Reinforcement experiments and in-situ breeding systems with Margaritifera margaritifera in the Armorican Massif (France)

Programme LIFE+ NAT FR 000583 / 1st September 2010 - 31st August 2016

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2nd International Meeting on Biology and Conservation of Freshwater Bivalves

Buffalo NY, USA
4-8 October 2015

The LIFE programme « Conservation of the freshwater pearl mussel from the Armorican Massif » (2010-2016) aims to save the six remaining populations of Margaritifera margaritifera in the West part of France (Figures 1a and 1b).

The main issue on each river (Table 1) is the non-recruitment in juvenile since several years. While restoration actions of river habitat were conducted, a reinforcement of juvenile from a breeding farm started in 2012.

We use cylindrical tubes (like hair curler, or « bigoudis » in french) to test the efficacy of these reinforcements, through 2 experiences presented in this poster.

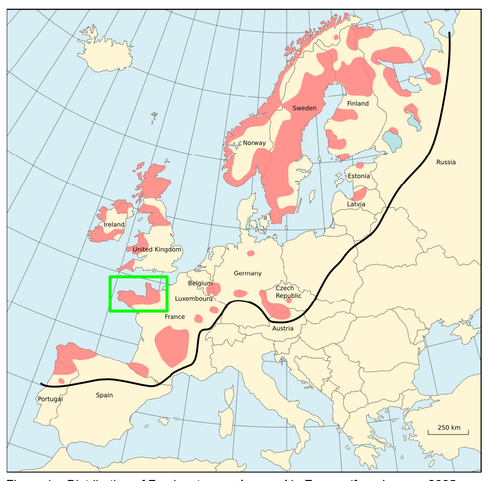

Figure 1a. Distribution of Freshwater pearl mussel in Europe (from Larsen, 2005, modified). The green rectangle shows the LIFE programme area

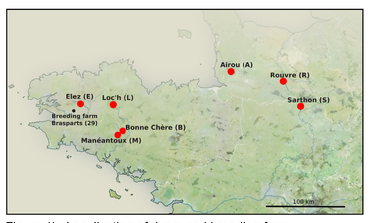

Figure 1b. Localization of rivers and breeding farm

Table 1. Main hydromorphological and physico-chemical characteristics of the distinct rivers (standard deviation into brackets) (E : Elez, L : Loc'h, B : Bonne Chère, M : Manéantoux, A : Airou, R : Rouvre, S : Sarthon)

River	Upstream catchment (km²)	Total length of the river with tributaries (km)	Nitrates N-NO3 (mg/L)	pH	Conductivity at 20°C (µS/cm)	Mussel population in 2011-2014
E	28	30	0.57 (0.26)	6.1 (0.5)	62 (18)	1,200 – 1,300
L	19	29	2.67 (0.83)	7.0 (0.5)	126 (20)	150-200
B	17	27	4.99 (1.01)	6.6 (0.3)	142 (23)	2,300 – 2,400
M	5	10	3.99 (1.03)	6.9 (0.3)	135 (24)	0
A	115	90	4.45 (0.54)	7.3 (0.6)	194 (78)	200-250
R	324	361	4.04 (1.52)	7.7 (0.4)	219 (50)	90-100
S	120	128	3.60 (1.98)	7.2 (0.5)	105 (13)	150-200

1. Materials & methods

For the experiences, young mussels from the breeding farm are placed into cylindrical tubes made of stainless steel (« bigoudis ») (Figure 2). For the two experiences, young mussels were 1 year old except for the river L where they were 2 years old. They were selected manually one by one to have individuals between 2 and 3mm long. These tubes are of 5cm long and 1.1cm diameter, with a mesh of 0.42 or 0.80mm (made by the French company called Gantois www.gantois.com). Aquarist gravels are placed into the tubes and put in the streams before the experiences which permit the biofilm developpement. Nylon strings are connected to the top of the tubes to find it at the end of the experiences. This technique was elaborated by the research team of the Agronomic National Research Institute (INRA, France) to test the embryonic survival of salmonid eggs (Dumas & Marty, 2006).

On each river, some hydro-morphological and physico-chemical criterias helped us to find favourable stations : riffle top, dissolved oxygen > 10mg/L, redox potential at 0 and 5cm > 300mV. On each station, 4 tubes were installed : 2 with a mesh size of 0.42 mm and 2 others with a mesh size of 0.80mm.

At the beginning of the experiences and at each checking, the shell length is measured from photographies (Figure 3), with the software ImageJ (http://rsbweb.nih.gov/ij/). The alive mussels are counted during this checking. Between each checking, tubes are not cleaned up or controlled.

