



**HAL**  
open science

## Link between muskrat (*Ondatra zibethicus*) density and freshwater mussel predation

Marie Vanacker

► **To cite this version:**

Marie Vanacker. Link between muskrat (*Ondatra zibethicus*) density and freshwater mussel predation. [Internship report] Université Paul Verlaine Metz. 2010. hal-02920178

**HAL Id: hal-02920178**

**<https://hal.science/hal-02920178>**

Submitted on 24 Aug 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

VANACKER Marie  
Year 2009-2010  
Masterwork, 2<sup>nd</sup> year of Master EA  
Speciality BEE, CRB

# Link between muskrat (*Ondatra zibethicus*) density and freshwater mussel predation

---



Realized from 01/03/2010 to 31/08/2010

In National Museum of Natural History in Luxembourg

under the direction of:

Frank RICHARZ and Edmée ENGEL, NMNH of Luxembourg

and

Jean-Nicolas BEISEL, University of Metz

Memory supported on the 9th September 2010

*National Museum of Natural History in Luxembourg*





## Table of Contents

Acknowledgments .....	6
Abbreviations .....	6
National Museum of Natural History .....	7
Résumé .....	8
Introduction .....	8
I/ Species Presentation.....	10
1.1.Freshwater mussels.....	10
1.1.1. Freshwater pearl mussel ( <i>Maragaritifera margaritifera</i> ).....	10
1.1.2. Thick shelled river mussel ( <i>Unio crassus</i> ) .....	10
1.1.3. Swollen river mussel ( <i>Unio tumidus</i> ) and Painter's mussel ( <i>Unio pictorum</i> ).....	10
1.1.4. Duck mussel ( <i>Anodonta anatina</i> ) and Swan mussel ( <i>Anodonta cygnea</i> ).....	10
1.1.5. Asian clams ( <i>Corbicula fluminea</i> ).....	10
1.2. Freshwater mussel predators .....	14
1.2.1. Muskrat ( <i>Ondatra zibethicus</i> ).....	14
1.2.2. Raccoon ( <i>Procyon lotor</i> ) .....	14
II/ Materials and Methods .....	14
2.1. Freshwater mussels.....	18
2.2. Capture-Mark-Recapture Procedure.....	18
2.2.1. Sites and periods.....	18
2.2.1.1. Our River .....	18
2.2.1.2. Sûre River.....	20
2.2.2. Method of Capture-Mark-Recapture (CMR).....	20
2.2.3. CMR Analysis .....	21
2.2.3.1. CloseTest program.....	21
2.2.3.1. Density population analysis.....	24
2.3. Predation of freshwater mussel by raccoons and muskrats .....	266
III/ Results .....	28
3.1. Middens of Freshwater Mussels .....	28
3.1.1. Freshwater pearl mussel ( <i>Maragaritifera margaritifera</i> ).....	30

3.1.2. Thick shelled river mussel ( <i>Unio crassus</i> ) .....	31
3.1.3. Painter's mussel ( <i>Unio pictorum</i> ) .....	32
3.1.4. Duck mussel ( <i>Anodonta anatina</i> ) .....	32
3.1.5. Swan mussel ( <i>Anodonta cygnea</i> ).....	34
3.1.6. Asian clams ( <i>Corbicula fluminea</i> ).....	34
3.1.7. Biostatistical test.....	35
3.2 Muskrat Capture .....	36
3.2.1. In Our Valley .....	36
3.2.2. In Lower Sûre.....	36
3.2.3. In Upper Sûre .....	36
3.3. CMR analysis .....	38
3.3.1. CloseTest analysis.....	38
3.3.2. Muskrat population in Our Valey (Rodershhausen) .....	38
3.3.3. Muskrat population in Lower Sûre site (Dillingen).....	38
3.4. Predation of freshwater mussel by raccoon and muskrat .....	40
3.4.1. By raccoons .....	40
3.4.1. By muskrats .....	40
IV/ Discussion .....	40
4.1. Freshwater predation .....	42
4.1.1. Muskrat predation.....	42
4.1.2. Raccoon predation .....	425
4.2. Muskrat ( <i>Ondatra zibethicus</i> ) .....	45
4.2.1. Population density in CMR sites .....	45
4.2.2. Daily and Seasonal activities.....	457
4.3. Link between muskrat density and freshwater mussel predation .....	48
Conclusion .....	50
References .....	40

## Chronological List of Tables

Table I. Results of statistical test for each species in link between predation signs and shell mensuration. a= Anova test. b= Kruskal-Wallis test.....	65
Table II. Results of CloseTest analysis (Stanley & Richards, 2005) .....	37

## Chronological List of Figures

Figure 1. Cycle life of <i>M. margaritifera</i> in Gum & Geist, 2009 .....	11
Figure 2. Cycle life of <i>U. crassus</i> in Gum & Geist, 2009 .....	12
Figure 3. Picture of a muskrat ( <i>Ondatra zibethicus</i> ) .....	14
Figure 4. Picture of a raccoon ( <i>Procyon lotor</i> ).....	16
Figure 5. Sites of CMR in Luxembourg: Our Valley (1), Lower Spure (2) and Upper Sûre (3) .....	22
Figure 6. Traps areas in Our valley, in Lower Sûre and in Upper Sûre. Each trap is separated of 250 m.....	23
Figure 7. Pictures of muskrat traps and extractor .....	24
Figure 8. Map of freshwater mussels collected in different middens in Luxembourg .....	27
Figure 9. Graphs of shell mensuration of different species : a: total length (mm), b: thickness (mm), c: height (mm), d: swelling (mm) and e: weight (g).....	28
Figure 10. Graph of shell number of different species with different predation marks.....	29
Figure 11. Pictures of middens of <i>M. margaritifera</i> (a) and <i>C. fluminea</i> (b) found in Our Valley and <i>U. crassus</i> shells found in Upper Sûre (c).....	29
Figure 12. Pictures of predation marks on <i>M. margaritifera</i> .....	30
Figure 13. Proportion of predation signs on <i>M. margaritifera</i> .....	30
Figure 14. Pictures of predation marks on <i>U. crassus</i> .....	31
Figure 15. Proportion of predation signs on <i>U. crassus</i> .....	32
Figure 16. Pictures of predation marks on <i>U. pictorum</i> .....	62
Figure 17. Proportion of predation signs on <i>U. pictorum</i> .....	62
Figure 18. Pictures of predation marks on <i>A. anatina</i> .....	63
Figure 19. Proportion of predation signs on <i>A. anatina</i> .....	63
Figure 20. Proportion of predation signs on <i>C. fluminea</i> .....	64
Figure 21. Pictures of predation marks on <i>C. fluminea</i> .....	64
Figure 22. Map of CMR results in Our Valley (1), in Lower Spure (2) and in Upper Sûre (3). The first number corresponds to first-capture number and the second to recapture number .....	68

Figure 23. Pictures of raccoon eating a mussel of <i>U. pictorum</i> .....	40
Figure 24. Pictures of predation marks by raccoons on <i>Unio sp.</i> (a and b) and on <i>A. anatina</i> (c) .....	41
Figure 25. Pictures of predation marks by muskrats on <i>U.tumidus</i> .....	41

## Chronological List of Annex

Annex A. Results of CMR procedure in Our Valley (Roderhausen): details of each muskrat captured (a), history data file for CloseTest analysis (b) and details for population density (c) .....	55
Annex B. Results of CMR procedure in Lower Sûre (Dillingen): details of each muskrat captured (a), history data file for CloseTest analysis (b) and details for population density (c).....	56
Annex C. Results of CMR procedure in Upper Sûre (Martelinville) E: escaped, trap was found destroyed; C: captured .....	58
Annex D. Number of shells with one valve or intact for each freshwater mussel species .....	59
Annex E. Table representing predation marks on different species of freshwater mussel .....	59
Annex F. Table of shell mensuration for <i>M. margaritifera</i> (a), <i>U. crassus</i> (b), <i>U. pictorum</i> (c), <i>A. antina</i> (d), <i>A. cygnea</i> (e) and <i>C. fluminea</i> (f).....	60
Annex G. Authorization by the Government of the Grand-Duchy of Luxembourg for the capture, the tagging and the release of muskrats .....	62
Annex H. Statement against plagiarism .....	63

## Acknowledgments

I thank Mr. Frank Richarz for his help: this study takes place in his PhD based on muskrat. I also thank Mrs Edmée Engel, conservator of vertebrate zoology, Mr. Georges Bechet, director of the museum and everybody in the National Museum of Natural History in Luxembourg who help me during my masterwork. Moreover, I thank Mr. Jean-Nicolas Beisel, teacher-researcher in the university in Metz, for his help.

## Abbreviations

ANF: “Administration de la Nature et des Forêts » of the Grand Duchy of Luxembourg

CMR: Capture-Mark-Recapture

IUCN: International Union for Conservation of Nature

## National Museum of Natural History

National Museum of Natural History of Luxembourg belongs to the Ministry of Culture, of Higher Education and Research. It has been created in 1850 and has opened its doors to the public in 1854. The creation of the scientific research center in the National Museum of Natural History was effectuated the 10<sup>th</sup> November 1982 by the grand-ducal regulation. Thus, the scientific research center permits to study every domain of the natural patrimony (botany, ecology, geology-mineralogy, geophysics-astrophysics, paleontology, population biology and zoology) and to contribute to its conservation.

Georges BECHET

Director of National Museum of Natural History in Luxembourg



Edmée ENGEL

Conservator of vertebrate zoology



Frank RICHARZ

PhD on muskrat (*Ondatra zibethicus*) as an alien species in Luxembourg



# Résumé

---

Une collecte d'amas de coquilles vides le long des berges de la rivière Sûre, de l'Our et de l'Alzette a été effectuée et six espèces de bivalves ont pu être identifiées (*Margaritifera margaritifera*, *Unio crassus*, *Unio tumidus*, *Anodonta anatina*, *Anodonta cygnea* et *Corbicula fluminea*). Après analyses de chaque coquille, des signes de prédation telles que des morsures, des traces de dents sur les valves, de griffures, ainsi que des coquilles cassées ont pu être observés. Une expérience a été menée en parallèle de deux espèces d'unionidés, et a ainsi permis de caractériser les marques de prédatons faites par les rats musqués (*Ondatra zibethicus*), ainsi que celles faites par les ratons laveurs (*Procyon lotor*). Il a alors été possible de dire que le rat musqué a prédaté une bonne partie des bivalves retrouvés, mais que le raton laveur est aussi un bon prédateur de moules d'eau douce. En effet, là où la densité de rats musqués est très faible et celle de raton laveur très forte, beaucoup de coquilles vides de type *Unio crassus* ont été collectées. De plus, là où la densité de rats musqués est plus importante, très peu de bivalves vides ont été trouvés.

# Introduction

---

Most of freshwater mussels comes from the families of *Margaritiferidae* and *Unionidae*, and are considered as endangered species. In North America, nearly 75 % of mussel species are threaten of extinction risk due to their complex life cycle that makes them particularly vulnerable to environment changes (Cudmore et al, 2004). Freshwater mussels need to live in good water and are also used for water quality indicators. Furthermore, they are generally consumed by a variety of small mammals, thus they are very important for aquatic ecosystem (Tyrell & Hornbach, 1998).

The freshwater pearl mussel (*Margaritifera margaritifera*) is considered as endangered over the world by the International Union for Conservation of Nature (IUCN) (Bensettiti & Gaudillat, 2004). Human degradation of mussel living environment has considerably increased the last

decades and is the main cause of the freshwater pearl mussel extinction in Upper Sûre area in Luxembourg (Schmitz & Krippel, 2007). The last population of *M. margaritifera* in Luxembourg is situated in the Natura 2000 area in the “Vallée supérieure de l’Our et affluents de Lieler à Dasbourg” (Richarz, 2009). This population is old which struggle to renew itself and a LIFE program was established in 2005 for its reintroduction (Arendt et al, 2008). In the same area in 2006, an accumulation of visually dead freshwater pearl mussel (more than two hundreds) was found on the Our river bank in discrete piles (called also middens), suggesting that they were eaten by an animal. The deposition of empty shells in middens is a sign of muskrat (*Ondatra zibethicus*) predation and provides evidence that muskrat eat them. Muskrat trapping campaigns by the “Administration de la Nature et des Forêts” (ANF) of the Grand Duchy of Luxembourg, were established in 2007 in the Our Valley after the empty shells discovery (Richarz, 2009). Muskrat trapping was also established in Upper Sûre (Schmitz & Krippel, 2007) due to another pic of freshwater mussel predation. In fact, the major predation in this site is on the thick shelled water mussel (*Unio crassus*) which is still estimated from 30.000 to 35.000 animals in Upper Sûre (Schmitz and Krippel, 2007). However, thick shelled water mussel is also considered as an endangered species by IUCN (Bensettiti and Gaudillat, 2004). At long term, populations in Upper Sûre are threatened of extinction due to its important predation (Schmitz and Krippel, 2007). However, in this site we cannot say if muskrats are responsible of the totality of the *U. crassus* predation. In fact, another major predator of freshwater mussel the raccoon (*Procyon lotor*) is also present. Muskrat and raccoon are both alien species and potentially threat freshwater mussels due to they may disturb ecosystem function (Nummi et al, 2006). Muskrats eat a greater variety of food and the consumption of animal matter is often considered as a no significant part in muskrat diet (Neves and Odom, 1989).

The main goal of this study is to give an overview of the mussel predation in Luxembourg and determine a link between freshwater mussel predation and the density population of muskrats. So mapping and collects for empty shells along river banks were realized and an evaluation of muskrat density by capture-mark-recapture (CMR) procedure was effectuated at three locations in Luxembourg in Our Valley, in Upper Sûre and in Lower Sûre. Additionally, an experiment with alive raccoons and muskrats was realized by given directly two unionids and thus, get information about marks predation on shells.

# I/ Species Presentation

---

## 1.1. Freshwater mussels

Reproduction of freshwater mussels is nearly similar within species. Male mussels release sperm into the water column which floats downstream and pass through female siphons. Then, ova are fertilized and developed into the mother shell at an intermediate larval stage called glochidium. The glochidia are released into the water column when the temperatures change. However, glochidia are parasite; they need to be attached at a fish species-specific host. They encyst in the host's tissue to complete their metamorphosis to the juvenile stage. After the transformation, the juvenile detaches from its host and falls into the substrate to complete its development into an adult. Both stages, glochidia and juvenile, are very vulnerable; the success of glochidia to pass to the following step is estimated at 0,004 % to as low as 0,000001 %. At the end adult mussels live at the water bottom and filter their feeding (Cudmore et al, 2004).

Shells of different freshwater species were collected in this study and species of them are described in following parts.

### 1.1.1. Freshwater pearl mussel (*Margaritifera margaritifera*)

- Presentation

The freshwater pearl mussel belongs to the Order of *Unionoida* and the Family of *Margaritiferidae*. Juveniles are brown yellowish and they have green streaks (Gum & Geist, 2009). Adult freshwater pearl mussels have their periostracum black matte and shell top is generally eroded (Vrignaud, 2004). Shells are elongated and reniform. Maximum size is about 14 to 15 cm. They can live until 100 – 120 years in Central Europe and until more than 200 years in northern Europe (Gum & Geist, 2009).

- Cycle life:

Freshwater pearl mussels may be hermaphrodites with a high fertility and begin to be sexually mature between 12 and 15 years. More than 4 million of glochidia are released one time per years during July to September (Figure 1). Two fish species-specific hosts are known for *M. margaritifera*, brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*). Glochidia pass 9 to 10 months on gills of its host and after, will be juveniles for 5 years buried into sediment (Gum &

Geist, 2009).

- Habitat :

They live into upland streams, oligotrophic (nutrient-poor). They are situated on primary rock areas where calcium and conductivity are low (<150  $\mu\text{S}/\text{cm}$ ). They live on stable beds (gravel, sand and cobbles) and into the water where interstitial are well-oxygenated (limiting factor for juvenile development). Often the freshwater pearl mussel is found with other *Unio sp.* or *Anodonta sp.* (Gum & Geist, 2009).

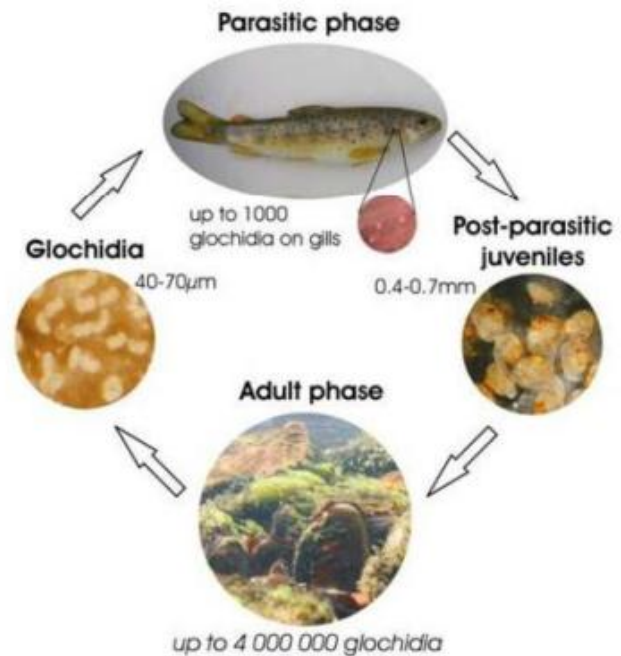


Figure 1. Cycle life of *M. margaritifera* in Gum & Geist, 2009

- Status:

Freshwater pearl mussel belongs to the Annex II and Annex V of the Directive 92/43 “Wildlife-Flora-Habitat” (Natura 2000 areas) and in the Annex III of Bern Convention to ensure population survival by limiting sampling strategy. In IUCN red list *M. margaritifera* is endangered over the world (Bensittiti & Gaudillat, 2004).

