Report of the Workshop of a Planning Group on the Monitoring of Eel Quality under the subject “Development of standardized and harmonized protocols for the estimation of eel quality” (WKPGMEQ)

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# Contents

Executive summary ........................................................................................................ 4

1 Introduction .................................................................................................................. 6
   1.1 Main tasks ............................................................................................................... 6
   1.2 Participants .......................................................................................................... 7
   1.3 The eel and its stock decline .............................................................................. 7
   1.4 Assessments to meet management needs ...................................................... 7
   1.5 Other international legislative drivers ............................................................ 8
   1.6 Monitoring and studies at national level ...................................................... 8
   1.7 Absence of guidance ....................................................................................... 9
   1.8 Conclusion ...................................................................................................... 10

2 General issues on eel sampling for contaminants and/or diseases. Treatment of the sampled eels on site and in the laboratory. ........................................ 11
   2.1 Introduction, making maximal use of sampled eels ...................................... 11
   2.2 Species ............................................................................................................ 11
   2.3 Life stage ......................................................................................................... 12
   2.4 Gender ............................................................................................................ 13
   2.5 Site selection .................................................................................................. 14
   2.6 Length, weight and age ................................................................................ 15
   2.7 Size of the sample, number of eels to be analysed. ..................................... 16
   2.8 Season of sampling ...................................................................................... 17
   2.9 Sampling methods ........................................................................................... 17
   2.10 Detailed description about sampling methods, sampling sites, habitat characteristics and date ................................................................. 18
   2.11 Holding and hygienic measures for eel manipulation ................................ 18
   2.12 Labelling, storage and preservation of samples and subsamples ........... 18

3 Indices for measuring eel condition and fitness ...................................................... 19
   3.1 Eel condition .................................................................................................. 19
   3.2 Lipids .............................................................................................................. 20
   3.3 Gross energy .................................................................................................. 22
   3.4 Gonadosomatic Index (GSI) ......................................................................... 22
   3.5 Hepatosomatic Index (HSI) ......................................................................... 22
   3.6 Reproduction Index ....................................................................................... 23

4 Sampling and analysis of contaminants ................................................................. 23
   4.1 General aspects after eel sampling .............................................................. 23
   4.2 Killing and anesthetizing .............................................................................. 24
   4.3 Tissue sampling ............................................................................................. 25
4.4 Storage of the samples .................................................................25
4.5 General treatment of samples and subsampling .........................26
4.6 Subsample treatment/storage and measurements .......................26
4.7 Detection limits ........................................................................28
4.8 Quality assurance and quality control .......................................28

5 Interpretation, visualisation and assessment ..................................29
5.1 Eel Quality Classes ..................................................................31
5.2 Eel Quality Index (EQI), derived from the Eel Quality Classes .......33
5.3 IMBI: individual mean (multi-metal) bioaccumulation index ........37
5.4 The Eel Patho-Index (EPI), a Health Status Classification System ..37
5.5 Reproductive potential in silver eels...........................................38
5.6 Effective Spawner Escapement as evaluated in Irish rivers ..........39
5.7 Conclusion ...............................................................................40

6 Diseases .....................................................................................40
6.1 Parasitic diseases .....................................................................40
  6.1.1 Anguillicoloides crassus .................................................... Error! Bookmark not defined.
  6.1.2 Other parasites ...................................................................41
  6.1.3 Reporting results ...............................................................42
6.2 Viral and bacterial diseases .......................................................42
  6.2.1 General introduction ...........................................................42
  6.2.2 Viral diseases ....................................................................43
  6.2.3 Bacterial diseases ...............................................................45

7 Perspectives of using biomarkers of effects to assess eel health ......47

8 International context and perspectives ..........................................50
8.1 Eel Regulation ..........................................................................50
8.2 Data Collection Framework (DCF) .............................................51
8.3 Water Framework Directive (WFD) ...........................................51
8.4 Marine Strategy Framework Directive .......................................52
8.5 Human health issues (Food safety) ............................................53
8.6 Reports of the Joint EIFAAC/ICES Working Group on Eels (WGEEL) .................................................................53
8.7 OSPAR ....................................................................................54
8.8 Results availability and need of an international database ..........54

9 Conclusions ...............................................................................55

10 Recommendations ......................................................................57

Annex 1: References ........................................................................58
Annex 2: Participants list ..................................................................75
Annex 3: Agenda, timetable and meeting tasks .................................79
Annex 4: WKPGMEQ terms of reference.................................................................81
Annex 5: Glossary.................................................................................................83
Annex 6: Eel Quality Database – general structure.............................................87
Annex 7: Country Reports: Report on the methods and assessment of eel quality (contaminants and diseases).................................................................88
Executive summary

The Planning Group on the Monitoring of Eel Quality met at the Research Institute Nature and Forest (INBO), Brussels, Belgium, on 20–22 January 2015 during its first Workshop (WKPGMEQ) under the subject “Development of standardised and harmonised protocols for the estimation of eel quality”, chaired by Claude Belpaire (Belgium) and Olga Haenen (The Netherlands). There were 31 participants (21 attendees and 10 remote participants) representing 13 countries.