Two different experiences were conducted and their characteristics are synthesized in Table 2.

Figure 2. Tubes with a mesh size of 0.80 (top) and 0.42mm (bottom)

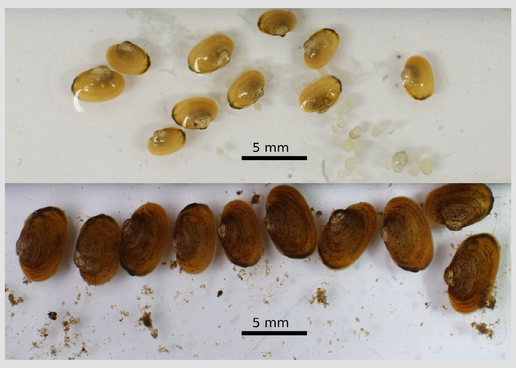

Figure 3. Freshwater pearl mussels at t0 and t+2 months (river A, tube A21)

Experience 1

During this first experience, we used two different methods to fix tubes in the rivers.
In Brittany (E, L, M), the tubes were put together, buried in a gravel pile or stones, horizontally (Figure 4). In Lower-Normandy (A, R, S), they were buried one by one, on riffle habitats, vertically, in a hole digged with a crowbar.
Tubes were installed at the beginning of July 2014 (t0) and checked at the end of September 2014 (t+3 months). To continue the experience, we made some groupings and the tubes were verified in March 2015 (t +9 months), July 2015 (t+12 months) and September 2015 (t+14 months).

Experience 2

During this second experience, a same method for all stations was used to fix tubes: together, buried in a gravel pile or stones, horizontally (Figure 4).
The 3 stations which were studied during the first experience were used again during this new one, added of others ones. Two control stations were put at the breeding farm: one of them stayed all the time at the farm (T), and the other travelled by car the day of the installation of the tubes in each river (V).
Tubes were installed at the beginning of July 2015 (t0) and checked at the beginning of September 2015 (t+2 months)

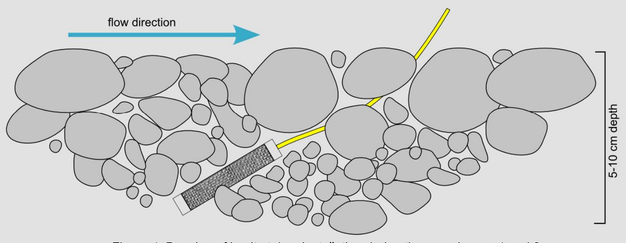

Figure 4. Drawing of in-situ tubes installation during the experiences 1 and 2

Table 2. Caracteristics of the experiences 1 and 2

River destination	Mussel lineage	French region	Experience 1: Nb of stations per river	Experience 1: Nb of mussel per tube	Experience 1: Nb of tubes per station	Experience 1: Total nb of mussel	Experience 2: Nb of stations per river	Experience 2: Nb of mussel per tube	Experience 2: Nb of tubes per station	Experience 2: Nb of stations at the farm	Experience 2: Total nb of mussel
E	E	Brittany	3	5	4	60	4	10	4	2	240
L	L	Brittany	3	5	4	60	8	10	4	2	400
M	B	Brittany	3	5	4	60	4	10	4	2	240
A	B	Lower-Normandy	3	5	4	60	5	10	4	2	280
R	B	Lower-Normandy	3	5	4	60	5	10	4	2	280
S	B	Lower-Normandy	3	5	4	60	5	10	4	2	280

Tubes checking and public sensibilization

2. Results

Experience 1

After 3 months, the percentage of surviving mussels were the best on rivers E, L and M (Table 3). The percentage of surviving mussels vary from 6.7% (river S) to 95.0% (river L). Mean growth is higher on rivers E, L, M and A (Table 4) and vary from 0.09mm (river S) to 0.56mm (river L).
After 14 months, only two rivers (L and M), still have alive mussels in tubes (respectively 26 and 23 individuals, for a growth of 1.26 and 1.77 mm since t0) (Figure 5).

