SPSD II

IMPACT ASSESSMENT AND REMEDIATION OF ANTHROPOGENIC INTERENTIONS ON FISH POPULATIONS (FISHGUARD)

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ATMOSPHERE AND CLIMATE

MARINE ECOSYSTEMS AND BIODIVERSITY

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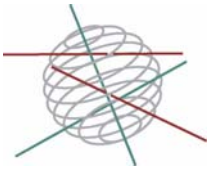
(SPSD II)



Part 2:
Global change, Ecosystems and Biodiversity

FINAL REPORT

IMPACT ASSESSMENT AND REMEDIATION OF ANTHROPOGENIC INTERVENTIONS ON FISH POPULATIONS (FISHGUARD)

EV/31

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TABLE OF CONTENTS

ABSTRACT **7**

1. INTRODUCTION **9**

2. MATERIALS AND METHODS **13**

2.1. ECOLOGY 13
2.1.1. Water courses and physical barriers investigated *13*
2.1.2. Analysis of fish communities in the model systems *13*
2.1.3. Quantification of actual migration of a selection of species in case studies *18*
2.2. ECOPHYSIOLOGY 19
2.2.1. Swimming capacity *19*
2.2.2. Leaping capacities under laboratory conditions *21*
2.3. GENETICS 23
2.3.1. DNA extraction and microsatellite amplification *23*
2.3.2. Genetic data analysis *24*
2.3.3. Geographical information *26*
2.3.4. Hypothesis testing *26*

3. RESULTS **29**

3.1. ECOLOGY 29
3.1.1. Analysis of fish communities in the model systems *29*
3.1.2. Quantification of actual migration of a selection of species in case studies *30*
3.2. ECOPHYSIOLOGY 39
3.2.1. Swimming capacities *39*
3.2.2. Leaping experiments *44*
3.3. GENETICS 45
3.3.1. Regional population structure and genetic diversity *45*
3.3.2. Estimation of the impact of restocking *54*
3.3.3. Geographical analysis of disruption of connectivity *56*

4. DISCUSSION **63**

4.1. ECOLOGY 63
4.1.1. Development of a work protocol and selection of the model systems *63*
4.1.2. Analysis of fish communities in the model systems *63*
4.1.3. Quantification of actual migration - case studies *65*
4.2. ECOPHYSIOLOGY 70
4.2.1. Swimming performance and energy use *70*
4.2.2. Leaping capacities in artificial constructions *74*
4.3. GENETICS 75
4.3.1. Non-stocked species *75*
4.3.2. Stocked species *78*

5. CONCLUSIONS & RECOMMENDATIONS **83**

6. REFERENCES **87**

7. PUBLICATIONS ORIGINATING FROM THE PROJECT **97**

ABSTRACT

The disruption of longitudinal river connectivity by man-made obstacles and the stocking of fish communities with non-indigenous species or genotypes threaten the fish fauna of Belgian rivers to various extents. Obstacles impede migrations between habitats that are vital for populations, and they may restrict the gene flow between populations, thereby reducing the effective size and genetic diversity of populations and increasing the risk of local extinction. Restocking programs often involve the introduction of non-indigenous genotypes in native populations. Moreover, although stocking programs lead to the temporal and superficial enrichment of local fish communities or gene pools, they generally result in a loss of biodiversity on a regional or international scale through the homogenisation of communities and the breakdown of genetic differentiation between populations. Thus, stocking programs cannot compensate for the loss of free migration by artificial obstructions. The impact of these changes on fish populations remains largely unknown, which complicates the prioritisation of spots to preserve and spots to restore. Here, we present an integrated study on Belgian waters, both in Flanders and Wallonia, which analyses fish communities, gene flow and migration patterns in the field, as well as the swimming and leaping performances of fishes under controlled conditions.

Keywords: fish, migration, barrier, stocking, lowland river, upland river, population structure, genetic variation, swimming capacity.

1. INTRODUCTION

Fish species have evolved strategies that enable them to optimise their fitness by using different types of habitats, hence movements between habitats are frequent. When these movements extend over long distances and concern a substantial proportion of the population, they are generally termed 'migrations' [1, 2]. However, the term 'migration' clearly applies to individual fishes too since it benefits to or reduces the fitness of individual fishes. Movements do not need to be long or involve journeys between biomes to result in a change of the fish's fitness [3]. Diadromous migrations [4, 5], have ruled for a while our perception of mobility in fishes, i.e. non-diadromous species have been deemed to be resident, and largely excluded from management schemes aiming at the restoration of the free circulation of fishes in river systems. Nowadays, there is a bulk of evidence that most so-called resident freshwater fish species undertake potamodromous migrations, either systematically or in a series of river systems [for review see 3]. The underestimation of these migrations owes largely to the limited accuracy of the methods employed to investigate them [e.g. 6]. Mobility in fishes serves a series of purposes (ontogenetic, refuge, feeding, spawning …). It also enables the recolonisation of habitats where the fish community has suffered catastrophic events of natural origin (e.g. spates) or anthropogenic origin (e.g. pollution). The free circulation of fishes is thus a prerequisite to colonisation processes, as well as to maintain a dynamic equilibrium within the fish community. Mobility is also a key component of evolutionary processes that lead to speciation and genetic diversity. The exchange of genes between spawners might favour genetic diversity, whereas the reduced exchange of genes might lead to speciation in the long term [7].

The growth of human population, the development of agricultural practices and industries, and the accompanying modifications of landscapes for accommodating these activities, have resulted in a series of impacts on the environment. Large rivers have been dredged for navigation, and the banks of several rivers and streams have been modified for hydraulic control, thereby resulting in a loss of essential habitats. Dams and weirs have been erected for navigation, flow control, hydro-power and irrigation [reviews in 8, 9, 10]. Huge dams are impassable obstructions that result in local extinction of populations, extirpation of species and depauperate communities in the upstream reaches [11]. In the River Meuse, for example, all long-distance migrants have gone extinct over the past two centuries [12], and in Flanders, eight of the 13 species with long distance migrations have gone extinct, while the remaining five species are very rare [13]. Weirs and dams, in addition to their direct effect on blocking or delaying upstream migrations, may have a series of side effects, owing to modifications of river flow, water temperature and other physico-chemical aspects, which may impact more or less on migrants, depending on the tightness of their mi-

gratory schedule. Small obstacles (small weirs, levees, culverts) might have the same impact as dams and weirs for species with lesser swimming and leaping capacities [14], and they have been rendered responsible, together with habitat loss and fragmentation, for the decline of populations and endangerement of fish species [15, 16, 17]. In a series of case studies, fish communities have remained seemingly unchanged after obstructions were built. However, genetic tools revealed that the construction of obstacles may affect the fine structure of fish populations [18, 19]. Population fragmentation due to the artificial obstacles results in lower effective population sizes, reduced variability and heterozygosity, higher chances for genetic drift and bottlenecking, increased inbreeding and hence reduced fitness, which compromises long-term survival [7]. Hence, small population size and genetic 'melt down' increase the probability of extirpation of the species, strain or population from the river basin, stream or stretch.

Most mitigation efforts have concerned fishways that enable migrants to negotiate huge dams during their upstream movements, and more recently in their downstream movements [20, 21, 22, 23]. The adequacy of fishways depends on two major characteristics, namely their attractiveness, which minimises the time and energy wasted by migrants before they find the entrance of the fishway, and the feasibility component, i.e. whether the fishway can be successfully crossed by targeted species. The knowledge on the swimming capacities of diadromous species exceeds that for all freshwater species. These generally display a broader range of behaviour and performance than diadromous migrants, which possess greater swimming and leaping capacities [synthesis in 24]. Fish can travel at a series of speeds that range from zero to the burst speed, i.e. the speed at which the fish uses all muscle fibres (red, pink and white), but which can be maintained for a few seconds only. The sustained swimming speed, for which only aerobically fuelled red muscle fibres are involved, is substantially lower. In practice, swimming performance is determined from the critical swimming speed U_{crit}: the fish is placed in a water tunnel or flume and is forced to swim against water currents of increasing velocities until it fatigues [24]. U_{crit} is an indicator for predicting the effects of environmental factors on fish swimming performance in the wild [25]. It also gives an estimate of the maximal aerobic swimming velocity of fishes, and is assumed to reflect the maximum oxygen consumption capability [26]. U_{crit} also provides a way of estimating the leaping capacities of fishes [14]. Information is available on the swimming capacities and associated energy expenditure in a limited number of fish species [24], but not for the vast majority of the European freshwater fish species.

Another attempt to mitigate anthropogenic effects is restocking wherever the free circulation or quality of habitat is insufficient for species to complete successfully their life cycle. Fish are introduced in public or private waters (rivers, ponds, dams, lakes and brooks) for various reasons, such as recreation and sport fisheries, the re-

establishment of extinct populations, ecosystem manipulation, immediate mitigation within the context of fisheries, or for historical or esthetical motivation [review in 27]. Most typically, stocked fish have been extracted from natural populations, and further bred and raised in closed systems locally, although they may also be imported from remote basins. Because of this immediacy, little attention has been paid, in most circumstances, to a series of side effects that have contributed to make stocking increasingly controversial [28]. These include the introduction of alien species, possible introduction of new pathogens, community effects (competition with or predation on native species) as well as the loss of genetic integrity of native populations wherever restocked fish that originate from distinct genetic pools reproduce. Their offspring increase the local genetic diversity, hence counteracting the effects of physical obstructions, which are generally responsible for a loss of genetic diversity. The two effects may or may not compensate, depending on whether restocked fish do or do not originate from the same gene pool. As for the issue of free circulation, most information at hand concerns salmonids [review in 27, 29, 30]. These issues raise further concerns about the usefulness of restocking, keeping in mind that restocking is expensive and that little information is at hand on the fate of restocked fishes, to what extent they contribute to recruitment, and to their long-term impact on the fitness of local populations where introgression may take place.

The aim of this study was to investigate the effects of migration constraints on fish populations in Belgium, both in Flemish lowland rivers and in Wallonian upland rivers. For this purpose we selected five key-species of intrest with contrasting migration behaviour and stocking histories: trout *Salmo trutta* (migrating, stocked, upland), roach *Rutilus rutilus* (migrating, stocked, lowland) three-spined stickleback *Gasterosteus aculeatus* (migrating and non-migrating morph, not stocked), gudgeon *Gobio gobio* (limited migration, limited stocking), and bullhead *Cottus gobio* (very limited migration, not stocked, threatened in Flanders). Within this study, we used three different approaches: 1) a more ecological approach, studying differences in fish communities and quantifying actual migration of the selected species, with the use of physical tags and biotelemetry transmitters, 2) a physiological approach, quantifying swimming and leaping capacities of different European species under controlled laboratory conditions, and 3) a genetic approach using microsatellites to examin the genetic differentiation of communities of the selected fish species.

2. MATERIALS AND METHODS

2.1. Ecology

2.1.1. Water courses and physical barriers investigated

Field studies of fish geographical distribution and movements were carried out in several rivers belonging to the Scheldt and Meuse basins (Figure 2.1.1 Table 2.1.1**).** These rivers were critically selected to represent a wide range of ecological situations for lowland and upland Belgian watercourses (Table 2.1.2 and 2.1.3). Different types of artificial obstructions (dams, weirs, culverts, siphons, water mills, micro hydro-power plants, shipping locks, fish-passes, *etc*.) are included in the selection. It was attempted to start the sampling at the first barrier on a stream (when achievable, e.g. Merelbeke on the Scheldt, Angleur-Liège on the Ourthe) followed by all the first barriers on major tributaries (e.g. Dendermonde on the Dender, Bomal on the Aisne) and a selection of barriers on the stretch of one or two of these tributaries (e.g. Demer/Dijle, Grote Nete, Aisne). Sufficient amounts of (some of) the target species were expected to be available (50 individuals for the genetic study). According to the obstacles, fishes are sampled downstream of each obstacle.

Data of localisation, type and general description of potential physical obstructions to fish movement were obtained from comprehensive surveys recently performed by the regional public authorities in Flanders (Research Institute for Nature and Forestry) and Wallonia (Direction des Cours d'eau non navigables). In several cases additional information about obstacles was directly measured on the field, focusing on variables such as height of a vertical or sub vertical fall, depth of the plunge-pool below a fall, length and slope of a chute and water velocity [31].

2.1.2. Analysis of fish communities in the model systems

Fish communities in the model systems were sampled by fyke netting or electrofishing (DEKA, 2.5 kVA) downstream of obstacles or caught in fish-passes at weirs or in fish-traps installed on their migration route during spawning period. Fish were anaesthetised in a solution of 2-phenoxy ethanol (depending on species) then fork length was measured (to the nearest 10 mm). Fish were weighted up to an accuracy of 1% and sexed when possible. Simultaneously genetic samples (fin clip) were taken from target species for at least 50 individuals. Before releasing in the wild, fish were tagged if required. Physical and chemical characteristics of the river at the sampling station were recorded: water level, water temperature, water quality, turbidity. Data were analysed in order to determine the potential impact of obstructions at the fish community level.

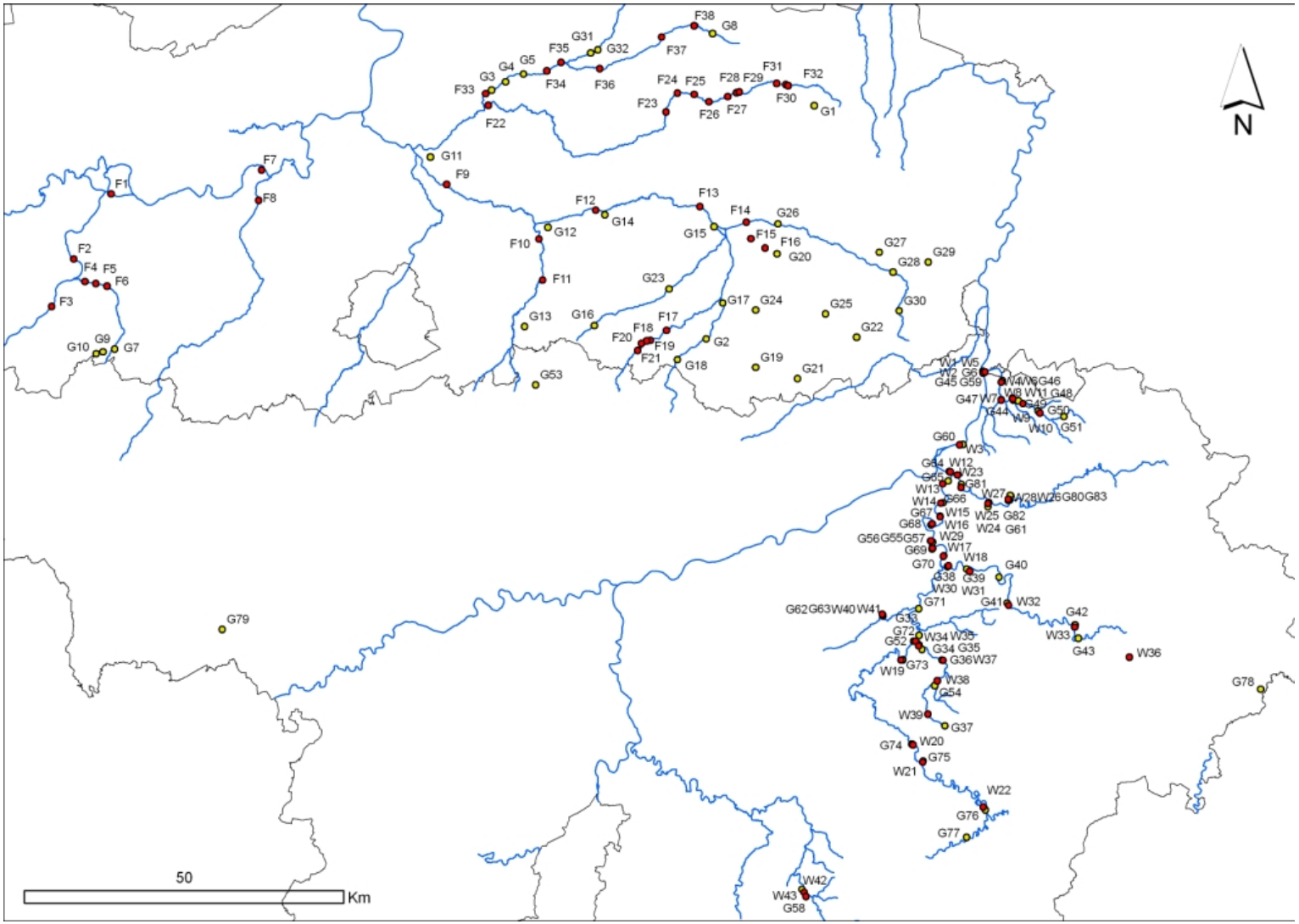

Figure 2.1.1: Map with the selected sampling sites in Belgium (From OC GIS Vlaanderen and AMINAL Water).

Table 2.1.1: Overview of the model sites in the lowland and the upland rivers. The numbers correspond with the numbers in Figure 2.1.1.

Flanders					
F1	Scheldt weir – shipping lock	F14	Demer weir	F27	Grote Nete water mill
F2	Scheldt weir – shipping lock	F15	Herk water mill	F28	Grote Nete siphon
F3	Scheldt weir – shipping lock	F16	Herk water mill	F29	Grote Nete water mill
F4	Zwalmbeek water mill	F17	Grote Gete water mill	F30	Grote Nete water mill
F5	Zwalmbeek weir	F18	Grote Gete fish-pass	F31	Grote Nete siphon
F6	Zwalmbeek water mill	F19	Grote Gete fish-pass	F32	Grote Nete weir
F7	Dender weir - shipping lock	F20	Grote Gete fish-pass	F33	Kleine Nete siphon
F8	Dender weir – shipping lock	F21	Grote Gete fish-pass	F34	Kleine Nete siphon
F9	Dijle branch weir	F22	Grote Nete siphon	F35	Kleine Nete weir
F10	Dijle water mill	F23	Grote Nete siphon	F36	Kleine Nete weir – fish-pass
F11	Dijle culvert	F24	Grote Nete weir	F37	Kleine Nete water mill
F12	Demer water mill	F25	Grote Nete weir	F38	Kleine Nete water mill
F13	Demer weir	F26	Grote Nete water mill		
Wallonia					
W1	Meuse Lixhe échelle	W16	Ourthe Hony barrage	W43	Lhomme Poix S-Hub barrage 4
W2	Meuse Lixhe barrage	W17	Ourthe Poulseur barrage	W31	Amblève Raborive barrage
W3	Meuse Monsin barrage	W18	Ourthe Chanxhe barrage	W32	Amblève Lorcé barrage
W4	Berwinne Berneau échelle	W19	Ourthe Barvaux barrage	W33	Amblève Coo cascade
W5	Berwinne Mouland barrage	W20	Ourtrhe Bardonwez barrage	W34	Aisne Bomal barrage
W6	Berwinne Berneau barrage	W21	Ourthe Jupille barrage	W35	Aisne Juzaine barrage
W7	Berwinne Dalhem seuil	W22	Ourthe Nisramont barrage	W36	Aisne Aisne barrage
W8	Berwinne Mortroux barrage	W23	Vesdre Chênée barrage	W37	Aisne Aux Roches barrage
W9	Berwinne Neufchâteu barrage	W24	Vesdre Chaudfontaine Carobel B	W38	Aisne Fanzel barrage
W10	Berwinne Val Dieu barrage	W25	Vesdre Trooz barrage	W39	Aisne Ammonines barrage
W11	Asse Mortroux barrage	W26	Vesdre Nessonvaux barrage	W40	Néblon CILE barrage aval
W12	Ourthe barrage	W27	Mosbeux barrage confluent	W41	Néblon CILE barrage amont
W13	Ourthe Campana barrage	W28	Vaux barrage confluent	W42	Lhomme Poix S-Hub barrage
W14	Ourthe Tilff barrage	W29	Haze barrage confluent		
W15	Ourthe Mery barrage	W30	Amblève Belle Roche barrage		

Table 2.1.1 (continued): Extra sampling sites for genetic sampling

G1	GNE01	Grote Nete	G29	B13b	Demer	G57	HAZ03	Haze
G2	KGE	Kleine Gete	G30	B14	Demer	G58	LHO01	Lhomme
G3	KNEP01	Kleine Nete	G31	AASA	Aa	G59	MEU01	Maas
G4	KNEP02	Kleine Nete	G32	AASO	Aa	G60	MEU02	Maas
G5	KNEP03	Kleine Nete	G33	AIS01	Aisne	G61	MOS01	
G6	MEU00	Maas	G34	AIS02	Aisne	G62	NEB05	Néblon
G7	VER	Verrebeek	G35	AIS03	Aisne	G63	NEB06	Néblon
G8	WNE	Witte Nete	G36	AIS04	Aisne	G64	OTH01	Ourthe
G9	ZWA13	Zwalm	G37	AIS07	Aisne	G65	OTH02	Ourthe
G10	ZWA14	Zwalm	G38	AMB01	Amblève	G66	OTH03	Ourthe
G11	B4a	Nete	G39	AMB02	Amblève	G67	OTH04	Ourthe
G12	B5a	Dijle	G40	AMB03	Amblève	G68	OTH05	Ourthe
G13	B5b	Dijle	G41	AMB04	Amblève	G69	OTH06	Ourthe
G14	B6a	Demer	G42	AMB05	Amblève	G70	OTH07	Ourthe
G15	B9a	Demer	G43	AMB06	Amblève	G71	OTH08	Ourthe
G16	B9b	Demer	G44	ASS01	Asse	G72	OTH09	Ourthe
G17	B9c	Demer	G45	BER01	Berwinne	G73	OTH10	Ourthe
G18	B9e	Demer	G46	BER02	Berwinne	G74	OTH11	Ourthe
G19	B9f	Demer	G47	BER03	Berwinne	G75	OTH12	Ourthe
G20	B9g	Demer	G48	BER04	Berwinne	G76	OTH13	Ourthe
G21	B9h	Demer	G49	BER05	Berwinne	G77	OTH14	Ourthe
G22	B9i	Demer	G50	BER06	Berwinne	G78	OUR02	Our
G23	B9j	Demer	G51	BER08	Berwinne	G79	TRO	Trouille
G24	B9k	Demer	G52	BOM	Aisne	G80	VAU02	
G25	B9l	Demer	G53	DIJLpb	Dijle	G81	VES02	Vesdre
G26	B11a	Demer	G54	EVE	Aisne	G82	VES03	Vesdre
G27	B12a	Demer	G55	HAZ01	Haze	G83	VES04	Vesdre
G28	B13a	Demer	G56	HAZ02	Haze			

Table 2.1.2: Main characteristics of the lowland rivers.

CHARACTERISTICS	SCHELDT	ZWALM	DIJLE	DENDER	DEMER
Elevation source (m)	95	48	36	40	90
Elevation - confluence (m)	0	10	2	5	8
Length (km)	350	22	64	65	85
Drainage area (km²)	21863	144	1122	1384	2280
Average slope (p/1000)	0.27	1.73	0.53	0.54	0.50
Width in lower course (m)	650	-	-	70	25
Average annual discharge (m³/s)	110	1.35	6.32	13.34	10.84
Water temperature (°C)	19.3	7.0	10.4	15.9	16.9
Dominant Huet fish zone	grayling bream	barbel	barbel	bream	bream
Dominant fish species (kg)	roach	gudgeon	gudgeon	eel	carp
Level of global water quality	bad	good	medium-bad	medium	medium
CHARACTERISTICS	HERK	GR GETE	GR NETE	KL NETE	
Elevation source (m)	112	54	62	27	
Elevation - confluence (m)	23	28	2	6	
Length (km)	44	23	101	51	
Drainage area (km²)	300	245	1013	596	
Average slope (p/1000)	2.02	1.13	0.59	0.41	
Width in lower course (m)	-	-	-	-	
Average annual discharge (m³/s)	1.68	1.24	5.17	6.74	
Water temperature (°C)	16.9	10.6	14.6	15.7	
Dominant Huet fish zone	bream	bream barbel grayling	bream	bream	
Dominant fish species	gudgeon	roach	Eel	roach	
Level of global water quality	medium	medium	High	high	

Table 2.1.3: Main characteristics of the upland rivers. Data relate to the lower course of each stream. *tributaries of the River Ourthe; **lower and upper limit of typological classes.

CHARACTERISTICS	OURHTE	AMBLEVE	AISNE	NEBLON	VESDRE
Elevation source (m)	507	586	600	255	626
Elevation - confluence (m)	63	102	135	120	64
Length (km)	175	93	35	18.3	72
Drainage area (km²)	3672	1083	184	78.7	702
Average slope (p/1000)	2.54	5.20	13.29	7.7	7.8
Width in lower course (m)	30-50	30-50	5-10	5.	30-50
Average annual discharge (m³/s)	67.4	21.7	2.6	09	11.4
Mean water temp in July (°C)	19.9	19.4	15.3	15.0	17.1
Dominant Huet fish zone	barbel	grayling	trout grayling	trout	grayling
Dominant fish species (kg)	barbel	barbel	trout		gudgeon
Level of global water quality	high	high	excellent	high	medium
CHARACTERISTICS	MEHAIGNE	OXHE	BERWINNE	LHOMME	
Elevation source (m)	180	260	270	475	
Elevation - confluence (m)	68	65	53	153	
Length (km)	66	13.9	29	50.5	
Drainage area (km²)	360	45.3	131	479	
Average slope (p/1000)	1.7	14.0	7.48	6,3	
Width in lower course (m)	5-10	5	5-10	5-10	
Average annual discharge (m³/s)	3.0	< 0.5	2.4	1.78	
Mean water temp in July (°C)	17.6	16.7	18.6	14.9	
Dominant Huet fish zone	grayling barbel	trout	trout grayling	grayling	
Dominant fish species (kg)	roach/chub	trout	chub	trout	
Level of global water quality	medium	high	medium	high	

2.1.3. Quantification of actual migration of a selection of species in case studies

To assess the mobility (migration/movement) of fish through artificial obstructions, to investigate the influence of fragmentation on the fish population structure (species richness) and to demonstrate whether the obstructions are passable for fish or not (gene dispersion), fish were batch-marked using fin clipping or injecting coloured elastomers into the skin. Individual recognition of fish was achieved by PIT-tagging (Passive Integrated Transponder) i.e. injecting a small electronic transponder into the body (dorsal muscle or belly cavity). Recapture of PIT-tagged fish was checked by manual examination (portable detector) or an automatic detection of fish passage through a fixed antenna (electrode) installed in a fish-pass and connected to a recording device and a computer (CIPAM system). These methods were used in two sites: the Bomal fish-pass on the lower River Aisne and at two canal siphons in the River Kleine Nete.

