

The experimental lake platform of PLANQUA

The experimental lake platform (ELP) is located on the CNRS-ENS field station near Paris (www.foljuif.ens.fr). Figure 1 presents an aerial view of the lake platform with its 16 experimental lakes, connected by groups of 4 by 10-m long dispersal canals (these canals are closed at this time). The two large lakes are:

- A 4000-m³ storage lake (south), which is used to homogenize water before distribution to experimental lakes and to make mesocosm experiments on a floating pontoon (this reservoir contains fishes [roach and perch]),
- A 4000-m³ drainage lake (north) that collects used water at the end of experiments and may also be used for experiments (this reservoir is fishless and generally contains large Daphnia (*Daphnia pulex*) and *Chaoborus* larvae).



Fig. 1. Aerial view of the experimental platform: the storage lake is visible in the foreground, while the drainage lake is visible in the background, behind the 16 experimental lakes. The mesocosm platform is visible on the eastern side of the experimental lakes.

I - The experimental lakes

Each 15 X 30 m experimental lake is structured with two shallow (30 cm of water) littoral zones and a deeper (3-m deep) central zone (Fig. 2), yielding a volume of 700 m³. The bottom of littoral areas is constituted by 20 cm of arable soil. As visible in figure 3, dense communities of macrophytes (mainly composed of *Typha* spp. and *Juncus* spp.) cover the littoral areas. Just at the end of the littoral areas, the pelagic zone begins, the slope of the edges is 45°. The bottom of the central area is composed by 30 cm of sand of the River Loire.

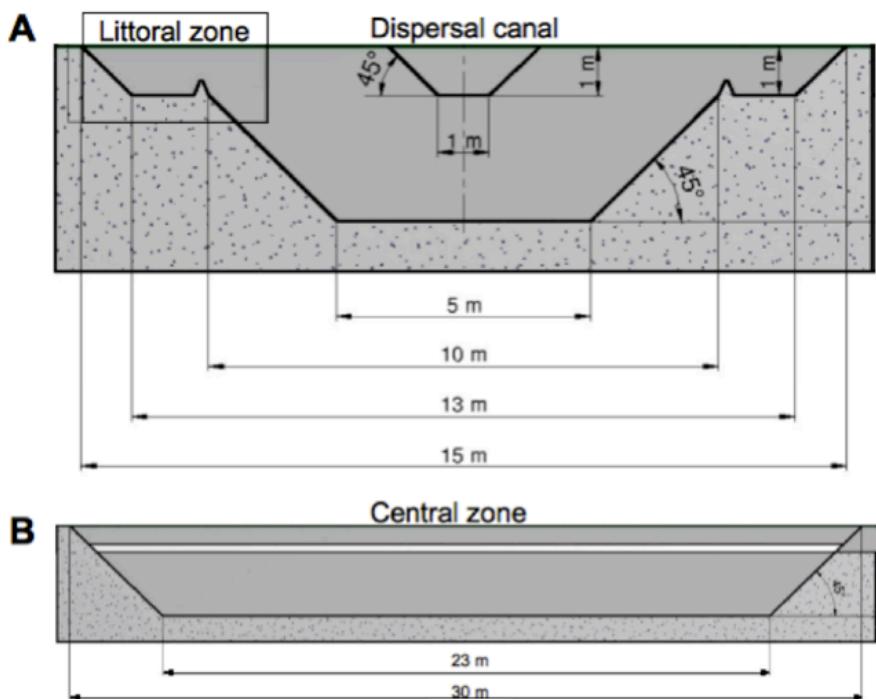


Fig. 2. Detailed scheme of an experimental lake:
 (A) Cross section showing the littoral vs. central structure with angles and dimensions. Also visible is the dispersal canal (dispersal canals are closed during this experiment).
 (B) Longitudinal section of an experimental lake.