Table 3. Mussel number and survival percentages during the experience 1

Rivers	t0	t+3 months		t+9 months			t+12 months			t+14 months		
	Nb of mussels	Nb of mussels	Survival %	Nb of mussels	Survival % since t+3	Total survival % since t0	Nb of mussels	Survival % since t+9	Total survival % since t0	Nb of mussels	Survival % since t+12	Total survival % since t0
E	60	46	76.7%	6	13.0%	10.0%	-	-	-	0	-	-
L	60	57	95.0%	53	93.0%	88.3%	42	79.2%	70.0%	26	61.9%	43.3%
M	60	42	70.0%	24	0.0%	40.0%	23	95.8%	38.3%	23	100.0%	38.3%
A	60	5	8.3%	0	-	-	-	-	-	-	-	-
R	60	11	18.3%	0	-	-	-	-	-	-	-	-
S	60	4	6.7%	0	-	-	-	-	-	-	-	-

Table 4. Mussel length and growth during the experience 1

Rivers	t0	t+3 months		t+9 months			t+12 months			t+14 months		
	Mean length (mm)	Mean length (mm)	Growth (mm)	Mean length (mm)	Growth (mm) since t+3	Total growth (mm) since t0	Mean length (mm)	Growth (mm) since t+9	Total growth (mm) since t0	Mean length (mm)	Growth (mm) since t+12	Total growth (mm) since t0
E	2.09	2.55	0.46	2.74	0.19	0.65		-	-			
L	2.88	3.43	0.56	3.62	0.19	0.74	-	-	-	4.14	0.52	1.26
M	2.46	2.86	0.40	3.16	0.30	0.70	4.00	0.84	1.54	4.23	0.23	1.77
A	2.72	3.22	0.51	-	-	-	-	-	-	-	-	-
R	2.73	2.86	0.14	-	-	-	-	-	-	-	-	-
S	2.68	2.77	0.09	-	-	-	-	-	-	-	-	-

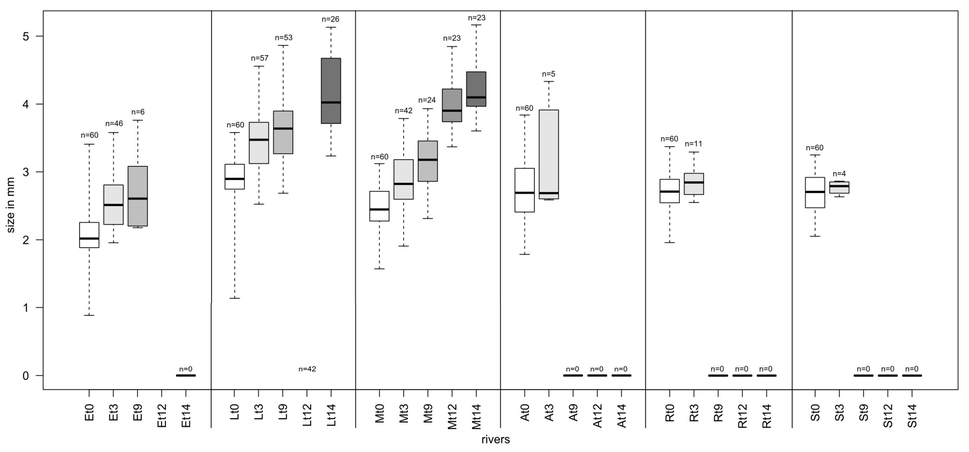

Figure 5. Whisker box of the mussel length for each river during the experience 1 (t0, t+3, t+9, t+12, t+14 months)

Experience 2

Mean survival percentage seems to be high at t+2 months (87.3% for L river to 98.9% on A river) with a mean growth which is very different depending on rivers (0.19 mm on M river to 1.21 mm on A river) (Table 5, Figure 6).
If we only consider the stations in the breeding farm we have a mean survival percentage of 95.8% and a mean growth of 0.15 mm. Comparing to all the stations in river, we have a mean survival percentage of 94.6% and a mean growth of 0.77 mm. Mussels seems to grow faster in rivers than in the breeding farm (Table 6).

Table 5. Mussel number per river, mean length, survival percentage and mean growth between t0 and t+2 months during experience 2 (standard deviation into brackets)

River	t0		t+2 months			
	Nb of mussels	Mean length (mm)	Nb of mussels	Survival %	Mean length (mm)	Mean growth (mm)
E	239	3.12 (0.33)	231	96.7%	3.64 (0.38)	0.52 (0.14)
L	400	3.83 (0.45)	349	87.3%	4.31 (0.47)	0.53 (0.16)
M	240	3.37 (0.34)	233	97.1%	3.56 (0.35)	0.19 (0.06)
A	278	3.52 (0.39)	275	98.9%	4.72 (0.52)	1.21 (0.16)
R	280	3.45 (0.31)	271	96.8%	4.09 (0.44)	0.66 (0.24)
S	280	3.40 (0.37)	271	96.8%	3.89 (0.40)	0.47 (0.12)