### 1.1.2. Thick shelled river mussel (*Unio crassus*)

- Presentation

Thick shelled river mussel belongs to the Order of *Unionoida*, family of *Unionidae*, and genus of *Unionidae*. Shells have oval form which color is green to brown. On the periostracum, they have several clearer green stripes situated generally on the posterior side and shell top is often eroded (Vrignaud, 2004). The thick shelled water mussel measures mostly up to 8 cm, but rarely more than 9 -10 cm (Gum & Geist, 2009).

- Reproduction:

Female and male are distinct and are sexually mature between three to four years. They can release more than 60.000 glochidia with several spawning during April to July. During four weeks, glochidia parasite gills of different fish species like chub (*Squalius cephalus*), minnow (*Phoxinus phoxinus*), bulhead (*Cottus gobio*), stickleback (*Gastrosteus sp.*) or others species (Figure 2). Juveniles are buried into sediment for one to two years (Gum & Geist, 2009).

- Habitat:

Thick shelled water mussels live into meso and oligotrophic water, with different limestone and primary rock areas with sable beds (fine sand and gravel). They live into calcareous water and high conductivity (>200  $\mu\text{S}/\text{cm}$ ). Interstitial water need to be well-oxygenated, otherwise it is a limiting factor for juvenile stage (Gum & Geist, 2009).

- Status:

*Unio crassus* is in annex II and V of “Wildlife-Flora-Habitat” Directive. In IUCN red list *U. crassus* is nearly endangered (Bensittiti & Gaudillat, 2004).

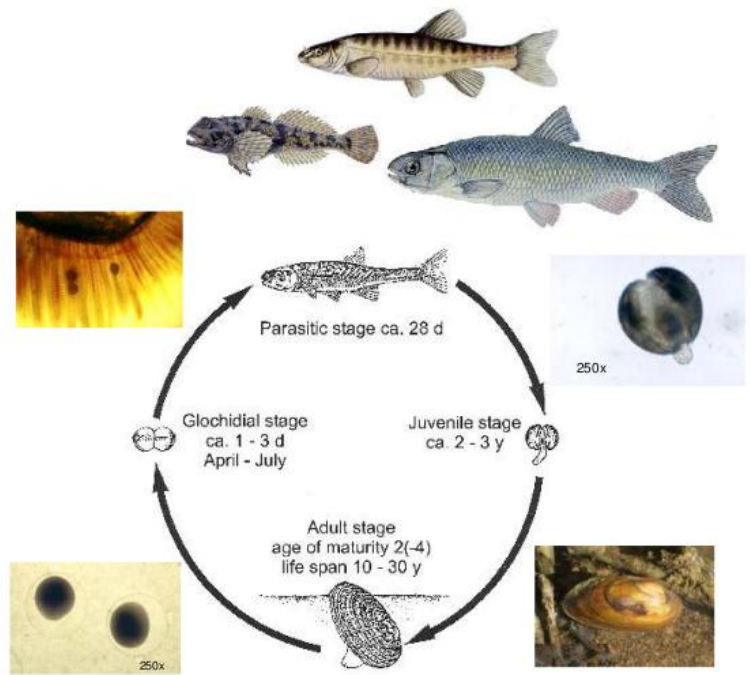


Figure 2. Cycle life of *U. crassus* in Gum & Geist, 2009

### 1.1.3. Swollen river mussel (*Unio tumidus*) and Painter’s mussel (*Unio pictorum*)

- Presentation

Swollen river mussel and painter’s mussel belong to the Order of *Unionoida* and family of *Unionidae*. Both are common and not protected.

Swollen river mussel (*Unio tumidus*) is ovoid, elongated, and dark brownish. Total length may reach 9.5 cm (Vrignaud, 2004).

The big difference between both species is that the painter’s mussel (*Unio pictorum*) is less ovoid and more elongated than *U. tumidus*. Thus *U. pictorum* is a little longer than *U. tumidus* and may measure up to 11 cm. Its posterior and inferior sides are parallels. Shell color for *U. pictorum* may vary from yellowish to dark brownish (Vrignaud, 2004).

- Reproduction and habitat:

Both species have more fish hosts than *U. crassus*, and fish hosts depend on which fish species are presents in the water. More than seventeen fish species were found by Blazek and Gelnar in 2006, especially in cyprinid species as roach (*Rutilus rutilus*). Both live in calm water and stagnant water (Vrignaud, 2004).

#### ***1.1.4. Duck mussel (Anodonta anatina) and Swan mussel (Anodonta cygnea)***

- Presentation:

As other species, duck mussel (*Anodonta anatina*) and swan mussel (*Anodonta cygnea*) belong to the Order of *Unionoida*, family of *Unionidae* and genus of *Anodonta*. They are not protected.

Duck mussel (*A. anatina*) is recognizable because of its big posterior-dorsal side. The shell is a few elongated and its inferior side is convex. Big shells are thick, and are very strong. Total length may reach 14 cm and the color is from greenish to brownish (Vrignaud, 2004).

Swan mussel shell is oval more elongated than duck mussel shell. The most difference between the both species is that *A. cygnea* has superior and inferior sides parallels or convex. Shell color is from yellow to green and the total length may measure up to 20 cm. Contrary to *A. anatina*, *A. cygnea* is less thick and the shell breaks more easily (Vrignaud, 2004).

- Reproduction and habitat:

As *Unio tumidus* and *Unio pictorum*, *Anodonta sp.* has many fish species-specific hosts. Blazek and Gelnar in 2006 listed ten potentially fish hosts to *A. cygnea* and *A. anatina* with eight cyprinid species and two from *Percidae*. *Perca fluviatilis* from *Percidae* will be their favorite host species (Blazek and Gelnar, 2006). Both *Anodonta* species are found in stagnant waters. However, *A. anatina* is also found in streams.

#### ***1.1.5. Asian clam (Corbicula fluminea)***

- Presentation

Asian clams or Asiatic clams are small with a maximum length to 65 mm, but are usually less than 25 mm. Shells are oval, thick with concentric and evenly spaced ridges. Color shell is from yellowish brown to black (ISSG, 2005). The life span of *C. fluminea* varies from 1 to 5 years. They grow very rapidly because of their high filtration and assimilation rates (Sousa, 2008).

- Reproduction:

*C. fluminea* is generally hermaphrodite and it is capable of self-fertilization (ISSG, 2005) but sometimes it can be dioecious (Sousa, 2008). Thus, contrary to other freshwater mussels, *C. fluminea* doesn't need a fish host. Sperm is released into water column to another clam (ISSG, 2005). The interlamellar junctions serve as protective period and alternative source of nutrition for embryos. After, larvae are released into the water and bury into the substratum. Juveniles can be

mature after 3 to 6 months. Most of their energy is devoted for growing and reproduction and a little part is for the respiration (Sousa, 2008).

- Habitat :

Asiatic clams occur in different habitat as lakes, streams, but also they tolerate strong salinities (13 ppt) and are also found in estuarine habitats. However, *C. fluminea* are usually not tolerant of pollution. They are generally found in stream where dissolved oxygen is high (ISSG, 2005).

- Status:

This species is considered to be one of the most important invasive species in aquatic systems over the world (rapid growth, short life span, high fecundity, earlier sexual maturity). Moreover, the Asian calm is very easily dispersed with human activities. Its presence in Europe was described for the first time in 1981 but some *Corbicula* genus was also present before the last glaciation in Europe (Sousa, 2008).

## 1.2. Freshwater mussel predators

### 1.2.1. Muskrat (*Ondatra zibethicus*)

- Presentation



[http://hillsteadblog.files.wordpress.com/2010/04/common-muskrat\\_jpg.jpg](http://hillsteadblog.files.wordpress.com/2010/04/common-muskrat_jpg.jpg)

Figure 3. Picture of a muskrat (*Ondatra zibethicus*)

Class: *Mammalia*  
Order: *Rodentia*  
Superfamily: *Muroidea*  
Family: *Cricetidae*  
Subfamily: *Arvicolinae*  
Tribe: *Ondatrini*  
Genus: *Ondatra*  
Species: *O. zibethicus*

Muskrat (*Ondatra zibethicus*) is the only species within the genus *Ondatra*. The animals have a « rat-like » appearance (Figure 3), however they are more closely related to voles *Microtus spp* and lemmings *Lemmus spp*. An adult animal weigh on average more than one kilogram. The body length is about 50 cm. The tail is characteristic of muskrat species; it is naked, laterally flattened and measured about 25 cm (Danell, 1996). Furthermore, its life

span is estimated at less than one year because of high mortality in juvenile and the loss of over half individuals in winter that don't spend two winters. They may eat a large variety of plants as cattail (*Typha spp.*), bulrush (*Scirpus spp.*), sedge (*Carex spp.*), arrowhead (*Sagittaria spp.*) and many other species (Hadlec et al, 2007). Its animal matter consumption is considered as no significant part (Neves & Odom, 1989) while plant consumption is for an adult muskrat about 82 g dry biomass per kg per day (Hadlec et al, 2007).

- Habitat/Density:

Muskrat species can survive in many habitats over the world, from subtropical rivers and coastal marshes to arctic tundra and deltas (Danell, 1996). They always live near by a river, lakes, streams, ponds or swamps, rich in vegetation. *Ondatra zibethicus* can come in mountain areas by following the river (Schley, 2002). Muskrat seems prefer to burrow into banks, unless if the population is very dense, also it builds lodges (Engeman and Whisson, 2003).

The reproduction of *O. zibethicus* varies following authors, but many say that their reproduction is seasonal. They reproduce from twice to three times between April and October and have generally from five to seven juveniles. The flexibility of their reproduce system is an important factor which permits to colonize the most countries in Europe (Schley, 2002).

- Situation in Europe

*Ondatra zibethicus* is native from neonartic area and is more common in North America. Muskrat has been introduced in Europe in 1905 in Czech Republic (Schley, 2002). Most of the animals which were introduced in Europe, were escaped for fur production (Becker, 1972). The central European muskrat population was established through the first animals released from a private estate situated about 40 km southwest of Prague (Bohemia, the Czech Republic) (Danell, 1996 ; Becker, 1972). Exactly, two males and three females were released from Prince Collorado Mannsfeld (Becker, 1972). These individuals spread to neighboring countries. « The muskrat invasion » was also facilitated by other releases, intentional releases (like in Finland, Russia and Lithuania), or individuals escaped from fur farms (like France, Belgium and Poland) (Danell, 1996 and Schley 2002). In 1926, about 100 million of individuals were estimated in Europe (Schley, 2002). Muskrat density is relatively high in Europe, from three to six individuals per ha in the North of Europe and 28 to 55 animals per ha in the South (Nummi et al, 2006). Currently, *Ondatra zibethicus* lives in all the country in Europe, excepted British islands, Island, Italy, Iberian Peninsula and the most part of Sweden



and Norway. In Britain, muskrat population was introduced in 1927 for fur farming and escaped, but they have been rapidly eradicated in 1939 (Schley, 2002 and Danell, 1996).

In Luxembourg, muskrat apparition is dated from the 1950's. Since 1959, muskrats are subjects to intensive campaigns by the ANF. Intensive campaigns were realized with the Order of the 10<sup>th</sup> march 1959 for the destruction of harmful and pest animals. Between 1959 and 1970, the number of muskrats captured by the ANF was increased dramatically (Schley, 2006). The 27<sup>th</sup> July 1971 another order was declared by the Grand Duchy of Luxembourg for actions to prevent the introduction and spread of pest animals (Schley, 2002 and Schley, 2006).

### 1.2.2. Raccoon (*Procyon lotor*)

- Presentation:

Raccoon belongs the Order of *Carnivora*. Its fur is grey and brown with a large and white head, a sharp snout, eyes black and short ears (from 4 to 6 cm). Raccoon is recognizable because of its large black patches around the eyes, black stripe on the nose and the tail is grey striped (Figure 4). An adult raccoon measure about 80 cm. (Rosatte, 2010).



Marie Vanacker, 2010  
Figure 4. Picture of a raccoon (*Procyo lotor*)

Class: *Mammalia*  
Order: *Carnivora*  
Suborder: *Caniformia*  
Family: *Procyonidae*  
Subfamily: *Procyoninae*  
Genus: *Procyon*  
Species: *P. lotor*

- Habitat/Density:

*Procyon locor* is able to swim and climb on trees, thus its diet is wide. It can eat insects, mollusks, amphibians, small mammals, eggs, birds, rats, fishes, fruits, cereals and chicken. Moreover, its favorite diet is muskrat juveniles (Schley, 2002). No many predators are known to kill raccoons, only fox and owl can kill them, only where they are juveniles. Moreover,

many of juveniles don't survive after their first winter because of undernourishment (Schley, 2002). Size of vital area depends to the availability in food and lodging and thus varies between 144 ha to 4950 ha. Their favorite habitats are forest areas near a waterbody, and where are dead trees with cavities. They may live in urbanized areas where they are parks and gardens (Schley, 2002).

- Situation in Europe:

Raccoons are native from North America. They have been introduced in Europe in 1927 and 1934 in Germany for fur farm production like muskrats and since their introduction, their range and density increase significantly. Their density is of one to two animals per km<sup>2</sup> in an optimal biotope and may up to a density of 115 individuals per km<sup>2</sup> in urbanized area (Schley, 2002).

Raccoon is not protected and can be killed in Luxembourg with the Order of the 10<sup>th</sup> march 1959 for the destruction of harmful and pest animals and also with the Order of the 21<sup>th</sup> January 1980 for raccoon widespread and introduction (Schley, 2002).

# II/ Materials and Methods

---

## 2.1. Freshwater mussels

The assessment of the impact of freshwater mussel predation is based on monitoring of empty shells left on the river banks or in the water. In the trapping areas of muskrat, all shells are collected. Other sites were prospected randomly in Alzette, Our and Sûre rivers. All middens found were collected and muskrat signs near to the middens were noted (feeding signs on vegetation, nests, dungs, tracks, or direct muskrat observation). To ensure that these shells are the results of muskrat predation, we looked for signs of mussel predation under a magnifying glass and under the light to bring out scratches or teeth marks. Predated mussels have characteristic signs: scratches on the periostracum on one or both valves, valves broken or teeth mark observed on the valve. In each midden, shells were cleaned, identified, counted, weighted and measured with a calliper (only if the shell was intact). Thus, shell mensuration was determined with the total length (anterior-posterior axis), shell swelling, shell height and valve thickness.

Statistical tests, an Anova or Kruskal-Wallis test, were effectuated by using the R software. These tests permit to evaluate the significance between shell mensuration and predation of muskrat. An Anova test (Analysis of Variance) was used when its residuals followed the normal law. If it is not, Kruskal-Wallis test was used. For both test, there is significance if the p-value is inferior or equal to 0.05.

## 2.2. Capture-Mark-Recapture Procedure

### 2.2.1. Sites and periods

To find out population densities, capture-mark-recapture (CMR) procedure was done with live trapping and individual marking. The choice of trapping areas was effectuated by looking for good conditions for trapping and hotspot of muskrat predation. Good areas to install

all traps are sites, where bank slope is not high and where the prospection during the day and night is facilitated (easiness to check each trap). The methodology of the study will be based on field investigations during two weeks in each site, but not during the week end. After animal first-capture in Our River, individuals were brought to the veterinary for an operation. An implant was put into the gastric cavity to follow each animal by radio-telemetry (however radio-telemetry data are not in this study).

### **2.2.1.1. Our River**

One CMR was effectuated along the Our River from the 15<sup>th</sup> March to the 26<sup>th</sup> March 2010, e.g. site 1 in the Figure 5. Our River passes through Belgium, Germany and Luxembourg along 78 km and serves as a border between Germany and Luxembourg. It flows into the Sûre River in Wallendorf, and so is a sub-tributary of Rhine River. In Dasbourg, Our River may reach in summer a depth of 25 cm. However, water level varies during day from 25 cm at midday, to 45 cm during night ([www.inondations.lu](http://www.inondations.lu)). On the contrary, during CMR procedure, water level was very important. For this study, the CMR site is situated in Our Valley at the border between Germany and Luxembourg in the Our Naturepark which was created in 1992. It includes today thirteen communities, covers a surface of 327 km<sup>2</sup> with 13.700 inhabitants. In 2008, in the natural park, a Natura 2000 area “bassin de la vallée supérieure de l’Our et ses affluents” (BE33062) was created for the habitat protection, and for the flora and fauna ([www.naturpak-our.lu](http://www.naturpak-our.lu)). CMR area is also localized just in the south of the perimeter of the LIFE project for the reintroduction of freshwater pearl mussel, *Margaritifera margaritifera*. LIFE (« L'Instrument Financier pour l'Environnement », the Financial Instrument for the Environment) is a financial tool in Europe which permits to develop a politic and legislation in the environmental field. LIFE-Nature projects are especially localized habitat restoration in Natura 2000 area where Birds Directive and Habitat-Flora-Fauna Directive are installed. The LIFE-Nature perimeter begins in Dasbourg, ([www.margaritifera.eu](http://www.margaritifera.eu)) where the last trap C15 is localized (Figure 6). In Our River, *M. margaritifera* is disappearing due to an insufficient recruitment rate with several threaten over these populations as the habitat destruction by the deterioration of the water quality, the net decrease of the trout population and the direct destruction by introduced predators (Arendt et al, 2008). That’s why campaigns against muskrats were effectuated to decrease impact on freshwater pearl mussel populations.