Reliable assessment of the eel stock quality and its quantitative effect on the reproductive stock is currently not possible, due to insufficient spatial and temporal data coverage (ICES 2009). This has emphasised the urgent need to establish a comprehensive overview with improved spatial coverage of the quality of the eel population across Europe as an essential and urgent requirement. Understanding the reproductive potential of the international spawning stock is a key component to predicting the effects on stock recovery of changes to silver eel escapement, arising from management actions implemented within Eel Management Plans.

To address this need, ICES 2012b recommended that Member States implement routine monitoring of lipid levels, contamination and diseases. Many countries have started compiling data on the health status of eels in their water bodies. Objectives for these monitoring actions are diverse and are not restricted to the framework of eel recovery. Eel quality is also monitored for different purposes, which include human health considerations and to meet requirements of the Water Framework Directive. Hence, there is a large amount of information collected by EU member countries. However, procedures with respect to sampling, analysis and reporting are not harmonised, hindering stock wide assessments and risking inefficient use of resources. Consequently, ICES (2009a) identified the need to develop standardised and harmonised protocols for the estimation of eel quality, so that national data would be comparable between Member States and could be reliably incorporated in international stock assessments.

The objective of WKPGMEQ was to document standardised and harmonised protocols for the estimation of the quality of the European eel Anguilla anguilla, with regard to the bioaccumulation of contaminants and the presence of diseases, including parasites.

WKPGMEQ took advantage of the preparatory work of the participants who in advance of the workshop drafted reports describing the framework and methods used in their countries for the assessment of contaminants and diseases in the eel. At the start of the meeting Member States’ country reports were presented. Two subgroups, covering contaminants and diseases respectively, further discussed the practical issues surrounding the sampling, assessment procedures, diagnostic approaches and reporting related to measuring contaminants and diagnosis of eel parasites and other diseases. As far as possible, common procedures and guidelines were described.

The report starts with an overview of the current eel quality assessments in the Member States, and further discusses general issues on sampling of eel quality assessments. It includes a chapter on the assessment of eel condition in terms of fitness and lipid levels. In further chapters best practices to (sub)sample, analyse, report and visualise contaminants in the eel are described. The disease sections focus on parasitic diseases (including the
swimbladder parasite *Anguillicolaides*), and on viral and bacterial diseases. Possible ways to integrate data and to implement them into eel quality indices have been suggested. The workshop also discussed the future perspectives of using biomarkers of effects to assess eel health. Finally the report concludes describing the international context and future perspectives in eel health assessments.

Several recommendations were made to facilitate the further development of a framework to integrate eel quality assessments into the quantitative management of the eel stock. Member States should apply harmonised methods for eel quality assessments and reporting, and routine monitoring and reporting of lipid levels, contamination and diseases needs to be integrated in the requirements within the Eel Regulation. Raw data should be made available to the international community and the management of the Eel Quality Database needs a structural basis. There is an urgent need for an internationally coordinated research project aiming at improving the understanding and quantification of the effects of contaminants on the reproductive success of the European eel, to allow integration of quality indicators in stock wide assessments.
1 Introduction

1.1 Main tasks

The ICES Workshop of the Planning Group on the Monitoring of Eel Quality [WKPGMEQ] (chaired by: Claude Belpaire, Belgium and Olga Haenen, The Netherlands) met at the Research Institute Nature and Forest (INBO) in Brussels, Belgium between 20-22 January 2015 to consider the development of standardised and harmonised protocols for the estimation of eel quality, following the terms of reference (ToR) set by WGEEL.

The workshop was preceded by a chair coordination meeting on 23 October 2014 in Brussels. The workshop was opened at 10:00h on Tuesday 20 January 2015, and closed at 16.30h on Thursday 22 January 2015 (the workshop agenda is provided in Annex 3). The following ToRs were defined:

a) Design standardised and harmonised protocols for the estimation of eel quality with regard to the bioaccumulation of contaminants (including sampling, analysis and reporting).

b) Design standardised and harmonised protocols for the estimation of eel quality with regard to diseases (including sampling, analysis and reporting).