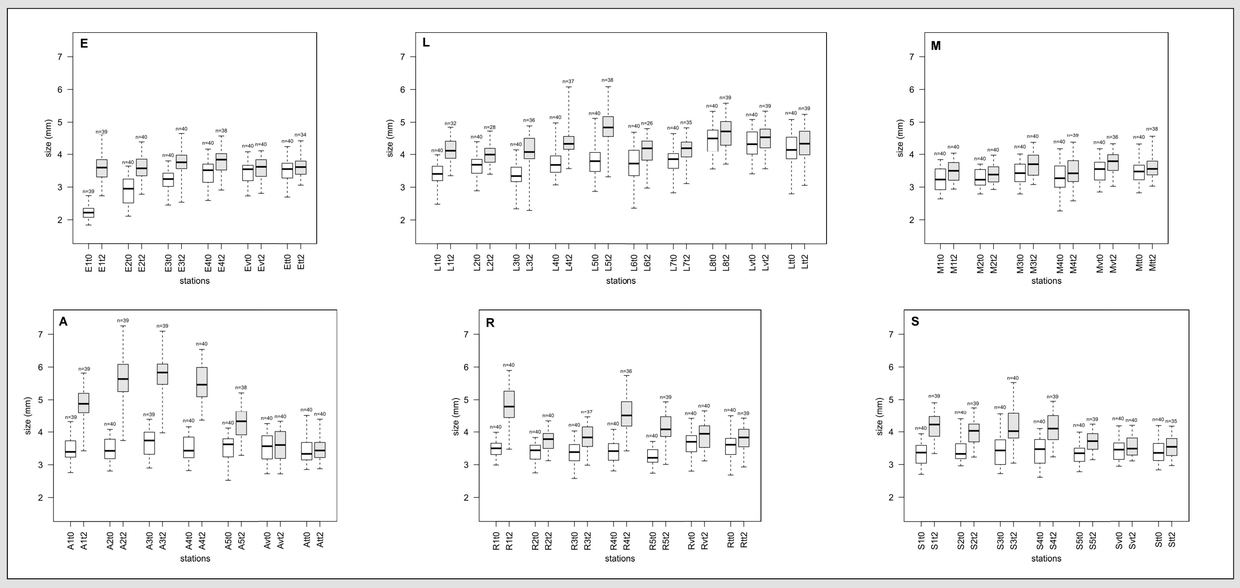

Figure 6. Whisker box of mussel length for each river and for the breeding farm (V and T) during the second experience

Table 6. Mussel number per station, mean length, survival percentage and mean growth between t0 and t+2 months during experience 2

River stations	t0		t+2 months			
	Nb of mussels	Mean length (mm)	Nb of mussels	Survival %	Mean length (mm)	Mean growth (mm)
E1	39	2.22 (0.21)	39	100.0%	3.58 (0.40)	1.37 (0.03)
E2	40	2.9 (0.42)	40	100.0%	3.57 (0.39)	0.67 (0.45)
E3	40	3.21 (0.31)	40	100.0%	3.76 (0.43)	0.55 (0.18)
E4	40	3.45 (0.37)	38	95.0%	3.78 (0.39)	0.33 (0.07)
E6 (V)	40	3.45 (0.34)	40	100.0%	3.55 (0.35)	0.10 (0.05)
E7 (T)	40	3.5 (0.33)	34	85.0%	3.62 (0.32)	0.13 (0.03)
L1	40	3.4 (0.33)	32	80.0%	4.15 (0.37)	0.69 (0.11)
L2	40	3.65 (0.37)	28	70.0%	3.99 (0.32)	0.32 (0.03)
L3	40	3.31 (0.46)	36	90.0%	4.05 (0.58)	1.00 (0.19)
L4	40	3.72 (0.44)	37	92.5%	4.42 (0.52)	0.80 (0.19)
L5	40	3.77 (0.50)	38	95.0%	4.84 (0.55)	1.07 (0.09)
L6	40	3.7 (0.56)	26	65.0%	4.08 (0.49)	0.39 (0.15)
L7	40	3.79 (0.39)	35	87.5%	4.12 (0.39)	0.52 (0.31)
L8	40	4.47 (0.46)	39	97.5%	4.67 (0.50)	0.20 (0.06)
L9 (V)	40	4.33 (0.45)	39	97.5%	4.49 (0.46)	0.05 (0.22)
L10 (T)	40	4.13 (0.59)	39	97.5%	4.30 (0.56)	0.29 (0.24)