### **2.2.1.2. Sûre River**

Two study sites were determined in the Sûre: in the Lower Sûre, e.g. site 2 in Figure 5 and in Figure 6 from Dillingen to Bollendorf-Pont, and in the Upper Sûre River in Martelinville, e.g. site 3 in Figure 5 and in Figure 6. In Upper Sûre in Martelinville, CMR was effectuated from the 19<sup>th</sup> April to the 30<sup>th</sup> April 2010. CMR in the Lower Sûre, from Dillingen to Bollendorf-Pont, was realized from the 29<sup>th</sup> March to the 9<sup>th</sup> April 2010. Lower Sûre CMR is not situated in a natural park, and so there is no protection there. Sûre River has its source in Belgium and crosses the entire width of Luxembourg to flow into Mosel River at the border with Germany. For both site, there are differences in water level, in Upper Sûre water depth reach 25 cm in summer whereas in Lower Sûre the water level is about 70 cm.

The site of Martelinville is situated in Upper Sûre Natural Park and is a peak of freshwater mussel predation recommended by the ANF, the most left shells on the bank were collected. Upper Sûre Natural Park was created in 1999 and covered on 184 km<sup>2</sup>; it was the first natural park created in Luxembourg. It groups six municipalities (Boulaide, Esch-sur-Sûre, Heiderscheid, Lac de la Haute-Sûre, Neunhausen and Winseler) and counts about 5.500 inhabitants. In the Upper Sûre Natural Park, more than 50% is covered by the forest which is higher than the national mean. It also includes the Upper Sûre Lake created by a dam and extends over 380 ha. The creation of the natural park permits to valorize resources, to promote the region development and an environmental education. A LIFE-project for the reintroduction of the European otter (*Lutra lutra*) is realized into the natural park.

### **2.2.2. Method of Capture-Mark-Recapture (CMR)**

Traps permit to catch one animal each time. In each trapping area, fifteen traps were installed during two weeks, fixed every 250 meters (Figure 7). The fifteen traps were put on the shore the Monday of each week and were taken off each Friday morning after the trapping campaign. During each week end, no muskrat were trapped. Controls of every trap were effectuated two times per day, at the dawn and dusk. During the CMR procedure, water level was monitored by online level, so expected damages by high-water events could be eliminated.

Traps are fabricated in wire; they were fixed on the bank where entrance pointed to the water. Two pieces of apple were deposit on a plate inside the trap. When a muskrat would take a piece of apple and came on the plate inside the trap, a system was triggered and the muskrat

would have been trapped (e.g. Figure 7). To get biometric information about the muskrat, a smaller trap (extractor) have been used, placed just to the entrance of the trap. This extractor has a funnel form, thus when the muskrat went out of the trap, it came into the extractor, and so muskrat was blocked with a wooden plate (Figure 7). Each muskrat captured the first time, was marked, its sex was determined, and biometric data were collected (weight and tail length). The weight was determined when muskrat was in the extractor by subtracting the total weight (muskrat and extractor) by the extractor weight. Each captured muskrat was individual marked with a passive integrated transponder. These transponders are small capsules with chip inside which are programmed with a unique identification code (Bourgault, 2008). A capsule is inserted in muskrat by subcutaneous injection. The chip-reader, a small portable player, permits to read the chip number when it is close to the muskrat skin.

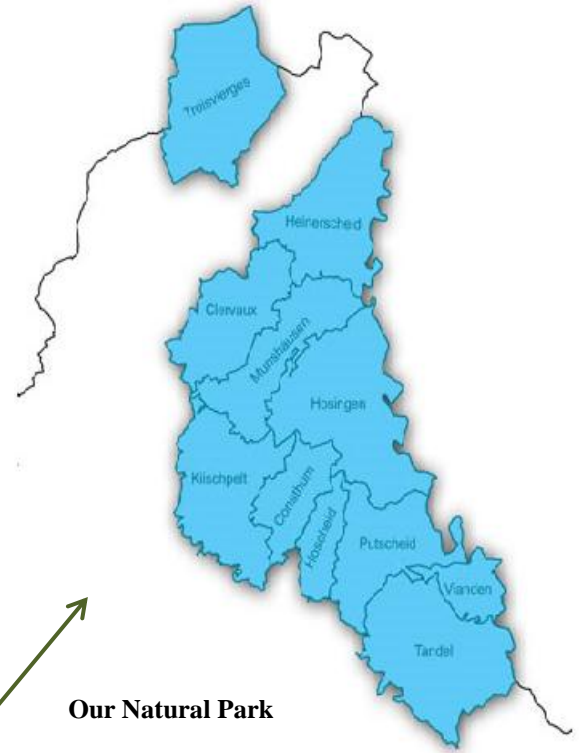
### ***2.2.3. CMR Analysis***

#### ***2.2.3.1. CloseTest program***

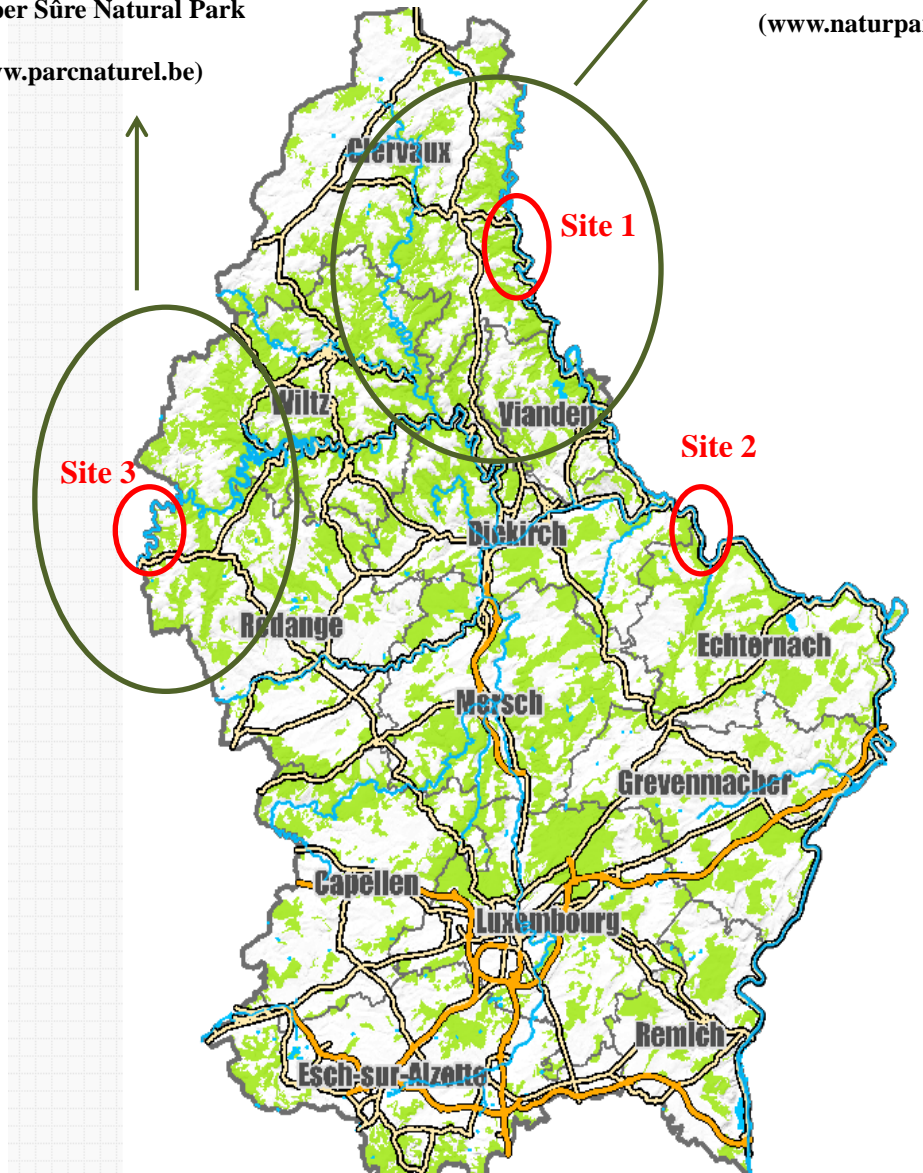
A closed population has no dead or births and no emigrations or immigrations (Potvin, 1994). To get the population density after the results of the CMR, we can suppose that the muskrat population is closed, because the capture time (two weeks) which is short. Additional the results of CMR have been analysed by CloseTest, a tool for testing the hypothesis of demographic closure for capture-recapture analysis data (Stanley & Burnham, 1999). It permits to test closure population with a chi-square test developed by Stanley and Burnham in 1999. This test represents the closed population capture-recapture model with time variation in capture probabilities (Stanley & Burnham, 1999). The test is very sensitive to permanent emigration but less for temporary emigration (Stanley & Burnham, 1999). To complete analysis, another statistical test was added to chi-square test, and represents a z-value. Low p-values for both, chi-square and z test, suggest that the population is not closed (CloseTest by Stanley & Richards, 2005). To use CloseTest, a history data file of capture is created in .txt extension which contains data with 1 and 0 only (Annex A(b) and Annex B (b)). 1 represents the capture of the animal and 0 its absence. Each line is the capture history of one animal, and columns represent recapture campaign (CloseTest by Stanley & Richards, 2005).



**Upper Sûre Natural Park**  
 ([www.parcnaturel.be](http://www.parcnaturel.be))



**Our Natural Park**  
 ([www.naturpark-our.lu](http://www.naturpark-our.lu))



**Figure 5. Sites of CMR in Luxembourg: Our valley (1), Lower Sûre (2) and Upper Sûre (3)**

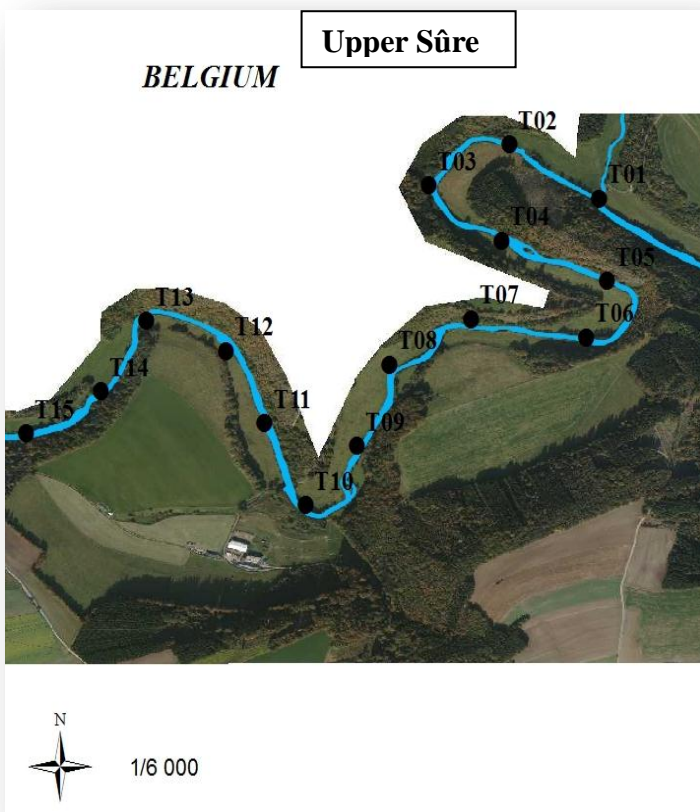
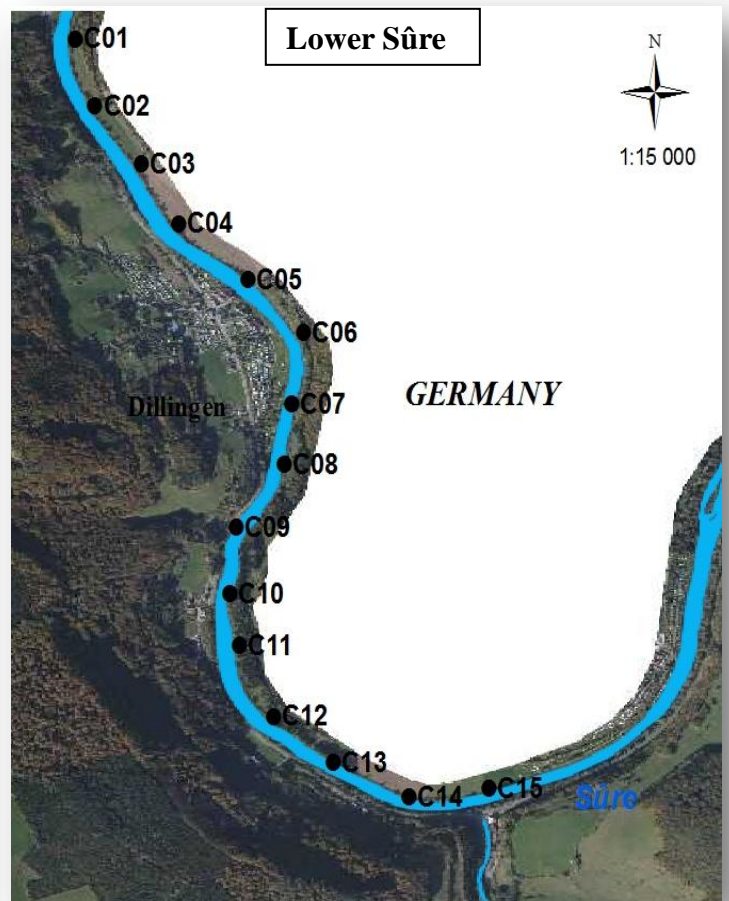
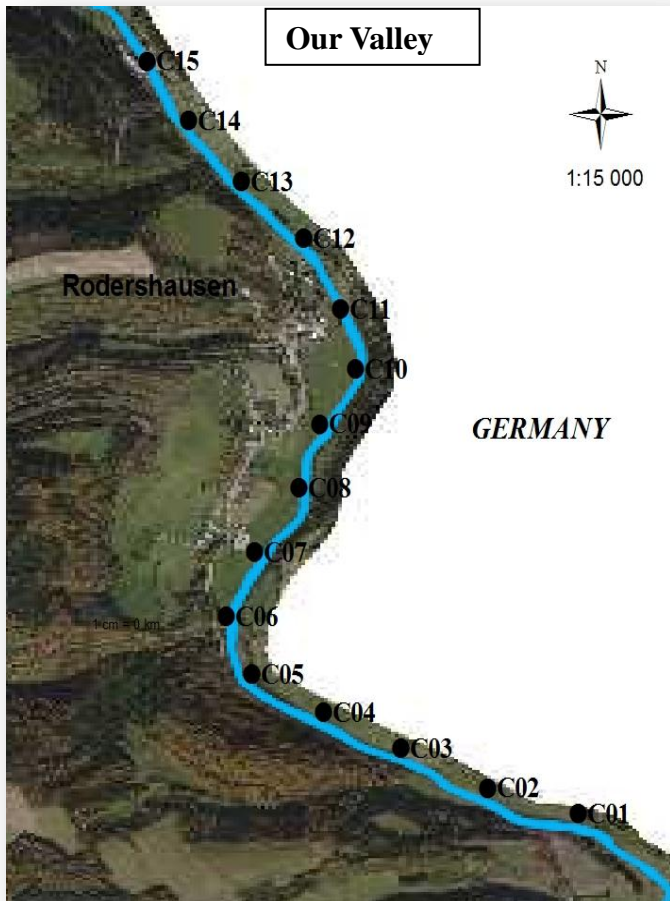


Figure 6. Trap areas in Our valley, in Lower Sûre and in Upper Sûre. Each trap is separated of 250 meters.





**Figure 7. Pictures of muskrat traps and extractor**

### ***2.2.3.2. Density population analysis***

Following the results of CloseTest program, Schnabel and Schumacher-Eschmeyer methods for close population were used to determine the densities of muskrat population in this study:

- Schnabel method (Daget, 1971 and Busson, 2003):

This method consists to analyse the population density with successive captures. To use this method, some conditions must be respected (Busson, 2003):

- muskrat population must be stable
- capture probability of muskrat must be the same for each animals
- recapture must be a random sampling
- mark must be healthy, without influence of the recapture probability

The arithmetic was given by Schnabel in 1938:  $N = \frac{\sum(n_i \times m_i)}{\sum r_i}$

where N is the number of individuals estimated in muskrat population,  $n_i$  is the number of individuals captured in trapping number i,  $m_i$  is the number of individuals marked before the trapping number i and  $r_i$  is the number of marked individuals recaptured in trapping number i. The estimation N by Schnabel is not the best; it is generally superior than in the reality. Thus Chapman in 1952 (Busson, 2003) created a Schnabel method adjusted:

$$N = \frac{\sum(n_i \times m_i)}{\sum r_i + 1}$$

The variance can be calculated with  $S^2 = \frac{R}{\sum(n_i \times m_i)^2}$

where R is the total number of recaptured individuals (sum of  $r_i$ ).