Fish selected for radio-tagging (> 150 mm FL and > 150 g) were placed ventral side up into a V-shaped support adjusted to their morphology. A mid-ventral incision was made between the pelvic girdle and the anus and an alcohol sterilised transmitter (40 to 42 MHz, internal implant radio transmitters (ATS Inc.) with internal or external coiled antenna) was inserted into the body cavity. The weight of the transmitter ranged from 4 to 20 g depending on fish body weight, making sure that the transmitter to fish body weight ratio in air would not exceed 2.0 %. The incision was closed by two to five separate stitches, 9-10 mm apart, using sterile plain catgut or vicryl on cutting needles. Fish were released precisely at their capture site (or upstream of the fish pass where they were caught) as soon as they had recovered posture and spontaneous swimming (about 5 min after surgery). This methodology minimises the possible biases originating from long term post-operative care.

In the course of this study 64 fish belonging to 3 species (N = 24 roach; N = 8 chub, N = 15 brown trout) were tracked for different periods of time. In addition, data from previous radio-tracking studies of trout and grayling in upland rivers were considered for further ecological and genetical analysis [32]. Fish in the field were located at least two times a week until the end of the transmitter battery life or loss of the signal. Locations were made by triangulation using an ATS R2000 or a mobile Fieldmaster radio receiver with a loop antenna (ATS Inc.) from labelled marks lining the banks of the river. Locations were made with an accuracy of 1 to 10 m², depending on river size and distance between the fish and the observer.

Water temperature was monitored hourly by data loggers (TidBit, Onset Computer Corp.) and water flow was recorded hourly (data from the Water Division) in each river. To take into account the differences in intervals between fish locations in the different rivers, the movements were standardised to 'weekly mean distance trav-

elled' to perform statistical analysis. It corresponds to the mean distance travelled by fish during a one-week interval.

2.2. Ecophysiology

2.2.1. Swimming capacity

Animal holding: To validate the protocols for the different sizes used, we first tested the effect of flume size on the performance of fish. Larger fish performed better in a flume with a longer swimming section [33] and for further experiments larger fish were tested in a large Brett style swimming flume available at Flanders Hydraulics Research in Antwerp while smaller fish were tested at the University of Antwerp in 8 Blazka style swimming respirometers. More details can be found in Tudorache and co-workers [33]. Fish (the key species trout (*Salmo trutta*), three-spined stickleback (*Gasterosteus aculeatus*), gudgeon, (*Gobio gobio*), bullhead (*Cottus gobio*), roach (*Rutilus rutilus*) and some other common species such as common carp (*Cyprinus carpio*), stone loach *(Barbatula barbatula*) and perch (*Perca fluviatilis)*) of ca. 5 to 10 cm body length (Bl) were kept at the University of Antwerp in 200 L tanks in Antwerp City tap water at a constant temperature of 15 ± 2°C for at least one month before experiments started. Tanks were set up in flow-through and water was partially renewed with a turn over rate of 100 L per day. Additional filtering occurred by means of a circular triple filter consisting of a cotton filter, an active carbon filter and a lava stone filter. Fish of the sizes 5 – 30 cm were kept at the Hydrological Institute in Antwerp in 300 L tanks in Antwerp City tap water at a constant temperature of 15 ± 2°C for at least three weeks before experiments started. The water was permanently filtered by means of a mechanical filter and a closed circuit with a turn over rate of 100 L per day. All fish were fed with 'Pond Sticks' (Tetrapond, Henckel, Germany) three times a week at a 2 % body weight ratio.

Determination of critical swimming speed (U_{crit}) and oxygen consumption (MO_2) in a Blazka-type swim tunnel: Swimming performance is tested under laboratory conditions by means of increased velocity tests where the swimming potential of individual fish is determined by means of critical swimming speed (U_{crit}; [34]). U_{crit} is a common estimation of prolonged swimming performance [35] or maximum sustained speed, the nominal speed at which it is generally assumed that maximum oxygen uptake occurs [26, 36, 37, 38]. It also has been defined as the highest maintainable swimming speed for a period equal to the time interval used in the test [39]. As U_{crit} is a good indicator for the capacity of an upstream migrating fish to swim through strong currents, it can be used as a measure for the impact of environmental challenges such as temperature, hypoxia, diseases or toxicant exposure [40, 41, 42, 43, 44]. This test is performed in swimming tunnels, where swimming speeds are kept constant for a certain time period and then changed, according to the protocol.

For determination of U_{crit}, eight fish from the size groups of approximately 5 to 10 cm were placed in individual separate Blazka-style swimming respirometers with a vol-

ume of 3.9 L. The sizes for the inner tube are 35 x 6 cm and 50 x 11 cm for the outer tube. Velocity was set to 5 cm s^{-1}. At this speed, the fish orient themselves towards the current and swim gently. For temperature control, respirometers were submerged on a wet table in a room acclimated to the same temperature as the water in the tanks, and a head tank provided a continuous flow of water saturated with oxygen through each respirometer at a rate of 4 l min^{-1} (total volume of the recirculating system approximately 2 x 225 l). These conditions were kept overnight to allow the fish to acclimate to the respirometers. The next day water velocity was then increased in increments of 5 cm s^{-1} at intervals of 20 minutes, until fish fatigued. Fatigue was determined as the situation where the fish could no longer maintain position against the current and were swept downstream. They were held against a mesh screen at the downstream end of the tunnel. Speed was then lowered for a short time to allow fish to restart swimming, and when fish were swept downstream for a third time, they were considered really fatigued and the performance test was terminated. U_{crit} was calculated [34]. The absolute values (in $cm.s^{-1}$) were converted to relative swimming speeds in body lengths per second ($BL.s^{-1}$). After U_{crit} measurements were finished, fish were removed and the body length, height and width were measured and fish were weighed. Speeds were corrected for the solid blocking effect, i.e. the speed that is measured is lower than the speed that the fish actually swims [45]. The burst swim moment, which is the time at which the first three gait transitions from cruise swimming to burst swim occurred within one speed interval, was determined.

Fish were allowed to recover from the U_{crit} determination overnight by swimming constantly at a gentle velocity of 5 cm s^{-1} and respiration measurements were performed the next morning. Respirometry measurements were started by closing the respirometers for a 1-hour period. During measurements, fish were swimming at different percentages of U_{crit} to determine the MO_2 at different swimming speeds using WTW-O_2-electrodes (oxi340i, WTW, Germany) connected to the computer program Windmill (Jill Studholme, Windmill Software Ltd, 1996). After 1 hour of measurement at the lowest speed, respirometers were reconnected to the continuous flow of water saturated with oxygen mentioned above and fish were given one hour to recover. Subsequently the procedure of MO_2 measurement was repeated at a higher velocity. After MO_2 measurements were finished, fish were removed and fork length was determined as well as the weight and the volume of the fish.

Determination of U_{crit} in a Brett-type swimming tunnel: Eight fish from the size groups of approximately 10, 20 and 25 cm were placed individually in a Brett-style swimming tunnel with a swimming section of 480 l (3 x 0.4 x 0.4 m). The total content of circulating water volume was approximately 8000 l. Velocity was set to 5 cm s^{-1}, at which the fish was allowed to acclimate for two hours. The protocol of the Blazka-type set up was repeated. As the cross sections of the tested fish were not bigger than 20 percent of the cross section of the tunnel, the solid blocking effect could be ignored.

U_{opt} determination: The optimal swimming speed (U_{opt}) is the speed at which the lowest relative oxygen uptake occurs, i.e. where the amount of work per meter reaches a minimum [46, 47]. This speed can be calculated from measurements of energy expenditure as MO_2 over a range of speeds [48]. Optimal swimming speed is the swimming speed at which a fish can do the highest amount of physical work, measured in terms of respired oxygen, per meter swam, and it can be also given in terms of percentage of the critical swimming speed and thus of percentage scope for activity, an indication of the costs to swim advantageously compared to the costs for other metabolic activity functions. This swimming speed gives an indication of the water speed that is passable by a fish on a longer distance. Optimal swimming speed was determined [48].

U_{max} determination: U_{max} is the maximal swimming speed. It is used for short burst swimming (e.g. to cross a short section with high water velocity) and in escape responses. Since it is mainly fuelled by anaerobic energy, it can only last for a few seconds. For small fish (5 -10 cm) experiments were carried out in a round white plastic tank with a diameter of 40 cm and a height of 55 cm. For larger fish (10-30 cm), experiments were carried out in a Plexiglas tank of 150 by 150 cm and a height of 50 cm. Water depth was 15 cm for small and 25 cm for large fish, supplied with the same flowing filtered freshwater as in the holding tanks. A reference grid (5 × 5 cm) was drawn on the bottom of the tank for the accurate determination of fish position during escape sequences. Escape responses were induced with a mechanical stimulus, a cubic weight, which was released from a height of 1.5 m above the water surface. Fish were allowed to move freely for at last 15 min before the release of the stimulus. A PAL video camera (Sony Corporation DCR-HC39E) was positioned directly above the experimental tank to film the burst swim event. All individuals were filmed three times. Video in PAL consists of 25 frames per second. Each frame can be split into two fields hence the video sequence can be converted into 50 fields per second – 20 ms apart. For this purpose the sequences of escape responses of individual fish were imported into Adobe premiere 6.0 (as AVI files) and deinterlaced. All recordings were analysed, but only the sequence producing the fastest velocity was chosen for further analysis [49]. Analysis was carried out on 20 fields for each individual and commenced on one field prior to the stimulus contacting. Each sequence was imported into Vernier Logger Pro 3.3 and the XY coordinates of the centre of mass (CoM) were determined. Velocity was determined by calculating the movement of CoM from each field over time. Length specific velocity was calculated in $L_B\ s^{-1}$.

2.2.2. Leaping capacities under laboratory conditions

Natural barriers in streams often consist of minor waterfalls due to weirs, debris jams, culverts and others. To have a successful upstream passage at these locations, fish species often may have to leap. Because in Flanders most species are weak swimmers, the leaping capacity is expected to be rather limited. Except this rather general

statement, very little detail is known about the fish swimming performance and their corresponding leaping capacity. Often the leaping capacity of fish is constrained by unfavourable conditions of plunge pool depth, high stream velocities and/or shallow depths at the crest of the fall. Bell [50] suggested that for determining fish passage success over waterfalls, some percentage of the upper limit of burst speed will be used, depending on the fish condition. Theoretical leaping length and height were determined accordingly from the results obtained in the swim performance tests.

In this part of the study we want to test the actual leaping capacity of different fish species in an experimental setup in combination with their swimming performance. The objectives of the leaping experiments are to 1) evaluate the impact of different parameters V (stream velocity above the weir), H (leaping height), S (shape of the weir) and D (depth of the plunge pool) on the ratio of success; 2) evaluate differences among fish species and within fish species, based on different length classes, and 3) evaluate leaping curves of different fish species, based on determination of swimming capacity.

Experimental setup: Within this project leaping experiments were done in 2 different periods: 2004 and 2005. Work during the 2004-series has focussed on the development of a good experimental set-up. In the 2005-series the scope was narrowed, aiming at a more detailed analysis. In the 2004 series, the setup consisted of a flume divided in two parallel, 1.2 m wide similar sections containing each a different type of weir (V-shaped weir and rectangular weir). The dimensions of the flume were length: 56 m, width: 2.40 m, height: 1.45 m, maximum discharge: 0.600 m³/s. Different levels of leaping height were tested during several days while pool depth and discharge were set to feasible values, based on literature. A nine week schedule for the first series of experiments has been elaborated with the factors shown in table 2.2.1. In both the 2004 and 2005 series a rectangular and V-shaped weir were tested. However there was a difference in the V-shaped weir type used in 2004 and 2005. The 2004 V-shaped weir had a larger angle ($\alpha = 24°$), while the 2005 V-shaped weir had a smaller angle, according to recent applications (fishways) on the field ($\alpha = 8°$). At the upstream side of the weirs a bow net was located in order to catch the fish that successfully leaped over the weir and to prevent them from swimming back. Every day a factor level combination was tested, starting from 16 pm until 10 am the next day. From 10 am the successful fish were collected and scanned before replacing them in the downstream part. The most difficult combinations were tested at the beginning of the experiments, in order to prevent exhaustion of the fish becoming too important.

In the 2004 series, 200 fish from the following species were equipped with a PIT-tag: gibel carp (*Carrasius auratus gibelio*) (42 individuals), gudgeon (*Gobio gobio*) (47 individuals), rudd (*Scardinius erythrophthalmus*) (56 individuals), ide (*Leuciscus idus*) (39 individuals) and tench (*Tinca tinca*) (16 individuals). In the 2005 series, 42 fish

from the following species were equipped with a PIT-tag: roach (*Rutilus rutilus*) (24 individuals), gibel carp (*Carrasius auratus gibelio*) (11 individuals), common carp (*Cyprinus carpio*) (7 individuals). Each fish was measured (fork length and total length) and weighted. Fish were divided in 2 groups with comparable species composition, which were placed in the left or right section of the flume. Both groups have tested every combination of the above parameters, in order to have repeated measurements for each factor-level combination.

Table 2.2.1: Overview of all factor levels.

Factor	Levels 2004-series	Levels 2005-series
Leaping height	0.05 - 0.15 - 0.25 m	0.05 - 0.15 - 0.25 m
Pool depth	0.40 - 0.60 m	0.20 - 0.40 m
Discharge	0.050 - 0.175 - 0.300 m³/s	0.100 - 0.200 - 0.300 m³/s
Weir shape	v-shaped_2004– flat	v-shaped_2005 – flat

Analysis of results: Differences in success ratio due to different levels of the factors velocity classes, pool depth, leaping height and shape of the weir were statistically analyzed for all fish species together and per species, for 2004 and 2005 separately (S-PLUS 2000). Kolmogorov-Smirnov tests and Kruskal-Wallis rank sum tests were used in order to detect significant factor levels, respectively for 2 or more sample comparisons. For the analysis, measured discharges were converted to (calculated) mean velocities on top of the weir, because velocity is a better link with the swimming experiments. Differences in success between fish species and within species (length-classes) were investigated through comparison of mean successes p_1 and p_2 [51].

2.3. Genetics

2.3.1. DNA extraction and microsatellite amplification

Genomic DNA was extracted from fin clips using a silica-based purification method [52] or the CTAB protocol for adipose fins [53] in case of brown trout. In the latter species seven microsatellite loci were selected; Str 15, Str 60, Str 73 [54], Ssa 85, Ssa 197 [55], SsoSL 438 [56], Str 85 [57]. For roach six loci were analysed (Lid-1, Lid-2, Lid-4, Lid-11, Rru-2, Rru-3 and Rru-4: [58]) and for stickleback we used six loci (Gac5196, Gac2111, Gac4170, Gac1097, Gac7033, Gac1125 [59]). In bullhead we selected six loci (Cgo310, Cgo56, Cgo1016, Cgo91, Cgo1033, Cgo1114, from [60]) and 12 loci (LCE27, CottE6, LCE22, LCE59, CottE23, LCE279, LCE48, CottES21, Cott686, CottE10, LCB16, LCE219). For brown trout the loci were amplified with standard PCR reagents (1 to 3 mM of $MgCl_2$, 100 µM of each dNTP, 0.6 unit of Taq gold DNA polymerase (Applied Biosystems), 60 ng of DNA and 0.25 to 0.5 µM of each primer). An initial step of 10 min at 94°C (enzyme activation) was followed by one step of 30 to 35 cycles of 45 s at 94°C, 45 s at annealing temperature, elongation of 30 s at 72°C, and ended by a final elongation step of 10 min at 72°C. Annealing temperature were 55°C for Ssa197, SsoSL438 and Str85, 58°C for Str15 and Str73 and 60°C for Str60 and Ssa85. One primer of each primer pair was end-labeled

with fluorescent dye (blue, green or yellow). PCR products were electrophoresed on a 4.25% acrylamide/bisacrylamide gel (29/1) together with a red labeled internal size standard (400HD). Gels were analyzed with GENESCAN software (Applied Biosystem). Couples of loci were multiplexed in the PCR (Str60 with Ssa85, Str15 with Str73, Ssa197 with SsoSL438, and Str85 alone). Sets of loci with non overlapping allele sizes were analysed in multiplex on the automated sequencer (Str60, Ssa85, Str15, Str73 on one gel and Ssa197, SsoSL438 and Str85 on another gel). For stickleback, roach and bullhead sets of loci were amplified simultaneously with the Qiagen® Multiplex PCR Kit (Qiagen). The 12.5 µl PCR contained 1-100 ng genomic DNA forward and reverse primer, 1× Qiagen Multiplex PCR master Mix (3 mM $MgCl_2$) and RNase-free water. The reaction consisted of an initial activation step of 15 min at 95 °C, followed by 30 cycles of 30 s at 94 °C, 90 s at 55 °C and 1 min at 72 °C. A final elongation step of 10 min at 72 °C was performed. PCR products were visualized on an ABI3130 Avant Genetic analyzer (Applied Biosystems). Allele sizes were determined by means of an internal GeneScan 500-LIZ size standard and genotypes were obtained using genemapper 3.7 (Applied Biosystems). Genotypes were checked for scoring errors that might be attributable to stutter-products, large allele dropout or to the presence of null-alleles, using the software micro-checker 2.3 [61].

2.3.2. Genetic data analysis

Genetic diversity was evaluated based on genotype and allele frequencies, the level of polymorphism, and the observed and unbiased expected heterozygosity (H_O and H_E) using GENETIX 4.04 [62]. Allelic richness was quantified in FSTAT 2.9.3.2 [63] and averaged over loci. Population differentiation was quantified in GENETIX using the standardized allelic variance F_{ST}, estimated as θ [64]. Overall and pairwise F_{ST}'s were tested for significance against 10^4 random permutations of the data. A multidimensional scaling (MDS) procedure as implemented in STATISTICA 6.0 was subsequently used to represent the relationships among populations, based on Reynolds genetic distance matrix (*D=-ln(1-Fst)*, [65]. The factorial correspondence analysis was carried out in GENETIX.

Evidence of recent population bottlenecks was tested using the coalescent approach [66]. The Wilcoxon signed-ranks procedure was used to test whether observed heterozygosity exceeded the values expected at mutation-drift equilibrium under a two-phase mutation model with 70% single-step mutations (TPM). Finally, we estimated the number of first-generation immigrants (i.e. "dispersers") with GENECLASS 2.0 [67]. We used the L_home/L_max likelihood computation, the Bayesian method of classification [68], and the Monte Carlo algorithm [69] to simulate 10 000 genotypes. The number of dispersers between two populations in both directions were pooled because of the low interpretability of dispersal direction [70], weighted for sample size, and $\log_{10}(x + 1)$ transformed.

The Bayesian inference approach [71] was used to estimate 1) the number of gene pools (K) represented by the different hatchery samples and 2) the individual's admixture coefficient q (i.e. the proportion of an individual's genome derived from one or another population) for wild but potentially introgressed fish as suggested by [72]. K was first estimated for eight hatcheries, assuming a model where individuals represented from 1 to 8 populations and using a burn-in of 100.000 followed by 1.000.000 MCMC replicates. The relative probability of each number of populations was calculated [71]. The highest probability was detected for K=1, which allows us to group all the hatcheries in a single group (so-called hatchery group) in the following analyses.
Analyses of the individual's admixture coefficients q were performed separately for all the potentially introgressed populations including one wild population and the hatchery group (being the potential source of introgression). We considered that hatchery fish have a known origin (popflag set to one for these fish) whereas wild fish have an unknown origin (popflag set to zero for the wild fish). This way the model considers the hatchery fish as "pure hatchery fish type" i.e. non-admixed individuals, and river fish (potentially introgressed fish) as admixed individuals. Estimated admixture coefficients and their 90% probability intervals were calculated assuming a model with two populations (K=2) and using a burn-in period of 50.000 steps followed by 200.000 MCMC replicates. As populations may be closely related, the option "correlated alleles frequencies" was chosen [73].
From the individual admixture coefficient, a population level of admixture (q mean) was estimated by calculating the mean of the individual admixture coefficients per population. The variance of the individual admixture coefficient (var(q)) per population was also calculated. Var(q) represents the degree of homogeneity of the level of introgression in each population. A low var(q) corresponds to an identical level of introgression for every fish from a single population whereas a high var(q) corresponds to the presence of different level of introgression within a population. Var(q) versus q mean was plotted to help visualize the different situations observed in our dataset.
In order to assess the power of STRUCTURE for identifying pure hatchery type fish, pure river type fish and admixed individuals, we considered a theoretical population composed of 50 real river type fish (from LFA population), 50 hatchery type fish (from HMR population) and of 50 simulated hybrids between these two latter populations. We chose to use the 50 fish of the LFA population because previous results showed that this population can be considered as the most preserved. 50 F1 hybrids between LFA and HMR were simulated using the program HYBRIDLAB [30]. This way the so-constituted theoretical population encompasses only fish of known origin. The same scheme of analysis of the individual admixture coefficients as presented above was used i.e. considering that fish from the theoretical population have an unknown origin whereas the hatchery group has a known origin. Note that this time the hatchery group is composed of all the hatcheries but HMR.

2.3.3. Geographical information

Geographical information was obtained from a digital map of the river system, and from a digital map containing the migration barriers on the main river channels [74]. We carried out complementary field surveys with a Global Positioning System (Etrex, Garmin) to locate sampling sites, and to type and digitize additional barriers on some unexplored river sections between sampling sites. We also registered the width of the stream at each sampling site, calculated as the mean of two independent measurements. Migration barriers consisted of several types but were classified in three main categories. The first category was water mills, which can be considered as constructions with a long history (100-500 years). The second category (weirs) consisted of more recent (< 100 years) hydraulic artefacts and inappropriately constructed bridges and culverts. The last category were tunnels, which are no physical barriers but which can be up to several kilometers long, and sluices, which are only temporal barriers. Fishes have difficulties to pass the steep drop-off that characterizes most barriers. The vertical heights of these drop-offs were obtained from [74], or were measured during our own field surveys. As the Scheldt basin is a watershed with lowland rivers, there are no natural rapids hindering dispersal or gene flow.

All geographical information was combined in a Geographical Information System (Geomedia professional 5.2, Intergraph Co.) and rasterized in Geomedia Grid 5.2 (Intergraph Co.). Using the standard cost analysis tool in Geomedia Grid we calculated upstream distances (defined as the maximal upstream distance along waterways from each sampling site), effective geographic distance (km), total number of barriers, number of each type of barrier or total vertical barrier height (m) as friction. We used PATHMATRIX to calculate pairwise distances along the river using the cost distance algorithm. Pathmatrix is an extension to the geographical information system (GIS) software ARCVIEW 3.2. The pairwise genetic distances [Fst/1-Fst] were calculated using Genetix [62]. We used Genetix to perform a Mantel test [75] and compute the Mantel correlation coefficient between the pairwise geographical distances and the pairwise genetic distances. The significance of this correlation was assessed by a permutation scheme (5000 permutations).

Using the same method, isolation by weir was also tested by calculating the correlation between the Fst/1-Fst distance matrix and the number of weir (between pair of sites) matrix. Pathmatrix considered that going from A to B is equal to going from B to A, which is not correct in our case. Because going upstream to downstream is easier than downstream to upstream

2.3.4. Hypothesis testing

Analyses focussed on genetic diversity, genetic differentiation and dispersal. For all tests, variables were inspected for normality and $\log_{10}$-transformed when necessary. Assessment of significance followed a parametric approach in the case of genetic

diversity, whereas matrix permutation methods [75] were applied in the case of genetic differentiation and dispersal.

Firstly, we tested the impact of geographic distance, barrier characteristics, upstream distance and habitat width on allelic richness. Effective geographic distance, total number of barriers, total barrier height and the number of each barrier type were calculated starting from the most downstream population. Associations with allelic richness were tested with simple and partial correlations (based on 21 populations) in statistica 6.0. Partial correlations controlled for upstream distance as a measure for the geographical range of riverine populations [76], accounting for the magnitude of genetic drift (or N_e) under natural circumstances. The results on allelic richness were also compared with the results on the F_{ST} values measuring the genetic differentiation with the most downstream population. Secondly, we tested the impact of pairwise geographic distance, barrier characteristics, the binary directionality matrix, log-transformed averaged upstream distance and habitat width on genetic differentiation and dispersal based on pairwise matrix correlations. Simple and partial correlations with pairwise F_{ST} and the number of dispersers (210 pairwise combinations) were calculated and tested with a Mantel test module [75] programmed in S-plus. Here, partial correlations controlled for geographic distance accounting for natural gene flow, i.e. as expected in the absence of barriers.

3. RESULTS

3.1. Ecology

3.1.1. Analysis of fish communities in the model systems

Since the beginning of the project in 2003, scientific fishing was performed in 9 lowland and 15 upland rivers and numerous stations representative of a wide range of situations downstream and upstream of physical barriers. A total number of 17.291 fish were caught belonging to 34 species in the lowland rivers and 58.447 fish were caught belonging to 33 species in the upland rivers. In these samples the five target species amounted to 8.023 and 19.889 individuals respectively and were distributed among river systems as indicated in Table 3.1.1. Fish were sampled for genetic studies and subsamples of fish were used for physiological research.

Table 3.1.1: Number of target species caught with electrofishing in the lowland and upland river systems in 2003-2004.