After a plenary informative session with lectures, intensive group discussions were held. The different chapters (tasks) were written by the task groups during the workshop.

The report starts with an introduction and an overview of the current eel quality assessments in the Member States (Chapter 1), and further discusses general issues on eel sampling developed for eel quality assessment (Chapter 2). It includes a chapter on the assessment of eel condition in terms of fitness and lipid levels (Chapter 3). In Chapter 4 best practices to (sub)sample, analyse, report, interpret and visualize contaminants in the eel are described. Chapter 5 describes how data collected during eel quality assessment may be used to calculate eel quality stock indicators. The disease sections (Chapter 6) focus on parasitic diseases (including the swimbladder parasite Anguillicoloides), and on viral and bacterial diseases. Possible ways to integrate data and to implement them into eel quality indices are suggested. The future potential of using biomarkers of effects to assess eel health were also discussed (Chapter 7). The international context, future perspectives in eel health assessments and the need of an international database are discussed in Chapter 8. The report ends with conclusions (Chapter 9) and recommendations (Chapter 10).

In response to the ToR, WKPGMEQ considered 10 Country Report Working Documents submitted by participants in advance of the workshop (Annex 6). Other references cited in the Report are given in Annex 1. Additional information was supplied by correspondence by those Working Group members unable to attend the workshop. A glossary of terms and list of acronyms used within this document is provided in Annex 5.
1.2 Participants
Thirty-one experts (21 attendees and 10 remote participants) representing 13 countries, participated in the WKPGKMEQ. A full address list for the attendees and remote participants is provided in Annex 2.

1.3 The eel and its stock decline
The European eel (*Anguilla anguilla*) is a long lived semelparous species distributed across the majority of coastal waters in Europe and North Africa. The species has a complex life history, spending most of its time in continental fresh and brackish waters, but thought to migrate as silver eels to the Sargasso Sea for spawning. We refer to WGEEL (ICES, 2014) for a more detailed description of its life cycle and ecology. The stock recruitment indicator (the amount of glass eels arriving in continental waters) declined dramatically in the early 1980s and the stock is considered as critically endangered (see recent ICES WGEEL reports). The reasons for this decline are uncertain but may include overexploitation by fisheries, predation, pollution, non-native parasites and other diseases, migratory barriers and other habitat loss, mortality during passage through turbines or pumps, and/or oceanic-factors affecting migration (ICES, 2014).

In recent years WGEEL has considered the risks of reduced biological quality of (silver) eels. The reduction of the fitness of potential spawners, as a consequence of (specific) contaminants and diseases, and the potential mobilisation of high loads of reprotoxic chemicals during migration, might be key factors that decrease the probability of successful migration and reproduction. Also, some diseases have been put forward as significantly impacting the eel. An increasing amount of evidence indicates that eel quality would be important to understanding the reasons for the decline of the species (ICES, 2014).

1.4 Assessments to meet management needs
To enable a better assessment of the impact of contaminants and diseases which may affect spawner biomass and reproductive success of eels, the implementation of national routine monitoring programs is required (ICES, 2012b). ICES further recommended taking up an obligation of the Member States (MS) for the realisation of routine monitoring of lipid levels, contamination and diseases as part of the Eel Regulation. In particular WGEEL (ICES, 2013) gave some specific advice for planning future reporting of the assessment of the quality of local stocks, specifically for calculating the Reproductive Potential of silver eels and the Eel Quality Index (EQI) of yellow eels in each Eel Management Unit (EMU). Minimal requirements for silver eels are the percentage lipids, muscle concentrations of the sum of 6 indicator PCBs, and prevalence (%) and abundance (n) of *Anguillicolaides crassus*. In yellow eels, the basic requirements for assessing the quality and calculating the EQI, are the levels of ∑6 PCBs, ∑DDTs, cadmium, lead and mercury, and for *A. crassus* prevalence (%) and abundance (n) (see Chapter 8 for more details).
1.5 Other international legislative drivers

Apart from the need to measure levels of contaminants in eels in support of the stock restoration, data on contaminant levels in eel may also be recorded in the context of the Water Framework Directive (WFD). In cases where local eels stocks are targeted by recreational or professional fisheries, contaminants may be measured in the context of human health issues and the protection of the consumer.