The standard variation  $\sigma = \sqrt{S^2}$

- Schumacher-Eschmeyer method (Potvin, 1994, Daget, 1971):

This method was given by Schumacher and Eschmeyer in 1943 with the following arithmetic (Potvin, 1994 and Daget, 1971):

$$\frac{1}{N} = \frac{\sum(m_i \times r_i)}{\sum(n_i \times m_i)^2} \quad \text{and} \quad N = \frac{\sum(n_i \times m_i)^2}{\sum(m_i \times r_i)}$$

N is the muskrat number in the population,  $m_i$  is the number of marked muskrat before the trapping i,  $n_i$  is the number of animals captured in the trapping i, and  $r_i$  is the number of individuals marked captured in i.

$$\text{Var}(S^2) = \frac{\sum(r_i^2/n_i) - (\sum r_i \times m_i)^2 / \sum(n_i \times m_i^2)}{n - 1}$$

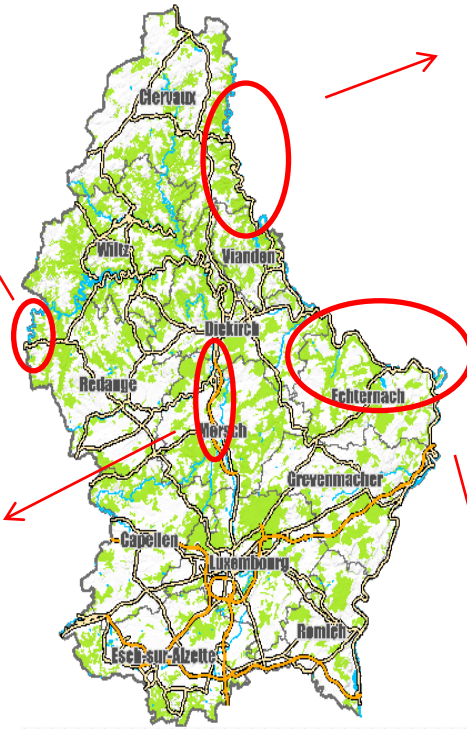
where n is the number of trapping (Potvin, 1994).

Standard variation  $\sigma = \sqrt{S^2}$








### 2.3. Predation of freshwater mussel by raccoons and muskrats

An experiment was effectuated by collecting 34 swollen river mussels (*Unio tumidus*) and painter's mussel (*Unio pictorum*) in Mosel River in Metz (France). These mussel species are very similar to the *Unio crassus* and should be an alternative for this protected species (see chapter 1.1 Freshwater mussel species). The principal aim of this experiment is to give these mussels to their natural predators like muskrat (*Ondatra zibethicus*) and raccoon (*Procyon lotor*) to find out which signs in form of scratches and broken shells will be obvious after feeding. Thus, fourteen mussels of *Unio sp.* and three *Anodonta anatina* (from the lake situated next to Wildlife center) were given directly to ten raccoons from Wildlife center in Saarburg (Germany).

Two muskrats were caught for this experiment and put into two cages with a small park and a bucket of water. The experiment during was about one week. Some mussels were put into the water and other out of water. Contrary to raccoons from Wildlife center, both muskrat caught for this experiment, were not accustomed to seen human in wildlife.



**Legend**

-  *Anodonta anatina*
-  *Anodonta anatina* + *Unio crassus*
-  *Anodonta anatina* + *Anodonta cygnea* + *Unio pictorum*
-  *Corbicula fluminea*
-  *Unio crassus*
-  *Margaritifera margaritifera*
-  *Unio crassus* + *Anodonta anatina*

**Figure 8. Map of freshwater mussels collected in different middens in Luxembourg**

# III. Results

## 3.1. Middens of Freshwater Mussels

A total of 2244 shells were found along Azette, Sûre and Our rivers. Middens were collected and six species were identified: *Margaritifera margaritifera*, *Unio crassus*, *Unio pictorum*, *Anodonta anatina*, *Anodonta cygnea*, and *Corbicula fluminea* (e.g. Figure 8). Many of middens were situated into water because of high water level occurring March and April. Different signs of muskrat activities were observed near by the localization of middens. However no signs muskrat activities were observed for several middens and no feeding signs on vegetation were observed.

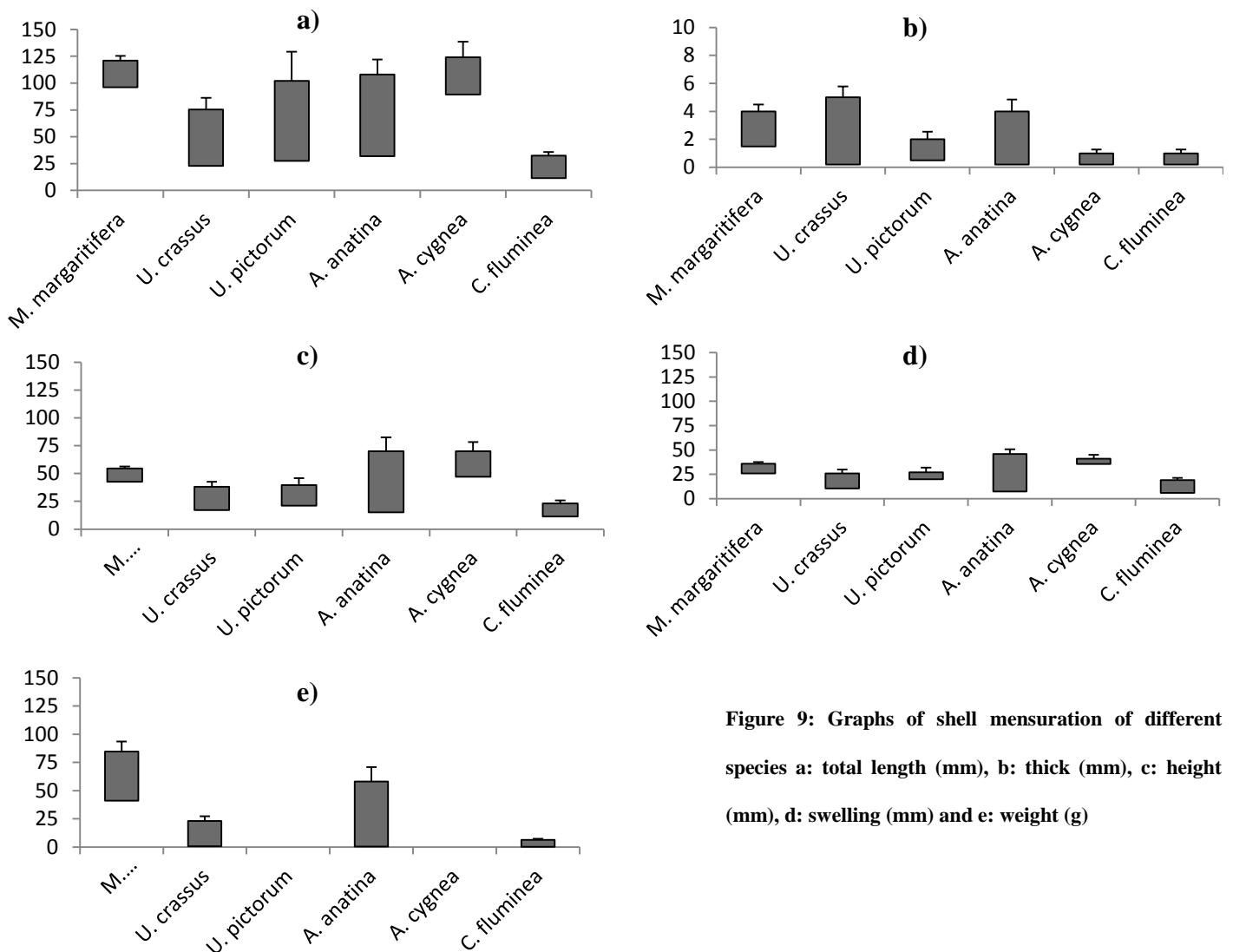


Figure 9: Graphs of shell mensuration of different species a: total length (mm), b: thick (mm), c: height (mm), d: swelling (mm) and e: weight (g)

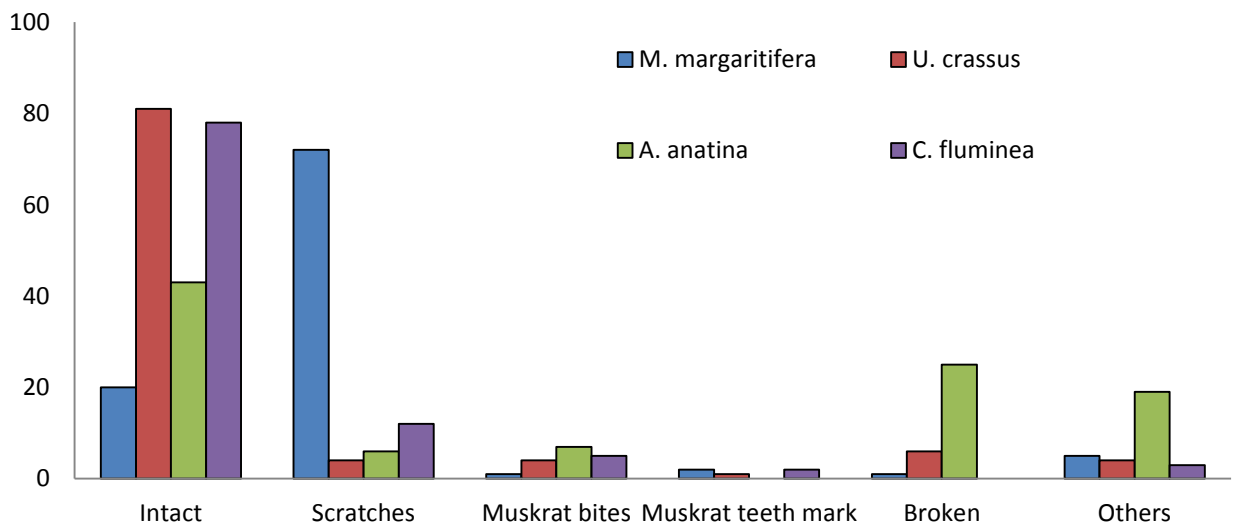


Figure 10. Graph of shell number of different species with different predation marks



Figure 11. Pictures of middens of *M. margaritifera* (a) and *C. fluminea* (b) found in Our Valley and *U. crassus* shells found in Upper Sure (c)

### 3.1.1. Freshwater pearl mussel (*Margaritifera margaritifera*)

The totality of freshwater pearl mussels was collected in 2006 in different middens (Figure 11, a) in the same area in Our River, next to Kalborn in 2006 (Figure 8). No sign of muskrat activities were noted. 213 freshwater pearl mussels were found where 43 shells have only one valve and 170 with both valves. Shell mensuration has been taken on all *M. margaritifera*s; however 49 shells have been not weighted (the 43 shells with the one valve only and six because of the shell degradation). The total length varies from 96 mm to 121 mm (Figure 9), with an average of 110.6 mm (Annex F, a). The shell weight varies from 41.1 g to 84.8 with an average of 61.42 g. Pictures of marks on freshwater pearl mussels are presented in Figure 12. Freshwater pearl mussel is the species where the most scratches are found (Figure 10) where more than 72 % of shells have scratches on them (e.g. Figure 11). However, 43 shells which represent 20 % of all freshwater pearl mussels have no signs of predation. Only one shell is broken, one other has only muskrat bite on it, and four shells (2%) have teeth mark of muskrat. 5 % (11 shells) have more than one sign of predation on them.

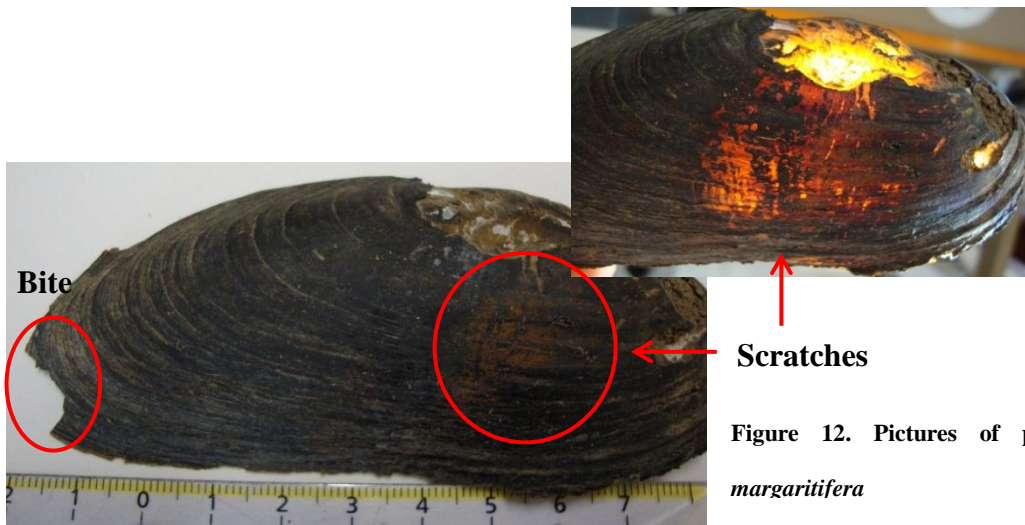


Figure 12. Pictures of predation marks on *M. margaritifera*

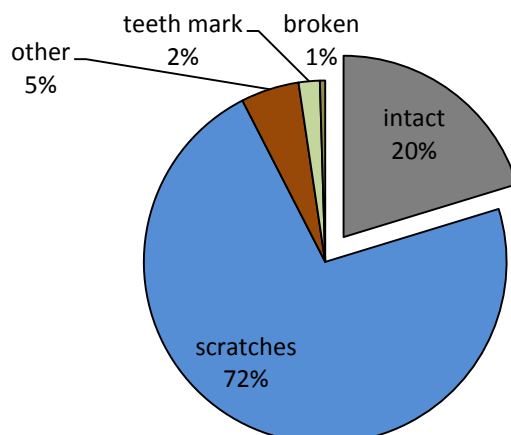


Figure 13. Proportion of predation signs on *M. margaritifera*

### 3.1.2 Thick shelled water mussel (*Unio crassus*)

Most of thick shelled water mussel were pick up along the Upper Sûre, but not in a distinct midden and were extended over hundreds meters (Figure 11, c). All mussels in Upper Sûre were collected during CMR procedure and a camera was installed in a site where most shells were collected. Two weeks after, there were still some shells of *Unio crassus*, however, no picture was taken by the camera. In Upper Sûre, muskrats were captured in this area, but no signs of muskrat activities were noted next to middens. Nearby middens of Mersch, dungs and tracks were picking up. In total, 1405 thick shelled water mussels were found where 135 shells have only one valve and 1270 with both valves. 118 thick shelled water mussels have not been measured because of their shells are broken or degraded. The total length varies from 23 mm to 68.5 mm (Figure 9), with an average of 48.3 mm (Annex F, b). 571 shells have been not weighted. The shell weight varies from 0.7 g to 9.8 g with an average of 7.25 g. 81 swelling shells and 126 shell heights have been measured.

Pictures of predation signs on the thick shelled water mussel are presented in Figure 14. 1130 mussels which represent 81 % of totally shells have no signs of predation on them (e.g. Figure 15). For the 19% of remaining shells, the majority are broken (more than 10 % which corresponds to 87 shells) or have scratches on them (more than 4 %, corresponding to 60 shells). Also, 4% (57 shells) have muskrat bites and only 1 % (19 shells) has only teeth marks of muskrat. Moreover, 52 (4%) thick shelled water mussels have more than one sign of predation on their shell.