Number of fish caught	*S. trutta*	*C. gobio*	*R. rutilus*	*G. aculeatus*	*G. gobio*	Other	TOTAL
LOWLAND RIVERS							
Scheldt	-	30	145	-	-	875	1.050
Zwalm	-	315	155	213	-	91	774
Dijle	1	154	140	118	-	333	746
Dender	-	-	131	1	-	481	613
Demer	-	201	215	62	-	681	1.159
Herk	-	110	14	110	-	409	643
Grote Gete	6	154	47	153	-	234	594
Grote Nete	-	162	152	40	8	363	725
Kleine Nete	-	1.113	3.841	22	210	5.801	10.987
Subtotal	7	2.239	4.840	719	218	9.268	17.291
UPLAND RIVERS							
Berwinne + trib.	332	668	129	157	638	9.935	11.859
Ourthe, main course	510	1.100	1.265	122	1.409	14.653	19.059
Ourhte, small trib.	1.078	2.061	9	7	103	2.974	6.232
Vesdre + trib.	2.119	1.805	494	1.351	893	3.552	10.234
Amblève + trib.	888	1.639	29	548	15	7.444	10.563
Lhomme	130	390	-	-	-	-	520
Subtotal	5.057	7.663	1.926	2.185	3.058	38.558	58.447
TOTAL	5.064	9.902	6.766	2.904	3.276	47.826	75.738

To visualise the global impact of barriers on fish populations in the lowland rivers, the mean number of fish species in relation to the presence of migration barriers between sampling stations and the North Sea was plotted (Flanders, N=38). Shipping locks, siphons, and fish-passes are considered as passable for fish. A negative relationship (R^2= 0.84; Figure 3.1.1) is shown.A river-by-river preliminary analysis did not reveal significant effects of fragmentation by weirs and dams on the patterns of target non–migratory fish species geographical distribution and population abundance. This is partly due to the major influence of fish stocking on local distribution of brown trout (*Salmo trutta trutta* L.) and roach (*Rutilus rutilus* (L.)).

In the lowland rivers, especially the Scheldt, Kleine Nete and Grote Nete, that have free access to the North Sea, the upstream migration of adult river lamprey (*Lampetra fluviatilis* (L.)) and juvenile flounder (*Platichthys flesus* (L.)), appeared to be influenced by successive obstacles. In the Scheldt, tide is noticeable upto Ghent, i.e. 160 km from the North Sea. These hydrographical and morphological characteristics make it likely that important migrations take place in this river. At different places in the downstream part of the river, the entrance of glass eel is still observed [77].
In most cases dams and weirs are impassable whereas shipping locks are temporally passable for migrating fish [78, 79]. It must be remembered that the fragmentation of the Dutch and Belgian river Meuse by modern dams from 1925 to 1932 caused the extinction of all anadromous migratory fish in this river basin [80]. In the range of upland rivers studied, only the diadromous European eel (*Anguilla anguilla* (L.)) was noted present in reaches upstream of some impassable barriers although they cause an obstruction to migration of juvenile yellow eel in their process of colonizing continental areas from the North sea. This kind of effect was observed in reaches upstream of large dams (Nisramont dam on the River Ourthe) and in small streams as well, for example the Mosbeux, a small tributary of the River Vesdre (Mos-08, Figure 3.1.3). The eel status is disturbed by the fact that wild young eels are available on the market for stocking and that such fish have been translocated (particularly in the past as glass eels with a life time of 15-20 year [81]) into artificial lakes (Robertville and Bütgenbach Lakes on the River Warche) and so may be found in stream reaches upstream of an impassable barrier.

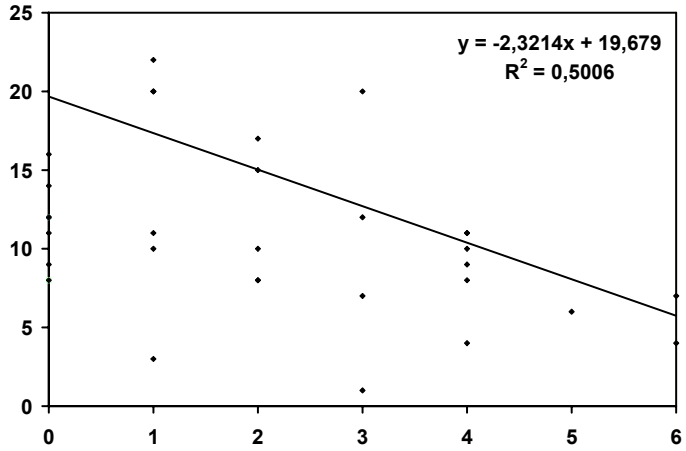

Figure 3.1.1: Influence of successive obstacles on the presence of different species in the sampled sites of lowland rivers.

3.1.2. Quantification of actual migration of a selection of species in case studies

Mobility of brown trout (*Salmo trutta trutta* L.):

This salmonid species is widely distributed and abundant in upland rivers. Several previous studies [32, 82, 83, 84] have described its migration behaviour and docu-

mented its high capacity to clear physical obstacles in a range of upland rivers types. Data for the Aisne have been used for a re-analysis in relation with the genetical part of the present project, based on samples of adipose fins collected in 2003. Complementary data on trout movements were collected in 2003-2005 using biotelemetry, PIT-tagging and catch of upstream migrating fish in a fish-pass or a fish-trap. New investigations carried out in 2003-2005 mainly focused on how trout move and migrate for reproduction within dam-fragmented medium-sized watercourses (Ourthe, Vesdre, Amblève and Lhomme) and into small spawning tributaries from large (Berwinne as a tributary of the Meuse) and medium-sized rivers (Mosbeux as a tributary of the Vesdre, Haze as a tributary of the Ourthe).

Table 3.1.2: Characteristics and summary of the movements of brown trout radio-tracked from October 1995 to June 2001 [32] and in 2003-2006.

River	N	FL±SD (mm)	W±SD (g)	Tracking period	% upsteam migrants	Km travelled (mean±SD)
Aisne	19	332 ± 72	428 ± 44	Oct. 1995-Dec.1998	88%	8.8 ± 2.0
Méhaigne	9	404 ± 71	884 ± 524	Sept. 2000-Feb. 2001	75%	2.0 ± 3.7
Néblon	4	311 ± 11	342 ± 60	Oct -Dec. 1999	25%	0.80
Oxhe	6	332 ± 56	420 ± 198	Sept. 2000-Jan. 2001	17%	0.25
Ourthe	9	480 ± 78	1.354 ± 474	Nov. 1995-Sept. 2000	78%	23.2 ± 11.2
Vesdre	10	394 ± 78	736 ± 476	Sept. 2004–June 2005	40%	1.34 ± 98
Lhomme	5	285 ± 19	261 ± 45	Oct. 2003-Jan. 2004	40%	1.45 ± 1.48
Amblève	7	320 ±41	377 ±142	Sept. 2005–Jan. 2006	28%	0.4 ± 0.14

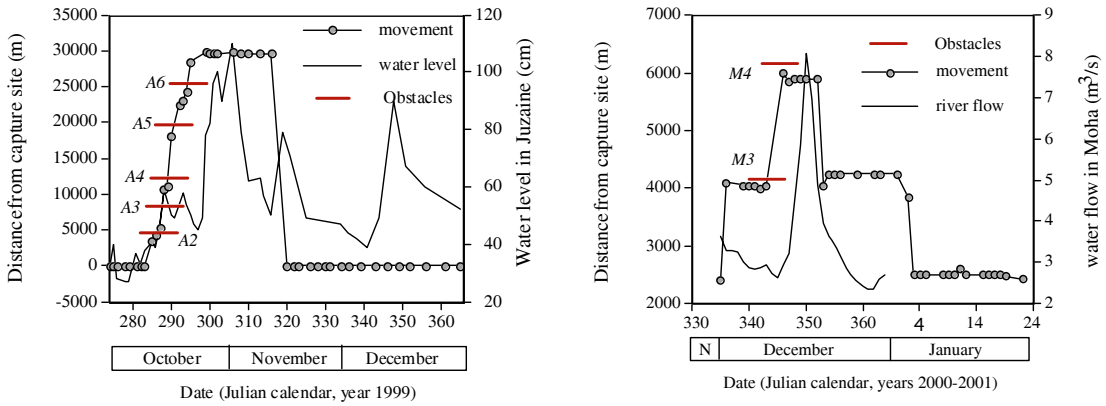

Figure 3.1.2: (A) Example of a typical migratory behaviour of wild trout in an equilibrated stream (low level of anthropisation). Spawning migration of a female brown trout (250 mm FL) and water flow in the Aisne stream. The alphanumeric codes refer to the five obstacles that this fish cleared. (B) Example of a typical migratory behaviour of wild trout in a disturbed stream (high level of anthropisation). Spawning migration of a female brown trout (511 mm FL) and water flow in the Méhaigne stream. The alphanumeric code M3 refers to the obstacle that this fish cleared.
The fish never passed through the obstacle M4.

(a) Patterns of brown trout migration in sub-natural and fragmented upland rivers: First studies on trout movements were conducted from October 1995 to February 2001 in 5 upland watercourses and additional data were collected in 2003-2004 in

the rivers Vesdre, Amblève and Lhomme (Table 3.1.2). These studies all together were based on 69 radio-tagged brown trout and provided a good overview of the spawning migration patterns of this salmonid species.

The proportion of the upstream migrants and the distance travelled by brown trout were particularly important in the Ourthe and its tributary the Aisne where obstacles rarely impeded movements of the fish (Figure 3.1.2.A). The proportion of upstream migrants was also high in the Mehaigne (75%), but most fish were blocked below an obstacle during their upstream migration (Figure 3.1.2.B). In the rivers Néblon and Oxhe, only one trout (in the Oxhe), migrated significantly upstream and was confronted with an obstacle. This low tendency to move for spawning in these two small streams could be linked to the trout population being composed of a high proportion of domesticated fish issued from mass stocking for years with hatchery reared eggs and fingerlings. The same phenomenon was observed at a higher degree in the intensively stocked lower course of the Amblève. In the Vesdre and upper Lhomme, 40% of the radio-tracked trout moved upstream and travelled over relatively short distances in average, probably because of the presence of obstacles reducing their free movements.

(*b) Movement of trout from the River Vesdre into the Mosbeux during spawning time:*

On September 12, 2004, a sample of 71 trout were caught by electrofishing below the impassable dam of La Fenderie in the River Vesdre in Trooz, near the confluence of the Mosbeux (Figure 3.1.3). 54 individuals (17-57 cm FL) were PIT-tagged and 5 of them were radio-tagged with the hope that some of these adult trout will migrate into the small tributary for spawning. A population of 64 migrating adult trout were intercepted in the fish-trap at a distance of about 800 m from the Mosbeux-Vesdre confluence. Among these 64 trout, there were:

- 2 tagged fish originating from the N= 29 individuals tagged in the lower Mosbeux (recapture rate 6.9%)
- 9 PIT-tagged (among which 2 radio-tagged) individuals originating from the River Vesdre (recapture rate 16.7%)
- 53 untagged fish likely originating from the River Vesdre .

These patterns of movement clearly demonstrated: i) the role of the Mosbeux as a typical spawning tributary for the R. Vesdre trout population, and ii) the capability of trout to pass over several low-height (< 0.7 m) physical obstacles in the lower course of the Mosbeux. After their capture in the fish-trap, un-tagged trout were PIT-tagged and released above. Several fish were observed continuing their upstream migration over various distances while clearing diverse obstacles with a maximum height of 0.8 m. But all migrating fish (among which 4 individuals previously intercepted in the fish-trap and originating from the Vesdre) were blocked by an impassable barrier consisting of a 1.8 m high waterfall located 2.4 km from the mouth. This barrier was suspected to fragmentise the trout population in the Mosbeux in two parts: a lower Mos-

beux population strongly connected with the Vesdre population and an upper Mosbeux population completely disconnected from those in the lower Mosbeux and the Vesdre. Samples of adipose fin were taken on trout from those different populations identified (migrant trout from the R. Vesdre, resident trout from the Mosbeux downstream of the impassable barrier, and resident trout from the Mosbeux upstream of the impassable barrier).

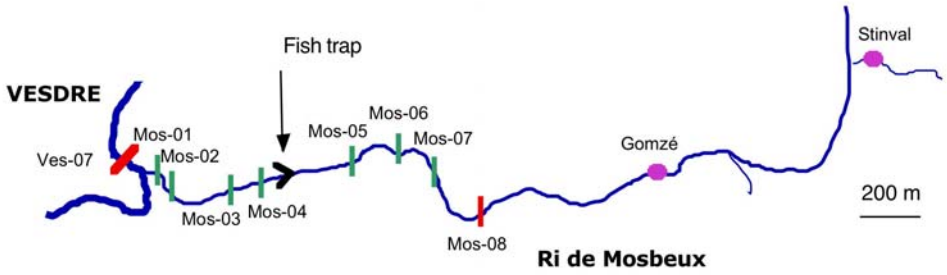

Figure 3.1.3: Situation of barriers to upstream trout migration and fish-trap on the Mosbeux, a small tributary of the R. Vesdre. Red = unpassed barrier; green = passed barrier; point = fishing sites upstream of impassable barrier.

Mobility of bullhead (*Cottus gobio* L.)

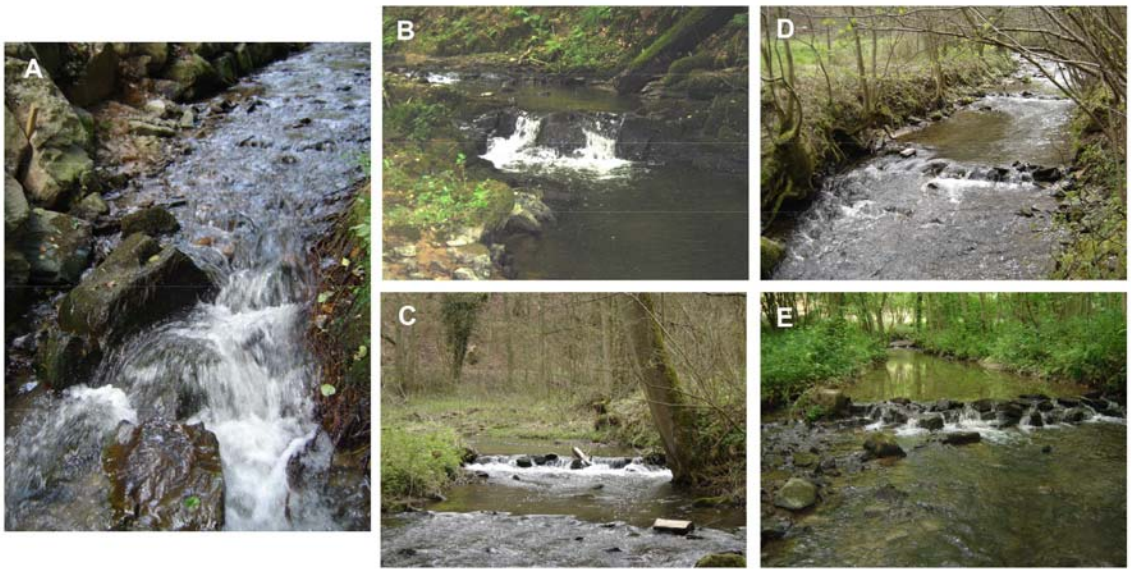

Figure 3.1.4: Views of small physical obstacles in sectors S1 and S2 of Ruisseau d'Oxhe in spring 2006. Obstacles A, C, D and E were passed by upstream moving PIT-tagged bullhead, obstacle B not.

An intensive mark-release-recapture experiment on bullhead mobility was conducted in two stations (S1= 300 m long and 1107 m²; S2= 105 m long and 637 m²) of the Ruisseau d'Oxhe, a small well preserved salmonid brook tributary of the River Meuse near Tihange (Figure 3.1.4). Each station was subdivided in successive 5 m long

sectors (60 in S1 and 21 in S2) where (> 3.3 g, 7-13 cm) bullhead were electrofished, PIT-tagged and recaptured during 4 sampling periods from February to June 2006. A total number of 1030 bullhead were PIT-tagged, representing about 50% of the population in place (multiple recapture estimate 731 individuals in S1 and 1.333 in S2). The numerous recapture data produced information on movements of fish during each fishing interval. In S1 (Figure 3.1.5), most bullhead displayed very restricted movements with 30% of recaptures encountered in the 5 m sector of origin (= last recapture), 23% in the two immediately adjacent sectors (11% upstream and 12% downstream), and 74% in the range -20/+20 m. Despite such a high sedentarity (average displacement 3.5 m downstream) of most fish, a few hypermobile (transient) individuals exhibited longer movements with a maximum of 205 m upstream and 375 m downstream. In S2, the average displacement was +4.5 m with a maximum of 375 m upstream and 50 m downstream. According to these results the short term circum-reproduction mobility of bullhead (March-June) may concern a population living in a 0.8 km reach of the brook and possibly more if long distance movements will be discovered by further sampling in summer.

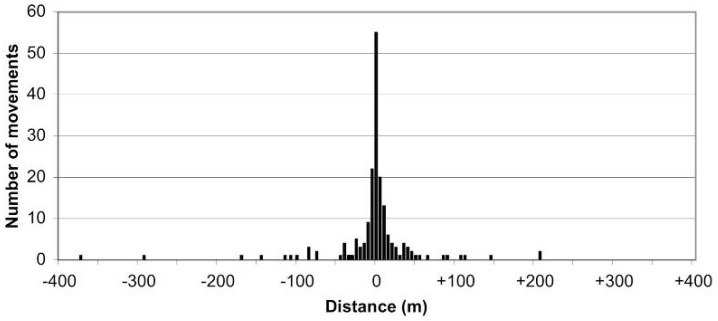

Figure 3.1.5: Distance and direction (+: upstream; -: downstream) that PIT-tagged bullhead moved during spring 2006 in the Oxhe Station S1. The figure refers to all movements corresponding to 184 single recapture data of PIT-tagged fish in the 300 m long initial study zone (composed of 60 5-m long sectors) and immediately upstream and downstream this zone.

Both stations contain several low irregular man-made weirs, made of blocks and stones which easily retain woody debris. All of these small semi-natural obstacles were successfully passed by some individual bullhead. These passable obstacles are characterised by a high transversal heterogeneity with minimum values of 0.25 m for the height and 0.3 m/s for the water velocity at relatively low discharge (0.17 m³/s). We have nevertheless identified about 250 m upstream of S1, a natural cascade (height 0.6 m and minimum water velocity 0.6 m/s) that seems quite impassable for bullhead and could prevent the fish from freely dispersing more upstream. Further research will be centred on this issue which is very important in relation to the ecological genetics of the species.

Mobility of roach (*Rutilus rutilus* (L.))

(a) An integrated biotelemetry study of roach movements in lowland and upland highly fragmented rivers: Roach is a dominant cyprinid fish in many rivers throughout the Eurasian mainland. It is common in rivers, lakes, canals and reservoirs, in streaming as well as in standing waters. It is a benthopelagic, potamodromous species that can survive in poor quality and fragmented rivers. Regardless of its strong tolerance to water disturbance, roach populations have rarely been protected and minimally studied [13]. Roach is widely distributed and stocked in Belgian rivers. That allowed the performing of comparative studies of its mobility and reproductive migrations in lowland rivers (Kleine Nete and Grote Nete) and in an upland river (Vesdre).

Twenty-four adult roach (> 150 mm FL and > 150 g) from 2 lowland (Grote Nete and Kleine Nete) and 1 upland river (Vesdre) were tagged with surgically implanted radio transmitters. Their seasonal movements were observed from March to August 2004 (circum reproduction period) in river stretches delimited by two physical barriers. The three rivers studied were highly fragmented and got different characteristics (Table 3.1.3 and Figure 3.1.6). The study site in the River Kleine Nete was situated in the slow flowing middle reach of the river in between two weirs of which the most upstream weir is equipped with a fish-pass. The distance between both weirs is 7 km. One tributary, the River Aa, enters into the Kleine Nete just upstream of the downstream weir. In the River Grote Nete the study site was situated in the slow flowing middle reach of the river, in a 3 km long river stretch between a weir and a water mill. The study site in the River Vesdre was situated in the lower reach of the river in between two dams at a distance of 1.2 km.

Roach displayed similar patterns of movements in all three rivers which were mainly influenced by the date of observation (movements increased in late April-May) and the water temperature (travel distances were more important when water temperature ranged between 10-14°C). When grouping the weekly mean distance travelled by roach in the three rivers (Figure 3.1.7), it appeared that movements increased from the beginning of April until mid-May when water temperature fluctuated between 10°C and 14°C. From mid-May, when water temperature rose above 14°C and the water flow decreased, the weekly mean distance travelled by the fish decreased and roach were less active. On 25 July and 15 August, two striking peaks (in weekly mean distance) corresponded to increased upstream distances travelled by three fish in the rivers Kleine Nete and Grote Nete. Roach movements were most important in the 10-14°C water temperature intervals that mainly occurred during April and May (potential spawning period) except for the two peaks in July and August. A significant difference in the weekly mean distance travelled was observed when grouping the roach movements (of the three water courses) into three categories of water temperature (< 10°C; 10-14°C; > 14°C; Kruskal-Wallis, $p<0.05$). Similar analyses were

performed to test the influence of water flow on roach movements, but no statistical relationship was found.

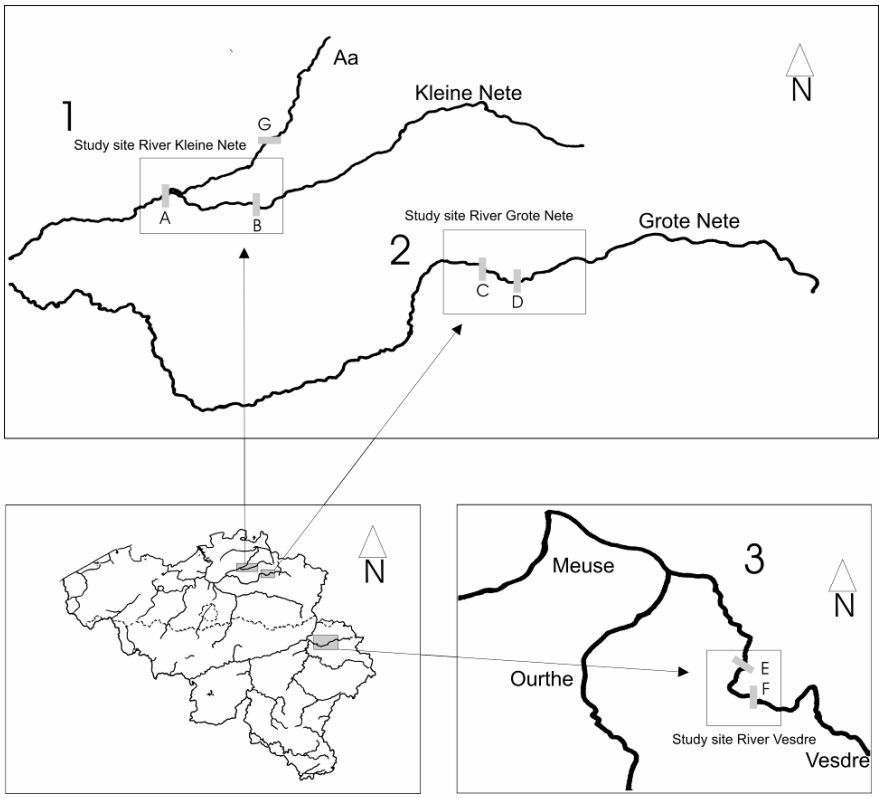

Figure 3.1.6: Location of the three study areas in Belgium: (1) the River Kleine Nete, (2) the River Grote Nete and (3) the River Vesdre. Transverse bars represent the barriers that may interfere with the free circulation of fish. (A) weir Grobbendonk, (B) weir Herentals equipped with a fish pass, (C) weir Meerhout, (D) water mill Meerhout, (E) weir Chaudfontaine, (F) weir Hauster and (G) weir on the River Aa, a tributary of the River Kleine Nete.

Table 3.1.3: Characteristics of the three studied rivers.

	Kleine Nete	Grote Nete	Vesdre
Length (km)	50	60	72
Tracking distance (km)	7	3	1
Mean width (m)	12	8	30
Mean discharge (m^3/s)	2.80	2.02	11.35
Mean slope (‰)	0.55	0.42	7.8
No of physical obstacles	10	13	29
Fish community	30 species	25 species	21 species

The weekly mean distances travelled by roach during the overall study period were different between rivers (Figure 3.1.8). In the River Kleine Nete, where fish migration is unobstructed for the first 14 km of the reach (first barrier in the River Aa), the mean distance travelled was about 475 m. In the rivers Grote Nete and Vesdre, the free migration reach is limited to respectively 3 and 1 km, by which the weekly mean distance travelled diminished to 145 and 86 m, respectively. Weekly mean distances travelled were significantly different between the rivers Kleine Nete and Grote Nete ($p<0.0001$; Scheffe f-test) and between the rivers Kleine Nete and Vesdre ($p<0.0001$;

Scheffe f-test). No differences were observed between the rivers Grote Nete and Vesdre (p=0.48; Scheffe f-test).

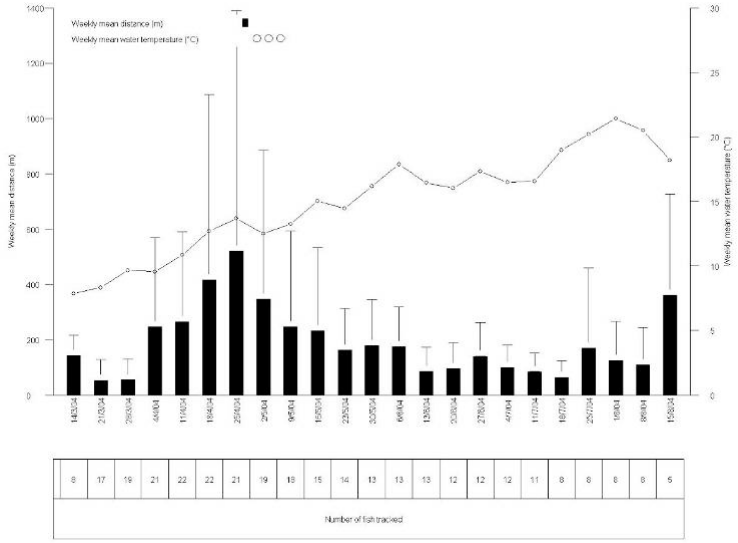

Figure 3.1.7: Weekly mean distance (m) travelled by roach, in the three rivers, in relation with the mean weekly water temperature (°C) of the three rivers. Error bars represent standard deviation.