Regardless of some restrictions of using eels due to their protected status, contaminant analyses are carried out by several MS under the WFD and may constitute an important source of information about eel quality. The WFD commits European Union member states to achieve good ecological and chemical status of all water bodies by 2015. Directive 2013/39/EU requires monitoring chemical status of surface waters, through analysis of fish tissues for specific substances. Considering the excellent performance of the eel as indicator species for local environmental pollution, some MS used the eel for analysis (see ICES, 2012b).

Regulation (EC) No 93/315 of 8 February 1993 (and Regulation (EC) No 1881/2006, amended by Commission Regulations, 420/2011, 835/2011, 1259/2011) established the principle that maximum levels should be set for contaminants in foodstuffs in order to protect public health (European Commission, 1993, 2006, 2011a, b)). Food containing contaminants at levels not compliant to these regulations may not be marketed. Considering the high market value of eel, the volume of its fisheries and the propensity of the species for bioaccumulating pollutants, the sanitary control of this species and its levels of regulated contaminants is a specific focus. Again, these data may generate valuable information on the eel quality in specific EMUs (see Chapter 8 for more details).

1.6 Monitoring and studies at national level

After analysis of the country reports (CRs) it was concluded that in most of the MS a routine monitoring network measuring contaminants and diseases in eels is not yet in place, despite the international need to estimate silver eel quality. The perceived reason is that reporting the quality of eels in the specific EMUs is not (yet) a (formal) requirement within the Eel Regulation, despite the recommendations of ICES (2012a) and OSPAR (2010). Similarly, Data Collection Framework (DCF) does not (yet) include the requirement to report on eel quality indicators (as recommended in ICES 2012b). However, the implementation of the new EU-Data Collection Multi Annual Programme (DCMAP) may envisage in its future strategy the inclusion of eel quality data.

Nevertheless, some MS have collected eel quality data relatively routinely, mostly targeted towards contamination. In The Netherlands annual and national monitoring of contaminants in eel has been conducted since 1978, and was initiated for the assessment of the status of pollutants in the environment and for food safety reasons (as requested by the Ministry of Economic Affairs) (De Boer et al., 2010). Also in Flanders, Belgium a monitoring network for contaminants in eel was conducted between 1994 and 2009 (as requested by the Ministries of Environment and of Human Health). Eel contaminant monitoring in Belgium was initially directed towards human safety in the framework of recreational fisheries (Bilau et al., 2007), but soon generated results useful to monitor the environmental status of contaminants for local policy (Maes et al., 2008) or in the context of WFD (Belpaire & Goemans, 2007b). Moreover, the network triggered studies showing
evidence of the detrimental effects of contamination in the eel through physiological, genomic and proteomic work. In France, national monitoring of PCBs was conducted from 2008-2012 in different fish species, including eels, as requested by the Ministries of Ecology, Agronomy and Health (http://www.pollutions.eaufrance.fr/pcb). Regarding diseases, routine monitoring is only carried out in France and the Netherlands. Since 2010, the glass eel quality (parasites and viruses) has been monitored as part of the eel restocking programme along the Atlantic French coast, and for decades, the Dutch yellow eels have been monitored for clinical disease and presence of A. crassus.

Other significant parts of data sets exist at international level which originate from ad hoc studies and research projects funded by regional or national administrations. This was the case in most of the MS, for both contamination (e.g. Amilhat et al., 2014; McHugh et al., 2010; Szlinder-Richert et al., 2014a, b; Pujolar et al., 2013; Sühring et al., 2013; Roland et al., 2014; Gravato et al., 2010; Esteve et al., 2012; Jürgens et al., 2013) and diseases (e.g. Becerra-Jurado et al., 2014; Lorin-Nebel et al., 2013; Quadrini et al., 2012; Wysujack et al., 2014; Haenen et al., 2009; Hermida et al., 2008; Mayo-Hernández et al., 2015; Armitage et al., 2013). For a complete review we refer to the WGEEL reports (ICES, 2008, 2009a, 2010, 2011, 2012b and 2013). These projects include a range of different objectives including the assessment of human health related to the risk of exposure to contaminants in eel, the assessment of habitat quality, and the environmental status of contaminants, the distribution and spread of eel diseases and parasites, and the evaluation of the quality of yellow eel or silver eels (spawner) stocks.