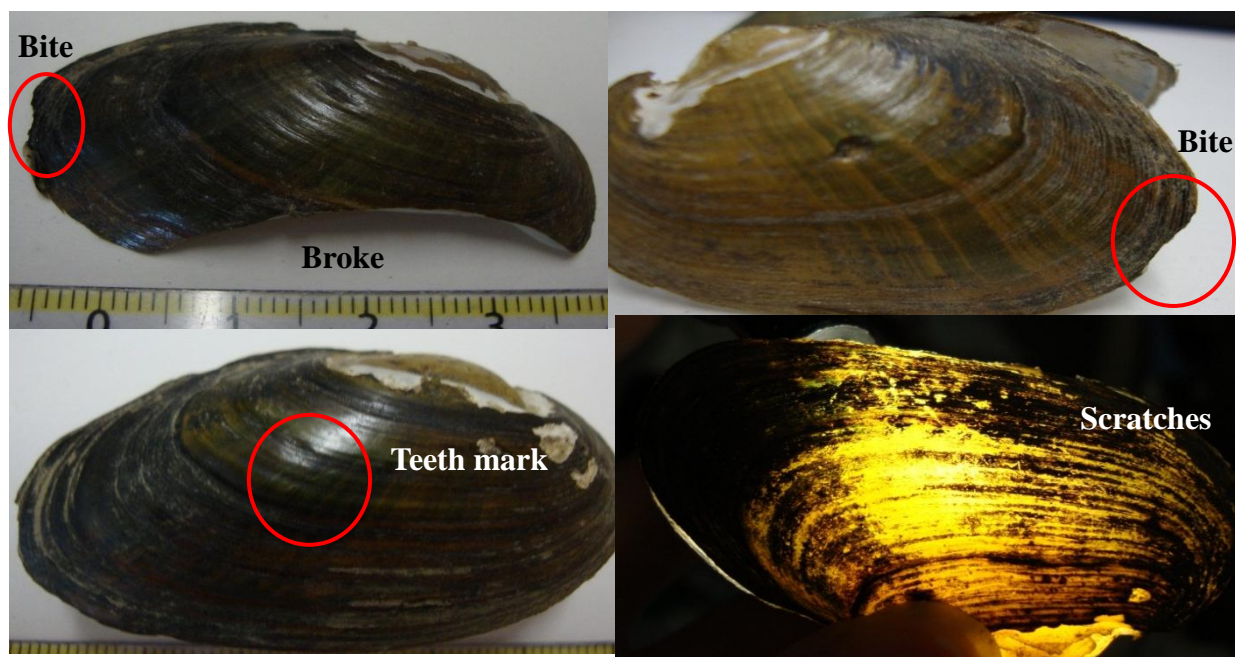


Figure 14. Pictures of predation marks on *U. crassus*



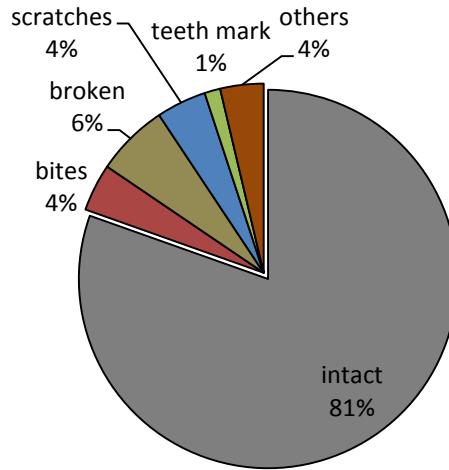


Figure 15. Proportion of predation signs on *U. crassus*

### 3.1.3 Painter's mussel (*Unio pictorum*)

Painter's mussels were found in Echternach Lake (Figure 8) and no sign of muskrat activities was noted. Only six shells were found, four have only one valve, and two have both parts. Only one shell was weighted and two have a shell swelling noted. The total length varies from 27.5 mm to 102 mm (Figure 9) with an average of 58.7 mm. The shell height varies from 21 to 39.5 and the valve thickness from 0.5 to 2 mm.

For the predation, only one shell was intact, which represents 16% (e.g. Figure 17). The others have predation signs on them: four have muskrat bites (Figure 16) and one other has both, broken and muskrat bite.



Figure 16. Picture of predation mark on *U. pictorum*

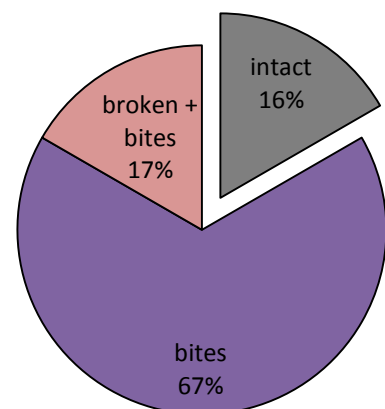


Figure 17. Proportion of predation signs on *U. pictorum*

### 3.1.4. Duck mussel (*Anodonta anatina*)

Duck mussels were found in Echternach Lake where no sign of muskrat activities was observed, and in CMR areas in Lower Sûre and in Upper Sûre where muskrats were

caught (Figure 8). In total, 299 shells of duck mussel were found where 242 with both valves and 57 with one valve. There is a big difference for shell mensuration, the bigger shells were found in Echternach Lake and the smallest were found in river. The duck mussels measure from 32 cm to 119.5 cm, shell swelling from 7.5 to 48 mm, and shell height from 15 to 70 mm (e.g. Figure 9). They weighed from 0.4 to 58.1 g. Shell majority are not very thick (average of 1.4 mm) but can go to 4 mm for the duck mussel found in Echternach lake.

The majority of shells have predation marks on them (Figure 19) and picture of different marks are in Figure 18. More than 57 % of duck mussels have signs of predation on their shell. The majority (25 % i.e. 74 shells) are broken, and 19 % are more than one sign of predation on their shells, and the majority of this 19 % are also broken (e.g. Figure 19). 22 and 18 duck mussels (i.e. 7 % and 6 %) have scratches or muskrat bites on their shell.



Figure 18. Picture of predation marks on *A. anatina*

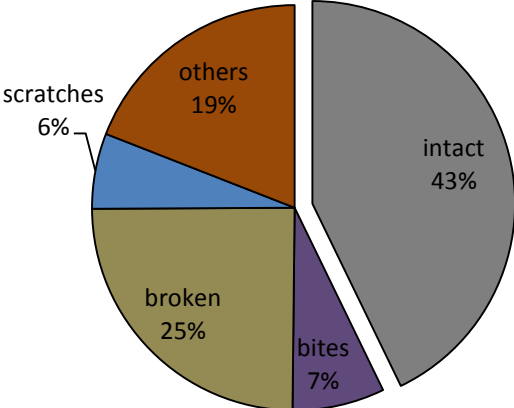


Figure 19. Proportion of predation signs on duck mussels (*A. anatina*)

### 3.1.5. Swan mussel (*Anodonta cygnea*)

Swan mussels (*Anodonta cygnea*) were collected with *A. anatina* and *U. pictorum* in Echternach Lake (Figure 8), where no sign of muskrat activities were observed. Eight swan mussels were found and all shells, except one, were broken. At the difference of the duck mussel, they are thicker but longer (Figure 9), and the shell swelling is weaker. On the eight swan mussel found, seven were broken and one has no sign of predation on it shell.

### 3.1.6 Asian clam (*Corbicula fluminea*)

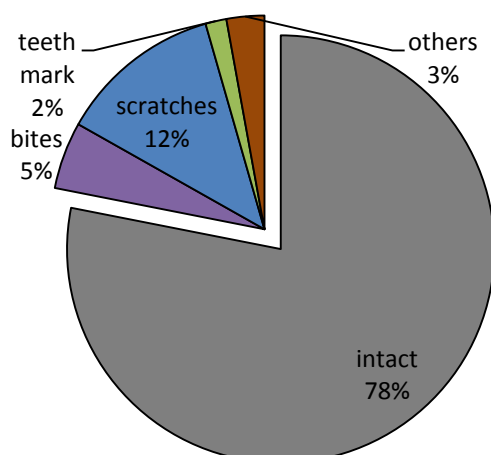


Figure 20. Proportion of predation signs on Asian clams (*C. fluminea*)

The invasive species *C. fluminea* was mainly collected in one big midden, with 269 shells (Figure 12, b). They have been picked up near to Vianden in Our River (Figure 8), but no sign of muskrat activities was observed.

The majority of *C. fluminea* were intact (Figure 10). 313 Asian clams were found where only nine mussels have one valve. At the

difference of other shells, Asian clams are small (average of 18.55 mm and 1.85 g), and thin (average of 0.49 mm). It is very difficult to open *Corbicula fluminea* because of the joints which are very tight. Shells are very thick, and no shell was broken. Thus, 246 of the 313 Asian clams found are intact (i.e. 78 %) (Figure 20) and 12 % (i.e. 39 shells) have scratches on them. However, at the difference of other species, scratches on Asian clam are inside of the shell. 16 shells (i.e. 5%) have smaller bites, 2% have muskrat teeth mark and 3% have more than one predation sign. Picture of predation marks are in Figure 21.

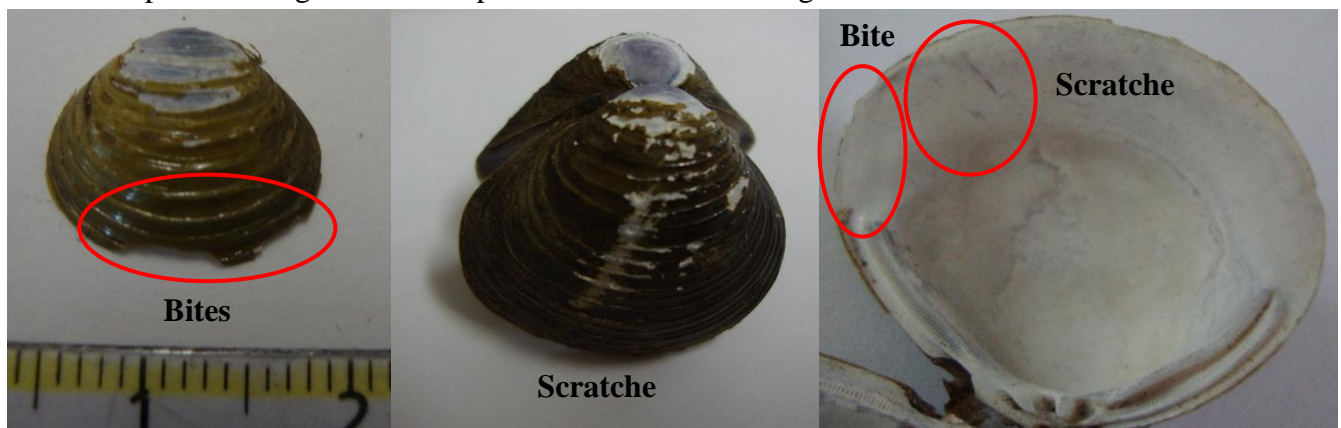


Figure 21. Picture of predation marks on *C. fluminea*

### 3.1.7 Biostatistical test

There is no link between shell mensuration and signs (intact, scratches, muskrat bites, muskrat teeth mark, broken or other signs) for shell of *M. margaritifera* and *Unio crassus* (Table I). However, p-value of weight of shell for *U. crassus* is quasi equal to 0.05 (0.0575), thus it is interaction between the weight of *U. crassus* shells and predation marks. In fact, we found more predation marks on shells when they have lower weight.

For *A. anatina* total length is significant and valve thickness, shell swelling and shell height are very significant with predation signs. There is only no link with shell weight for duck mussels. In fact, there are more intact duck mussels when they are smaller. In addition, they are more predation signs when they are longer as muskrat bites and broken shells. For the thickness, they are more muskrat bites and broken shells when mussels are thin (lower than 2.5 mm). Superior to this value, there are no many predation signs. For the shell swelling and shell height, when the values are small, shells are generally intact, no many predation signs were observed.

For *C. fluminea* only valve thickness and shell height are significant. Most of predation signs (bites and scratches) were observed when values of shell height and valve thickness are low.

	<b>Total length</b>	<b>Valve thickness</b>	<b>Shell swelling</b>	<b>Shell height</b>	<b>Weight</b>
<i>M. margaritifera</i>	0.5595 <sup>a</sup>	0.733 <sup>b</sup>	0.205 <sup>b</sup>	0.499 <sup>a</sup>	0.2236 <sup>a</sup>
<i>U. crassus</i>	0.247 <sup>b</sup>	0.0647 <sup>b</sup>	0.511 <sup>b</sup>	0.7718 <sup>b</sup>	0.0575 <sup>*b</sup>
<i>A anatina</i>	0.052 <sup>*b</sup>	3.791e-06 <sup>***a</sup>	5.694e-05 <sup>***b</sup>	1.638e-07 <sup>***b</sup>	0.06225 <sup>b</sup>
<i>C. fluminea</i>	0.1194 <sup>b</sup>	0.01454 <sup>*b</sup>	0.528 <sup>b</sup>	0.04441 <sup>*b</sup>	0.4688 <sup>b</sup>

**Table I: Results of statistical test for each species in link between predation signs and shell mensuration. a = Anova test. b = Kruskal-Wallis test**

## 3.2 Muskrat Capture

### 3.2.1. *In Our valley*

Each individual caught during the morning campaign were brought to the veterinary just after their capture to put an implant into gastric cavity for radio tracking. Because of general anesthesia, muskrats were released during the night campaign of their capture day. During this campaign, eight animals were captured with four females and four males, and three were recaptured (one female and two males). Males weight more than one kilogram, while females weight about eight hundreds grams. Only one female, was very small, and weight 678 g (e.g. Annex A). On the map 1 in Figure 22, only one muskrat was captured in the South of Dasbourg-Pont and two muskrats in Rodershausen. The five last muskrats captured and all recaptured muskrats were trapped in the south of the trapping area.

### 3.2.2. *In Lower Sûre*

Thirty-one animals were captured with fifteen recaptured muskrats (map 2 in Figure 22). There were nine females and twenty-two males; at the end of the trapping campaign, new captured muskrats were only males. Most of muskrats were trapped during morning trapping and the majority was effectuated in the North of Dillingen. None muskrat recapture was realized in the last three traps, only one muskrat was recaptured in trap number 14. In the trap number 4, ten muskrat recaptures were realized, it is because of the muskrat number one (e.g. Annex B) recaptured nine times in the trap 4. The trap was set under a tree where the muskrat one has its nest. On the contrary of Our valley, male and female muskrats had no difference in weight and tail length, three females weighted more than one kilogram. Differences between live trapping and weather conditions (cloudy, rain and sunny) could be noted during CMR in Lower Sûre (where the most of individuals were caught). We can noted that they are less capture in dusk where the day was sunny (three captures could be noted during the two weeks) whereas in a cloudy or in rainy day eight captures could be noted for both last weather conditions. On the contrary in dawn campaign, more muskrats were captured during sunny day than in bad weather.

### 3.2.3. In Upper Sûre

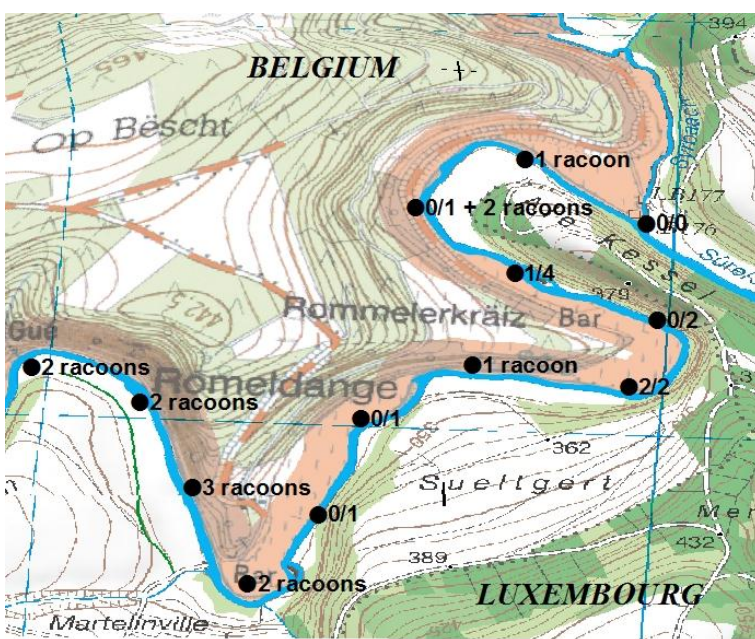
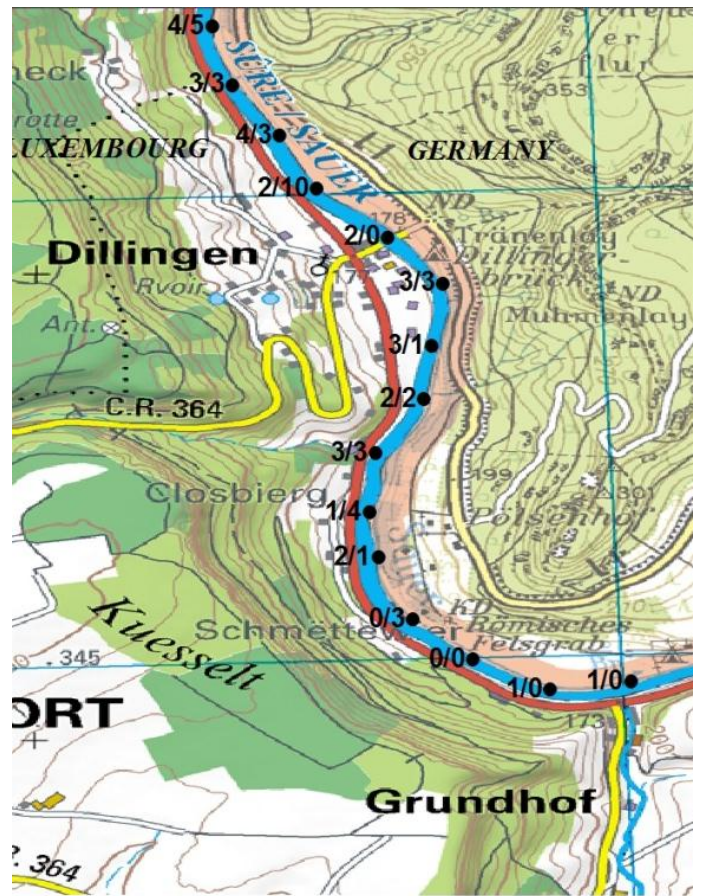
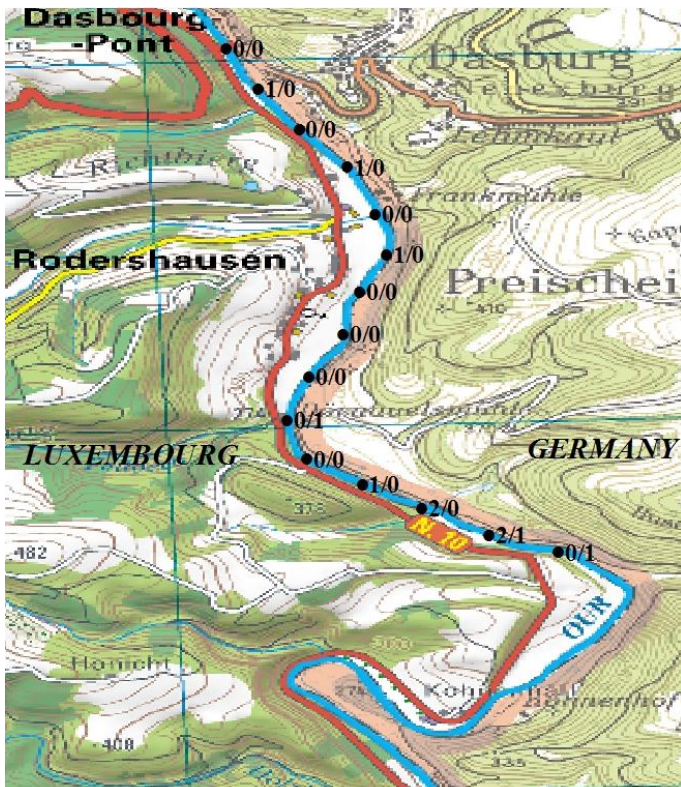


Figure 22. Map of CMR results in Our Valley (1), in Lower Sûre (2) and in Upper Sûre (3). The first number corresponds to first-capture number and the second to recapture number.