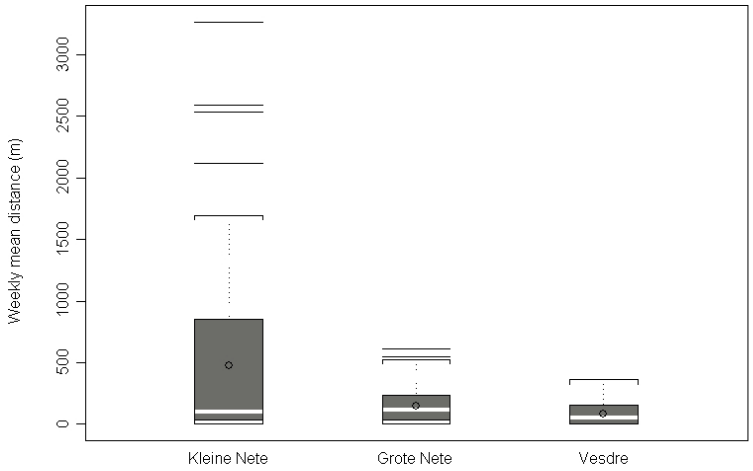

Figure 3.1.8: Weekly mean distance travelled by tagged roach in the three rivers during the overall study period. Values are median, percentiles 5, 25, 75 and 95. Bars indicate outlier values and circles indicate weekly mean distances.

Our results suggest that roach are no frequent obstacle leapers (at least in the up-stream direction), only in the River Kleine Nete, roach cleared a temporarily flat lying weir and a fish-pass. On the other hand, both in the rivers Kleine Nete and Vesdre,

downstream movements while passing barriers were observed. Results of this comparative study have been communicated in a paper by Geeraerts *et al*. [85].

(b) A study of movements of PIT-tagged roach in a lowland river: The inventory procedure for this study is designed to be an applicable, consistent method for investigating obstacles that impede passage of fish in streams. It tries to answer questions like "For which species does the siphon restrict the migration and movement and to what extent?" Full answers to these questions are essential to managing rivers and planning for restoration. Planning for restoring watersheds and setting priorities cannot logically proceed without considering how fragmented the aquatic habitat is and how important it is relative to the suite of restoration needs of the whole watershed.

The PIT-tag experiment was done at two canal siphons in the River Kleine Nete, in Lier and Grobbendonk, to assess the mobility (migration/movement) of fish through these two successive siphons. Fish were sampled by electric fishing every two weeks at 6 sampling sites from September 2003 till October 2004. During sampling 100 genetic samples were collected from gudgeon, bullhead and roach. Fish were fin clipped or PIT-tagged to investigate the influence of fragmentation on the fish population structure (species richness) and to demonstrate whether the siphons are passable for fish or not (gene dispersion). Afterwards, a second intensive linking survey at the siphon under the Albert canal was carried out. During two weeks fish were PIT-tagged or fin clipped and translocated downstream the siphon. Fyke nets were used to close up the river upstream and downstream the siphon.

The River Kleine Nete meanders strongly between the siphon under the Albert canal and the weir in Grobbendonk but is, at the same time, completely embanked. The brinks consist of stones, the bed of sand. The shore revetment exists of exuberant vegetation that only reaches the water level with high tide. At low tide sandbanks are visible at both sides of the river. The BBI (Belgian Biotic Index) is 8, which points to a good water quality. At the first fish migration barrier (from the North Sea), the weir in Grobbendonk, large amount of fish and many fish species (27) were caught. An explanation for this phenomenon is simple: all fish were blocked by the 1.50 m high weir. Only European eel sometimes climb up the wall near the weir (personal communication with the miller).

5838 fish of 29 species were collected. The most common species were roach (40%), European eel (15%), perch (12%), gudgeon (3%), stone loach (3%), bullhead (3%), and ide (3%), white bream (2%), and chub (2%). The presence of stone loach, bullhead and spined loach make it likely that the water quality of the Kleine Nete is good. Most fish were captured downstream the siphon under the Albert canal and downstream the weir in Grobbendonk (32% and 31% respectively; Figure 3.1.9). The species diversity per sampling site is comparable for all sites but the number of fish considerably varies. Roach is the most common species at all sites, followed by European eel. Four diadromous species were found in the River Kleine Nete: floun-

der, European eel, river lamprey and three-spined stickleback (*Gasterosteus aculeatus*). Only low numbers were caught of these species, nevertheless migration through the siphons could be confirmed as they appear at the weir in Grobbendonk, upstream both siphons. Only 3% fish were recaptured of which 2.5% was recaptured on the same spot. Migration of roach, perch (*Perca fluviatilis*), European eel, stone loach (*Barbatula barbatula*), bullhead and gudgeon (*Gobio gobio*) was observed but migration through the siphons only was seen for 14 fish (0.2%) of three species: roach, perch and gudgeon. It concerned mainly downstream migrations except for one roach (FL = 12.2 cm) that swam upstream and passed both siphons. A second detailed study showed 8 species swimming (N= 24) through the siphon (in upstream direction): perch, roach, gibel carp (*Carassius auratus*), stone loach, chub (*Leuciscus cephalus*), rudd (*Scardinius erythrophthalmus*), and tench (*Tinca tinca*). In general, the species distribution downstream and upstream the two siphons is comparable.

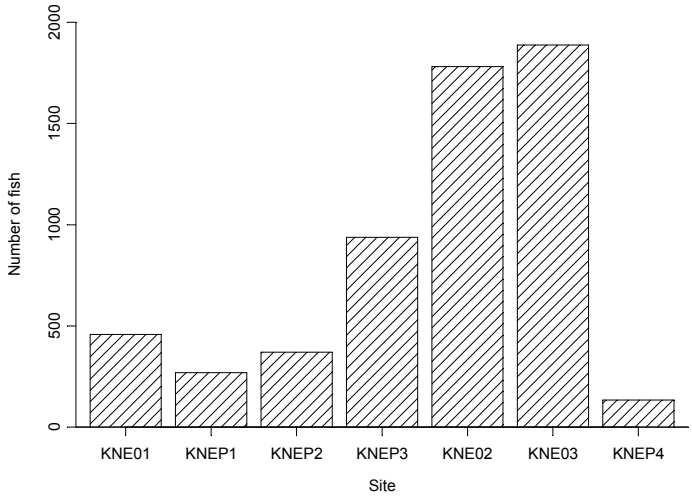

Figure 3.1.9: The number of fish caught at each capture site. Most fish were found at the siphon under the Albert canal and downstream the weir in Grobbendonk.

Mobility of three-spined stickleback (*Gasterosteus aculeatus* L.)

This species was sampled for genetical studies in lowland and upland rivers but none of the sites offered good conditions for a detailed investigation of fish mobility. Further studies on this small fish species will require a specific sampling methodology.

3.2. Ecophysiology

3.2.1. Swimming capacities

When comparing swimming performances in different sizes of flumes, results indicate that fish in longer swimming tunnels perform better than fish in small tunnels and that the major factor contributing to this finding are the longer periods of burst and glide

swimming and behaviour, as ground speed analysis indicated [33]. This effect became gradually more important with increasing fish size. It proves that absolute numbers such as U_{crit} must be applied with caution and that swimming sections in tunnels should be at least 3.5 Bl of the fish tested to obtain optimal performance.

Swimming performance and energy use were determined for the key species, being trout (*Salmo trutta*), three-spined stickleback (*Gasterosteus aculeatus*), gudgeon, (*Gobio gobio*), bullhead (*Cottus gobio*), roach (*Rutilus rutilus*) and some other abundant species such as common carp (*Cyprinus carpio*), stone loach *(Barbatula barbatula*) and perch (*Perca fluviatilis)*. Three-spined stickleback has a migrating (*trachurus*) and non-migrating morph (*leiurus*). They differ both in migrating behaviour and morphological characteristics. A more detailed study on the swimming capacity and energy use of these two morphs was performed simultaneous with the work presented here [86]. It allowed a comparison between a migratory morph and a non-migratory morph in the same geographical area, habitat, temporal window, species and even population and confirmes that the migrating morph in three-spined stickleback show better physiological adaptations to migration in terms of swimming performance and energy stores compared to the non-migrating morph (for details [86]).

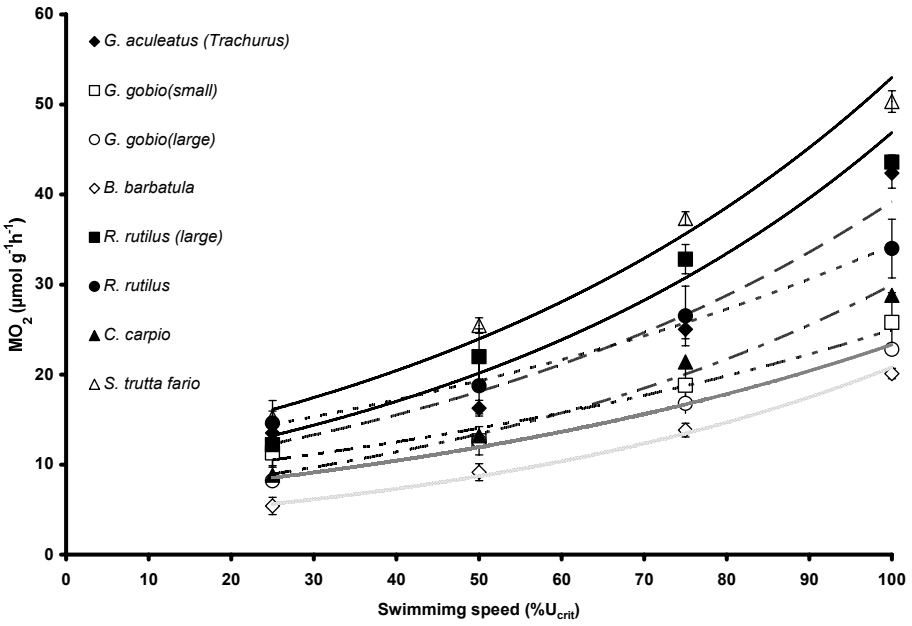

Figure 3.2.1: Oxygen consumption at different swimming speeds.

Energy expenditure, measured as oxygen uptake, at different swimming speeds is given in figure 3.2.1. Swimming speeds are presented as percentage critical swimming speed and oxygen uptake (MO_2) is given in µmol $g^{-1}h^{-1}$ in order to make values comparable across species. Thus energy expenditure is not given at the same absolute speed, but rather at the same level of effort for each species.

The active metabolic rate (AMR), represented by the oxygen uptake at U_{crit} and extrapolation to standard metabolic rate (SMR), scope for activity and optimal swim-

ming speed as percentage of scope of activity for *G.gobio*, *B.barbatula*, *R.rutilus*, *G. aculeatus* and *S. trutta* are presented in table 3.2.1.

Table 3.2.1: Standard metabolic rate (SMR), scope for activity and optimal swimming speed as percentage of scope for some European fish species.

	SMR ($\mu mol\ g^{-1}h^{-1}$)	**AMR** ($\mu mol\ g^{-1}h^{-1}$)	**Scope of Activity** ($\mu mol\ g^{-1}h^{-1}$)	**MO_{2opt}** **(%Scope of Activity)**
G. aculeatus (lei)	5.75±1.67	34.52±3.52	28.77±2.55	55.57±19.59
G. aculeatus (tra)	8.364±0.83	42.36 ± 1.67	39.60±3.26	59.21±7.47
C. carpio (small)	5.98±0.79	34.79±1.21	28.81±0.91	45.05±9.46
G. gobio (small)	7.88±1.53	25.79 ± 3.31	17.90±2.42	75.72±9.84
G. gobio (large)	6.1±0.72	22.8 ± 0.66	16.69±0.69	62.76±9.84
B. barbatula	3.64±0.94	20.1 ± 0.58	16.45±0.76	57.47±9.84
R. rutilus (large)	9.58±0.21	35.59 ± 0.77	26.00±0.49	63.36±9.84
R. rutilus (small)	8.79±2.46	34.0 ± 3.26	23.16±2.86	80.43±9.84
S. trutta fario	10.83±2.37	50.33±3.21	39.5±3.46	60.35±3.31

Critical swimming speed, optimal swimming speed, maximum swimming speed and Fulton condition index of the different species at different sizes and at different temperatures are presented in table 3.2.2. From these data it is obvious that temperature plays an important role in swimming performance, and thus capacity to cross barriers, in these species. 15°C seems to be an optimal temperature to maximise swimming capacity. When feasible, theoretical leaping lengths and heights were calculated from the maximal swimming performances obtained at 15°C. However, we did not calculate these data for the more bottom dwelling species such as *G. gobio*. Despite the fact that they showed high maximum swimming speeds, their ecological niche and behaviour makes it highly unlikely that they would perform high leaps at migration barriers.

Besides temperature, also the effect of ammonia was examined as an environmental factor that could affect swimming behaviour, especially fast starts. Fish fast starts are used for escape and predation and are therefore an ecologically important movement pattern. Escape response and predation strikes were investigated in brown trout (Salmo trutta fario) of 8 and 25 cm body length exposed to elevated (1 mg l-1) ammonia concentration for 24 and 96 hours. In C-starts, ammonia affected cumulative distance, maximum swimming speed and turning radius and directionality of escape

Table 3.2.2: Measured critical swimming speed, optimal swimming speed, maximum swimming speed and Fulton condition index of European fish, as well as theoretically calculated leaping lengths and heights.

Species	*C. gobio*	*G. gobio* small	*G. gobio* large	*B.barbatula*	*R.rutilus* small	*R.rutilus* large	*R.rutilus* very large
Body Length (cm)	7.43±0.93	9.96 ±0.29	12.3±0.35	7.16 ±0.48	4.60±0.16	7.36±0.33	15.74±1.55
U_{crit} ($cm.s^{-1}$)	---	54.16 ±2.02	60.17±1.17	28.25±0.32	45.78±2.1	59.45±1.27	110.75±6.71
U_{crit} ($BL.s^{-1}$)	---	5.43 ±0.27	4.89±0.61	3.94±0.20	9.95±0.24	8.11±0.38	7.04±0.76
U_{opt} ($cm.s^{-1}$)	---	47.09±2.41	51±2.05	18.46±4.47	30.93±6.61	41.49±13.07	---
U_{opt} ($BL.s^{-1}$)	---	2.26 ±0.02	2.08±0.03	4.94±0.32	4.06±0.07	3.68±0.14	---
U_{opt} (% U_{crit})	---	74.62±15.02	86.95±0.98	57.47±0.75	86.95±1.58	59.17±1.12	---
Fulton condition	1.35±0.02	1.48 ±0.03	1.42±0.06	1.40±0.15	1.58±0.02	2.81±0.20	1.48±0.07
U_{max} ($cm.s^{-1}$) 10°C	112.46±6.72	---	117.61±1.34	108.04±1.52	55.12±1.37	---	139.5±1.64
U_{max} ($BL.s^{-1}$) 10°C	15.13±1.94	---	9.56±1.45	15.09±1.63	11.98±0.98	---	8.86±1.43
U_{max} ($cm.s^{-1}$) 15°C	90.43±5.74	---	136.78±1.53	83.54±1.46	62.37±0.43	---	133.25±1.53
U_{max} ($BL.s^{-1}$) 15°C	12.17±2.54	---	11.12±1.74	11.66±1.35	13.55±0.52	---	8.46±1.73
U_{max} ($cm.s^{-1}$) 20°C	82.63±3.24	---	116.74±1.87	72.73±1.57	64.87±0.26	---	126.00±1.36
U_{max} ($BL.s^{-1}$) 20°C	11.12±4.24	---	9.49±1.64	10.16±1.67	14.10±0.72	---	8.00±1.86
Leaping length (m, α=60°, T=15°C)	---	---	---	---	0.17±0.03	---	0.78±0.21
Leaping height (m, α=60°, T=15°C)	---	---	---	---	0.14±0.03	---	0.67±0.21

Table 3.2.2 (continued).

Species	*S.trutta fario*	*C. carpio* small	*C. carpio* large	*C. carpio* very large	*P. fluviatilis* small	*P. fluviatilis* large	*G. aculeatus* (*trachurus*)	*G. aculeatus* (*leiurus*)
Body Length (cm)	7.84±0.02	4.875±0.08	10.72±0.22	22.76±3.92	10.11±0.16	17.83±0.44	5.37±0.14	5.21±0.12
U_{crit} ($cm.s^{-1}$)	65.43±0.54	43.31±2.15	62.30±4.15	87.09±5.24	80.56±1.5	113.04±1.37	44.60±0.68	35.42±0.22
U_{crit} ($BL.s^{-1}$)	8.34±0.49	9.24±0.71	5.83±0.59	3.90±0.54	7.97±0.16	6.34±0.12	8.33±0.24	6.80 ± 0.21
U_{opt} ($cm.s^{-1}$)	31.64±0.53	30.59±4.36	---	---	---	---	25.72±3.89	22.01 ± 4.91
U_{opt} ($BL.s^{-1}$)	4.03±0.52	3.59±0.23	---	---	---	---	4.61±0.12	4.16 ± 1.04
U_{opt} (% U_{crit})	48.35±0.34	62.11±2.13	---	---	---	---	64.93±0.74	62.14±0.74
Fulton condition	1.68±0.53	2.96±2.20	2.94±0.85	2.01±0.32	1.04±0.42	1.93±0.34	2.11±0.17	1.72 ± 0.14
U_{max} ($cm.s^{-1}$) 10°C	125.86±0.58	---	98.43±0.42	126.25±1.45	---	---	78.32±1.74	---
U_{max} ($BL.s^{-1}$) 10°C	16.05±0.36	---	9.18±0.52	5.55±1.21	---	---	14.58±1.54	---
U_{max} ($cm.s^{-1}$) 15°C	93.74±0.38	---	103.42±0.34	134.23±1.52	---	---	82.68±1.53	59.28±1.23
U_{max} ($BL.s^{-1}$) 15°C	11.95±0.28	---	9.64±0.32	5.89±1.32	---	---	15.39±1.34	11.38 ± 1.67
U_{max} ($cm.s^{-1}$) 20°C	---	---	97.34±0.63	125.42±1.25	---	---	77.78±4.20	---
U_{max} ($BL.s^{-1}$) 20°C	---	---	9.08±0.37	5.51±1.45	---	---	14.30±1.54	---
Leaping length (m, α=60°, T=15°C)	0.39±0.02	---	0.47±0.03	0.79±0.01	---	---	0.30±0.03	0.15±0.05
Leaping height (m, α=60°, T=15°C)	0.33±0.09	---	0.40±0.03	0.68±0.01	---	---	0.26±0.03	0.13±0.04

performance was at random after 96 hours of exposure. Predation strikes also were affected. Distance, speed and turning radius were significantly different between exposed and non-exposed fish. Predator behaviour was also altered and the number of captured prey was reduced. Thus, ammonia exposure affects brown trout escape response mainly through impairment of directionality and fast start velocity. In addition to reduced strength of response, ammonia exposure may reduce fish elusiveness facing a predator.

3.2.2. Leaping experiments

In 2004, the overall success ratio was very low. The ratio of success was higher at smaller leaping heights. The most successful experiments seemed to occur at around 0.9 to 1.2 $m.s^{-1}$ water velocity on top of the weir, which was the maximum speed tested. The different tested pool depths of 0.4 m and 0.6 m downstream of the weir did not seem to have great influence on the success ratio. A more detailed statistical factor analysis of the results of 2004 only showed a significant effect of the stream velocity on general success ratio for all species, divided into classes of 0.2 $m.s^{-1}$ ($p = 0.0131$). Also for ide ($p = 0.0003$), especially the smaller ones (5-15 cm) ($p = 0.0008$) a signifficant effect occured. The rectangular weir seemed to improve passage success in general ($p = 0.056$), probably because the narrow angle of the 2004 V-shaped weir resulted in water speeds that were too high for the fish to pass. Leaping height significantly influenced rudd success ratio ($p = 0.0066$), especially for small rudd (5-15 cm) ($p = 0.0058$). With the plunge pool depths used within this experiment, we did not observe a significant contribution to passage success.

Because of the low general response of the different species tested, we found no significant differences in passage success ratio between the used species. Tench never leaped successfully. Rudd and ide were more successful, although mean success ratio was still very low. Gudgeon and gibel carp hardly had any successful leap. Gudgeon only reached leaping heights of 0.05 m and they never leaped successful over the V-shaped weir. Gudgeon and gibel carp only used the deepest pool for successful leaping, possibly indicating that this was a minimal pool depth. The leaping capacity of rudd and gibel carp seemed to be limited at 0.15 m. Within the species (i.e. between different length classes) only a few interesting trends could be noticed, without being significant ($p \geq 0.1$). Focusing on rudd, the smaller ones (5-15 cm) prefered leaping heights of 0.05 m. For ide, stream velocities of about 1 $m.s^{-1}$ improved passage success of especially small ide species (5-15 cm). Small gibel carp (5-15 cm) never passed the V-shaped weir.

In 2005 stream velocity above the weir was changed to 0.8 - 1.2 $m.s^{-1}$ since this was a significant contributing factor in 2004. This was the maximum feasible velocity in the flume. Pool depth was changed to 0.2 and 0.4 m. The V-shaped weir was changed into a slighter V-shape, conform recent applications in the field. The overall passage success ratio of the experiments in 2005 was again low but slightly better

than in 2004. In contrast with 2004, the shape of the weir does not seem to influence passage success, indicating that the narrow angle of the V-shaped weir in 2004 caused indeed a problem. The ratio of success is again higher at smaller leaping heights. A more detailed analysis of the results of the 2005 experiments for factors shape of the weir and velocity (classes of 0.2 $m.s^{-1}$) indicated that neither of these 2 factors influenced the passage success ratio significantly ($p \leq 0.05$) for the 3 fish species that were used. The leaping height had a significant influence on passage success of gibel carp ($p = 0.0149$) and of small common carp ($p = 0.0417$). Significant differences in success ratio between the used species were not found since the general response of the different tested species was low.

Stream velocities over 1.2 $m.s^{-1}$ limited a successful passage of common carp. Within the species (i.e. between different length classes) some interesting non significant trends could be noticed. Small roach (5-15 cm) were less successful leapers ($p = 0.0618$) in general. They never used the V-shaped weir and only passed when water velocities were below 1.0 $m.s^{-1}$. Focusing on gibel carp, smaller individuals (5-15 cm) did not have successful leaps, even at 0.05 m, while the maximum achievable leaping height for the larger ones (15-25 cm) was limited to 0.15 m. For common carp, leaping heights from 0.15 m and higher seemed to limit a successful passage.

3.3. Genetics

3.3.1. Regional population structure and genetic diversity

The genetic diversity and differentiation indices for bullhead (*Cottus gobio*), roach (*Rutilus rutilus*), three-spined stickleback (*Gasterosteus aculeatus*) and brown trout (*Salmo trutta*) are summarized in Table 3.3.1.

Bullhead and roach populations from Scheldt and Meuse basin strongly differentiated with pairwise Fst of 0.24 and 0.22 respectively. Individuals from both basins form distinct clusters in the factorial correspondence analysis of the multilocus genotypes (Figure 3.3.1). In both species the observed heterozygosity in Meuse and Scheldt was very similar, approximately 0.70 for roach and 0.42 for bullhead. In case of roach the mean number of alleles (MNA) and allelic richness (AR) was substantially higher in the Scheldt (MNA= 15.50; AR= 16.20) compared to the Meuse (MNA= 11.83; AR= 14.70). In bullhead the Meuse was the most diverse in terms of alleles. In the latter basin we observed on average 21.85 alleles per locus (AR= 16.91) whereas this was only 12.77 (AR= 12.02) in the Scheldt basin.

Table 3.3.1: Summary of the genetic diversity and differentiation indices for the four target species: bullhead (Cottus gobio), roach (Rutilus rutilus), three-spined stickleback (Gasterosteus aculeatus) and brown trout (Salmo trutta) for the Scheldt and Meuse basin.

	Observed het-ero-zygosity (Ho)	Mean number of alleles (MNA)	Allelic rich-ness (AR)	overall Fst	N	# loci
Gasterosteus aculeatus						
Scheldt	0.734	27.5	27.4	0.123	1266	6
Rutilus rutilus						
Scheldt	0.680	15.5	16.2	0.006	737	8
Meuse	0.717	11.8	14.7	0.033	530	8
Cottus gobio						
Scheldt	0.433	12.8	12.0	0.219	389	13
Meuse	0.420	21.8	16.9	0.109	1009	13
Salmo trutta						
Meuse	0.599	8.7	7.6	0.073	1277	7
Hatchery	0.674	7.0	7.0	0.027	357	7

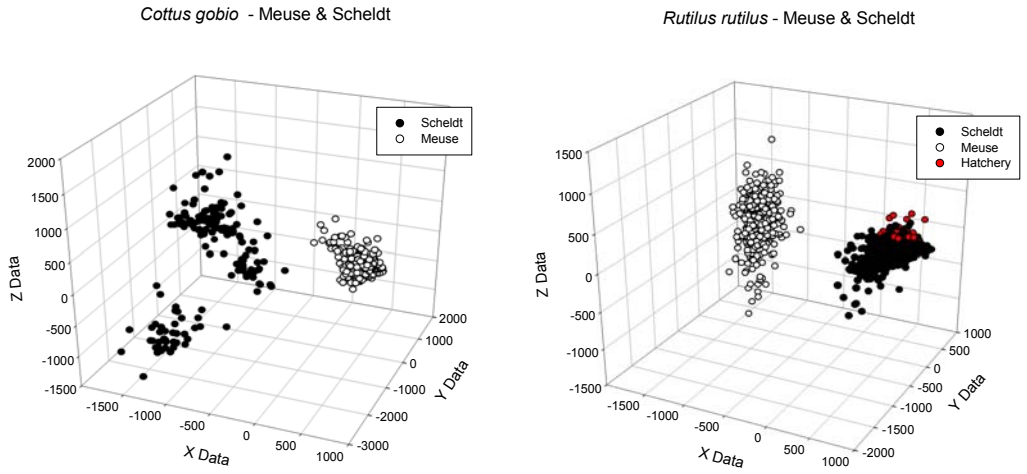

Figure 3.3.1: Factorial correspondence analysis of the multilocus genotypes of bullhead (*Cottus gobio*) and roach (*Rutilus rutilus*) from the Meuse and Scheldt basin.

Within the Scheldt basin the overall differentiation for stickleback, bullhead and roach was very different (Table 3.3.1). The overall Fst values of bullhead and stickelback, respectively 0.22 and 0.12, suggest that both species are structured within the Scheldt basin. The overall differentiation for roach (0.006) suggests a single panmictic gene pool for the Scheldt River.

The different genetic structure is illustrated by the factorial correspondence analysis in Figure 3.3.2.