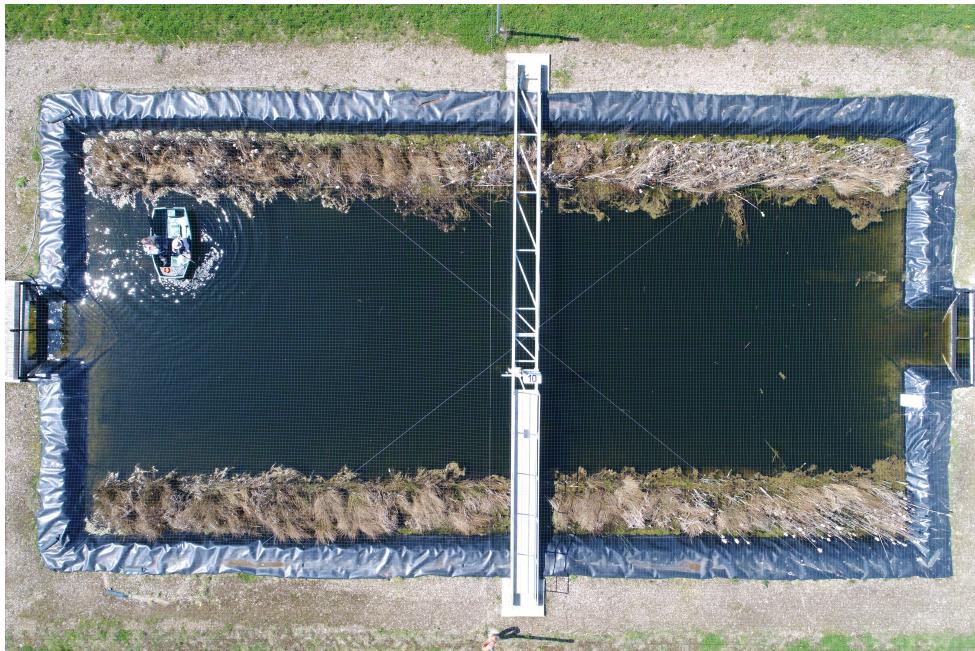


Fig. 3. View of an experimental lake (30 x 15 m) with its two littoral areas, and the bridge that supports the automatic probe system. A small boat (with two persons sampling water) is visible on the upper left part of the picture. A net with a 10-cm mesh size protects the whole lake platform against large birds.

II – The main steps of the creation of the experimental lakes platform

The period going from Autumn 2013 to Summer 2014 was devoted to the construction of the artificial lakes (Figs. 4 & 5).



Fig. 4. Creation of an artificial lake, with a littoral area on each side of the greater length. The beginning of a dispersal canal is visible in the background.

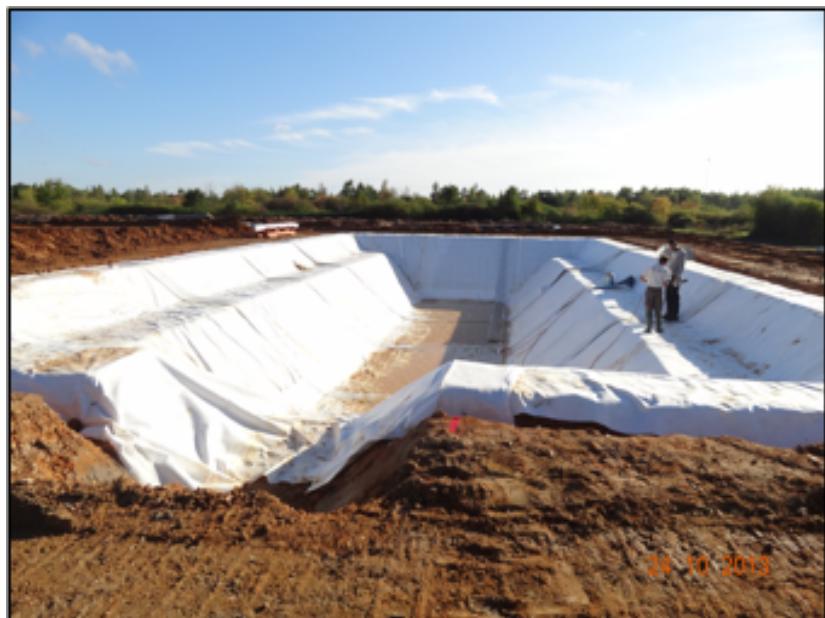


Fig. 5. The bottom and the walls of each artificial lake are protected by two geotextile layers (visible in white in the picture). After this stage, They will be protected and waterproofed by a 15/10 HDPE liner (the liner is visible in figure 6).

After installing the liner to seal each experimental lake, PVC tubes (30 cm in diameter) were installed along the entire length of the lake at the boundary between the littoral zone and the pelagic zone. The two tubes were covered by HDPE liners, which were heat-sealed on the sealing liner in order to isolate two littoral cavities (30 cm deep, 1.5 m wide, and 29 m long). These shallow cavities were created to retain the substrate of the littoral zones.

III – From lake construction to life colonization

The ELP provides replicated macrocosms, which ecological structures are similar to that of real systems, including secondary carnivores. Such complexity required a careful ecosystem construction, in order to allow the progressive construction of trophic networks up to top carnivores. Hence, we decomposed ecosystem settlement into several steps.