At the difference of previous CMR areas, in the Upper Sûre site (Martelinville, map 3 in Figure 22) only three animals were trapped during the two weeks and two were recaptured (Annex C). One of both recaptured muskrats was a juvenile, it weighted 560 g. It was recaptured eight times in five different traps and traveled more than 1250 meters. Because of the rate weak of capture, it is not necessary to analysis CMR in Martelinville area. Many raccoons were also captured in this area which majority was in the West of Martelinville. These animals sometimes visited the traps, and destroyed them to escape. Destroyed traps were immediately replaced.

### 3.3. CMR analysis

#### 3.3.1 CloseTest analysis

Low p-value inferior to 0.01 suggests that the population is not closed i.e. Stanley & Burnham test and Otis et al. test (Stanley & Richards, 2005).

In Our valley, the results in Table I show that population is closed. Indeed, p-values of Stanley & Burnham test and Otis et al. test are higher than 0.01 (reciprocally 0.298 and 2.00). There is no evidence of additions or losses in the population because all p-values are very high.

In Lower Sûre, p-value of the Stanley & Burnham test (0.012) is superior to 0.01. It is similar for Otis et al. test where p-value is 0.546. Thus, both tests show that muskrat population in the Lower Sûre is closed. Like in Our valley, there is no significance in losses and additions of individuals in muskrat population.

	Our valley (Roderhausen)	Lower Sûre (Dillingen)
N_hat <sup>a</sup>	12	35
p-value of Stanley & Burnham Test	0.298	0.012
p-value of Otis et al. Test	2.00	0.546
NR vs JS <sup>b</sup>	1.00	0.168
M_t vs NM <sup>c</sup>	1.00	0.022
M_t vs NR <sup>d</sup>	0.298	0.016
NM vs JS <sup>e</sup>	1.00	0.107

a: N\_hat is the maximum likelihood estimate of animals number at risk of capture on the first capture occasion.

b: NR vs JS permits to evaluate the evidence of additions by testing the fit of the No-recruitment model against the Jolly Seber model.

c: M\_t vs NM evaluates whether there is evidence of additions by testing the fit of the closed-population model against the No-mortality model.

d: M\_t vs NR evaluates the evidence of losses from the population by the fit of the closed-population model against the No-recruitment model.

e: NM vs JS permits to evaluate the evidence of losses from the population by the fit of the No-mortality model against the Jolly-Seber model.

**Table II. Results of CloseTest analysis (Stanley & Richards, 2005)**

### 3.3.2 Muskrat population in Our Valley (Roderhausen)

- **Schnabel method:**

$$N = \sum(n_i \times m_i) / (\sum r_i + 1) = \mathbf{11.75}$$

$$\text{and Var}(S^2) = R / (\sum(n_i \times m_i))^2 = \mathbf{0.00136}$$

$$\sigma = \sqrt{S^2} = \mathbf{0.04}$$

Following Schnabel method, muskrat population is about 12 individuals. The standard deviation is very low (0.04) and the variance too (0.00136).

- **Schumacher - Eschmeyer method:**

$$N = \sum(n_i \times m_i)^2 / \sum(m_i \times r_i) = \mathbf{12.41}$$

$$\text{Var}(S^2) = (\sum(r_i^2/n_i) - (\sum r_i \times m_i)^2 / \sum(n_i \times m_i^2)) / (n-1) = \mathbf{2.25}$$

$$\sigma = \sqrt{S^2} = \mathbf{1.5}$$

Muskrat population is approximately of 12 individuals with Schumacher – Eschmeyer method in the Our valley. Standard variation is of 1.5 individuals, thus we can say that muskrat population is between 11 and 14 individuals.

### 3.3.3 Muskrat population in Lower Sûre site (Dillingen)

- **Schnabel method:**

$$N = \sum(n_i \times m_i) / (\sum r_i + 1) = \mathbf{32,97}$$

$$\text{and Var}(S^2) = R / (\sum(n_i \times m_i))^2 = \mathbf{2,36 \times 10^{-5}}$$

$$\sigma = \sqrt{S^2} = \mathbf{0.00486}$$

Following Schnabel method, muskrat population in Dillingen is equal to 33 animals and standard deviation is near to zero.

- **Schumacher - Eschmeyer method:**

$$N = \sum(n_i \times m_i)^2 / \sum(m_i \times r_i) = \mathbf{34,39}$$

$$\text{Var}(S^2) = (\sum(r_i^2/n_i) - (\sum r_i \times m_i)^2 / \sum(n_i \times m_i^2)) / (n-1) = \mathbf{25,46}$$

$$\sigma = \sqrt{S^2} = \mathbf{5,05}$$



For Schumacher – Eschmeyer method, muskrat population is about 34 individuals, with more or less five individuals. Thus, there are between 34 and 39 individuals in Dillingen area.

### 3.4. Predation of freshwater mussel by raccoon and muskrat

#### 3.4.1. *By raccoons*

Starting the experiment, raccoons were directly flocked to the mussels. They opened the given mussels with their teeth between the two front-legs (Figure 23). With their powerful jaw, raccoons opened mussels after few minutes. All mussels were broken at the end and had net scratches on them (e.g. Figure 24). Scratches were very visible and white, and are mainly due to raccoon teeth. Moreover, a majority part of shells had also bites on them, and three mussels had a hole in the shell due to raccoon teeth. However, majority of raccoons was not able to separate both valve and eat the meat. With their legs, they caught a part of meat until they didn't reach the rest of the food and after let the shell (picture on the right, Figure 23).

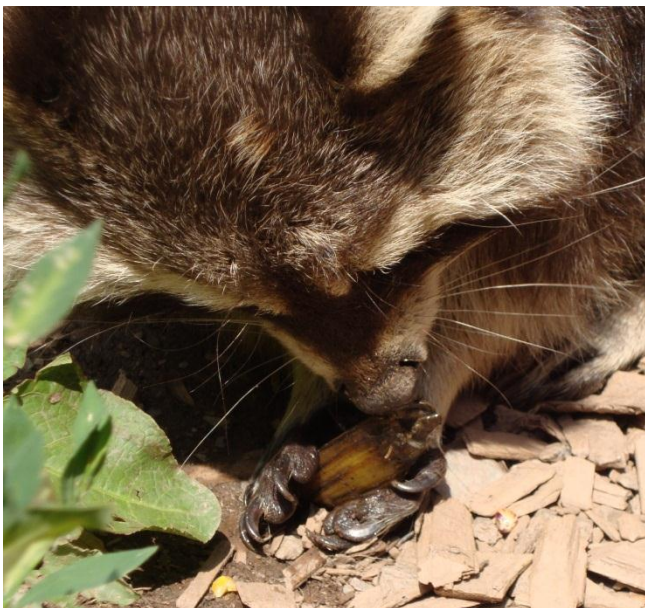


Figure 23. Pictures of raccoon eating a mussel of *Unio pictorum*

#### 3.3.2. *By muskrats*

Muskrats eat shells more slowly as raccoons. Thus, the muskrat experiment was during five days. At the beginning, muskrats didn't eat mussels. Two days after the beginning of the study, one mussel was cut, opened and put into each cage muskrat. The following day,

both muskrat have eaten this mussel. In each cage, muskrats put all mussels out of the water, but only in one cage two mussels were eaten. Other mussels put out of the water, were still alive, and had also some marks on them. All of shells had bites and scratches on them (only one didn't have bites). Muskrat scratches are different than raccoon scratches, on the one hand the scratches are smaller and on another hand, the majority of scratches were less visible and clearly appeared with the light (Figure 25). The four mussels eaten by muskrats, had detached valves.

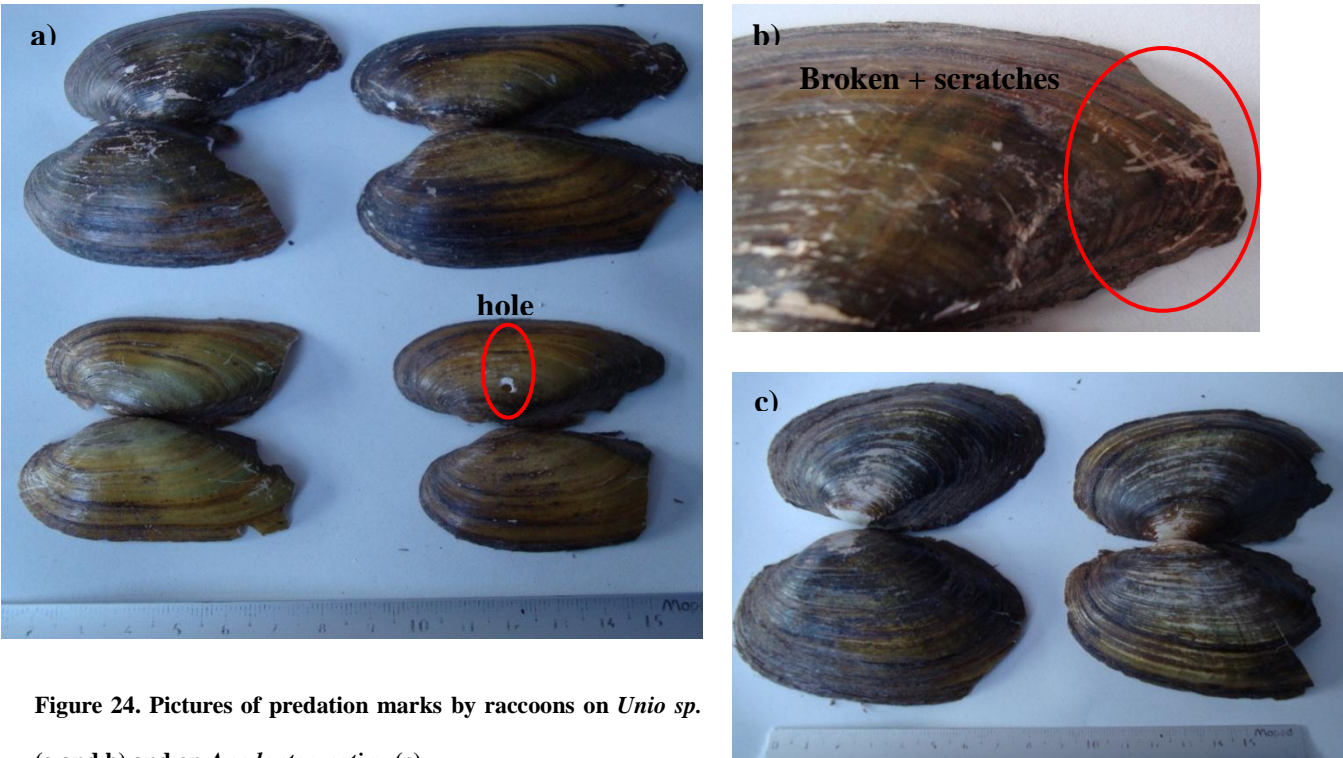


Figure 24. Pictures of predation marks by raccoons on *Unio sp.* (a and b) and on *Anodonta anatina* (c)

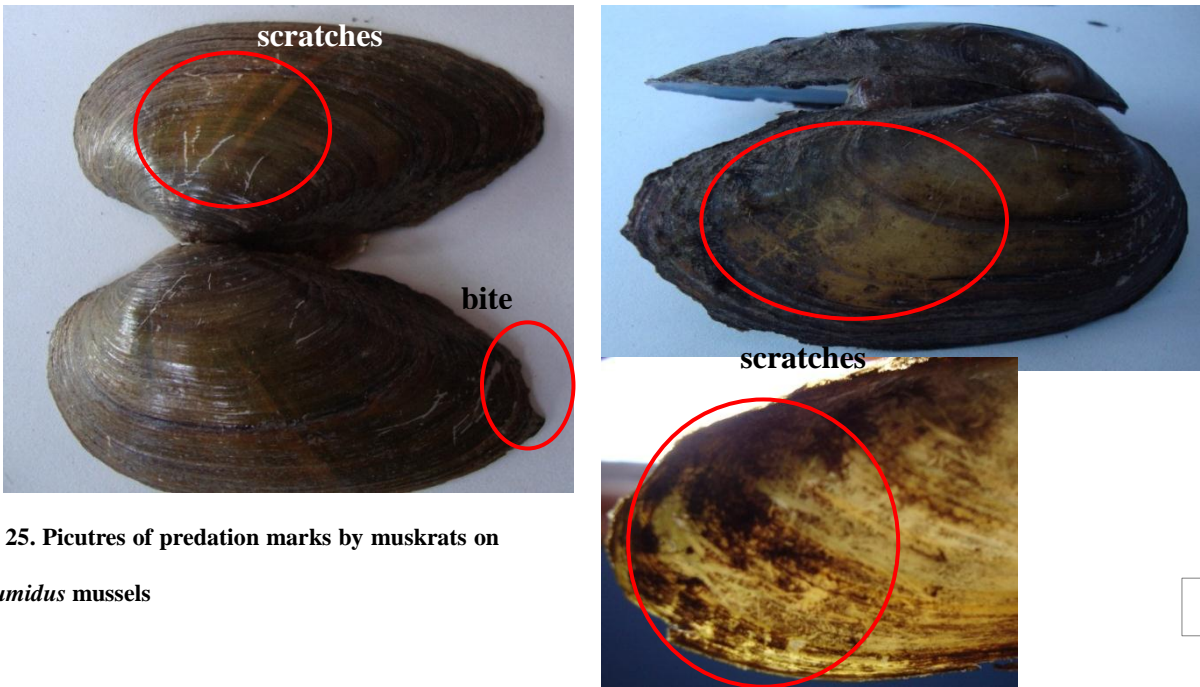


Figure 25. Pictures of predation marks by muskrats on *Unio tumidus* mussels

# IV/ Discussion

---

Like other invasive species, muskrats (*Ondatra zibethicus*) have large native range, high abundance, high viability and relatively short generation time. Introduced in Europe due to fur farm production, muskrats have spread rapidly. In Luxembourg, the presence of muskrats was registered in 1950's. They are considered as harmful and pest animals and can be killed since 1959 (Schley, 2002). As all invasive species, they may have negative effect on aquatic habitat structure (Danell, 1996). For example they may contribute to the spread of another alien species, the Canadian waterweed (*Elodea canadensis Michx*) which are facilitated by open areas created by muskrats (Nummi et al, 2006). However, in most wetland habitats with abundant water due, they may be considered as a “keystone” species. When they are present, many scientists noted an increase of the diversity of the habitat structure and plant species richness (Danell, 1996). However, muskrat is a good predator of freshwater mussel, its predation mainly retarding species recovery and contribute to further declines of endangered population (Neves and Odom, 1989). Different freshwater mussels were also collected and predation signs could be observed and are detailed in following parts.

## 4.1. Freshwater predation

### 4.1.1. Muskrat predation

Empty shells of freshwater pearl mussels (*M. margaritifera*) were found in different big middens created by muskrats (Neves and Odom, 1989). Predation signs on shells were observed and compared to marks of *Unio tumidus* and *Unio pictorum* eaten by muskrat (i.e. muskrat bites, teeth marks and specific scratches). Mussels predated by muskrats have often characteristic signs on them (Neves and Odom, 1989). When we gave the both unionids in cage of both muskrats, they picked up mussels to put them out of water. Then they waited that mussels begin to die to more easily open them. Muskrats scratched shells with their legs and don't generally remove the periostracum and so marks are not really visible (it is possible to better see their scratches under the light). Mussels predated by muskrats have often characteristic signs on them (Neves and Odom, 1989). When we gave *Unio tumidus* and *Unio*

*pictorum* to muskrats in cage, they picked up mussels to put them out of the water. Then they waited that mussels begin to die to more easily open them. Muskrats scratched shells with their legs and don't generally remove the periostracum and so marks are not really visible (it is possible to better see their scratches under the light). Moreover, on *U. tumidus* and *U. pictorum* shells, characteristic bites of muskrats were found. In comparison of shells empty of freshwater pearl mussel, the same marks could be observed. Most of these shells have scratches on them and muskrat bites were also observed. No small shells of *M. margaritifera* were found due to the aging population in the Our River.