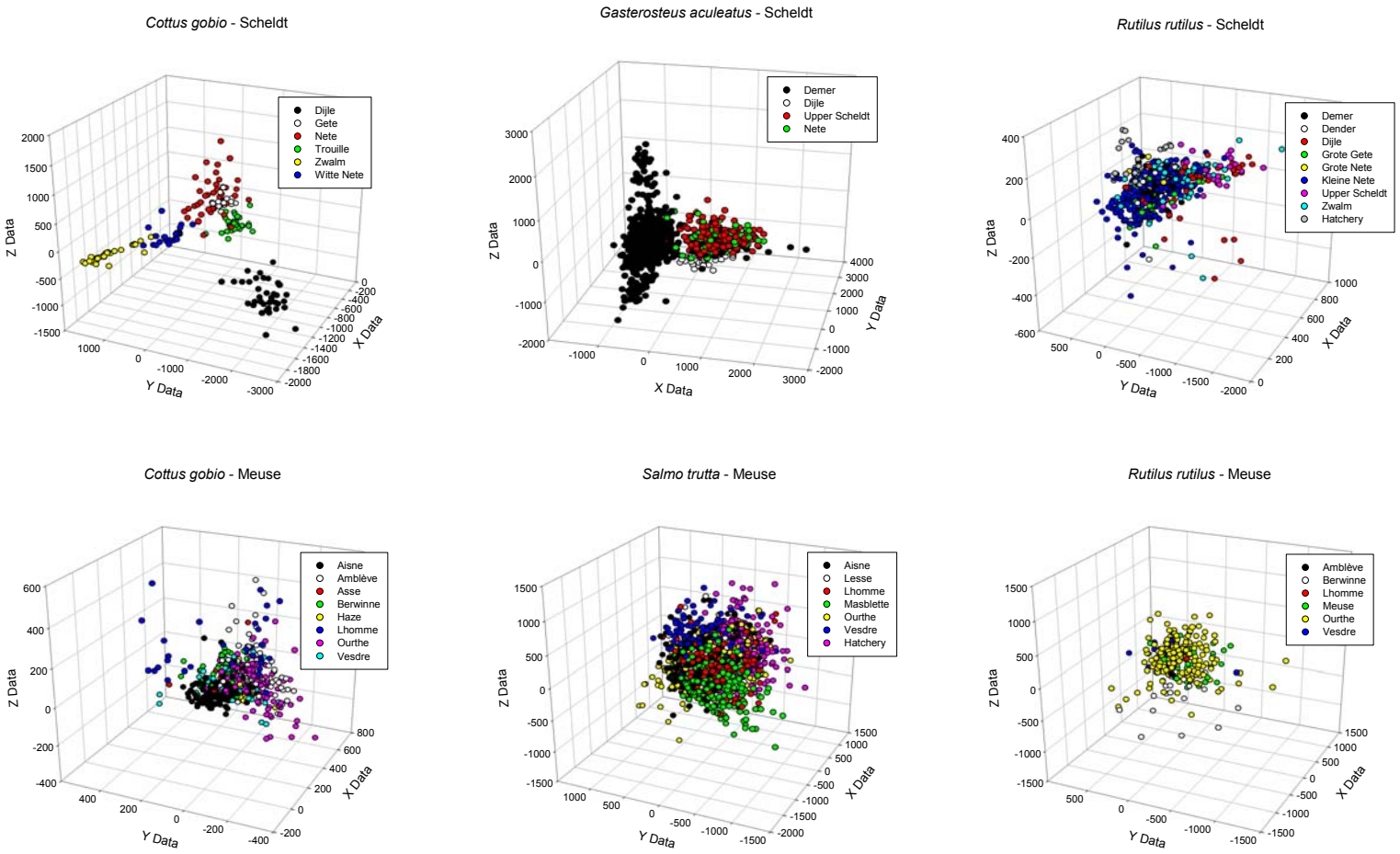

Figure 3.3.2: Factorial correspondence analysis of the multilocus genotypes from the four target species: bullhead (*Cottus gobio*), roach (*Rutilus rutilus*), three-spined stickleback (*Gasterosteus aculeatus*) and brown trout (*Salmo trutta*) for the Meuse and Scheldt basin.

Bullhead displays substantially lower genetic diversity indices compared to roach and stickleback. The latter species is the most diverse, as well in terms of heterozygosity as in terms of number of alleles. In the Meuse basin the Fst values (Table 3.3.1) point out that bullhead (Fst = 0.11) and brown trout (Fst = 0.07) are clearly structured. For roach we observe an Fst of 0.03. Although this is substantially lower than for the other two species in the Meuse basin it is almost an order of magnitude higher that the genetic differentiation observed for roach in the Scheldt basin.

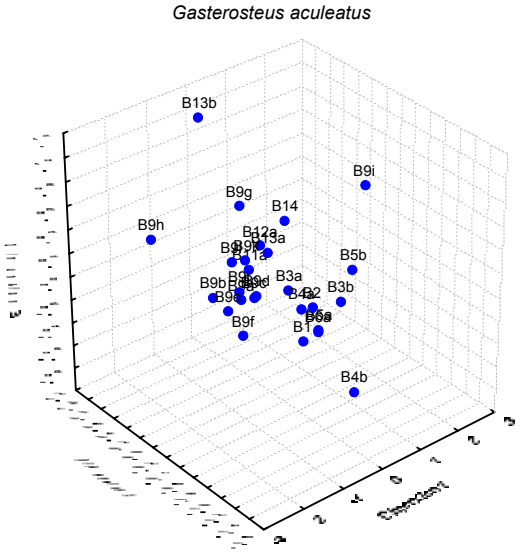

Figure 3.3.3: Multidimensional scaling (MDS) plot based on pairwise Reynolds genetic distances between populations of *Gasterosteus aculeatus* from the Scheldt basin.

In three-spined stickleback the mean allelic richness was maximal (10.33) in B4a, the most downstream population, and minimal (3.46) in B9i, one of the most upstream populations. The decrease was significantly related to the geographic distance along waterways starting from B4a (r= -0.62; P= 0.0029).

Observed and expected heterozygosity, both ranging between 0.43 and 0.83, revealed a similar decrease from downstream to upstream populations. There was no evidence for systematic scoring errors according to MICROCHECKER. Evidence of recent population decline was found in populations B9g, B13a and B13b according to the least conservative TPM. B13a and B13b are located upstream, but only B13b originates from a small tributary. Overall genetic differentiation was high (F_{ST} = 0.15). Only two of the 210 pairwise F_{ST} values were not significant after Bonferroni correction (B9a vs. B9j - F_{ST} = 0.010; B12a vs. B13a - F_{ST} = 0.008). A MDS plot of pairwise Reynolds genetic distances (Figure 3.3.3) shows that each of the upstream population B4b, B5b, B9h, B9i and B13b was highly differentiated from a cluster grouping the three most downstream populations (B4a, B5a and B6a) and a cluster grouping a mix of downstream and upstream populations.

In the Scheldt drainage overall genetic differentiation between roach populations was very low (F_{ST} = 0.006) and many pairwise F_{ST} values were not significant. A MDS plot of pairwise Fst values (Figure 3.3.4) shows the differences between the investigated samples. The Nete samples cluster tightly together with DEM02, DEN01, ZWA01 and GGE04. However, the position of the latter is not reliable since it was only based on 4 individuals. The differentiation between samples from the Kleine and Grote Nete (KNE & GNE) were not significant after Bonferroni correction. However the differentiation between GNE04 and the samples from the Kleine Nete was more pronounced than the differentiation among KNE samples. All other samples, including the hatchery sample (VFF03), displayed a low but significant differentiation.

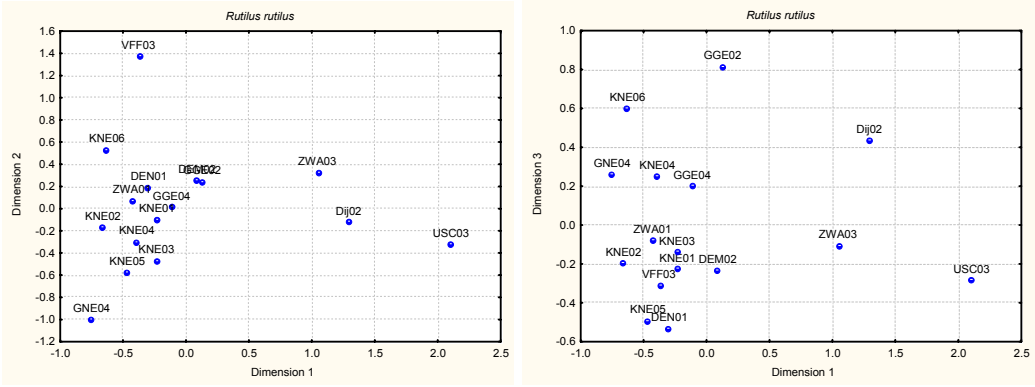

Figure 3.3.4: Multidimensional scaling (MDS) plot based on pairwise Fst values between populations of *Rutilus rutilus* from the Scheldt basin.

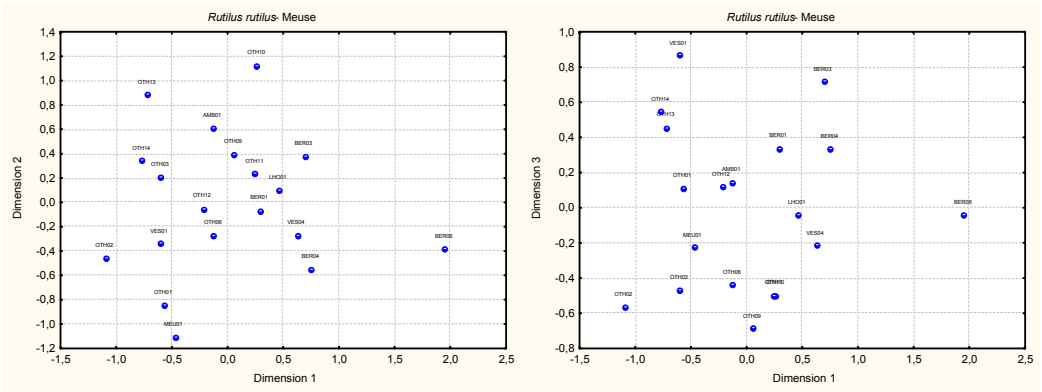

Figure 3.3.5: Multidimensional scaling (MDS) plot based on pairwise Fst values between populations of *Rutilus rutilus* from the Meuse basin.

In the Meuse drainage the overall genetic differentiation was five times higher then in the Scheldt drainage (F_{ST} = 0.03). However due to low sample sizes most pairwise comparisons showed no significant differentiation. For the other samples pairwise Fst is maximal 0.04 and if not zero mostly significant. A MDS plot of pairwise Fst values (Figure 3.3.5) shows the differences between the investigated samples.

For bullhead, overall genetic differentiation in the Scheldt drainage was high (F_{ST} = 0.22). Only the pairwise F_{ST} values between the samples from the Kleine and Grote Nete (KNE & GNE) were not significant after Bonferroni correction. A MDS plot of

pairwise Fst values (Figure 3.3.6) shows the differences between the investigated samples. The Nete samples, except for the upstream populations WNE, cluster tightly together. All other populations were strongly differentiated. The lowest significant differentiation was observed between ZWA13 & VER (Fst = 0.13). The remaining significant pairwise Fst values were all higher than 0.2, with a maximal differentiation between ZWA13 & GGE (Fst = 0.69).

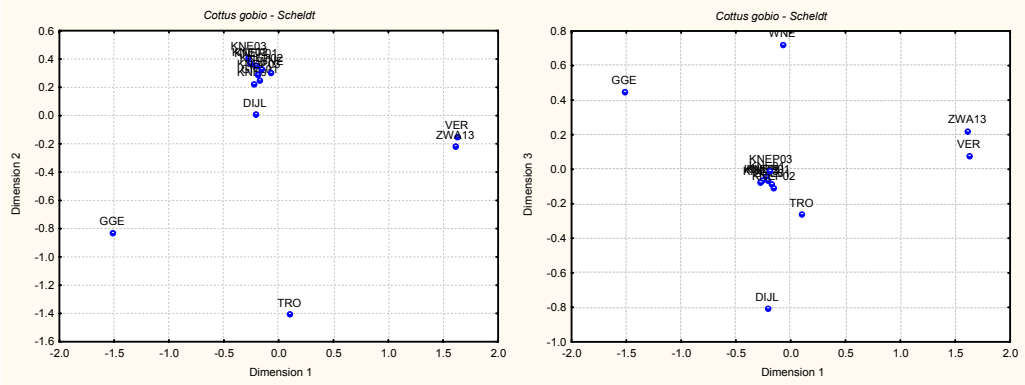

Figure 3.3.6: Multidimensional scaling (MDS) plot based on pairwise Fst values between populations of *Cottus gobio* from the Scheldt basis.

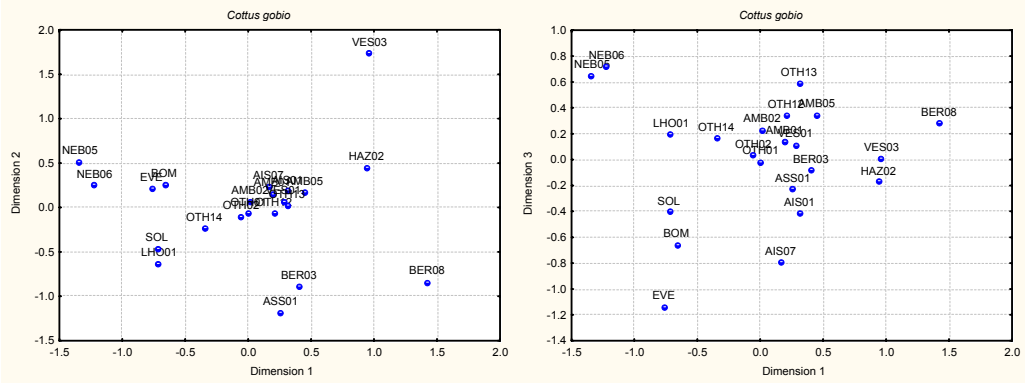

Figure 3.3.7: Multidimensional scaling (MDS) plot based on pairwise Fst values between populations of *Cottus gobio* from the Meuse basis.

In the Meuse drainage the overall genetic differentiation was lower then in the Scheldt drainage (F_{ST} = 0.11), but most pairwise comparisons showed significant differentiation. Except among samples from the Amblève (AMB), Neblon (NEB) and between samples BER03 & ASS01, OTH01 & OTH02 and OTH12 & OTH13. A MDS plot of pairwise Fst values (Figure 3.3.7) shows the differences between the investigated samples.

Highly significant genetic differentiation was observed among brown trout populations with an overall F_{st} value of 0.07. Pairwise F_{st} values ranged from 0 to 0.21. In three situations, neighbouring pairs of populations from the same river system were not significantly different, suggesting that these sites form a unique population. We decided to aggregate these samples (in the Ourthe river, OCM, OHO, OPC → ODO;

OBW, OJU, ONV → OUP; ORO, OFA, OER, OAG → AIS). Pairwise F_{st} integration of these aggregations is shown in Table 3.3.2.

For each river population, a pairwise F_{st} with the reference hatchery was calculated. This genetic differentiation between hatcheries and river populations was weaker for the Vesdre River (0.01 to 0.03) and the Ourthe River (from 0.01 to 0.07) whereas the Masblette River was more genetically distinct from the hatcheries (0.11 to 0.16).

The number of alleles per locus ranged from 6 to 13. $H_{e.}$ ranged from 0.52 to 0.71, H_{obs} from 0.50 to 0.74 and MNA from 3.46 to 5.67 (Table 3.3.2). Over all populations, the highest values of $H_{e.}$ were observed for hatchery samples (HMR, HGE, HJA). The lowest values of $H_{e.}$ were observed for sites of the Masblette basin, i.e., the non-restocked sites, whereas sites of the Ourthe and Vesdre river (intensively restocked and recolonized sites respectively) display the highest values of $H_{n.b.}$ among the river sites.

For rivers, the F_{is} per population was significantly different from 0 for four of the 19 populations of river (OJZ, AIS, OUP and LFO, from 0.05 to 0.9) due to a heterozygote deficiency (Table 3.3.2). A major part of the dataset structure is concentrated along the first axis of the factorial correspondence analysis (Figure 3.3.9). This axis is correlated with an admixture gradient from the non-restocked sites to the hatcheries.

Table 3.3.2: List of the river sites and hatchery sites (with the number of individuals collected and the sampling year); Diversity measures for each population (ODO, OHO and OPC forming a single population): expected and observed heterozygosity (H_{exp}, H_{obs}), unbiaised estimate of expected heterozygosity ($H_{n.b.}$), mean number of alleles per locus (MNA) and F_{IS} (P<0.05 = *, P<0.01 = **, P<0.001 = ***). The river sites are ranked by basin (Ourthe, first letter of the code O and then Lesse, first letter of the code L) and within each river, sites are classified from downstream to upstream position. The restocking intensity (RI) is given for each site and when that there is aggregate, RI is the average of the RI of the river samples.

Code		Site	River	N	Year	RI		H_{exp}	$H_{n.b.}$	H_{obs}	Na	Fis	
River samples													
OCM		Colonster	Ourthe	40	2003	0.7							
OHO	ODO	Hony	Ourthe	25	2003	1.5	1	0.66	0.67	0.64	5.31	0.04	
OPC		Poulseur	Ourthe	59	2003	0.8							
OHA		Hamoir	Ourthe	50	2003	0.4	0.4	0.65	0.66	0.66	5.06	0.00	
OBS		Bomal-Sy	Ourthe	64	2003	0.4	0.4	0.66	0.67	0.66	4.90	0.02	
OBW		Bardonwez	Ourthe	20	2003	0.1							
OJU	OUP	Jupille	Ourthe	36	2003	0.1	0.23	0.66	0.66	0.62	5.21	0.07	**
ONV		Nisramont1	Ourthe	49	2003	0.5							
ONM		Nisramont2	Ourthe	50	2003	0.4	0.4	0.62	0.62	0.63	5.01	-0.01	
OMC		Moircy	Ourthe	42	2003	0.2	0.2	0.64	0.65	0.61	4.97	0.06	
OFR		Fraipont	Vesdre	47	2003			0.64	0.65	0.65	5.05	-0.01	
ONE		Nessonvaux	Vesdre	36	2003			0.65	0.66	0.63	5.57	0.06	
OBO		Bomal	Aisne	49	2003	0.3	0.3	0.66	0.67	0.64	5.50	0.04	
OJZ		Juzaine	Aisne	50	2003	0.3	0.3	0.65	0.66	0.60	5.10	0.09	**
ORO		Aux Roches	Aisne	49	2003	0.3							
OFA		Franzel	Aisne	50	2003	0.3							
OER	AIS	Erezée	Aisne	49	2003	0.4	0.35	0.61	0.61	0.58	4.54	0.05	**
		Ammonine											
OAG		gué	Aisne	49	2003	0.4							
LRC		Lesse	Lesse	52	1998	0.5	0.5	0.61	0.61	0.56	5.08	0.09	*
LFO		Forrières	Lhomme	50	2003			0.66	0.67	0.65	5.32	0.03	
LGO		Gobaille	Lhomme	50	2003	0.5	0.5	0.66	0.67	0.66	5.33	0.01	
LMY		Masblette1	Masblette	50	2003	0	0	0.61	0.61	0.62	3.91	-0.01	
LMZ		Masblette2	Masblette	36	2003	0	0	0.56	0.57	0.56	3.85	0.02	
LDV		Donneuse1	Masblette	32	2003	0	0	0.58	0.59	0.54	3.57	0.08	
LPB		Pied de Bœuf	Masblette	45	2003	0	0	0.57	0.57	0.56	3.75	0.02	
LFA		Falgaude	Masblette	50	2003	0	0	0.52	0.52	0.50	3.46	0.05	
River baseline populations													
		Louba											
OGI		Gileppe	Vesdre	49	2001			0.39	0.39	0.39	3.13	0.00	
LDO		Donneuse2	Lhomme	49	2005			0.55	0.55	0.55	3.23	0.00	
OGI+LDO				98				0.54	0.54	0.47	3.57	0.13	***
REF				400				0.54	0.54	0.54	3.52	0.00	
Hatcheries													
HMR		Mirwart hatchery		50	2001			0.70	0.71	0.74	5.47	-0.05	
HGE		La Gernelle hatchery		50	2002			0.69	0.70	0.70	5.12	-0.01	
HJA		Rossart hatchery		50	2002			0.68	0.69	0.70	5.34	-0.01	
HFR		Freux hatchery		61	2002			0.66	0.67	0.67	5.58	-0.01	
HAC		Achouffe hachery		50	1995			0.68	0.68	0.67	4.84	0.01	
HBL		Blandiaux hatchery		48	2004			0.56	0.57	0.56	4.51	0.02	
HCO		Compère hatchery		48	2004			0.63	0.64	0.67	4.76	-0.04	
7 HAT				357				0.69	0.69	0.67	5.70	0.02	
REF				400				0.68	0.68	0.69	5.67	-0.01	

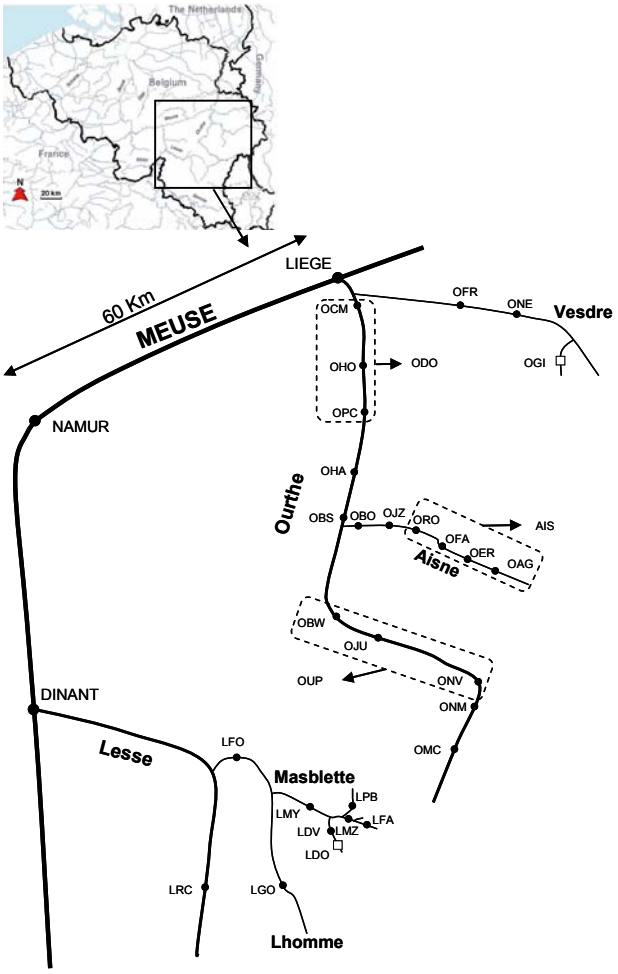

Figure 3.3.8: Schematic drawing of the prospected area. River samples are displayed as circles (●) and river baseline as rectangles (□). Box dotted indicate the sites which are gathered in a population because their genetic differentiations are non-significant.

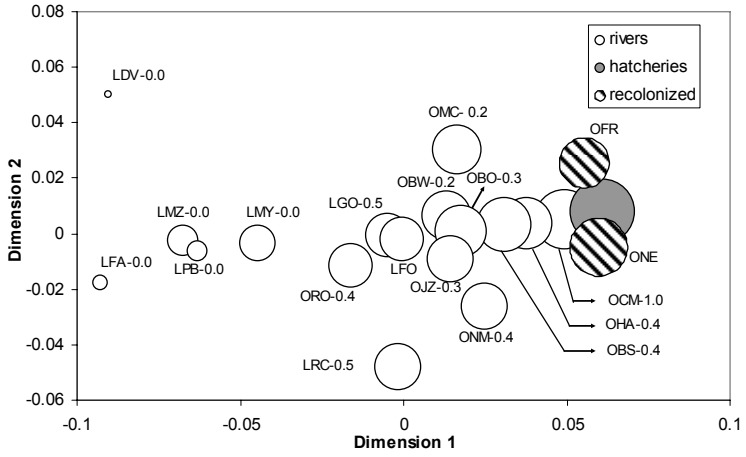

Figure 3.3.9: Plot of the multidimensional scaling analysis of Reynolds' distances between populations. The populations are marked according to their AC (area of the bubbles) and their RI (figure after the acronym) (dimension 1: 43.2% of variance, dimension 2: 2.3% of variance).

3.3.2. Estimation of the impact of restocking

Due to the low overall differentiation of roach populations and the non significant differentiation of hatchery fish from wild fish it was not possible to assess the impact of restocking activities for this species. As such this analysis was only carried out for brown trout. The impact of restocking was estimated at the individual level (IAC) and at the population level (AC and AP) (Figure 3.3.10 and Table 3.3.3). We used STRUCTURE to estimate the individual admixture coefficients (IAC) defined in the present study as the proportion of the genome of a fish derives from hatchery trout [71]. The Bayesian inference approach attempts to group individuals into clusters on the basis of their genotypes, minimising linkage and Hardy-Weinberg disequilibrium between loci within each cluster, while simultaneously estimating each cluster's alleles frequencies.

LEADMIX, a Fortran program based on the likelihood method developed by [87], is used to estimate the admixture proportion (AP) defined in the present study as the proportion of contemporary gene pools derived from hatchery gene pool.

IAC makes it possible to visualize the composition of each population, i.e. to know the type of fish which composes it and to estimate a population level of admixture, AC by calculating the mean of the IAC by population. On the level of the population, AC is thus the direct effect of the restocking. AP is used like control AC.

Estimations of IAC were performed separately for each population. Individual admixture coefficients are estimated assuming a model with two populations (K=2). We considered that fishes from the reference hatchery have a known origin (popflag set to one), as well as the fish from the reference river whereas the fish from the population to be tested have an unknown origin (popflag set to zero). This way, the model considers the hatchery fish and the reference river fish as "pure hatchery fish type", and "pure indigenous fish type" respectively i.e., non-admixed individuals, and river fish as potentially admixed individuals. Estimated IAC and their 90% probability intervals were calculated using a burn-in period of 50.000 steps followed by 200.000 MCMC replicates, which was considered as sufficient after preliminary tests. As populations may be closely related, the option "correlated alleles frequencies" was chosen [73].