Step 1: Introduction of sand and soil – This step (Spring and Summer 2014) consisted in covering the bottom of the central areas of lakes with 30 cm of washed sand (originating from the River Loire) in order to allow the progressive development of benthic life, and adding 25 cm of soil (taken locally) in the littoral zones in order to allow the future colonization by plants (Fig. 6). Note that we did not treat the soils, which were added with their living microorganisms, plant propagules or animal resting stages (e.g. ephippia of Cladocera).



Fig. 6. We observed a rapid natural colonization by aquatic invertebrates (in particular insect larvae) of the rainwater accumulated in the lakes (here, qualitative sampling of these macro-invertebrates). We also observed a rapid development of terrestrial vegetation in the littoral areas after soil addition.

Fig. 7. A few months after soil addition, the littoral areas were progressively colonized by aquatic macrophytes (cattails are visible in the foreground), due to rainwater accumulation in these littoral areas



Step 2: Lake filling – In summer 2014, all the rainwater of the artificial lakes (present in different quantities due to their progressive construction) was pumped into the storage lake and mixed with tap water. From the end of Summer 2014 to the end of December 2014, the water of the storage lake was then progressively and homogeneously distributed to the experimental lakes using pumps. During water pumping to experimental lakes, tap water was continuously added to the storage lake.

Step 3: natural colonization and enrichment of lakes (2015 and 2016) – During two years, we allowed the natural colonization of the artificial lakes, without adding voluntarily any organism. During these two years, we added progressively a total 100 µg of P-PO₄ per liter, through 20 successive additions of 5 µg P L⁻¹. We first decided to add only phosphorus because tap water was natural rich in N-NO₃.

Step 4 (December 2016 - February 2017) – In December 2016, we added a similar fish community in all the lakes:

- Cyprinidae:
 - o 120 roach individuals (*Rutilus rutilus*),
 - o 30 sunbleak individuals (*Leucaspis delineatus*)
 - o 35 gudgeon individuals (*Gobio gobio*)
- Percidae
 - o 25 perch (*Perca fluviatilis*),
- Gasterosteidae
 - o 10 ninespine stickleback (*Pungitius pungitius*)

In February 2017, we added to 2 pikes (*Esox lucius*), Esocidae in half of the lakes in order to cross two levels of nutrient addition to the presence or absence of top-piscivorous fish. All the roach, perch, and pike individuals were tagged with RFID PIT tags.

IV – First experiment (December 2016 - October 2018)

As indicated in the previous paragraph, the first experiment, programmed for the period 2017-2018, consisted in a two-factor experimental treatment that crossed:

- two levels of nutrient loading,
- the presence or absence of two (35-cm long) pikes at the top of the food webs.

Nutrient treatment – In 2017, we added a total 100 µg of P-PO₄ per liter, through 20 successive additions of 5 µg P L⁻¹, as done during the colonization step in the 8 previously enriched lakes. We observed only punctual and transient effects of phosphorus enrichment et observed that concentration of dissolved nitrogen had became very low in the experimental lakes (with littoral areas characterized by dense communities of macrophytes), compared to the concentration measured in the storage lake (without any macrophytes). This led us to suspect both denitrification at the bottom and immobilization of nitrogen by littoral areas. Thus we decided in 2018, to add progressively (20 successive additions) both nitrogen (1mg N L⁻¹) and phosphorus (100 µg L⁻¹), at a N/P mass ratio equal to 10/1.

Fish treatment - In order to understand the dynamics of fish communities, we realize each year a scientific fishing in October. This scientific fishing consists in three successive passages of a large trawl making the width of the pelagic zone and the entire height of water, while people are fleeing the fish that might be eventually present in the littoral areas towards the central zone. The first scientific fishing, carried out in October 2017, revealed the high efficiency of the fishing technique for capturing roach and perch individuals, while the technique was probably less efficient for fish juveniles and for small species (sunbleak, gudgeon, ninespine stickleback).

Moreover, while the survival of roach and perch appeared to be high in these artificial environments, we did not capture any pike in the lakes. This strongly suggested an absence of pike survival in the experimental lakes. Our conclusion on the probable absence of pike survival was strongly reinforced by the lack of visible effect of fish treatment on both fish community structure and other lake parameters. In order to rule out the possibility of a trophic dead end (refuge by size) preventing pike from catching their potential prey, we reintroduced larger pike individuals (40-cm long, 500 g wet weight) by December 2017. We also introduced 30 sunbleak individuals and 30 gudgeon individuals in each of the 16 experimental lakes, in order to allow for small prey. The second scientific fishing realized in October 2018 revealed again an absence of survival of pikes in the experimental lakes. The most probable explanations are (1) that the littoral vegetation is too dense and (2) that the depth of these littoral areas is often too low (in particular in summer) to offer suitable areas for these classical ambushed top predators. This led us to propose a new experimental factorial design from October 2018. The analyses on these two years of experimentation are in progress.