By biting on valve, large thin-shelled as *Anodonta sp.* are more easily eaten (Tyrrell & Hornbach in 1998) and with this technique muskrats don't break shells and valves are generally still attached by the hinge ligament. Another technique is sometimes muskrat force the opening of the shell and thus may break valves of thin-shelled (Neves and Odom, 1989). In this study, many broken shells of *A. anatina* were observed and had also scratches and muskrat bites on their shells. Thick shelled water mussels (*Unio crassus*) are morphologically near to *U. tumidus* and *U. pictorum*. Many of shells of *U. crassus* were dispersed on the shore and the majority didn't have marks of predation on them. On *U. crassus* shells when marks could be observed, muskrat bites, scratches and muskrats teeth marks were noted showing that these mussels were eaten by muskrats. Moreover, many shells were also broken, showing a raccoon predation.

Many scientists studied freshwater mussel predation by muskrat and reported a size-selection. For example, Diggins and Stewart in 2000 showed that shells in middens were significantly larger than mussels still alive in the river. The hypothesis is that larger freshwater mussels are easier seen in the water and are more susceptible to be predated. Moreover, larger mussels are also present in higher shell area and have better encounter rates to foraging muskrat in comparison to smaller mussels (Diggins and Stewart, 2000). In this study, there is no interaction between total length and marks of predation except for *A. anatina*. In fact, the duck mussel *A. anatina* is very big in Echternach Lake and shells have muskrat bites and are broken. Shell length varies within mussel species, thus variation for muskrat to eat a specific size range will vary depending of species composition in the site (Tyrrell & Hornbach, 1998). Furthermore, the size distribution of mussels in the bottom water may be influenced by muskrats (Tyrrell & Hornbach, 1998) and thus the average of large mussels increase with the distance of the shore. Large Unionids are generally too heavy to transport or too tightly to open (Neves and Odom, 1989; Sietman et al, 2003) and that's why, muskrats predate all sizes of mussels near shore and only smaller mussels when the distance is

more important (Sietman et al, 2003). Thus, it is better for muskrats to predate medium-size mussels, avoiding small size (due to the decrease of the visibility of some species in the bottom of the river) and large size individuals (due to energy cost) (Tyrrell & Hornbach, 1998).

For other mensuration of the shell, there is an interaction on *U. crassus* shells between the weight and predation marks. In fact, if mussels were lighter, there are more predation marks observed and we can suppose that they are more predated by muskrats or raccoons because the energy cost to carry them is lower. For duck mussels, there are links between total length, valve thickness, shell swelling and shell height with predation signs. Light and thin mussels have more muskrat bites on them and they are more broken. On the contrary for low values of shell swelling and shell height, shells are generally intact. In the West of New York, *Pygamodon grandis* is the most numerous unionid and the most part of predated mussel by *O. zibethicus*. On the other hand, *P. grandis* eaten by muskrat decreased significantly in length, height and growth rate, but not in shell weight. Thus, shell thickness plays a major role in selection prey within this species (Diggins and Stewart, 2000).

For Asiatic clams (*Corbicula fluminea*), most of predation signs were observed when values of shell height and valve thickness were low. At the difference, on empty shells of *C. fluminea* smaller scratches were observed inside or outside the shell and smaller bites. The main hypothesis is when values of shell thickness are low, muskrat more easily bite them. Asiatic clams are very small mussels and, when they are present in the river (as in Our River in Luxembourg, and in the Clinch River in Virginia), are strongly predated by muskrat (Neves and Odom, 1989). Asiatic clam is also an invasive species and is one of the most serious pests in US aquatic ecosystems with zebra mussel (*Dreissena polymorpha*). Both were introduced by human and cause serious problems of fouling and threats on native species. The addition of an introduced exotic species into predator's environment alters the predator's feeding behavior because of its abundance (very high) (Pimentel et al, 2001). Indeed, Asian clams when they are widespread are generally the most abundant bivalves (Neves and Odom, 1989). On the contrary to zebra mussels, Asian clams disperse and grow less rapidly (Pimentel et al, 2001). Moreover, zebra mussel may attach to most surfaces, especially on mussel's shell and thus prevent a good feeding, respiration, excretion and locomotion to its host (Cudmore et al. 2004). In fact, muskrats predated more introduced exotic species and so they become the primary food, reducing the consumption of native species (Sietman et al, 2003 and Neves and Odom, 1989). In Ohio River colonized by zebra mussels muskrats learned to eat them by selecting zebra mussels over Unionids. Muskrats not consume Unionid species after zebra

mussels were removed, but just zebra mussels (Sietman et al, 2003). That's why predation on zebra mussels may have very positive impact on survival Unionids due to the high rate of unionid mortality by zebra mussels (Sietman et al, 2003).

#### ***4.1.2. Raccoon predation***

Raccoons (*Procyon lotor*) as muskrats eat freshwater mussels but no many scientists studied raccoon predation on freshwater mussel and it is difficult to find study about that. Raccoon is able to swim but its predation on freshwater mussels is higher when water become shallow and mussels may be easier visible and more easily caught (Johnson et al, 2001). Their technique to open shells is different than for muskrats. They can open them more quickly with their powerful jaw and may break easily thick shells whereas muskrats will bite them. Many shells of *Unio crassus* were broken on a site where many raccoons were present and thus mussels could be eaten by raccoons. Moreover, raccoon marked shells especially with their teeth while their legs hold the shell. Thus, they may remove the periostracum and so marks of raccoon are whiter and very visible. Furthermore, a typical hole could be sometimes observed on shells. They have been creating by raccoon tooth which may break through the shell. On the other hand, many raccoons were not able to separate both valves and eat all mussel meat. In fact, raccoons tend to predate thin-shelled smaller species by broken shells at the posterior end and consuming the soft internal organs (Johnson et al, 2001).

## **4.2. Muskrat (*Ondatra zibethicus*)**

### ***4.2.1. Population density in CMR sites***

To detect if muskrats inhabit areas, scientists search feeding platforms, defecation sites, tracks or active houses. However, counts of muskrat houses and lodges do not permit to readily detect short-term population, they sometimes be left to neglect (Engeman and Whisson, 2003). In addition, the use of traps to catch muskrat is required to really know the population density. In this study, two methods of density population analysis, Schnabel and Schumacher-Eschmeyer methods, were used in the different localizations after muskrat captures. Through the both methods, results of population density don't show the same results

for each CMR site. The standard variation of the Schnabel method is very close to zero, and so the risk to have an error in population density is higher. For this method, the capture probability of muskrat must be the same for each individual (Busson, 2003). However, the muskrat capture showed that the probability of capture was not the same of each muskrats. Many of them were always captured in same traps. On the other hand, the standard variation of the Schumacher-Eschemyer method is stronger, and the risk is minimized, thus this method is better.

In Lower Sûre, the population was the highest with thirty-one individuals caught in the fifteen traps. The result for Schumacher-Eschemyer method showed that there are between 34 to 39 animals. Moreover, the population is under-evaluated due to the presence frequently of same individuals in the same traps. For example, the presence of muskrat number one captured nine times in the traps number 4. Thus, we can suppose that the muskrat population density is more close to 39 individuals in this area.

On the contrary, during CMR procedures in the Our valley and in Upper Sûre, less animals were captured in the traps, respectively eight and three animals. In Upper Sûre, it was not possible to calculate population density due to the low number of muskrats captured. However, in Our Valley, muskrat population was calculated and the result showed between 11 and 15 animals with Schumacher-Eschmeyer method. Our valley and in Upper Sûre in comparison to Lower Sûre, are both situated in natural parks and in Natura 2000 area. In both sites, *Margaritifera margaritifera* were present and trapping campaigns against muskrat (considered as the responsible of their disappearance) were begun (Schmitz and Krippel, 2007; Richarz, 2009). Moreover, in Our Valley, perimeter of the program LIFE for the reintroduction of the freshwater pearl mussel is directly situated in the North of the CMR site. Thus, the pressure against muskrat due to the trapping campaigns effectuated by the ANF may be the cause of differences of population density in comparison to Lower Sûre area. Furthermore, in Upper Sûre, many raccoons (*Procyon lotor*) were also captured. Their favorite diet is muskrat juveniles (Schley, 2002) and thus, raccoons may contribute at the low muskrat density by eating them.

Furthermore, the difference between population densities may be due to environment stability. Muskrats in Illinois and Ohio were more abundant in areas with stable water levels than in areas with fluctuating levels (Kinler et al, 1990). Water flooding was very important in Our valley and in Upper Sûre. At the difference, the river in Lower Sûre is more stable than in other sites. In fact, deep water levels not facilitate access to muskrat dwellings, and have an important effect from autumn to spring (Messier et al, 1990). When water level exceeds the

lodges top, the main problem is that juveniles cannot survive unless they are assisted by an adult. Often young litters are abandoned where dwellings become flooded. The probability for juveniles to survive is better if they can take refuge on floating lodges, vegetation or fates debris, only if they are more than twenty-four days old (Kinler et al, 1990). Another possibility is that muskrats don't like to live near to grazing area. In fact intensive grazing of livestock has detrimental effects on vegetative cover which decrease and has a direct effect on muskrat density. In Iowa, more than twice animals were caught along ungrazed banks (Walelen & Hoffman, 1984).

#### *4.2.2. Daily and Seasonal activities*

A weight of 300 g corresponds at a muskrat aged of about 50 days-old and a weight from 800 g to 850 g is reached in the fall to an age of about 200 days, which is maintained during winter and spring. At one year a muskrat weigh about one kilogram (Le Boulengé and Le Boulengé-Nguyen, 1981). According to these values, juveniles of the last year (inferior to 850 g) and muskrat adults were both presents in the three areas. The heaviest muskrat was a male of 1360 g captured in Lower Sûre, and the lightest muskrat was a male juvenile (560 g) caught in Upper Sûre. This juvenile was caught where the population density was very low (only three muskrats were caught). It has been caught many times in five different traps, traveling about 1250 meters. In contrary, others muskrats in denser populations, didn't travel so much between traps and generally were captured in same traps or next to the trap of their first-capture. This difference could be explained by the fact that it exists in small mammals an inverse relationship between population density and home-range size. In fact, high density increase intruder pressure by neighbors, floaters or immigrants and thus, animals restrict their movements to foraging. Moreover, during breeding season juveniles tend to have higher home range than female and male adult muskrats which have parental care (Marinelli and Messier, 1993) with the education of juveniles (Le Boulengé and Le Boulengé-Nguyen, 1981).

In Canada, two main muskrat movements appear each year, one peak in September-October due to juvenile dispersal and another due to breeding season in late April (Messier et al, 1990). In Europe, muskrats are physiologically ready to reproduce earlier in spring season (mid-March to mid-April), and a "spring migration" could be observed (Le Boulengé & Le Boulengé-Nguyen, 1981). In this study, CMR were effectuated during the breeding season. The high density of muskrat in Lower Sûre may correspond to this migration and the majority of muskrat captures was male muskrat, not many females were caught. Moreover, population



density may decline due to the beginning of the reproduction activity (Le Boulengé & Le Boulengé-Nguyen, 1981).

As other nocturnal mammals, muskrats have their daily activity directly associated to the light intensity. MacArthur in 1980 noted two main peaks in muskrat activity near to dusk and dawn (MacArthur, 1980). That's why trap statements in this study were effectuated in these moments. Differences between live trapping and weather conditions (cloudy, rain and sunny) could be noted during CMR in Lower Sûre (where the most of individuals were caught). We can noted that they are less capture in dusk where the day was sunny (three captures could be noted during the two weeks) whereas in a cloudy or in rainy day eight captures could be noted for both last weather conditions. On the contrary in dawn campaign, more muskrats were captured during sunny day than in bad weather. MacArthur in 1980 noted that muskrat activity may be influenced by patterns as air temperatures, mean daily wind speed and weather. The strongest correlation with weather occurs at the beginning of winter. In fact, wind speed would be most influence on muskrat activity in summer and early winter. Late fall and early spring are seasons where the muskrat is not really influenced by environmental patterns and is likely to be exposed to wind; low air and water temperatures. Muskrat daily activity which is more important in winter (about 6.2 h) than in summer (5.8 h) due to the intensity of the light (MacArthur, 1980).

### **4.3. Link between muskrat density and freshwater mussel predation**

The freshwater pearl mussel (*Margaritifera margaritifera*) was widespread in Europe, but in the 20th century, more than 90% of individuals were disappeared. The last population in Luxembourg lives at the border between Luxembourg and Germany in Our River. The population was about 3.000 in 1989, then it declines rapidly to get only 200 individuals in 2008 (Arendt et al, 2008). After analysis of predation marks on shells, there is no doubt about the origin of freshwater pearl mussel predation: they were eaten by muskrats which are contributed at a significant part of the decline population of freshwater pearl mussel in Our Valley. Moreover, for the CMR procedure in Our site, the density population is at maximum of fifteen individuals along 3.500 meters, and no many shells were found.

In CMR site in Lower Sûre (Dillingen area) not a lot of empty shells were found (twenty-three shells of *Anodonta anatina*). These shells were collected next to some traps but not in distinct middens. In addition, many muskrats were caught in the Lower Sûre and the population is near to 39 animals in this site. On the contrary, in the Upper Sûre, where only

three muskrats were caught, the most shells were collected which about two thousands shells of thick shelled water mussel (*Unio crassus*) and 121 shells of duck mussel (*Anodonta anatina*). A hypothesis is that there is less mussel predation in Lower Sûre than in Upper Sûre certainly due to water depth. The water depth reaches 25 cm in August in Upper Sûre in Bigonville, whereas in Lower Sûre in Bollendorf, it up to 70 cm ([www.inondations.lu](http://www.inondations.lu)). Thus, it is easy for mussels to be predated when they are situated shallower and thus they are more visible, and in parallel they may be easily take away by flooding. Moreover, this the low water deep, raccoons can extract easily mussels and predation marks of raccoon were identified on thick shelled water mussel. On the contrary, in Lower Sûre, the water depth is more important, and it will more difficult for muskrat to pick up mussels. Moreover, during the CMR procedure, there was high water and the flooding may took away shells and that's why not many shells were found.

# Conclusion

---

With more and more people traveling from one country to another, the probability of invasive species introduced into new environment increase significantly. The alien species *Ondatra zibethicus* is a serious predator of freshwater mussels. Six species of freshwater mussel (*M. margaritifera*, *U. crassus*, *U. pictorum*, *A. anatina*, *A. cygnea* and *C. fluminea*) were found empty along Our, Sûre and Alzette rivers. Characteristic muskrat marks were identified on shells of two species of Unionids and compared to other marks. Thus, sign of muskrat predation were found on empty shells of both *Anodonta sp.* and *U. pictorum* in Echternach Lake. With more than two hundreds shells found in different middens, it is sure that the endangered freshwater pearl mussels were eaten by muskrats and thus contributed to the net decline of *M. margaritifera* in Our River. Moreover, in Upper Sûre where the other endangered freshwater species *U. crassus* collected with more than one thousand found collected, not many muskrats marks were observed on shells, the majority was intact and raccoons marks are also reported. Thus, muskrats were not responsible of the totality predation but also a big majority was predated by raccoons. In this area, muskrat density was very low (only three were caught) and raccoons are mainly present. A contrario, where the most of muskrats were captured, fewer shells were found on the bank. This is certainly due to high water conditions: middens of muskrat are often washed away by the water. However, muskrat predation is not the main cause of the disappearance of freshwater mussels, they are generally threatened by human (habitat destruction especially due to pollution but also due to deforestation, canalization of rivers, removal of bankside vegetation, dams or draining of wetland) (Veron et al, 2008). These degradations of mussels living environment contributed of mussel species disappear as *M. margaritifera* in Upper Sûre (Schmitz and Krippel, 2007).

# References

---

- ARENDR A., HEINEN P., MASURA L., MOLITOR M., THIELEN F. (2008) Restauration des populations de moules perlières en Ardennes. Rapport intermédiaire (période du 1er septembre 2005 au 30 juin 2008). Projet LIFE-Nature LIF05 NAT/L/000116. Fondation Hëllef fir d’Natur. 5-6.
- BECKER K. (1972) Muskrats in Central Europe and their control. Vertebrate Pest Conference Proceedings Collection. Proceeding of the 5th Vertebrate Pest Conference.
- BENSETTITI F. and GAUDILLAT V. (2004) Cahiers d’habitats Natura 2000. Connaissance et gestion des habitats et des espèces d’intérêt communautaire. Espèces animales. La documentation française **Tome 7**, 353 pp.
- BLAZEK R. and GELNAR M. (2006). Temporal and spatial distribution of glochidial larval stages of European unionid mussels (Mollusca: *Unionidae*) on host fishes. Folia Parasitologica **53**, 98–106.
- BOURGAULT P. (2008) Techniques de capture et de marquage des micrommamifères. TP d’écologie (ECL 307) : volet écologie animale. Université de Sherbrooke.
- BUSSON S. (2003) Inventaire des populations d’écrevisse à pieds blancs en basse Maurienne. Stage de Maîtrise BPE, Université de Rennes 1. 34-57.
- CloseTest by STANLEY TR. and RICHARDS JD. (2005): <http://www.mesc.usgs.gov/Products/Software/cloctest/>
- CUDMORE B., MACKINNON CA. and MADZIA SE. (2004) Aquatic species at risk in the Thames River Watershed, Ontario. Canadian Manuscript Report of Fisheries and Aquatic Sciences, **2707**.
- DAGET J. (1971) L’échantillonnage des peuplements de poissons d'eau douce. Extrait de « Échantillonnage en milieu aquatique ».
- DANEL K. (1996) Introductions of aquatic rodents: lessons of the muskrat *Ondatra zibethicus* invasion. Wildlife Biology **2**, 213-220.