In order to assess the power of STRUCTURE for identifying pure hatchery type fish, pure river type fish and admixed individuals, we considered a theoretical population composed of 50 individuals of reference river, 50 individuals of reference hatchery and 50 hybrids simulated of two references, ie the simulated hybrids (F1) were generated using the program HYBRIDLAB [30]. This way, the so-constituted theoretical population encompasses the three types of fish that we may expect in our dataset, all of known origin. AP was calculated by using the river reference and the hatchery reference used in STRUCTURE to estimate individual admixture coefficient (IAC) in order to be able to compare AP with AC.

The results of individual admixture coefficients for the theoretical population proved the ability of the STRUCTURE program to distinguish between river fish (IAC<0.15), hatchery fish (IAC>0.80) and hybrid fish (0.15<IAC<0.80). The admixture coefficient (AC) by population is calculated by averaging the IAC per population (Table 3.3.3). Another estimate of the admixture at the population level is the admixture proportion (AP) based on the method proposed by Wang [87] (Table 3.3.3). For example, the AP for LRC is 0.49, i.e. 51% of the present population being of river origin or 49% ascribed to hatchery origin. The AC of the same river is 0.50. Both AC and AP are correlated with RI, r=0.82 (P<0.0001) and r=0.74 (P=0.0011) respectively. The correlation may be distorted by the extreme values ODO, but the correlation between AC and RI remains of 0.81 (P=0.0003) if ODO is removed. For a given range of RI, the AC is higher in the most downstream rivers (Figure 3.3.10). When RI is close to zero, the AC is below 0.11 except for LMY and LMZ which are populations on the main course of the Masblette. Their AC is higher due to the presence of many hybrids in the population. A individual of hatchery type is also identified in LMZ.

Table 3.3.3: Population level of admixture: % of fishes assigned to river = % river (i.e. fishes with IAC<0.15), to hatchery = % HAT and to hybrid = % hyb, the AC (mean de IAC) and admixture proportion AP (estimated with Leadmix). The restocking intensity = RI and N = size's population.

			LEADMIX	STRUCTURE				GENETIX			Fstat
Populations	N	RI	AP	AC	% river IAC<0.15	% HAT IAC>0.80	% hyb.	Hnb	Fis		Na
ODO	124	1.00	0.91	0.82	0	74	26	0.67	0.04		5.31
LRC	52	0.50	0.49	0.50	0	0	100	0.61	0.09	*	5.08
LGO	50	0.50	0.48	0.48	0	0	100	0.67	0.01		5.33
OHA	50	0.40	0.75	0.66	2	28	70	0.66	0.00		5.06
OBS	64	0.40	0.78	0.69	3	39	58	0.67	0.02		4.90
ONM	50	0.40	0.61	0.53	0	0	100	0.62	-0.01		5.01
AIS	197	0.35	0.21	0.45	0	0	100	0.61	0.05	**	4.54
OBO	49	0.30	0.70	0.61	0	14	86	0.67	0.04		5.50
OJZ	50	0.30	0.61	0.54	0	4	96	0.66	0.09	**	5.10
OUP	105	0.23	0.65	0.55	0	2	98	0.66	0.07	**	5.21
OMC	42	0.20	0.71	0.56	0	0	100	0.65	0.06		4.97
LMY	50	0.00	0.25	0.32	6	0	94	0.61	-0.01		3.91
LMZ	36	0.00	0.16	0.25	22	2	76	0.57	0.02		3.85
LDV	32	0.00	0.15	0.02	100	0	0	0.59	0.08		3.57
LPB	45	0.00	0.18	0.11	82	0	18	0.57	0.02		3.75
LFA	50	0.00	0.13	0.07	88	0	12	0.52	0.05		3.46
OFR	47	recolonized	0.76	0.58	0	0	100	0.65	-0.01		5.05
ONE	36	recolonized	0.82	0.75	3	47	50	0.66	0.06		5.57
LFO	50	/	0.46	0.47	2	8	90	0.67	0.03		5.32

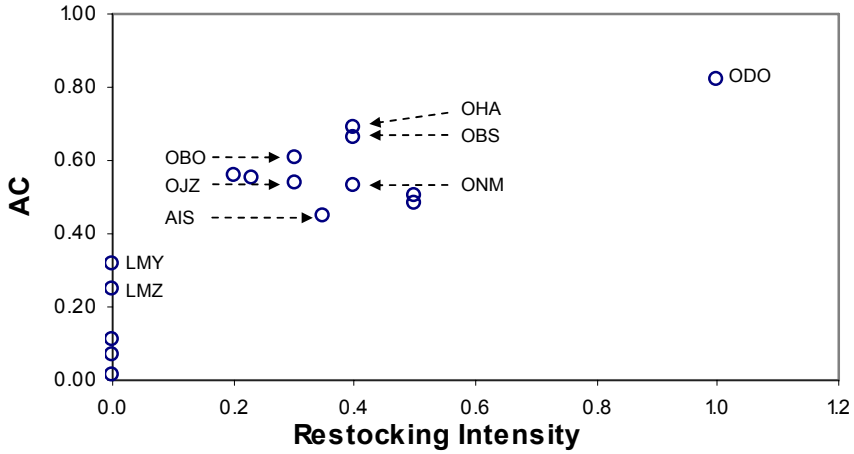

Figure 3.3.10: The relationship between the admixture coefficient (AC) and the restocking intensity (RI).

Our estimate of RI doesn't take into account the effect of selection on the restocked fish. In comparison, AC is a good indicator of the actual level of RI, integrating the initial level restocking (that was estimated by RI) and the impact of selection. AP is an estimator of the same type but as AC and AP are highly correlated ($r=0.93$, $P<0.0001$) and AP is not based on individual information, we will use AC as an estimate of the actual pressure of restocking in the rest of the text.

3.3.3. Geographical analysis of disruption of connectivity

The effects of migration barriers were assed for stickleback in the Demer River and for brown trout in the Masblette River for which the previous genetic analysis could not detect impact of restocking on the local genetic structure.

Three-spined stickleback

All geographic features and barrier characteristics showed significant simple correlations with allelic richness. Allelic richness strongly decreased with the total number of barriers ($r = -0.86$; $P < 0.0001$). Among barrier types, the correlation with the number of weirs was highest ($r = -0.82$; $P < 0.0001$). Upstream distance was positively linked to allelic richness ($r = 0.75$; $P < 0.0001$). Control for upstream distance did not affect the significance of the relation between total number of barriers and allelic richness. This was also the case for mills and weirs, but not for tunnels and sluices. The correlation of habitat width and allelic richness was positive but weak (partial correlation: 0.33; $P > 0.05$). Correlations between geographic features and the genetic differentiation with the most downstream population (B4a) were in general lower.

All geographic features and barrier types, except the number of sluices and tunnels, were significantly correlated with pairwise F_{ST}, and remained significant after control for effective geographic distance. F_{ST} strongly increased with the total number of barriers ($r = 0.70$; $P = 0.0003$). Among barrier types, the correlation with the number of

weirs (r = 0.68; P = 0.001) was higher than with mills (r = 0.53; P =0.0051). Pairwise average habitat width (r = -0.57; P = 0.0002) and pairwise average upstream distance (r = -0.37; P = 0.0146) were negatively associated with pairwise F_{ST}. Interestingly, variability in pairwise F_{ST} increased significantly with barrier height and the number of weirs and mills, and decreased significantly with habitat width.

Ninety-three individuals (9.3%) were identified as dispersers, and were detected between just 25% of all population pairs. Due to such low resolution, dispersal was considerably less explained by geography than pairwise F_{ST}. Main limitations to dispersal were geographic distance, the total number of barriers and the number of weirs.

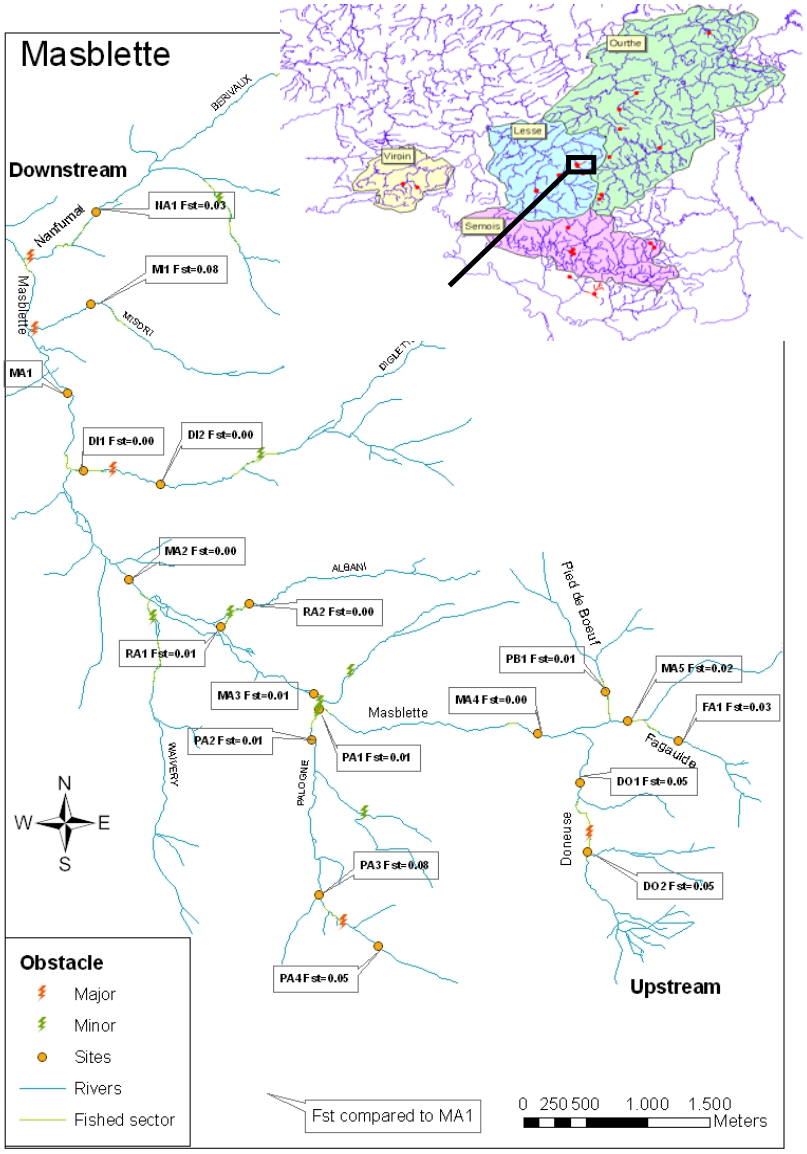

Figure 3.3.11 : Map of Masblette basin.

Brown trout

A factorial correspondence analysis-representation (FCA) gives an overall view and general information about the genetic differentiation between populations (Figure 3.3.12). The two first axes of the factorial correspondence analysis encompass 45.29% of the inertia of the data matrix. Most of the sites have a central position except 6 sites (DO1, DO2, NA1, PA3, PA4, MI1). The population of Misdri (MI1) and the two populations of Doneuse (DO1, DO2) are the more distinct. In the populations, the number of individuals sampled is very small.

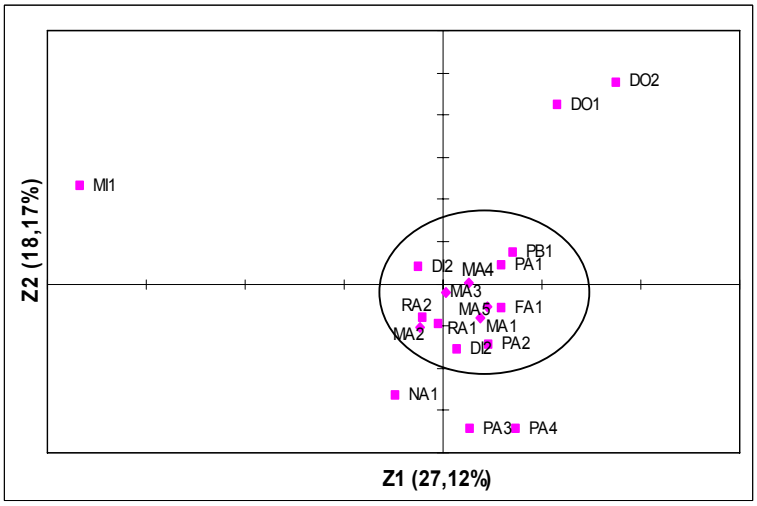

Figure 3.3.12: Factorial correspondence analysis-representation of the populations on the first two axes of the analysis. Similar sites are circumvented by circle.

A new FCA was calculated without MI1 (Figure 3.3.13). The two first axes of the factorial correspondence analysis without MI encompass 41.08% of the inertia of the variation. The most upstream sites are located at the bottom of the figure (circle).

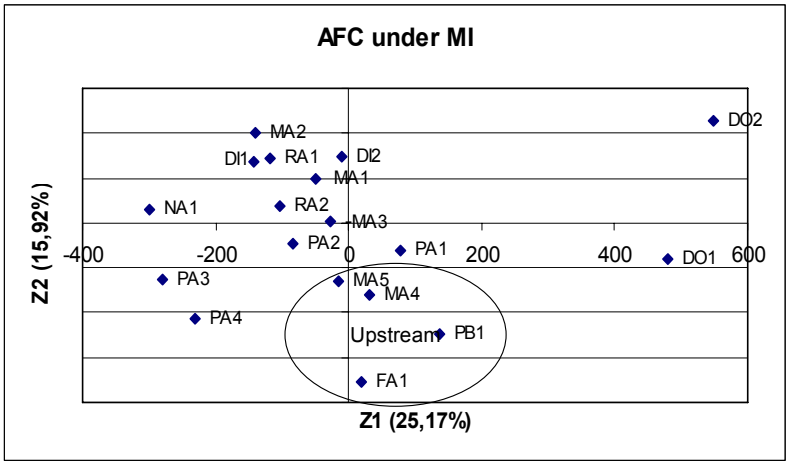

Figure 3.3.13: Factorial correspondence analysis-representation of the populations on the first two axes of the analysis, under MI.

In the tree of figure 3.3.14, the top part of the tree includes populations of down-stream rivers. Populations of upstream rivers and Palogne 1 (PA1) are at the bottom of the tree. The tributaries form branches which are articulated on the tree trunk. The important genetic distance between DI1 and DI2 (la Diglette) and between DO1 and DO2 shows the effect of major obstacle. The two populations of the "Doneuse" DO1 and DO2 seem rather genetically different from the other populations. In spite of the major obstacle between PA3 and PA4 (la Palogne), the genetic distance between them is not-significant. On the other hand, there are no obstacles between PA2 and PA3, and the genetic distance between PA2 and PA3 is important due to the combined effect of geographic distance and natural obstacles.

Relationship between the degree of genetic differentiation (Fst) and geographical waterway distance between samples is significant (Mantel test: P=0.0008, Pearson Coefficient r= 0.522). This may be partly explained by the fact that the most distant sites are also isolated by weirs. Fst increases with geographical waterway distance.

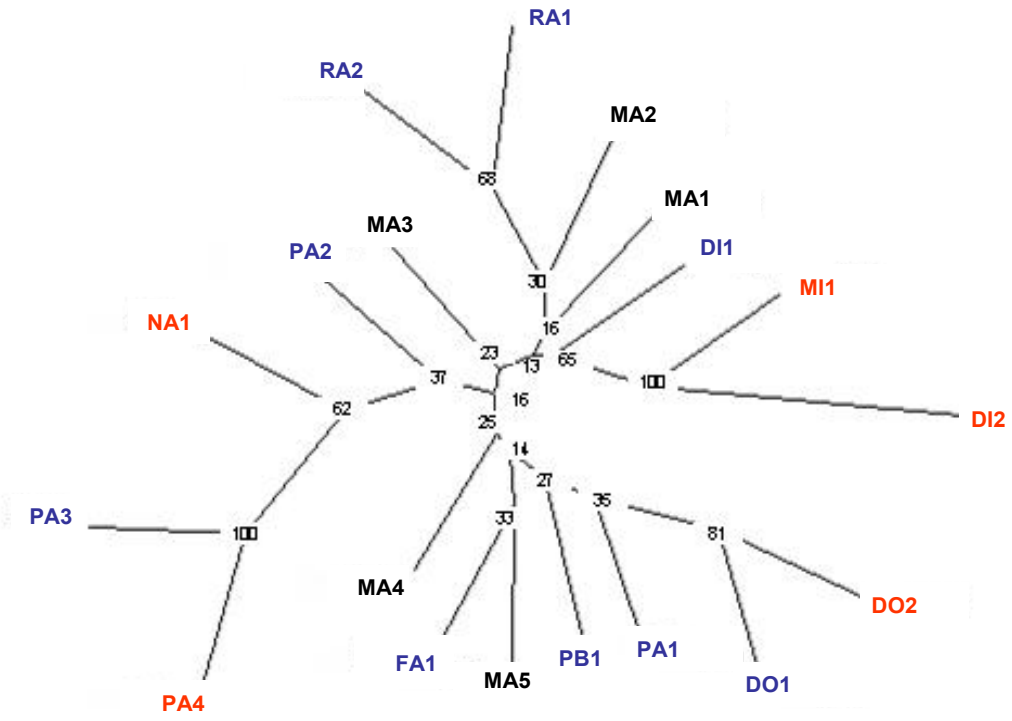

Figure 3.3.14: Tree of Masblette's population, Neighbour-joining tree based on Cavalli-Sforza and Edward's (1967) chord distances. Confidence in tree topology was assessed by bootstrapping over loci (1000 bootstraps).

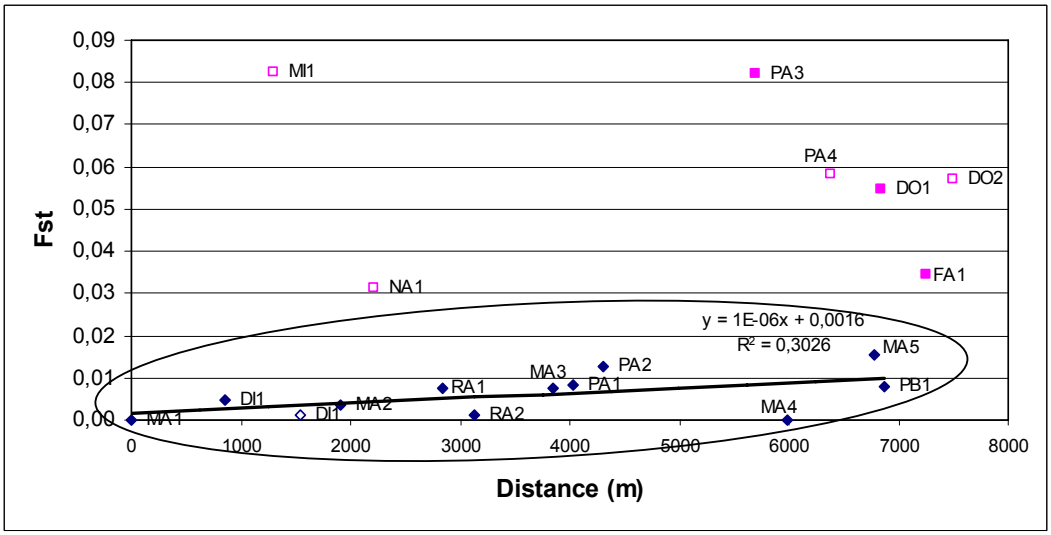

Figure 3.3.15: Isolation with distance. Relationship between degree of genetic differentiation (Fst) at microsatellite loci compared to MA-1and geographic distance of MA-1. Fst increases with distance in means. The white points are the sites separated by a major obstacle.

Figure 3.3.15 shows that the relation between geographic and Fst is the best for the sites that are circumvented (R^2=0.3026). For the other sites, the relationship is poor. Major obstacles in front of the the sites MI1, PA4, DO2 explains this low correlation. But, for PA3, DO1 and FA1, the cause of the low correlation between geographic and Fst distances is not known. A lack of correlation between Fst and geographical distance was already observed in other brown trout populations [88, 89, 90].

Fst increases with the number of obstacles (Figure 3.3.16). Mantel test for the effect of the number of major and minor obstacles on the genetic distance gives a Pearson coefficient of 0.396 and p=0.01.

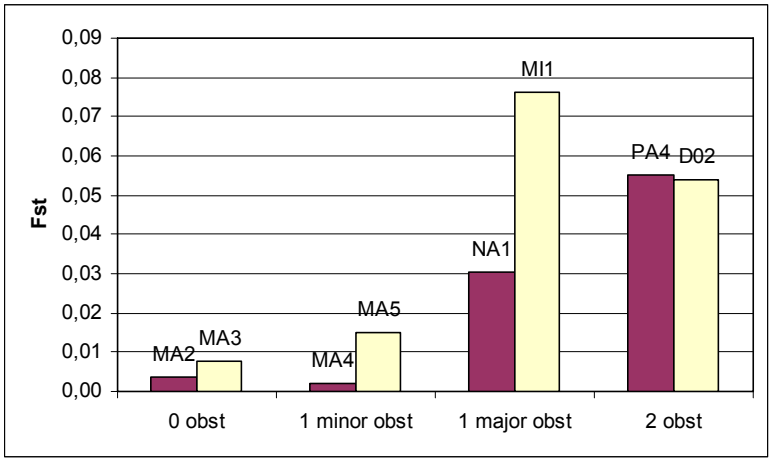

Figure 3.3.16: Fst genetic distance between Ma1 and MA2, MA3 (0 obstacle), and MA4 MA5 (1 obstacle), NA1, MI1 (1 insurmountable obstacle) and PA4, and DO2 (2 obstacles).

The Mantel test for major obstacles gives a Pearson coefficient of 0.539 and p= 0.021. The correlation between the number of major obstacles and Fst is higher than the relation between the combined number of major and minor obstacles and Fst.

The mantel test for minor obstacles shows that the relationship between minor obstacles and Fst is low (Pearson Coef. r=0.144; p=0.144). At this stage, we know that a relationship exists between the Fst and the geographical distance along the waterway (Pearson Coef. r= 0.522) and also between the Fst and the number of major and minor obstacles (Pearson coefficient of 0.539). In order to study the relationship between the Fst, the geographical waterway distance and obstacles together, obstacles should be converted in a value in meter. Different values in meter has been attributed to major and minor obstacles. We have added these values to the geographical waterway distance and we have ran the Mantel test in order to know the relationship between the Fst and the geographical waterway distance + major obstacles in meters. The results for different values are given in Table 3.3.4. This table shows that the best Pearson coefficient is obtained when we add 5000m to the geographical waterway distance for major obstacles and 500m for minor obstacles.

Table 3.3.4: Mantel test between Fst and geographic distance+ several values of obstacle (m)

Geographic distance+ Obstacle (m).		Coefficient	p
Major	Minor		
0	0	0.522	0.0008
1500m	0	0.575	0.0006
2000m	0	0.588	0.0014
2500m	0	0.597	0.0008
4000m	0	0.608	0.0030
4000m	500m	0.608	0.0024
4000m	800m	0.606	0.0038
5000m	0	0.609	0.0032
5000m	**500m**	**0.614**	**0.0028**
5000m	800m	0.613	0.0018
6000m	0	0.608	0.0032
10000m	0	0.597	0.0088

When sites are ordered as followed: sites of the main courses from downstream to upstream and then sites located on tributaries from downstream to upstream, we observe a gradient of diversity from upstream to downstream on the basis of Na (Figure 3.3.17 and 3.3.18): A) Diversity gradient from upstream to downstream on 5 sites of Masblette's means stems (Figure 3.3.17); B) Diversity gradient from upstream to downstream within each tributaries (Figure 3.3.18); C) Diversity gradient from upstream to downstream between sites connected of different tributaries.

Across all population, the number of alleles per locus ranges from 2.5 to 5.3 with a mean of 4. The number of allele per locus (Na) for PA3 and PA4 is the lowest. The measure of diversity: Hobs ranges from 0.688 to 0.496 with a mean of 0.59. The het-

erozygosity of PA3 and Fa1 is low. It is difficult to draw a conclusion for PA3 and PA4 because the sample sizes of these populations are small.

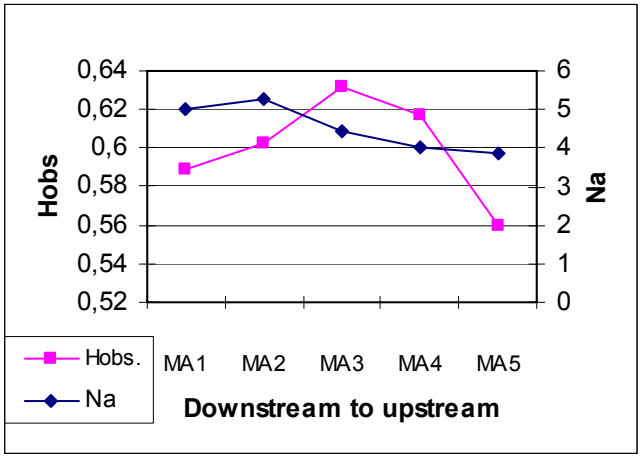

Figure 3.3.17: Hobs and Na from downstream to upstream in Masblette.

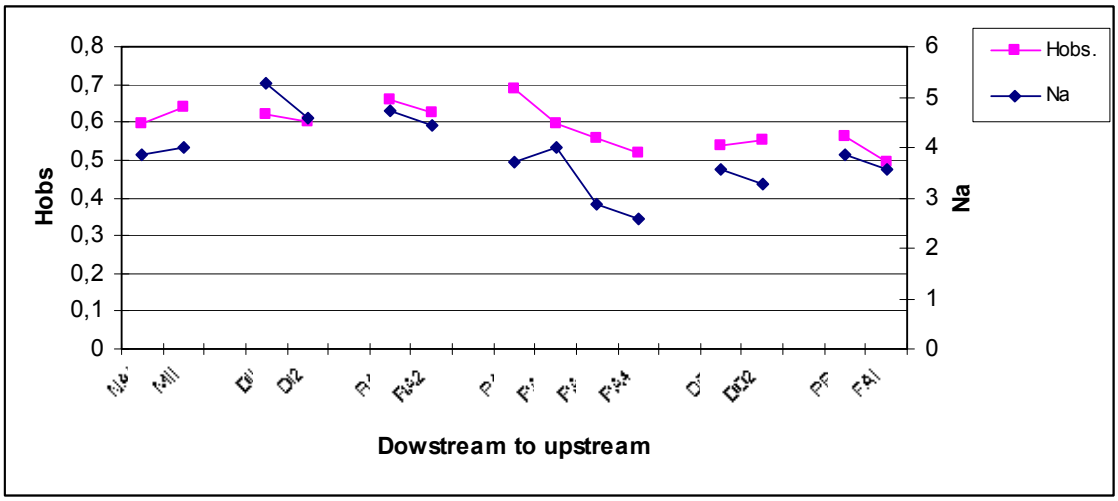

Figure 3.3.18: Hobs and Na of tributaries of Masblette from downstream to upstream.