1.7 Absence of guidance

In most countries national guidelines for eel sampling and the measurement of contaminant are not available or are only accessible on an institutional level. In some cases (e.g. in the UK, Lewin et al., 2014) the development of national guidance for eel health examination is in progress. Common procedures to estimate eel quality on an international level are not available, except for a sampling protocol used during an international FP7 research project which focused on silver eel (www.eeliad.com).

As a consequence, methods of sampling, sample handling procedures, the range of chemical contaminants and other parameters measured, and the protocols for data reporting differ significantly among studies. Similarly, results of these studies are presented in different formats (including scientific papers), but the raw data is usually unavailable to the international community. Depending on the country, data are compared to different threshold values. Examples include permissible limits of contaminants in food, quality classes from monitoring of eels in Belgium, EQSbota values set up in the WFD and EQI.

The compilation of available results and their utilisation for the holistic assessment of the quality of European eel spawners is hampered by significant variability in methods and reporting. The development and application of standardised protocols in the study of contaminants and diseases in eels conducted in different countries are of primary importance. It would significantly increase the potential use of data provided, and help progress understanding the effects of contaminants and diseases on eel health and reproduction success. Considering the need to integrate eel quality indicators in future stock assessment at a European level, standardisation of data collection is vital.
WKPGMEQ was setup to progress on the development of standardised and harmonised protocols to measure Eel Quality. This report is the result of this exercise. It is the intention that the recommended guidelines are used by MS for measuring the quality of local eel stocks, ensuring the use of national data in international eel stock assessments.

1.8 Conclusion

This report is a first step towards a harmonised methodological approach in assessing and reporting the quality of eel across its distribution area. It is part of a wider framework to allow the integration of eel quality indicators in international stock assessment. Figure 1.1 shows the representation of the future workflow. Inclusion of eel quality monitoring requirements in the European legal framework, implementation of monitoring strategies in the Member States, data management on an international level, and further research on the benchmarking of detrimental effects of contaminants and diseases are essential further steps, required for giving scientific advice on management of the international eel stock.

Figure 1.1. Workflow for integrating eel quality indicators in the international advice for stock management and restoration.
2 General issues on eel sampling for contaminants and/or diseases.

Treatment of the sampled eels on site and in the laboratory.

This chapter discusses issues related to the sampling of eels including sampling methods, targeting specific life stages, sampling numbers, size, gender and life stage, site description and characterisation of abiotic descriptors. Also, information regarding the holding of sampled eels on site, during transportation and in the laboratory.

2.1 Introduction, making maximal use of sampled eels

The eel is considered a critically endangered species by the IUCN (Jacoby & Gollock, 2014), hence destructive sampling for monitoring purposes should be kept to a minimum. However, despite its status as an endangered species, eels are still caught in considerable quantities in fisheries in many European countries. Europe wide, fisheries of yellow and silver eel currently amount to circa 3000 tonnes (ICES, 2013). In comparison to this, required yearly quantities for monitoring are low. For example, it has been calculated that the quantity required for the Flemish eel-monitoring network for contaminants, is less than 0.005% of the eels consumed annually by the Belgian population, and less than 0.07% of what is currently harvested by recreational fisheries in Flanders (Belpaire & Goemans, 2007a).

For scientific purposes, the European Data Collection Regulation (DCR) (European Commission 2008a, 2008b, 2010) requires the annual analyses for length, weight and age of 100 yellow and 100 silver eels. This is completed per river basin district on a national level. Within several member states, eels are also sampled for Food Safety, to support the progress of Eel Management Plans and for Water Framework Directive purposes.

To minimize culling of eels for monitoring purposes, we recommend synergy in monitoring actions, and making maximal use of the sampled eels, by combining studies of contaminant analysis and diseases. This approach will reduce the number of sampled eels and enable analysis of the synergetic effects of combined pressures.

Specific objectives may require adequate and adapted sampling strategies. What to analyse in the eel and in what tissues, will be determined by the purpose of the study, and many parameters should be taken into account when setting up a sampling protocol. Contaminants may have quite different behaviour regarding uptake and accumulation in the eels, depending on their chemical properties, which might impact the sampling strategy (see section 4.1). The setup of a standard protocol allows consistency for future studies.

