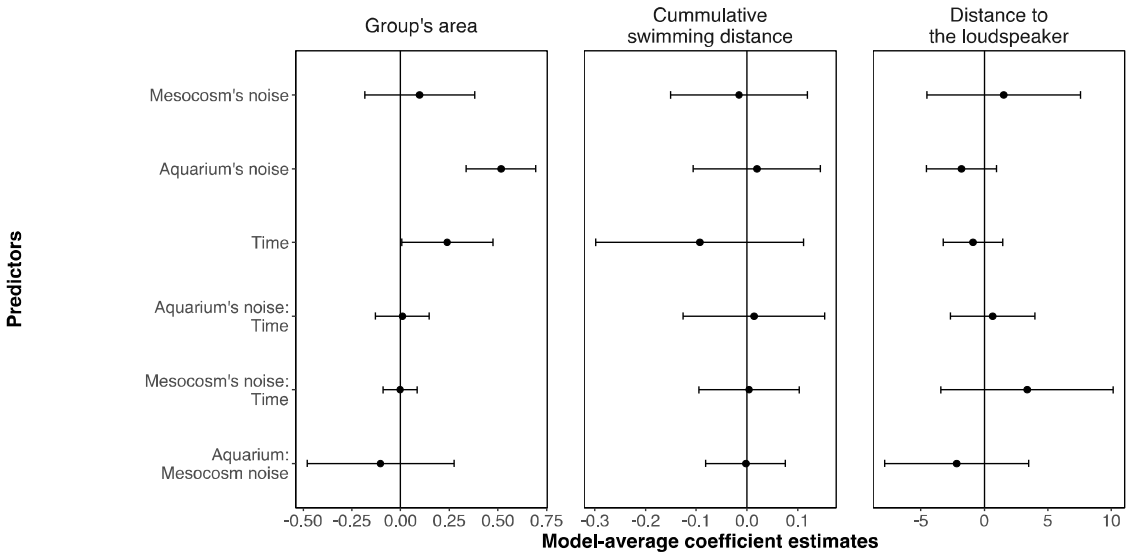

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739 **Fig. 5**