4. DISCUSSION

4.1. Ecology

4.1.1. Development of a work protocol and selection of the model systems

A protocol was written to ensure a maximum degree of standardization in the methodologies and to correctly obtain sampling data related to population density. The strategy for the selection of the model sites is defined by the partners, following the requirements listed in Materials and Methods and adapted to the research questions of the project. To ensure repeatability, fishing effort, fishing equipment and fishing protocols were the same on each sampling at the same site. In most cases fish were caught with electrofishing, wading or by boat, dependent of the water depth. In large rivers electrofishing for absolute estimates of fish populations is difficult. Therefore, a stratified sampling procedure is necessary. Qualitative, and to a lesser extent, abundance information can be obtained by using conventional electrofishing with hand-held electrodes in the river margins and delimited areas of habitat. In many cases, especially prior to the late 1980s, electrofishing had been considered not only the most effective but also the least harmful means to capture fish, particularly moderate to large-size specimens.

Over the past five decades, there have been considerable improvements and refinements in the methodologies and techniques for studying the spatial behaviour of fish [91]. Commonly used tools include physical tags (including PIT-tags), biotelemetry transmitters and genetic markers. Biotelemetry, for the detailed tracking of a limited number of individuals, and PIT-tagging, for the monitoring of large numbers of fish at dedicated sites, represent the ideal combination for investigating the mobility of fish in rivers and streams. Their use is best when studies of the behaviour of fish parallel investigations of their genetic characteristics.

4.1.2. Analysis of fish communities in the model systems

Since the beginning of the 19th century, the ecological quality of the water courses drastically changed, resulting from increasing industrialization, intensive agriculture and an increased population density. Rivers also changed because of the building of weirs and dams for various purposes (navigation, power generation, water stocking and abstraction), canalization and straightening in behalf of shipping traffic, embanking to increase land use and to protect residential areas. All this has threatened the fish population in Belgian rivers to various extents, causing the local extinction or reducing the population abundance of species most sensible to water pollution, physical habitat degradation and river fragmentation as a factor restricting migration and spawning activity, and consequently gene flow [13, 92]. The decline in species diversity in upstream direction is a normal feature looking at whole river basins. However our results were obtained over a relatively short stretch of the river and they still show

a negative relationship between the number of fish species and the presence of migration barriers between the sampling stations and the North Sea. This indicates the importance of migration barriers in the species composition in short stretches. The effect of other factors such as water quality and structural diversity are probably less important on this short distance.

Diadromous species in the Scheldt and Meuse basin: Several lowland rivers investigated in the Fishguard project are connected to the tidal part of the Scheldt and to the North Sea. Before the building of weirs and shipping locks, river systems like the River Nete and Dijle had open access from North Sea up to the spring. This was seen in the fish stock with facultative catadromous species like flounder (*Platichthys flesus*) and marine species like plaice (*Pleuronectes platessa*) and even sole (*Solea solea*)) beside anadromous species like sea trout (*Salmo trutta trutta*), allis shad (*Alosa alosa*), twaite shad (*Alosa fallax fallax*), and smelt (*Osmerus eperlanus*) [93].

During a period of strong water pollution in the River Scheldt estuary, most of these species had disappeared from this river and the lower part of its main tributaries. Since about two decades, water quality has improved in the Scheldt estuary and several diadromous species have recovered; particularly flounder, smelt, twaite shad and river lamprey [79]. In that context, it is particularly important to allow these migratory fish to move as upstream as possible in the main Scheldt channel and in the tributaries. In the River Kleine Nete, migratory species such as flounder can migrate till the first barrier (weir in Grobbendonk). In the River Scheldt migratory species can migrate till the weir/lock in Asper. To this account, they have to clear the weir/lock of Merelbeke, where the tidal weir is passable under certain hydraulic circumstances [79].

The relationship between the diadromous migratory fish and physical obstructions is quite different between the Meuse and Scheldt basins. Indeed most part of the Belgian Meuse basin is far from the North Sea and not directly influenced by tidal phenomena. Furthermore, the Meuse channel is fragmentised by numerous navigation weirs (7 in the Netherlands between Lith and Borgharen-Maastricht and 6 in Belgium between Visé-Lixhe and Namur) among which several (Linne, Borgharen, Lixhe, Monsin) are equipped of any joined ship-lock. The building of the seven navigation weirs on the Dutch Meuse from 1925 to 1932 caused the local extinction of all but sea trout, anadromous migratory fish (sturgeon, allis shad and twaite shad, sea lamprey and river lamprey, houting, and Atlantic salmon) that were prosperous before. Following the significant improvement of water quality that happened in the seventies in the Dutch and Belgian parts of the Meuse [12, 94], restoration programmes for the anadromous salmonids (Atlantic salmon and sea trout) were put forward in the eighties in both countries under the name 'Meuse Saumon 2000' in Belgium [95, 96] and 'Zalm terug in onze rivieren' in the Netherlands [97]. In the course of these programmes (supported by the BENELUX since 1996 and the International Meuse

Commission since 1999), all the weirs obstructing the navigable River Meuse in the Netherlands and in Belgium up to the confluence of the River Ourthe in Liège have been fitted with modern fish-ways in order to restore the free circulation of diadromous and accompanying potamodrous species. The second facet of the Salmon Meuse programme consisted in restocking Belgian upland salmonid streams (Ourthe, Amblève, Berwinne, Lesse, Samson) with hatchery-reared young salmon from foreign origins. These measures (improvement of water quality + building of modern fish-passes + reintroduction by stocking) resulted in the return of 15 adult (61-79 cm) Atlantic salmon in the River Meuse in Visé and the lower River Berwinne in Berneau in 2002-2003 [98].

Restoration of fish migration possibilities for migratory species between saltwater and freshwater, in the main course, and in the tributaries is a must. Conservation of meanders, restoration of nature friendly banks, restoration of the relation between river and wash land, decontamination of soil pollution are important action items for this century. Recently some important international initiatives are taken. The BENELUX order [99] foresees the guarding of migration routes for fish through the removal of fish migration barriers on all our water courses. In 2000 the European Water framework Directive [100] was published which foresees the achievement of clear quality objectives. This order attaches great importance to the ecological quality elements including fish.

4.1.3. Quantification of actual migration - case studies

Mobility of brown trout as representative of large rheo- and lithophilic fish

Brown trout is a rheophilic and lithophilic fish widely distributed and abundant in upland rivers from the River Meuse basin but rare in the River Scheldt basin. Previous studies started in 1988 as part of the Meuse Salmon 2000 project, brought many information about its migration behaviour in the Meuse channel and several tributaries and sub-tributaries with different types and degrees of disturbance of the longitudinal connectivity associated with physical obstructions [31, 32, 82, 83, 101, 102, 103, 104]. The Fishguard project offered the opportunity to carry on additional investigations and to increase knowledge about trout movements and migrations in a wide range of river types from the canalised River Meuse, used as migration route for anadromous sea trout to small brooks supporting a population of sedentary trout and receiving an autumnal run of spawners moving upstream from the main river. In this range of watercourses, various types of physical obstructions were encountered and behavioural responses of migrating trout were observed and analysed using 3 techniques: radio telemetry, catching-tagging and recapturing fish with electrofishing or in a trap or fish-pass and monitoring the fish passage in a fish-pass all year round.

Many of these data on trout movements were already analysed by Ovidio & Philippart [32] who proposed photos and a rough description of 28 obstacles cleared or not by radio-tracked migrating brown trout (and five other species: grayling, Atlantic salmon,

barbel, nase and pike) in upland rivers included in the Fishguard study area. Among the numerous obstacles examined up to now in upland rivers, only a very few number could be considered as quite impassable by upstream migrating brown trout, with a potential significant effect on fish population dynamics (reduction of recruitment). These are the high reservoir dam without any fish-pass facility erected on the Ourthe in Nisramont in 1966 and some fixed weirs, perched waterfalls (obstacle Mos-08 on Mosbeux), culverts and road-crossings on small salmonid nursery streams [105]. Most of the other obstacles were proven passable by trout for various raisons. In the navigable Meuse, trout can migrate through fish-passes, ship-locks or over mobile weirs totally or partially opened during floods. In the lower and medium course of the Ourthe upstream migration may proceed through fish-passes, automatic mobile weirs maintained open from October to March or fixed weirs when the height of the obstacle is lowered at times of elevated water discharge (summer spates and high fall and winter discharges). In small streams trout are able to pass through small obstacles by using a fish-pass or taking advantage of the structure of the obstacle in terms of low height and sufficient depth of the plunge-pool.

In practice, it was sometimes difficult to determine whether the lack of passage of an obstacle is due to an inability of the fish to surmount the blockage. Zones immediately downstream of blockages are often (particularly in non typical trout stream) propitious habitats for trout and other rheophilic and lithophilic species because of the abundance of food (forage fish for trout), well oxygenated water and presence of gravel beds to spawn. This may be why certain trout established their principal resting places in these areas. Apparent blockage of a trout below an obstacle may also result from the fish having attained the end point of its migration journey from downstream, particularly when the upstream migration implies moving from a main stream to a smaller tributary.

Finally, it must be remembered that the upstream migration of adult trout is basically governed by its reproductive homing behaviour, i.e. its return to or near the place where it was born and from where it emigrated early and moved downstream. Therefore the lack of upstream migration behaviour in individual trout might be explained by their birth in a fish farm which might lead to less migrant fish (see Genetics).

Clearance of an obstacle by trout can be temporary, depending strongly on water flow conditions. Water temperature is also important in the success with which trout pass obstacles because it affects the fish's muscular efficiency and thus its swimming and jumping capacities [106, and Ecophysiology section in this report]. Observations [32] show that the various obstacles are cleared in a thermal range from 4.6° to 19.8° C, with a preference for temperatures between 8° and 12°C. Individual trout cleared obstacles in summer when mean daily water temperature exceeded 16°C. The combined effects of flow and water temperature were observed several times. When trout arrive at the foot of an obstacle, they almost always attempt to clear it immediately. If

they are unable to do so, they go downstream dozens to several hundreds of metres and wait, sometimes several weeks, for environmental conditions to improve (increase in water level or temperature), which will allow them to clear the obstacle. This type of behaviour is very costly in terms of energy and the numerous jumping attempts could also result in injury. Moreover, obstacles cause delays that may constrain the fish to reproduce during non-optimal environmental conditions.

Within a given species like trout, clearing capacities can vary from one individual to another. This phenomenon is often observed in individuals of different sizes. Indeed, in function of their structure and characteristics (depth downstream, water height on the obstacle), some obstacles are more easily cleared by fish whose size is within a certain range. Ovidio & Philippart [32] have also observed that, for identical environmental conditions, individuals of the same size sometimes remain blocked for different periods of time at the foot of the same obstacle. Such inter-individual differences do not facilitate establishing precise norms for clearing capacities of fish.

Studies on mobility of bullhead, a small endangered litho- and rheophilic fish

Patterns of bullhead residency-mobility in the fast-flowing Oxhe are very similar to previous observations in a small spring creek in France [107], a sub-montane alpine stream in Austria [108], and in two lowland small streams (Laarse Beek, width: 4 m; Steenputbeek, width: 1 m) of the Scheldt basin in Flanders [109, 110, 111]. In all these small watercourses the great majority of bullhead stays at or very near the place of their capture and tagging. But some individuals display movements over relatively long distances ea. about 400 m (approximately 4000 times the body length) downstream and upstream. Maximum movements registered in the Oxhe are the highest compared to those in the other streams, particularly in the Scheldt basin: 365 m upstream and 375 m downstream in the Oxhe versus 260 m upstream and 160 m downstream in the Laarse Beek. But in both cases the bullhead population seems to be composed of a fraction of sedentary individuals and a fraction of mobile fish, as seen before [110]. Further research based on multiple-recapture data of PIT-tagged fish is needed to characterise over long periods of time (at least one year) the mobility status of individual bullhead in a range of various stream types in terms of importance (width, stream order), physical habitat features (discharge, flow velocity, sedimental bottom structure, presence of aquatic vegetation) and biotic factors (population density of bullhead and other species, *etc*.).

According to [112], artificial vertical obstructions with a height of 18-20 cm are impassable for upstream moving bullhead and thus act as migration barriers. In the Oxhe, bullhead succeeded in passing heterogeneous man-made stony weirs with a maximum of 25 cm height but seem quite incapable of moving upstream over a 0.6 m high natural cascade. The passage of bullhead over such small obstacles should be studied by using automatic recording systems of PIT-tagged fish as the CIPAM system installed in the fish-pass in the lower Aisne. The use of this technique with bull-

head nevertheless will be limited by the small size of the fish compared to the size of the PIT-tag to be chirurgically implanted into the belly. But we feel that it should work well with bullheads bigger than 12 cm.

When studying the mobility patterns of bullhead, special attention should be paid to downstream movements of fish, not only young-of-the-year fry (drifting) but also adults as revealed before [113] during a study of fish impingement on the water intake of the Tihange nuclear power plant on the River Meuse. In 2001-2004, a total number of 1221 bullheads (20 - 90 mm) were caught, confirming a similar observation of bullhead moving downstream through the turbines of the Linne hydropower plant on the Dutch River Meuse (n= 177; 5-9 cm) in Oct.-Dec. 1999 [114].

All these new ecological information on bullhead mobility undoubtedly will help to interpret results of genetic studies performed on samples of fish collected from a wide range of watercourses in upland and lowland Belgian rivers (see Genetics).

Studies on mobility of roach, an ubiquitous rather limnophilic species from both lowland and upland rivers

(a) An integrated biotelemetry study of roach movements in lowland and upland highly fragmented rivers: The integrated biotelemetry study on roach movements performed in the lowland rivers Kleine Nete and Grote Nete and in the upland Vesdre is the first investigation of this kind in Belgium. Earlier information was obtained from the monitoring of upstream migration of roach in fish-passes in the Meuse basin (Meuse at Tailfer [115], and Lixhe [116], Méhaigne in Moha [117] and in the Scheldt basin (Laarse Beek [110]).

The duration and dynamics of the roach movements were generally quite similar between individuals in the tracked upland and lowland rivers. Roach showed maximum activity from the beginning of April until the end of May and they were frequently located in the faster flowing parts of their study reaches during that time period. Even though spawning activity could not be observed, it can be assumed that these movements were related to spawning activity as this period corresponds to the reproduction time of the species in similar environments [118, 119, 120, 121]. Fast flowing zones in rivers were already described as potential spawning areas for roach [122, 123]. Outside of this period (during the pre- and post-spawning period) roach frequently moved between different locations but the net length of the daily journeys were generally smaller. Distances travelled by roach increased significantly when water temperature varied between 10°C and 14°C, which also corresponds to the late April-May period. Baade & Fredrich [120] noticed that there is a highly significant distinction in mobility rates and activity levels between April and May (when fish are most active) than during other times. Longer movements were observed during spawning season.

No statistical relationship was observed between water flow and distance travelled although some rare winter movements were observed during very high flow events.

For lake Årungen (south-eastern Norway), it was suggested that spawning activity is regulated by water flow and water temperature and roach spawned synchronously in years with rapid increases in temperature, whereas they had a prolonged spawning period in years with low or with slow increases in water temperature [118]. It has previously been demonstrated that photoperiod is the principal factor synchronizing the start of the spawning in roach [124]. In unstable environments the use of predictable cues such as day length will be positively selected. Temperature however is important in regulating the intensity and duration of the spawning [124].

Although the dynamic of the roach movements and the activity levels were similar in the three rivers, our results demonstrate that the extent of the movements observed was mainly related to the distance between the physical barriers in the study areas. Distances travelled were more pronounced in the Kleine Nete where the distance between the physical barriers is 14 km (first barrier on the River Aa tributary). Upstream from the downstream weir, free entrance in the Aa tributary is possible, and the upstream weir (in the Kleine Nete) is equipped with a fish-pass. On the opposite, in the Vesdre, where the distance between the physical barriers is only 1.2 km, the distances travelled were much shorter and the proportion of roach moving downstream during the reproduction period was more pronounced.

Our results suggest that roach were not frequent obstacle leapers (at least in the upstream direction) and they were able to complete all of their biological activities in limited stretches of rivers in highly fragmented environments. As well, they sometimes demonstrated up- and downstream movements during the reproduction period in search of available spawning habitat. In a 32 km unfragmented stretch of the Spree (Germany), it was observed that roach migrated up to 10 km upstream to spawn [120]. Some studies however showed that roach could sometimes clear small physical obstacles. Lucas *et al.* [121] demonstrated that radio-tracked roach ascended the Skip Bridge weir on the Nidd (United Kingdom) and moved further upstream to spawning areas. Svärdson [125] observed roach that was trapped in a wire-netting, jumping at least 15 cm above the water level.

(b) A study of movements of PIT-tagged roach in a lowland river: Both PIT-tag experiments have proven up- and downstream migration through the siphons of 32 individuals of 8 freshwater species: perch (*Perca fluviatilis*), roach (*Rutilus rutilus*)*,* gibel carp (*Carassius gibelio*), chub (*Leuciscus cephalus*)*,* tench (*Tinca tinca*)*,* gudgeon (*Gobio gobio*), rudd (*Scardinius erythrophthalmus*) and stone loach (*Barbatula barbatula*). Unfortunately, it was impossible to gain an idea of the part of the population able to migrate through the siphons. Only very few diadromous species (eel, flounder and river lamprey) were captured during the sampling period. For flounder and river lamprey, migration through the siphons could be confirmed as they appear at the weir in Grobbendonk, upstream the two siphons. This shows that, at least a few individuals are able to pass these siphons. It's difficult to determine the migration pattern

of eel because it is abundantly restocked and is able to climb up the wall near the weir. Two reasons for its distribution over the whole river.

4.2. Ecophysiology

4.2.1. Swimming performance and energy use

When comparing swimming speeds and gait transition in short and long swimming tunnels, it was obvious that length of the swimming section in the flume influenced the swimming performance of free swimming fish [33]. The longer the swimming tunnel section, the higher the critical swimming speed and the longer the period of burst swim. This effect gets larger with increasing fish size, meaning that the effect of forced confined conditions is more important for large fish than for small fish. It appeared to be important that fish could change between cruise and unsteady swimming whenever they needed to. This indicates that a regular and free change of swimming modes is important for a higher swimming performance which is comparable to earlier findings [126]. The total time of burst swim, measured from gait transition on, is significantly longer in the longer swimming section and again indicates that when given the possibility, fish use burst swimming mode longer, leading to a higher critical swimming speed. Larger individuals burst and coast relatively longer indicating that they use their white muscles for a longer period. This is interesting because fast twitch (anaerobically powered) muscles acidify quicker than slow twitch (aerobically powered) muscles. In this period fish show so-called burst and glide swimming, describing a way to propel the body by white muscle use and then use the obtained force to glide the rest of the way. An explanation can be found in the fact that the relative viscosity of water is higher for smaller fish than for larger fish. Zebra fish (*Danio rerio*) larvae, for instance, have a very low Reynolds number, with the effect that the same burst and glide movement, i.e. one active burst, does not bring them as far while gliding as adult Zebra fish individuals with a high Reynolds number [127, 128, 129]. For large fish this is only possible in long swimming sections. An ideal length of a swimming tunnel might be species-specific but it can be concluded from the results of this study that a flume should be definitely longer than 3.5 Bl, which has been taken into account when experiments were conducted.

Ground speed, i.e. the speed reached relatively to the ground when the fish was bursting, show significant differences at different water speeds for small fish, but larger fish reduce their differences between ground speeds at different water speeds [33]. This indicates that it is easier for larger fish to keep a certain ground speed over different water speeds. Therefore they are not so dependent on changes in water speed while migrating. The results provide us with a better understanding of the possible movements and speeds fish use while migrating in nature as ground speed is the speed that matters for the passage of distance in nature. Both observations indicate that migrating fish do not necessarily prefer the most 'economic' optimal swim-

ming speed, reducing the energy use while migrating. They rather select their preferred swimming speed to keep up a certain ground speed, depending on their body size, and for smaller fish depending on the water flow. Burst and coast activities are an essential strategy to maintain high swimming speeds, certainly in larger fish.
When comparing the different species, U_{crit} gave a decent indication of the migration capacities of the species. Although U_{crit} measurements are not a self-sufficient measurement and can not directly be extrapolated to populations swimming in the wild [25, 39, 44], critical swimming speed test can be used in order to test physiological or ecological parameters. It allows us to differentiate between 'good' or 'bad' long term swimmers. According to these results, brown trout is the best long term migrating species of those tested. Even small individuals reached high U_{crit} values, had a high scope of activity and U_{opt} was also high, when put side by side with comparably sized other species. Unfortunately, it was not possible to test large individuals holding conditions, but even a careful extrapolation to large individuals allows us to predict best results for this species. This statement is also supported by results found in literature [3, 13, 130]. Also leaping capacity, extrapolated from U_{max} results, reached high values and it can be stated that trout should be the best 'jumper' of the species tested. However, this species is very sensitive to oxygen concentrations and water temperatures, which affect its swimming capacities much stronger than the other species tested [3]. Our study also showed the effects of ammonia on swimming behaviour, especially fast starts necessary for jumps, escape and predation, and orientation were affected suggesting that migration would be impaired. Therefore it is vital to ensure a good water quality in order to reach high swimming performances in this species.
The best long term swimmers appear to be small *R. rutilus* and *C. carpio*. As these species are migrating only at older age and thus larger size, we compared size classes that resemble migrative ages. In large fish, high velocities are reached by *R. rutilus* and *P. fluviatilis* which are more long distance migratory species [3, 13, 131]. For technical reasons, critical swimming speed could not be measured for *C. gobio*. When set into the swimming tunnel, this species set its pectoral fins in such a position that it could not be swept downstream by the water, an adaptation to its natural habitat, where *C. gobio* lives between rocks and stones of fast streaming creeks and small rivers. Also, Johnston *et al.* [132] showed that the main part of *Cottidae* muscles is glycolytical which might be an explanation for the typical hopping movement they display. *C. gobio* does not show any cruise swimming behaviour but always bursts for locomotion. This is reflected in the relatively high maximum swimming speeds. Also, it is doubted that *C. gobio* is a migrating species although there is evidence for supporting this theory in some individuals (see Ecology results). Like *C. gobio*, *B. barbatula* also displaces itself by bursts rather than by continuous swimming. Also with this species, there is no evidence for any migrating behaviour. How-

ever, even though there might not be any evidence for long distance migration, every fish species displaces itself in its life cycle at any point. In order to avoid genetic fragmentation of the population and bottleneck effects it is of vital importance to construct passages that in their feasibility include also species of which it is not known if they migrate or not and even if, how far. Therefore, species as *C. gobio* and *B. barbatula* are included in our analysis.

Calculated optimal swimming speeds should be interpreted with care. Some non-migrating species might not really use the optimal swimming speed for displacement but will always swim with swimming speeds above or below U_{opt}. Also, as U_{opt} is a theoretical value, and as stated above, there is not much evidence for the actual use of this swimming speed. Actually, as fish pass difficult areas of their migration routes, speeds are often altered and energy saving strategies like burst-and-coast swimming are adopted, as this swimming behaviour has been proven to reduce aerobic energy demands for up to 60% [133]. Also migrating Sockeye Salmon (*Oncorhynchus nerca*) do not swim as expected, i.e. energy saving speeds as critical swimming speed or optimal swimming speed [134]. Especially while passing difficult passages on their migration route, "burst-an-coast" or "cruise-and-coast" swimming is adopted. The optimal swimming speed as a percentage of the critical swimming speed indicates the demand of energy for migration as percentage of the scope for activity. Here we can see that all species tested show comparable results. This can be deduced to comparable morphological and physiological aspects. Swimming capacity is very much connected to morphology and energy metabolism. It might be not possible to see these three factors independently. Optimal swimming speed (U_{opt}) is generally different in terms of absolute and relative values. Sometimes, the values for U_{opt} as percentage of U_{crit} are higher in smaller individuals than in larger individuals of the same species. The values that are interesting here are the absolute values in cm s^{-1} and the MO_{2opt} as percentage of the scope for activity. They show that smaller fish swimming at U_{opt} move slower than larger fish and use a higher percentage of their scope for activity. Thus, being large as a fish is energetically more advantageous.

Another consideration to take into account is the fact that that fish forced to swim use less energy than spontaneous swimming fish, measured by means of respirometry [126]. Krohn and Boisclair [135] tested the ability of a stereo-video method to provide estimates of the metabolic costs of spontaneous swimming by simultaneously video taping free-swimming fish in three dimensions and measuring their oxygen consumption. Spontaneous swimming costs, measured directly by respirometry, were on average six times higher than the costs predicted by the forced swim relationships measured in swimming tunnels for the same speeds. This difference suggests that the metabolic costs of turning and acceleration can be substantial. Fish also reached a higher speed when swimming spontaneously. In our case the swimming protocol of the fish tested is also forced in the sense that they cannot turn and change direction,

but fish are free to choose their speeds and gaits, as supported by [126]. Prolonged activity in nature is a relatively uneven activity and is frequently associated with periods of cruising and occasional bursts [136]. Constant speed swimming is rare and the analysis of routine swimming and its force balance shows that routine swimming is not quasi-steady as generally assumed [137]. Peake and Farrell [138] examined the swimming behaviour, gait transition, oxygen consumption and post-exercise metabolics in smallmouth bass (*Micropterus dolomieu*) in a spontaneous and a forced swimming set-up and found that fish switched between steady and unsteady swimming at intermediate speeds. They interpreted this behaviour as evidence that unsteady swimming was being fuelled by the limited supply of anaerobic substrates in the white muscle, supported by the fact that unsteady swimming fish showed significantly lower muscle glycogen levels, higher lactate concentrations (muscle and plasma) and higher post-exercise oxygen consumption rates compared with fish that used a steady gait. The reduction in passage time achieved by fish using an unsteady gait allowed them to ascend the raceway with relatively minor post-exercise metabolic imbalances.