2.2 Species

In most European studies on eel, determination of individuals on species level is not carried out and most field studies assume they deal with A. anguilla exclusively. However, it has been reported that among eel populations in Europe other species (mainly A. rostrata, the American eel) may occur. Frankowski et al. (2008) reported that among eels from free-draining European inland waters 2.6% are American eels. In addition to this hybrids have been found in Iceland where both species occur (Albert et al., 2006). Similarly, other Anguilla species have occasionally been stocked in Europe (Jacoby & Gollock, 2014).
Eel species identification (European eel and American eel only)

Individuals of *A. rostrata* have fewer vertebrae than *A. anguilla* (102-112, usually 106-108, vs. 111-119, usually 114-116). DNA analysis is the best tool to distinguish between European eels and other species and samples of muscle or fin tissue can be collected in >70% ethanol. For further analytical procedures refer to Frankowsky *et al.* (2008) and Trautner (2006).

2.3 Life stage

Sampling strategies to assess eel quality are very different dependent on the objectives of the study and the life stages must be targeted for these objectives. As pointed out by WGEEL (ICES, 2013) there is a need for future reporting on both, the quality of eel in local stock assessments and the quality of silver eel leaving the catchment for their journey to the spawning grounds (for the specific requirements see Chapter 8). For local stock assessment, studies should focus on the quality of the yellow eel stage, e.g. enabling the estimation of the EQI (see Chapter 5). In contrast, to estimate the Reproductive Potential of the eels in the EMU, silver eels should be targeted.

*Yellow eel*. As a long-living semelparous carnivore with high body fat content, the European eel (*Anguilla anguilla*) is probably one of the most vulnerable fish species for the accumulation of lipophilic contaminants during its continental feeding and growth phase. Yellow eels are considered primarily sedentary (Baras *et al.*, 1998; Lafaille *et al.*, 2005; Belpaire & Goemans, 2007a), although investigations on otolith microchemistry (at least for yellow eels in lower river stretches) are partially contradictory (Marohn *et al.*, 2013). Due to the life-long accumulation of lipophilic contaminants, the examination of yellow eels provides an overview of the contamination level they have been exposed to during their life. Yellow eels are most appropriate for the detection of local contamination sources within catchments and therefore habitat-specific contamination patterns. Various lipophilic contaminants may accumulate to very high levels in fat stores of the yellow eel, dependent on the pollution pressure of the habitat where they lived. They may become toxic when released in to the blood during the silver phase and the reproductive migration, and reach sensitive tissues such as the liver or the ovaries. During the continental growth phase, yellow eels are challenged by several diseases and parasites, of which some are reported to have serious impact on their host and its capacity to complete their migration. Yellow eels can therefore be regarded as most suitable for the general impact assessment of a specific habitat throughout the eel’s continental life. The assessment of yellow eel quality over the different EMUs enables an overview of the variability in quality over its distribution area. This will have implications for eel management measures (e.g. restocking practices) and allows possible inclusion of quality issues in the international stock assessment.

*Silver eel*. In contrast, silver eels, which have already started their downriver migration, are considered to have reached their maximum level of lipophilic contaminants. During migration, the less lipophilic contaminants can be (partially) excreted due to equilibrium processes (as modelled by Foekema, 2013), with residual levels being less than 10% of initial levels. The highly lipophilic com-
pounds, such as the highly chlorinated PCBs, dioxins and flame retardant (PBDE), will not be eliminated during migration.

As a result of their migration, silver eels cannot be considered sentinels for local contamination. However, they may be potentially representative of the whole watershed, if an appropriate sample number is assessed.

Assessment of the quality of silver eels leaving their EMU, potentially gives the best available information on possible impacts of contaminants and diseases on migration and successful reproduction. Fitness descriptors of potential spawners (e.g. size, condition, lipid levels) are required to estimate the Reproduction Potential (in terms of number of produced eggs) of female silver eels leaving the EMU. Also, parasites and diseases including heavy infections in the silver eels (e.g. by A. crassus) may lead to reproductive migration failure. As such, it is essential this information is included in eel quality data in the stock quantification models, used for the international advice.

Apart from the monitoring of quality in the context of the eel restoration, valuable information may be gained through eel quality measurements for WFD and under human health directives. Both frameworks use their own guidance towards their recommended methods (respectively European Comission, 2014 and European Commission, 2006). While sampling under the WFD is targeting sedentary (thus yellow) eels, analysis under sanitary control of fisheries products may target both life stages, dependent on the nature of the fisheries.

In some studies the life stage of eels is not reported. Some published papers combine data from silver and yellow eels. As such, inappropriate conclusions can be made regarding the habitat quality of a sampling site when silver eels have been assessed from hundreds of kilometers upstream. It is therefore essential to record and report on the stage of individuals chosen for quality assessment. It is recommended not to combine data from silver and yellow eels in the studies.