DEMOL T. (2000) Identification des moules non marines de Belgique. Document utilisé dans le cadre du Life-Nature B8590 «Conservation des habitats de la moule perlière en Belgique».

DIGGINS TP. and STEWART KM. (2000) Evidence of large change in Unionid mussel abundance from selective predation, as inferred by shell remains left on shore. International Review of Hydrobiology **85**, 505-520.

ENGEMAN RM. and WHISSON DA. (2003) A visual method for indexing muskrat populations. International Biodeterioration & Biodegradation **52**, 101-106.

GRANJON L., COSSON JF., QUESSEVER E. and SICARD B. (2005) Population dynamics of the multimammate rat *Mastomys huberti* in an annually flooded agricultural region of central Mali. Journal of Mammology **86** (5), 997 – 1008.

GUM B. & GEIST J. (2009) Distribution, abundance and conservation status of the critically endangered freshwater pearl mussel (*Margaritifera margaritifera*) and the thick shelled river mussel (*Unio crassus*) in Bavaria, Germany. Technische Universität München.

HADLEC RH., PRIES J. and MUSTARD H. (2007) Muskrats (*Ondatra zibethicus*) in treatment wetlands. Ecological Engineering **29**, 143-153.

ISSG (2005) *Corbicula fluminea*. Global Invasive Species Database. Invasive Species Specialist Group, IUCN : [www.issg.org](http://www.issg.org)

JOHNSON PM., LINER AE., GOLLADAY SW. and MICHENER WK. (2001) Effects of drought on freshwater mussels and instream habitat in Coastal Plain tributaries of the Flint River, southwest Georgia (July-October, 2000). Final Report presented to The Nature Conservancy Apalachicola River and Bay Project.

KINLER QJ., CHABRECK RM, KINLER NW., and LINScombe RG. (1990) Effect of tidal flooding on mortality of juvenile muskrats. Estuaries **13** (3), 337 – 340.

LE BOULENGE E. & LE BOULENGE-NGUYEN PY. (1981) Ecological study of a muskrat population. Acta Theriologica **26** (4), 47-82.

MACARTHUR RA. (1980) Daily and seasonal activity patterns of the muskrat *Ondatra zibethicus* as revealed by radiotelemetry. Holarctic Ecology **3**, 1-9.

MARINELLI L. and MESSIER F. (1993). Space use and the social system of muskrats. Canadian Journal of Zoology **71**, 869-875.

- MESSIER J., VIRGL JA. and MARINELLI L. (1990) Density-dependent habitat selection in muskrats : a test of the ideal free distribution model. Oecologia **84** : 380 – 385.
- MOTTE G. (2006) Cahiers « Natura 2000 ». Espèces de l'Annexe II de la Directive Habitats présentes en Wallonie. Centre de Recherche de la Nature, des Forêts et du Bois. 19 – 37.
- NEVES RJ. and ODOM MC. (1989) Muskrat predation on endangered freshwater mussels in Virginia. Journal of Wildlife Management **53** (4), 934-941.
- NUMMI P., VAANAMEN VM. and MALINEM J. (2006) Alien grazing: indirect effects of muskrats on invertebrates. Biological Invasions **8**, 993-999.
- PIMENTEL D., McNAIR S., JANECKA J., WIGHTMAN J., SIMMONDS C., O'CONNELL C., WONG E., RUSSEL L., ZERN J., AQUINO T., TSOMONDO T. (2001) Economic and environmental threats of alien plant, animal, and microbe invasions. Agriculture, Ecosystems and Environment **84**, 1-20.
- POCOCK MJO., FRANTZ AC., COWAN DP., WHITE PCL and SEARLE JB. (2004) Tapering bias inherent in minimum number alive (MNA) population indices. Journal of Mammology **85** (5), 959 – 962.
- POTVIN J. (1994) Importance de la prédation des cônes de l'épinette noire (*Picea mariana*) par l'écureuil roux (*Tamiasciurus hudsonicus*). Mémoire présenté à l'Université du Québec.
- R: R Development Core Team (2006). R: A language an environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>.
- RICHARZ F. (2009) Spatiotemporal pattern and other ecological aspects of the muskrat (*Ondatra zibethicus*) as an alien species in Luxembourg. International Seminar: Increased sedimentation, a widespread problem leading to degradation of freshwater communities and habitats. Clervaux (Luxembourg). 45.
- ROSATTE RC. (2010) Le raton Laveur, Faune et Flore du pays (Canada). [www.hww.ca/hww2\\_f.asp?pid=1&id=101&cid=8](http://www.hww.ca/hww2_f.asp?pid=1&id=101&cid=8)
- SCHLEY L. (2002) Expansion géographique de quelque espèces de mammifères en Europe : le rat musqué, le ragondin, le raton laveur, le chien viverrin, le vison d'Amérique, le castor d'Eurasie et le Lynx. Situation au Grand-Duché de Luxembourg et conséquences. Mémoire de Recherche.

SCHLEY L. (2006) [L'homme et les prédateurs de la faune sauvage.] La situation au Grand-Duché de Luxembourg: réflexions concernant les mammifères. Actes du 5ème colloque international de Saint-Hubert du 12 août 2005. 41 – 70.

SCHMITZ C. & KRIPPEL Y. (2007) Contrat de Rivière Haute-Sûre. Mise en place d'un plan de gestion transfrontalier des ressources en eau de la Haute-Sûre belgo-luxembourgeoise. Parc Naturel de la Haute-Sûre.

SIETMAN BE., DUNN HL., TUCKER JK. and KELNED DE (2003) Muskrat (*Ondatra zibethicus*) predation on zebra mussels (*Dreissena polymorpha*) attached to unionid bivalves. Journal of Freshwater Ecology **18**, 25-32.

STANLEY TR. & BURNHAM KP. (1999) A closure test for time-specific capture-recapture data. Environmental and Ecological Statistics **6**, 197-209.

STANLEY TR. and RICHARDS JD. (2005) CloseTest: A program for testing capture-recapture data for closure.

SOUSA RG. (2008) Factors contributing to the invasive success of *Corbicula fluminea* (Müller, 1774). Dissertação de doutoramento em Ciências do Meio Aquático. Universidade do Porto.

TUYTTENS FAM. (2000) The closed-subpopulation method and estimation of population size from mark-recapture and ancillary data. Canadian Journal of zoology **78** (2), 320 – 326.

TYRELL M. and HORNBACH J. (1998) Selective predation by muskrats on freshwater mussels in two Minnesota rivers. Journal of North America Benthological Society **17**, 301-310.

VERON G., PATTERSON BD, REEVES R. (2008) Global diversity of mammals (*Mammalia*) in freshwater. Hydrobiologia **595**, 607 – 617.

VRIGNAUD S. (2004) Clef de détermination des Naïades d'Auvergne. Margaritifera : Bulletin de liaison de l'Atlas des Mollusques de l'Allier. Numéro 4.

WALLEN AW. And HOFFMAN RD. (2004). Habitat suitability index models: muskrat. US Fish and Wildlife Services FWS/OBS – 82/10. **46**, 27 pp.

[www.inondations.lu](http://www.inondations.lu)

[www.eau.public.lu](http://www.eau.public.lu)

[www.margaritifera.eu](http://www.margaritifera.eu)

# ANNEX

---

## Annex A. Results of CMR procedure inn Our Valley (Rodershausen)

### A/ Detail of each muskrat captured

Animal				First capture		Recapture	
Name	Sexe	Weight (g)	Tail length(cm)	Date of catch	Trap	NB of recapture	Trap
1	F	850	21	16/03/2010	12		
2	M	1139	20,6	16/03/2010	14		
3	F	678	19,8	17/03/2010	2		
4	M	1112	19,5	17/03/2010	3	1	2
5	M	1083	21,4	17/03/2010	4	1	6
6	M	1019	20,9	18/03/2010	2		
7	F	866	21,8	19/03/2010	3	1	1
8	F	806	20,3	19/03/2010	10		

### B/ History data file for CloseTest analysis

```

1000000000
1000000000
0100000000
0101000000
0100000010
0010000000
0001001000
0001000000

```

### C/ details for population density

Date	$n_i$	$m_i$	$r_i$
16/03/10	2	0	0
17/03/10	3	2	0
18/03/10	1	5	0
19/03/10	3	6	1
23/03/10	1	8	1
25/03/10	1	8	1



## Annex B. Results of CMR procedure in Lower Sûre (Dillingen)

### A/ Detail of each muskrat captured

Animal				First capture			Recapture			
Animal Nb	sexe	weight (g)	Tail length (cm)	Date	Time	Trap	Nb of recapture	Trap RC	Morning RC	Night RC
1	M	1000	22,3	30.03.2010	06:45	C04	9	C04	4	5
2	M	980	19,5	30.03.2010	07:00	C03	2	C03	2	
3	F	1040	21	30.03.2010	07:40	C01	1	C02	1	
4	M	820	22,3	30.03.2010	08:35	C10				
5	F	1020	20	30.03.2010	09:30	C09	4	C09 + C10	4	
6	F	880	22,5	30.03.2010	18:45	C14				
7	F	820	19,5	30.03.2010	19:45	C04	1	C04		1
8	M	860	22,3	30.03.2010	20:15	C01	3	C01 + C02	2	1
9	M	1060	21,1	30.03.2010	20:45	C03				
10	M	970	22,2	31.03.2010	07:15	C03	1	C03	1	
11	F	860	20,7	31.03.2010	08:10	C07				
12	F	1120	21,2	31.03.2010	09:15	C11	3	C11 + C12	3	
13	M	900	21,2	31.03.2010	18:20	C07				
14	M	1000	21,5	01.04.2010	07:30	C09	2	C09	2	
15	M	1360	21,3	01.04.2010	09:20	C06	1	C06		1
16	F	980	20,3	01.04.2010	18:00	C11	1	C12	1	
17	F	1140	20,5	01.04.2010	19:20	C05				
18	M	920	19,5	02.04.2010	06:45	C01				
19	M	920	22,2	02.04.2010	07:45	C07				
20	F	920	21,6	02.04.2010	08:45	C15				
21	M	760	22,2	06.04.2010	08:05	C02				
22	M	840	21,2	06.04.2010	08:30	C01	3	C01	1	2

23	M	1200	22	06.04.2010	10:30	C06				
24	M	840	21,7	06.04.2010	11:05	C08	3	C07 + C08	3	
25	M	1140	21,4	07.04.2010	07:30	C05				
26	M	660	19,2	07.04.2010	18:50	C02				
27	M	860	20,9	08.04.2010	07:20	C09				
28	M	820	20,9	08.04.2010	10:30	C03				
29	M	720	21,3	08.04.2010	11:00	C02	1	C01	1	
30	M	1140	21,7	08.04.2010	12:00	C06	2	C06	1	1
31	M	860	20,9	09.04.2010	08:10	C08				

### B/ History data file for CloseTest analysis

10101111101110	00100010010001	00000001000000
10000001010000	00010000000000	00000001010101
10000000010000	00001000010001	00000000010000
10000000000000	00001100000000	00000000001000
10100001000101	00000100000100	00000000000100
01000000000000	00000100000000	00000000000100
01010000000000	00000010000000	00000000000101
01001010100000	00000010000000	00000000000101
01000000000000	00000010000000	00000000000001
00101000000000	00000001000000	
00100000000000	00000001111000	

### C/ Detail for population density

*(1) represents morning trapping and (2) represents night trapping.*

Date	ni	mi	ri
30.03.2010 (1)	5	0	0
30/03/2010 (2)	4	5	0
31/03/2010 (1)	5	9	2
31/03/2010 (2)	2	12	1
01/04/2010 (1)	5	13	3
01/04/2010 (2)	4	15	2
02/04/2010 (1)	6	17	3
06/04/2010 (1)	7	20	3
06/04/2010 (2)	3	24	3
07/04/2010 (1)	7	24	6
07.04.2010 (2)	4	25	3
08.04.2010 (1)	7	26	3
08.04.2010 (2)	2	30	2
09.04.2010 (1)	7	30	6

### Annex C. Results of CMR procedure in Upper Sûre (Martelinville)

**E: escaped, trap was found destroyed; C: captured**

Animal				First capture			Recapture			
Animal Nb	Gender	weight (g)	Tail length (cm)	Date	Time	Trap	Nb of recapture	Trap	Morning RC	Night RC
raccoon C				20/04/10	7h15	T11				
raccoon E				20/04/10	Morning	T02				
raccoon E				20/04/10	Morning	T14				
1	M	560	20,4	21/04/10	8h30	T04	8	T04 + T03 + T09 + T06 + T05	5	2
raccoon C				21/04/10	9h45	T11				
raccoon C				22/04/10	9h30	T13				
raccoon E				22/04/10	Morning	T10				
raccoon C				23/04/10	9h30	T15				
raccoon E				23/04/10	Morning	T14				
2	F	920	21,3	27/04/10	8h20	T06				
raccoon E				27/04/10	Morning	T10				
raccoon E				27/04/10	Morning	T03				
raccoon E				27/04/10	Morning	T12				
3	F	700	19,7	28/04/10	8h20	T06	2	T06 + T08	2	
raccoon E				28/04/10	Morning	T03				
raccoon E				28/04/10	Morning	T12				
raccoon E				28/04/10	Morning	T13				
raccoon E				28/04/10	Morning	T14				
raccoon C				30/04/10	8h00	T11				
raccoon C				30/04/10	9h00	T07				

### Annex D: Number of shells with one valve or intact for each freshwater mussel species

Species	1 valve	2 valves	Shells Number
<i>Anodonta anatina</i>	57	242	299
<i>Anodonta cygnea</i>	5	3	8
<i>Corbicula fluminea</i>	9	304	313
<i>Unio crassus</i>	135	1270	1405
<i>Unio pictorum</i>	4	2	6
<i>Margaritifera margaritifera</i>	43	170	213
<b>Total</b>	<b>253</b>	<b>1991</b>	<b>2244</b>

### Annex E. Table representing predation marks on different species of freshwater mussel

		M. margaritifera n = 213	U. crassus n = 1405	U. pictorum n = 6	A. anatina n = 299	A. cygnea n = 8	C. fluminea n = 313
<b>Intact</b>		43	1130	1	128	1	246
<b>Scratches</b>		153	60		18		39
<b>Muskrat bites</b>		1	4	4	18		16
<b>Muskrat teeth mark</b>		4	19				5
<b>Broken</b>		1	142		79	7	
<b>others</b>	<b>bites + scratches</b>	7	1		1		3
	<b>bites + Muskrat teeth mark</b>		1		1		1
	<b>bites + broken</b>			1	20		
	<b>Muskrat teeth mark + scratches</b>	2	2		1		3
	<b>broken + scratches</b>	1	44		30		
	<b>broken + muskrat teeth mark</b>		2		1		
	<b>bites + muskrat teeth mark + scratches</b>	1					
	<b>Muskrat bites + broken + scratches</b>				2		

**Annex F: Average of shell mensuration for *M. margaritifera* (a), *U. crassus* (b),**

***U. pictorum* (c), *A. anatina* (d), *A. cygnea* (e) and *C. fluminea* (f)**

(a)	Total length (mm)	Weight (g)	Shell swelling (mm)	Shell height (mm)	Valve thickness (mm)
<b>Min</b>	96	41,1	26	42,5	1,5
<b>Max</b>	121	84,8	36	54,5	4
<b>Average</b>	110,6	61,42	32,5	49	2,5
<b>N</b>	213	164	169	213	212

(b)	Total length (mm)	Weight (g)	Shell swelling (mm)	Shell height (mm)	Valve thickness (mm)
<b>Min</b>	23	0,7	12	17	0,2
<b>Max</b>	68,5	9,8	25,5	35,5	4,5
<b>Average</b>	48,3	7,25	17,5	25,3	1,6
<b>N</b>	1287	831	81	126	1342

(c)	Total length (mm)	Weight (g)	Shell swelling (mm)	Shell height (mm)	Valve thickness (mm)
<b>Min</b>	27.5	/	20	21	0.5
<b>Max</b>	102	/	27	39.5	2
<b>Average</b>	58.7	25.6	23.5	25.5	1.1
<b>N</b>	5	1	2	6	6

(d)	Total length (mm)	Weight (g)	Shell swelling (mm)	Shell height (mm)	Valve thickness (mm)
<b>Min</b>	32	0.4	7.5	15	0.2
<b>Max</b>	119.5	58.1	48	70	4
<b>Average</b>	62.5	9.19	22.4	42.3	1.4
<b>N</b>	210	108	101	159	86

(e)	Total length (mm)	Weight (g)	Shell swelling (mm)	Shell height (mm)	Valve thickness (mm)
<b>Min</b>	89.5	/	31.5	47	0.2
<b>Max</b>	124	/	41	70	1
<b>Average</b>	113.3	/	38.25	57.2	0.66
<b>N</b>	4	/	2	6	7

(f)	Total length (mm)	Weight (g)	Shell swelling (mm)	Shell height (mm)	Valve thickness (mm)
<b>Min</b>	11.5	0.3	6	11.5	0.2
<b>Max</b>	32.5	6.4	15.5	23	1
<b>Average</b>	18.55	1.85	10.9	18.15	0.49
<b>N</b>	313	303	310	28	313