As a fish accelerates in order to pass difficult passages with high water speeds, but also to predate upon prey or escape predators, maximum swimming speeds are used. The results show the capability of some species to accelerate quicker than others. However, species with a higher burst capacity are not necessary more successful in passing difficult passages. For example, *C. gobio* reaches very high burst speeds but is not known to leap out of the water. As this species does not have any swimming bladder, it is negatively buoyant and remains on the ground. This is also the reason, why there is no calculation for the leaping capacities of these ground dwelling species in the result section. Other species as *R. rutilus* or *C. carpio* can theoretically leap over longer distances and larger heights. Those also show good cruising capacities and in general are more actively moving in the water column.

When comparing migrating and non-migrating populations of the same species, the three-spined stickleback, the migration morph *trachurus* showed better physiological adaptations to migration in terms of swimming performance and energy stores than the non-migrating morph. Critical and optimal swimming speed differ significantly between the two morphs. *Trachurus* shows higher values than *leiurus*. As U_{opt} is the swimming speed at which the lowest amount of energy is consumed per distance swam, it is favourable for migrating fish over long distances. Swimming at U_{opt}, *trachurus* reaches higher speeds than *leiurus* but consumes higher amounts of oxygen, which is not very favourable for the migrating morph, arguing from a theoretical perspective. But when recalculating the absolute values into percentage of scope for activity, there are no differences between the two $MO_{2\ opt}$. These results suggest that there is no difference in relative oxygen consumption and thus that *trachurus* and *leiurus* swim at the same relative energetic level when migrating at U_{opt} but at differ-

ent speeds. To consume more oxygen and thus more energy is generally spoken not favourable but it might be the price to reach higher optimal and critical swimming speeds. A possible reason might be an increase in blood circulation in the migrating *trachurus* morph, carrying more nutrients and oxygen to the muscles. Extrapolation to zero reveals the SMR of the two morphs with higher values for *trachurus* than for *leiurus*. The higher SMR in the migrating group can be compared with earlier results [139] and reflects the values found for migrating sticklebacks in summer while the data for *leiurus* reflect the values found for sticklebacks outside the migration period in winter. The scope for activity is also lower for *leiurus* than for *trachurus* [139]. Theoretically, by adjusting the SMR, fish can potentially compensate for many features associated with migration [48] and in this case for the higher AMR in *trachurus*. The same SMR for both groups but a higher AMR in *trachurus* would have resulted in a higher scope for activity in *trachurus* but a lower optimal swimming speed, compared with *leiurus*.

The higher concentration of lipids in the livers of *trachurus* individuals correlates with the higher swimming performance that is powered by aerobic and anaerobic muscles. Lipids are the most compact form of energy stores and can be transformed into glycogen which is used to power also white muscles. The storage of energy in the form of lipids is ideal for migrating fish.

4.2.2. Leaping capacities in artificial constructions

The overall success ratios were rather low. In general, it can be said that the motivation of the fish to migrate under artificial conditions is too low. We advise to keep the acclimatisation period under laboratory conditions as short as possible. Although general response is low, some general statements from both experimental setups can be made. Stream velocities on top of the weir were of major importance in this study, with emphasis on velocities ranging from 0.8 to 1.2 m/s. Lower velocities were not attractive enough under these circumstances. Another possible explanation could be that at these velocities other factors are more favourable for passage, e.g. the depth of the water on top of the weir. Pool depths of 0.2 m seemed to be sufficient for successful leaping. A sharp v-shaped weir (type 2004) limited migration capacity of the fish species, while a slight v-shape weir (type 2005) worked as well as a rectangular one. A better result might still be obtained with a wider V-shaped weir: this would not only allow fish species to aim their direction before jumping, but perhaps also chose the best flow condition (faster but deeper in the middle, slower but narrower near the sides). Regarding leaping height, rudd, common carp and gibel carp seemed to be limited to 0.15 m leaping heights, which for gibel carp was stated both in the 2004 and 2005 experiments. Tench was not succesfull. Gudgeon was limited to 0.05 m, while ide was most successful, even at leaps of 0.25 m. Also roach species proved to have quite good leaping skills (up to 0.25 m). These heights are lower

than the calculated leaping heights from the swimming experiments, possibly reflecting low motivation as well.

4.3. Genetics

4.3.1. Non-stocked species

Three-spined stickleback

Levels of genetic divergence between three-spined stickleback populations of the eastern subbasins of the Scheldt River were high and significant, consistent with earlier studies indicating marked differentiation in freshwater sticklebacks within and among watersheds [140]. Within this network of lowland rivers, with pairwise geographic distances along waterways up to 116 km, we observed a significant isolation-by-distance pattern, indicating that sticklebacks are approaching genetic equilibrium. A non-equilibrium drift model was also very unlikely as revealed with 2MOD. On the other hand, genetic drift (as quantified with absolute residuals) apparently increased with other landscape variables rather than with geographic distance (see below). Nevertheless, we carefully conclude that equilibrium conditions were sufficiently approached to allow historical genetic signals to be ignored.

Bottleneck analysis indicated that three populations had experienced a recent population decline. One site was extremely isolated upstream, but the other sites were from main tributaries. This suggests that recent fluctuations in population size were rare but might have occurred all over the river system. It is not unlikely that this is the consequence of a period of extensive pollution of the Demer basin around 1980 (H. Verreycken, pers. comm.). Among populations, decreasing upstream distance was accompanied by a strong decline in genetic diversity, similar to bullhead [76, 141]. Genetic diversity can be reliably predicted from the position in the river system. Remarkably, correlations among allelic richness and upstream size in *Cottus gobio* [76, 141] and upstream distance in sticklebacks (this study) have nearly identical values (0.75-0.79). Such measures can be equally considered as measures for the geographical range of riverine populations accounting for effective population size or drift [76].

Barrier characteristics revealed strong univariate relationships with genetic diversity and genetic differentiation, and to a lower extent with dispersal. Within barrier characteristics, weirs were in general more influential than water mills. This is against expectations, because mills are thought to have affected stickleback genetic structure during a longer time period. Perhaps weirs were old enough or represent "modern" constructions that obstruct rivers more efficiently. Another possibility is that weirs are more influential because they were more numerous on small tributaries inhabited by small populations. From a conservation perspective, it should be realized that modern barriers (including some fish passages with a poor design) should not be neglected. The influence of tunnels and sluices on genetic diversity and genetic differ-

entiation was minor. It agrees with recent tagging studies (see above) and genetic simulations [109] demonstrating that tunnels are passable, at least to some individuals.

Allelic richness also correlated with habitat width, which may represent an alternative for upstream distance as a measure for effective population size or the magnitude of drift. However, it is also possible that one or both variables just reflect to what extent the position within the river system is isolated by distance. Habitat width and upstream distance, averaged over population pairs, correlated negatively with pairwise FST, suggesting that small (or geographically isolated?) populations are more differentiated. This effect remained significant after control for geographic distance, demonstrating that geographic distance is not the only natural factor influencing genetic differentiation.

In summary, the dependence of genetic diversity and genetic differentiation on the interplay between gene flow and genetic drift is obvious in our data set. Geography is an important determinant of stickleback genetic structure. The restricted models for both genetic levels (diversity and differentiation) pointed to a major effect of barriers, reducing allelic richness and long-term gene flow. In the case of genetic diversity, the effect of barriers was substantial and likely represented a true anthropogenic effect, as two covariables accounted for natural patterns (upstream distance and habitat width). In the case of genetic differentiation, confidence that barriers truly affect population structure was obtained after incorporation of geographic distance, habitat width and upstream distance. Habitat width also appeared to influence genetic differentiation, suggesting that the isolating effect of habitat size is important.

As pointed out before, the residual variation of geographic models for genetic differentiation will always be associated with drift. In the case of isolation by distance models, absolute residuals point to the magnitude of drift under migration-drift equilibrium [142]. Conversely, under migration-drift equilibrium the magnitude of drift should point to the most isolating geographical factor. The signal of drift in the error term of the multivariate model was strong, as revealed by the association of absolute residuals with allelic richness, but not significantly linked to any of the predictors separately. However, using absolute values of the univariate regression residuals, we showed that, due to drift, predicting FST becomes particularly more difficult when more barriers (mainly weirs) separate populations. To a lesser extent this was also the case for populations from smaller habitats, but as mentioned already, not for populations isolated-by-distance. Hence, isolation-by-barriers, rather than isolation-by-distance, may reflect the balance between genetic drift and gene flow under anthropogenic disturbance.

Anthropogenic barriers have a severe impact on three-spined stickleback population structure in the eastern subbasins of the Scheldt River. We convincingly showed that barriers not only affect gene flow, but that they also represent the main isolating fac-

tor promoting genetic drift. Therefore, barriers may disrupt stickleback population structure. Firstly, physical isolation increases the risk of stochastic population extinction, which is particular high in rivers [143]. Secondly, inbreeding depression caused by genetic isolation might lower survival and population sizes [144, 145]. Given the marine ancestry of sticklebacks and their natural history of extinction and fast recolonization [146, 147], this may not harm the species in an evolutionary perspective. However, it may result in temporarily impoverished populations. Moreover, sticklebacks represent one of the strongest species in a highly disturbed aquatic fauna; our results might also hold for much more vulnerable and obligate freshwater species that have less flexible population structures.

We acknowledged the complexity of the natural component of the variation in genetic diversity and differentiation by a landscape genetics approach, in order to estimate the contribution of barriers with high confidence. Extensive geographic modeling of genetic diversity, genetic differentiation and dispersal in riverine networks has several applications in ecological genetics. Crispo et al. [70] applied the method to differentiate between the impact of geography and natural selection. It may be further useful to distinguish barrier effects from other landscape predictors of downstream and upstream migration [141], and it may also apply to detect the effects of natural barriers in rivers of which the impact is important for conservation management [148]. Genetic connectivity could also be modeled in combination with data on dispersal and water velocity in the field, to distinguish between effects of recent and historical river landscapes [149], and to disentangle effects of isolation-by-distance and long-term divergence.

Bullhead

As expected, the bullhead populations from Meuse and Scheldt were genetically strongly differentiated. This was also true for the populations from the different rivers in the Scheldt basin. But surprisingly, we observed a relatively low differentiation between populations from the Ourthe river over a distance of more than 170 km. Some of these populations were certainly separated by impassible barriers. Overall most Ourthe samples were genetically very similar, except for 2 samples in the middle part of the river. The fact that the most downstream sample was genetically very similar to the most upstream samples bullhead populations suggests geneflow between these distant locations. This geneflow could have happened in the recent past or could be ongoing. However the latter is not possible since we observed differentiated populations in the middle part of the river. The influence of isolation (absence of geneflow) on the population structure depends of the time (number of generations) since isolation and the population size of the involved populations. Large populations will differentiate much slower then small populations. Most sample sites are separated by relatively young barriers (<100 years). This is too recent to affect the genetic profile of large populations. Only small populations will differentiate in this short time

frame. The sample sites on the Ourthe that differentiated were recently subjected to heavy pollution and were probably bottlenecked. This population reduction enchanced the differentiation process. Furthermore this suggests that these bottlenecked populations did not recently receive migrants from upstream or downstream of the separating migration barriers. As such the migration barriers do not allow a natural restoration of initial diversity.

This demonstrates that even the non-mobile bullhead can maintain large continious genepools over large distances if free migration is possible. Since most migration barriers in the Meuse basin are fairly recent and bullhead populations are relatively large there is no pronounced effect of habitat fragmentation on the population genetic structure. However, this is only a matter of time and populations size as illustrated by the bottlenecked populations. In the Scheldt basin the effect of habitat fragmentation is more pronounced.

4.3.2. Stocked species

Roach

As well roach (stocked) as bullhead (non-stocked) display a similar genetic differentiation between basins (Meuse vs Scheldt). In both species both basins are populated by distinct genepools. As such the genetic profile of an individual fish can be used to determine its origin. However, the genetic analysis did not detect any roaches that were translocated between Meuse and Scheldt.

The differentiation within the Scheldt basin was remarkably low, suggesting a single panmictic in this basin. In the Meuse basin we observed a higher differentiation but not a clear geographic pattern of differentiation. As such we did not observe a pronounced effect of migration barriers on the population genetic structure of roach. The homogenious structure could be due to the intensive restocking practice of the last decades. An alternative explanation could be that roach is able to maintain large population size and that the effect of fragmentation is too recent to affect the genetic composition of such large populations.

Brown trout

Our results provided evidence that restocking has a proportional effect on wild populations of brown trout through introgression of hatchery gene pool into river gene pool. The restocking increases the genetic diversity in the restocked sites.

The power of STRUCTURE to distinguish river, hybrid and hatchery type of fish was checked on a theoretical population and by comparison of estimate of AC with AP obtained by Leadmix. Väha and Primmer [150] studied the efficiency of STRUCTURE for detecting hybrid individuals under different hybridization scenarios (Fst = 0.03, 0.06, 0.12 and 0.21) and with different numbers of loci (6, 12, 24 and 48). In our study, the pairwise Fst between the hatchery reference and the river reference is of 0.17. For a Fst of 0.12 and 0.21 Väha and Primmer [150] obtained with 6 loci a efficiency of >60%, >80% and with 12 loci >80%, >95% respectively. The efficiency is

the proportion of individuals in a group that were correctly identified. Thus, with 7 loci and a Fst of 0.17, the efficiency should be between 70 and 80 %. From our theoretical population, we estimated the efficiency in the identification of the hybrid individuals to be 82% (41 hybrid individuals correctly identified as hybrids/50 hybrids actually in the sample).

The individual admixture analysis suggested that past and present restocking activities have led to introgression of the hatchery gene pool into the river gene pool. Many admixed individuals (i.e., with intermediate values of IAC) were indeed observed in our dataset (Table 3.3.3). Genetic contribution of hatchery fish into wild gene pool was also observed in the Danish Skjern river using similar analyses [151]. This contrasts with results on others Danish rivers, in which a very few number of admixed fish were observed or wherein admixed individuals could not be unambiguously identified due to the very wide probability intervals [151, 152]. These latter studies pointed to a limited effect of stocking on wild populations of trouts due to strong selection acting against hatchery fish. In our case, the strong introgression could be mainly explained by the fact that trouts are resident and not anadromous. Indeed, certain studies have shown that the impact of hatchery trouts is lower when they were introgressed in population of anadromous trouts [29, 153, 154].

As inferred by the results of individual admixture coefficients, trouts from the Meuse catchment belong to several categories of fish: (1) 'river type fish', (2) 'hatchery type fish' and (3) admixed individuals to various degrees. According to the proportions of the three types of fish, four types of rivers may be identified. The populations of Vesdre (recolonized, OFR and ONE) and the intensity restocked downstream section of Ourthe (ODO, OHA, OBS) seems nearly entirely composed of hatchery fish. The non-restocked populations of Masblette clearly shelter river fish. The intermediate situations may be classified in two categories : (1) population composed of 50/50 hybrids only (LRC, ONM, OMC) and (2) more heterogeneous population made up of fish going from the river type to the hatchery type (LFO, LGO, OUP, OBO, OJZ, AIS).

As RI and AC are strongly correlated, we infer that the restocking involves introgression. But the introgression will also depend on the localization of the restocking since with equal RI, a restocking in a downstream stretch of river will have more impact than a restocking on an upstream stretch of the same river (Figure 3.3.10). This effect had already been observed [155, 156] on trout and coho salmon [157]. The restocking may contribute to the homogenization of the genetic composition since sites like OCM and OPC 30 km apart formed a single population (pairwise Fst = 0.01, non significant) whereas wild populations of Masblette distant of 3km are distinct populations (pairwise Fst = 0.05 between LMZ and LDV, $P<0.001$). This effect of stocking was also observed earlier [156, 158].

The behaviour of trout varies from downstream to upstream stretches [159]. The trout living in downstream stretches spawn in the tributaries (because these sites are

more favorable for reproduction) whereas trout living in the upstream part of the river spawn locally. The homogenization observed in our data for OCM, OHO and OPC may partially be explained by a common spawning behavior in the same location.

The very strong correlation between AC and genetic diversity clearly indicates that the effect of restocking corresponds to an increase of genetic diversity within populations. This effect can also be seen via the relation between AC and the genetic differentiation with hatchery reference and along the first dimension of the MDS. This effect of introgression on the diversity was already observed before, although without explicitly quantifying it [154, 155, 156, 158, 160, 161, 162].

For hatcheries, the expected heterozygosity was high, ranging from 0.64 to 0.71. This high genetic diversity of hatcheries, which was also noticed in previous studies [155, 156, 160], can be explained by the long-term historical practice of exchanges between Belgian hatcheries. Exchanges also occurred among Belgian hatcheries and foreign hatcheries (Switzerland, Germany and Denmark).

Heterozygote deficiencies could be expected in populations where different types of fish (river type, hatchery type or hybrids) coexist without hybridization. This could be the case in populations where restocked fish have not yet interbred with local fish. The absence of heterozygote deficiency in the majority of the restocked sites (15 river populations out of 19) tends to support our hypothesis of hybridization. The results of heterozygote deficiency related to mixture of populations should however be carefully interpreted because mixture of non-interbreeding populations do not always result in heterozygote deficiencies [163]. The four populations with a heterozygote deficiency (between 5 and 9%) are OJZ, AIS, OUP and LFO. AIS and OUP are two populations resulting from the aggregating of several not differentiated sites. However the heterozygote deficiency cannot be attributed to the aggregation procedure as the heterozygote deficiency was already observed in the sites OJU and ONV (part of AIS) and ORO (part of AIS). Moreover, from the four populations with a heterozygote deficiency, three (OJZ, AIS and OUP) are populations made of a mixture of river, hybrid and river type fish as LGO. A possible explanation would be that there is hybridization for these populations, the mating between wild and restocked trout not being completely panmictic (i.e. restocked and wild trout, respectively, preferred to mate with individuals from their own group) [164].

We observed that the restocking involves an introgression, but that the admixture coefficient (AC) is not perfectly correlated with the level of restocking (RI) as the fitness of restocked fishes may vary from site to site. Several teams observed that the reproductive success of hatchery-produced and wild born trout may be rather similar [165]. This does not fit with other findings, showing that hatchery fish display low performances into the wild due to years of selection regime in captivity [151, 158, 166, 167, 168, 169, 170]. In these latter cases, strong selection against hatchery fish led to a limited long-term effect of stocking. The main sources of mortality after a restock-

ing event are predation by birds or fish, delay in adapting to wild food and running waters (see review in [171]). Survival rate also depends on stocking time, stocking location, acclimatization and age [171] with for example the 0+ age group having a higher chance of surviving [172]. In Belgium, the majority of the restocked fish are juveniles (0+, 1+) (even if different age classes may be used), which could lead to a better adaptation to local environment and to an increased survival. Our results showed that the performance of hatchery fish may depend on local situations (the selection against hatchery fish being less strong downstream than upstream). Our results can be compared to the model presented by Hansen [151], who considered the impact of restocking as a function of (1) immigration rate of hatchery fish (i.e. intensity of restocking, RI in our study) and (2) selection acting against hatchery fish (variable along the river). We hypothesize that the selection against hatchery fish is stronger in upstream river because the conditions in upstream river are more different than the hatchery condition: higher current water velocity, higher concentration of oxygen, higher predation, and higher quality of the habitat than in the downstream part.

It is generally accepted that stocking wild populations with non native hatchery fish is not a viable conservation option [152]. However, stocking is still very common in order to improve angling activity. Except when take-able fish are released, it is not clear whether restocking really provides an increase of the quality of angling. Conversely, disadvantages of restocking are numerous. In addition to the loss of genetic integrity of wild strain due to hybridization, restocking creates an important ecological disequilibrium in the river. This disequilibrium is related to an increase of fish density without any regards to the "carrying capacity" of the river, possible competition for food and habitat, predation [171]. Because of these threats, restocking should only be used in very particular situations, for example when population extinction occurred due to pollution, or when the conditions for recolonization by neighbouring wild populations are not met.

The major issue is to know how anthropogenic hybridization has to be considered in terms of conservation. Allendorf *et al.* [173] provided guidelines to answer this question and reviewed the different points of view encountered in the literature. It is sometimes argued that hybridization should not be a concern. Indeed, the introgression among populations of the same species should not create outbreeding depression, as these populations generally share alleles. Furthermore, the introduction of hatchery strain may be considered as beneficial because, as exposed above, it often involves an increase in genetic diversity in the restocked sites. However, this point of view denies years of selection in the wild. If it is true that restocking increases local diversity, it decreases at the same time the global species diversity as it erases the effect of local adaptation.

5. CONCLUSIONS & RECOMMENDATIONS

A major contribution of the project was to provide new data on the genetic structure of several fish species over a wide range of rivers in the Scheldt and Meuse basins, and to provide new insights in migration capacities and activities of some common fish species. These results should serve as first tools to set up or reorientate policy and actions in nature and biodiversity conservation (particularly for wild endangered and Natura 2000 classified bullhead) and fishery management (particularly for trout and roach).

For trout, restocking increases the genetic diversity and decreases the differentiation between populations through introgression of the hatchery gene pools into the river gene pools. These effects are proportional to the restocking level and more important in a downstream stretch of the river than the upstream stretch of the river. Some upstream populations of trout blocked by barriers are least contaminated by stocked fish (most indigenous, e.g. Masblette). These sites require consideration for conservation and more research is certainly needed on the size and fitness of this population. These non-restocked sites should be considered as good candidates for conservation and could be preserved from stocking activities. Considering the ability of populations to recover after stocking has ceased [174], restocking could be stopped in the slightly restocked sites where river type fish still exist. This would lead to the recovering of a more "river type population". Another possibility for sites, where river type fish still exist is to identify the remaining indigenous individuals and use them for supportive breeding, i.e., breeding in hatchery of local strains [29]. Overall, such a policy in the studied zone would result in prohibiting the restocking in the non-restocked sites (Masblette), give permission for restocking in the strongly affected sites (downstream of Ourthe and Vesdre) and allowing supportive breeding only in the intermediate sites.

Roach is genetically uniform within basins or watersheds despite the fact that they did not pass barriers in our telemetry studies. Barriers might be too recent and populations too large for observing an impact on the genetic structure. Although published results show that roach do cross, perhaps lower, barriers [121] the extent of movement in our study was related to the distance between the physical barriers suggesting that the barriers were impeding further migration. The duration and dynamics for migration of roach was very similar between upland and lowland rivers. Stocking of roach might be a confounding factor within this data-set, but this could not be confirmed. We observed a substantial difference between Meuse and Scheldt populations. There was no evidence of Scheldt fish in the Meuse (no hybrids or second generation hybrids), while the Meuse has been stocked with Scheldt fish. Scheldt populations also differed more from hatchery roach than from each other. Both these observations indicate that roach stockings might have failed.

For bullhead, the river Ourthe is very homogenous when comparing upper and lower part. This probably reflects the natural situation. The in between populations show signs of disturbance which can no longer be remediated now that barriers impede migration. This proves that barrier effects might only show if there is a disturbing factor on a short time scale, but even without such disturbing events, effects are certainly to be expected on the long time scale. Although few, the migrating bullhead are very important for the restoration of disturbed populations and thus bullhead should be taken into account when looking at the remediation of barriers. Even if passes can only be passed at certain periods this might be important from a genetic point of view. Given the high level of geographical genetic differentiation between most of the wild bullhead populations, any translocation of fish should be strictly forbidden or limited to rivers likely to be similar in their genetic pattern for this species.

While most barriers were passable by trout, they were not for roach, grayling or barbel. Water conditions (flow discharge, temperature and ammonia levels) are very important, especially for trout as was shown here. Therefore, barriers might be passable one year, but not on another year. If upstream populations are under pressure, this might be threatening for the recruitment in these populations. Measured swimming capacities were very good for trout, carp and roach. However, predictions of leaping capacities seem optimistic, as we did not see roach pass barriers >0.5 m. In our field survey, they did pass fish passes with jumps/swims of 15-20 cm, and in the laboratory roach and ide were able to cross barriers of 25 cm. Further research to determine their capacity to cross barriers >25 cm is warranted. Calculated leaping capacities were certainly overstated for burst swimmers such as gudgeon, and were therefore not reported. In the laboratory, gudgeon seemed to be limited to a 5 cm leaping height. However, in the river Mark, they have been seen to pass 18,5 cm when they can swim between rocks and rubble (J. Coeck, pers. comm) instead of jump. In the Nete, they pass the fishpass when they can do so while swimming. In the Oxhe, even bullhead can pass differences in height of 25 cm if it has rocks and debris providing resting places. It is clear that variation in flow regimes and substrate are extremely important for such species. Maintenance of variation in water discharge is therefore desirable. Also in the laboratory leaping experiments, the water velocity on top of the construction proved to be an important factor influencing success in clearing the obstacles. Highest success rates were obtained within the range 0.8–1.2 m/s. Pool depth seemed less important, and a pool depth of 0.2 m showed to be sufficient to allow successful leaps.

Most river systems are physically and biologically very different between Flanders (lowland) and Wallonia (upland) and require specific eco-regional approaches and technical solutions for conserving and restoring the surface water continuity for fish species. Nevertheless, the same fish pass technologies could be applied for the lowland rivers stretches both in Flanders and Wallonia. It is the species composition that

contributes most importantly to the type of management that is necessary. As seen here, it is important to evaluate remediations after construction, since small differences can affect efficiency a lot (e.g. Grote Gete - Kleine Nete, Grote Nete, Mark, Aa beek). As such, each site with an obstacle in a river system represents a particular and unique case that needs a specific individual treatment what the design and building of a fish pass is concerned. As often as possible, fish passes should be equipped with a fish trapping device (or designed in a way allowing the temporary installation of such a device) to be used as a tool for monitoring fish movements and biology at physical obstacles. This would greatly enhance our understanding of the passabilty of barriers to different species.

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7. PUBLICATIONS ORIGINATING FROM THE PROJECT

SCIENTIFIC PAPERS IN PEER REVIEWED INTERNATIONAL JOURNALS

- Van Houdt JKJ, Pinceel J, Flamand MC, Briquet M, Dupont E, Volckaert FAM, Baret PV. 2005. Migration barriers protect indigenous brown trout (*Salmo trutta*) populations from introgression with stocked hatchery. *Conservation Genetics* **6 (2)**: 175-191
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Submitted

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SPSD II (2000-2005)

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