**Determination of life stage**

Yellow and silver eels differ in a number of external morphological features (colour, eye size, pectoral fins, see for example Tesch 2003). In the field, without morphometric measurements the external visual distinction is not always conclusive and intermediate forms may occur. Several methods are used such as the Pankhurst Ocular Index (1982), and two qualitative criteria that specify the state of the lateral line (i.e. differentiated or not) and the color contrast (i.e. present or not) (Acou et al., 2005). We recommend using the staging system introduced by Durif’s silver index (Durif et al., 2005), see also section 3.6.

### 2.4 Gender

It is important to determine the gender of the eels targeted for quality assessment. Female eels, which may grow larger and may be older, may have higher infection levels of parasites than males. Similarly, large females might have bio-accumulated higher levels of contaminants, due to more time spent in polluted waters or through a shift in feeding regime (females tending to be more piscivorous than smaller males). Males may contain higher lipid levels compared to females of similar lengths, which may affect contaminant
levels. If male/female ratios differ between samples, this might need consideration in the interpretation of results.

To assess the Reproductive Potential (in terms of expected number of eggs produced) in an EMU, female silver eels should be analysed. But, eel quality assessments in males may also be used to calculate energetic contents in comparison of their requirements to fulfil migration and spawning.

The male/female ratio in the sample should be chosen so to reflect the male/female ratio as observed in the catch.

**Determination of the gender**

Eels above 46 cm can be considered as female. Eels smaller than 46 cm may be both, male or female. The male/female ratio may differ between locations. Targeting eels larger than 46 cm will result in eel quality assessments of females only, which may be required for avoiding sex related biases (e.g. related with different lipid levels between both sexes). In contrast, by targeting a sample with eels smaller than 46 cm a mixed gender sample can be expected.

For eels smaller than 46 cm, the gender has to be determined by macroscopic examination of the gonads and stage data recorded according to Durif et al. (2005). Gonads from female eels can usually be differentiated macroscopically from males (See pictures: Tesch and Thorpe, 2003, pp.: 36-37). For smaller yellow eels, distinguishing gender after macroscopic observation may not be so obvious. As such, in small eels it is necessary to collect a piece of gonad tissue and squash it under a coverslip on a microscope slide for direct observation under a light microscope (or fix gonads in a fixative such as buffered formaldehyde, Bouins or Davidsnsons for histology). Note that even after microscopic observation (of a fresh squash or of a histological preparation) distinguishing gender for small yellow eels remains problematic.

### 2.5 Site selection

Determining the location of a site for sampling eels in support of quality assessment, will be dictated by several limitations. Practical issues such as accessibility and sampling facilities usually govern site selection. For the sampling of silver eels, sites must be chosen along the migration route and samplers may take advantage of some local factors e.g. infrastructures such as fish-passes or fish traps located near hydroelectric stations or weirs. Site selection may focus on areas identified as model cases for a holistic approach in defining eel stocks, covering the assessment of many aspects relevant to eel (quantification of the stock, quality of the stock, quality of the habitat, environmental pressures, etc.).

For quality assessments in yellow eel over an EMU, site selection is of primary importance, as the eel reflects local conditions with regard to pollution pressure. The choice of the samples will be dependent on the objectives of the study. If the aim is to estimate the eel quality over an EMU, an a-select sampling scheme with a number of random sites is most appropriate. The number of sites to be selected will be dependent on the size of the catchment or EMU, and the heterogeneity of the pollution. Where eel assessment is targeted towards monitoring the impact of different pollution sources on the eel, sampling sites should be selected in the vicinity of pollution sources.
It has been reported that pollution profiles in individual eels along a river (even at distances <5 km) may be significantly different (Belpaire et al., 2008). Pollution profiles of eels of given sites are often very similar but differ from eels from other sites (even if nearby), reflecting variability in pollution pressure throughout the river. Significant differences in contaminant levels in eels, over relatively small distances, might mirror differences in environmental pollution occurring up or downstream of a point-source (which may be a diffuse source e.g. related to agricultural run off). For this reason, for some monitoring networks, a sampling site has been defined as a river stretch restricted to a maximum of 250 m (Flanders, Belgium).

Efforts to monitor the health status of eel in a certain country are generally not designed to be representative for the whole country or area. Therefore the results presented for one or several sites per country cannot be regarded as an overview of the environmental quality for specific countries (ICES, 2012b). We recommend that the eel quality assessment should be carried out for a representative number of catchments/habitats per country.