V – Second experiment (October 2018 - ...?)

The second experiment, programmed at least for the period 2018-2019, consists in a two-factor experimental treatment that crosses:

- two levels of nutrient loading,
- the occurrence of perch in the food webs.

While, we decided to maintain the nutrient treatment as previously, we removed all the perch captured in the lakes that were initially considered as no-pike ecosystems. All these perch individuals have been introduced only in the 8 lakes that had previously received pikes. Perch introduction have been done in order to insure similar abundances of and size structures of perch populations in the 8 lakes with perch. The roach individuals were released into the lakes where they had been caught. In order to begin this experiment with similar cyprinid densities, we shall add new roach individuals in the next days, in order to get similar total abundances and size structures of roach populations in the 16 experimental lakes. We cannot exclude the hypothesis that a few perch and roach individuals had not been caught during the scientific fishing of October 2018. However, this process should be of minor importance due to high catch efficiency of roach and perch by the trawl nets. Our aim is to contrast systems with absent of very low predation pressure on roach populations and systems with important predation pressure by piscivorous perch. The maximal size of perch individuals (25-28 cm) is sufficient to allow high predation pressure of perch and modify roach recruitment.

VI – Complementary information on the experimental lakes

Are there any lakes without fish on the experimental platform? – The only artificial lake without fish is the drainage lake (4000m³) situated in the northern part of the platform. This reservoir, without any vegetalized littoral area, contains invertebrate communities dominated by *Daphnia pulex* and *Chaoborus* larvae.

Do the lakes stratify during the summer period? – The lakes stratify slightly during summer (from June - July to August – September). Even a slight thermal stratification may be associated to a clear vertical oxygen gradient during the warm season.

Does hypolimnetic anoxia develop in the systems? – While the whole vertical profile of lakes is generally well oxygenated from late autumn to the end of spring (May - June), the periods characterized by the strongest oxygen deficits occur from July to September according to years. Due to technical constraints, the automatic probes installed on each lake do not go down to the sediment – water interface. This means that supplementary oxygen measurements will be necessary for analyzing with high precision vertical oxygen distribution and anoxia near the water –sediment interface.

Is macrophyte coverage similar across all lakes? – Macrophyte coverage is globally similar in all the experimental lakes, with dense communities of *Typha* spp and *Juncus* spp. The relative importance of *Typha* and *Juncus* may vary according to lakes, but does not depend upon fish or nutrient treatments. The littoral vegetation is sampled twice a year (spring and autumn) in order to have information of the relative importance of all species. Aerial pictures of lakes are taken each month. This not only furnishes information of the littoral vegetation but also on the lake fraction covered by filamentous algae, which can develop sporadically.

Zooplankton – Rotifers (Bdelloidea, Synchaetidae [*Polyarthra*], Lecanidae [*Lecane*], Brachionidae [*Keratella*, *Brachionus*], Euchlanidae [*Lepadella*], Testitudinellidae [*Hexarthra*, *Pompholyx*], Asplanchnidae [*Asplanchna*]...), Copepods (both Cyclopoida and Calanoida), and Cladocera (Chydoridae, Bosminidae, and Daphniidae) are present in the experimental lakes. Among Cladocera, Daphniidae (*Ceriodaphnia*), Bosminidae (*Bosmina longirostris*), and Chydoridae are frequently observed. *Daphnia*, which were rather abundant before fish introduction, are now rare (although present) in the experimental lakes. *Chaoborus* larvae, which were abundant before fish introduction, are now very rare in the 16 experimental lakes. *Leptodora* and *Bythotrephes* were never observed. Large insect larvae can be found among vegetation within the littoral area. This list is only indicative. A complete list of zooplankton will be available at the end of 2018.

Sampling and counting of zooplankton – All classical sampling gears (bottles, traps, nets) can be used in the lakes. Mesh size can be adapted according to experiments. The facility provides ethanol and Alka-Seltzer tablets for narcotizing zooplankton prior to sample preservation with ethanol. A standard zooplankton sampling is realized each month: 45 samples of zooplankton are taken (at all depths and all parts of the pelagic zone) of the lakes with a 2-liter zooplankton bottle. The samples are mixed, filtered on a 50-µm nylon screen, and preserved in ethanol. Sub-sampling systems and stereoscopic microscopes are available on site for counting the zooplankton.