River stations	t0		t+2 months			
	Nb of mussels	Mean length (mm)	Nb of mussels	Survival %	Mean length (mm)	Mean growth (mm)
M1	40	3.24 (0.34)	40	100.0%	3.47 (0.33)	0.23 (0.06)
M2	40	3.27 (0.26)	40	100.0%	3.38 (0.27)	0.11 (0.05)
M3	40	3.44 (0.33)	40	100.0%	3.68 (0.35)	0.24 (0.08)
M4	40	3.31 (0.43)	39	97.5%	3.48 (0.42)	0.18 (0.09)
M6 (V)	40	3.51 (0.35)	36	90.0%	3.74 (0.37)	0.21 (0.05)
M7 (T)	40	3.47 (0.35)	38	95.0%	3.62 (0.36)	0.16 (0.06)
A1	39	3.46 (0.39)	39	100.0%	4.84 (0.54)	1.41 (0.24)
A2	40	3.45 (0.37)	39	97.5%	5.60 (0.72)	2.15 (0.11)
A3	39	3.71 (0.43)	39	100.0%	5.76 (0.57)	2.04 (0.37)
A4	40	3.5 (0.38)	40	100.0%	5.50 (0.58)	2.00 (0.06)
A5	40	3.52 (0.40)	38	95.0%	4.27 (0.47)	0.71 (0.19)
A6 (V)	40	3.55 (0.41)	40	100.0%	3.62 (0.43)	0.07 (0.04)
A7 (T)	40	3.42 (0.35)	40	100.0%	3.47 (0.34)	0.05 (0.08)

River stations	t0		t+2 months			
	Nb of mussels	Mean length (mm)	Nb of mussels	Survival %	Mean length (mm)	Mean growth (mm)
R1	40	3.48 (0.24)	40	100.0%	4.80 (0.63)	1.41 (0.60)
R2	40	3.38 (0.30)	40	100.0%	3.73 (0.31)	0.34 (0.41)
R3	40	3.39 (0.33)	37	92.5%	3.84 (0.37)	0.44 (0.11)
R4	40	3.41 (0.31)	36	90.0%	4.55 (0.54)	1.11 (0.25)
R5	40	3.25 (0.25)	39	97.5%	4.08 (0.46)	0.90 (0.11)
R6 (V)	40	3.68 (0.37)	40	100.0%	3.87 (0.40)	0.19 (0.08)
R7 (T)	40	3.56 (0.40)	39	97.5%	3.77 (0.39)	0.22 (0.14)
S1	40	3.33 (0.35)	39	97.5%	4.16 (0.4)	0.83 (0.13)
S2	40	3.43 (0.35)	39	97.5%	3.99 (0.38)	0.45 (0.19)
S3	40	3.47 (0.49)	40	100.0%	4.15 (0.58)	0.68 (0.17)
S4	40	3.42 (0.42)	39	97.5%	4.12 (0.47)	0.69 (0.10)
S5	40	3.33 (0.79)	39	97.5%	3.73 (0.31)	0.40 (0.09)
S6 (V)	40	3.44 (0.32)	40	100.0%	3.56 (0.31)	0.12 (0.10)
S7 (T)	40	3.41 (0.35)	35	87.5%	3.56 (0.35)	0.11 (0.05)

3. Discussion

For the first experience, survival percentage and growth are better in Brittany (E, L, M) than in Lower-Normandy (A, R, S). This is probably due to the fixation method which was not the same between both regions. Maybe these results were affected by the fact that mussels used in Lower-Normandy were from a Brittany lineage (B), whereas in Brittany we used the mussels from each river. Moreover maybe, the different physicochemical parameters of rivers and their sediment could have affected these results.
For the second experience, survival percentage and growth are high for all rivers. The fixation method is probably the parameter which had an influence on the first experience. Lineage seems not to be a problem in this second experience.

Global synthesis

The analysis of all the datas is not over. However, these first results seems to be encouraging. The technique of tubes seems to be appropriate to test survival and growth of young mussels in-situ. In Europe, most of the in-situ tests of survival and growth have used the Buddensiek cages (Buddensiek, 1995). This technique needs a regular cleaning which is time-consuming whereas it is not necessary for the tubes. Moreover, living conditions of mussels during the tubes experiences seems to be closer of wild individuals, and of young mussel from the breeding farm directly released in the river without any control.

Note

During each experience water temperature was measured every hour on each river. Conductivity, pH, Nitrate, Orthophosphates and redox potential were also regularly measured. The analysis of the relation of these parameters with the growth and survival of the young mussels will be done soon. The analysis of the relation with mesh size will also be done soon.

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The LIFE+ programme is co-financed by the European Commission and by :

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