### 2.6 Length, weight and age

Morphometric data such as total length (L) and weight (W) are essential parameters required in eel quality assessment, other than gender and stage. L and W are needed for the calculation of condition indices (see Chapter 5), and Reproduction Potential. They are essential for recalculating body burdens of contaminants.

The size and age of eels are crucial for the interpretation of contaminant data as well as disease incidence. Some contaminants are taken up extremely slowly (very lipophilic, low levels in the foodchain) and hence older animals can contain higher levels. Larger/older animals can eat different, larger prey items (higher in food chain), influencing the uptake of contaminants by the eel and infection of more specific parasites (such as A. crassus) through predation of infected host species (Lefebvre et al., 2002b; Neto et al., 2010).

Weight (with an accuracy to 1 g) and length (with an accuracy to 0.1 cm) should be measured at the time of capture or directly before dissection. Measurements should be taken on euthanized or anesthetized eels. W and L should always be expressed in grams and cm.

In case it is not possible to measure L and W on live or freshly killed eels, it can be determined on frozen fish after thawing. If thawed specimens are measured, values should be corrected by assuming a reduction of 2.4% and 2.7% respectively for L and W according to Wickström (1986).

Age is crucial for understanding and interpreting eel quality data. For the determination of the eel’s age (Graynoth, 1999) and life history information through microchemical otolith analysis (Marohn et al., 2013), otoliths should be sampled. They may also be stored for later analysis. We refer to specific guidance documents for otolith collection, process and reading (ICES, 2009b and Vøllestad et al., 1988).
2.7 Size of the sample, number of eels to be analysed.

Considering the budgetary implications of the analyses and the endangered status of the eel, the numbers of eels sampled should be restricted to minimal requirement. Sample size will be dependent on local conditions and capture methods. Clearly the sample size needs to be considered within the specific objectives, and sample size should be sufficient to ensure data is representative.

To estimate the quality of silver eels leaving an area the size of the catchment or EMU should be taken into account when defining the sample size. The anthropogenic pressures over the catchment also need to be considered. The size and heterogeneity of the water basin may dictate the number of silver eels required for a proper estimation of eel quality. Large sized catchments will require larger sample sizes than in smaller ones. The sample should reflect the eel quality of the entire water basin. Catchments with a high variability in levels and types of anthropogenic pressures will result in a high variability within the silver eels leaving the system. This will increase demand for analysis of more individuals than in catchments with moderate or uniform pollution pressure. To assess the Reproductive Potential of female silver eels, the sample size must allow a fair representation of the length class in the local population of the EMU. If males need to be included in the assessment, this also has to be taken into consideration. The male/female ratio in the sample should be chosen so to reflect the male/female ratio as observed in the catch.

In the case of the resident yellow eels, sample size per sampling locality may be smaller, as in general, eels of the same site show similar pollution profiles (Belpaire et al., 2008). For example, studies in Flanders, Belgium examined five eels per site for contaminants. Analysis of pooled samples (per site) may be considered (for budgetary reasons), but one has to be aware that individual information is getting lost. In The Netherlands contaminant analysis is performed on pooled samples of 30 individual eels. To make a representative estimation of the quality of yellow eels over an EMU, a number of sites have to be assessed. Again, the number of sites should be proportional to the size of the catchment and the anticipated heterogeneity of the pollution pressure over the catchment.

With regard to disease diagnosis, any clinically diseased eel should be analysed as a priority. However, in the absence of clinical disease or for surveillance of specific pathogens, larger sample sizes would be needed. Testing for the absence or presence of a virus would need a maximum of 150 eels, depending on the population size according to Poisson (Simon & Schill, 1984).

In parasitological studies, when assessing the sample size, a number of factors should be considered in order to develop a clear and robust approach. This will depend on the type of examinations being conducted, the questions being asked, and the resources available to do such work. We encourage sampling at least 30 eels from each location. If no previous data exists for a particular environment the number of eels sampled should be increased (Simon and Schill, 1984).

Specific measurements in support of human health sanitary controls and the WFD may have separate requirements in terms of sample size. We refer to the specific guidance for this (respectively European Comission, 2006 and European Commission, 2014).
2.8 Season of sampling

For practical reasons, sampling is usually restricted to seasons when eels are active. In winter months sampling is not appropriate, as efforts with conventional gear will result in very low catches.

Sampling of silver eels during downstream migration is mostly restricted to the migration season, from July to late December (Tesch, 2003; Vøllestad et al., 1986). It is of note that environmental parameters (e.g. water discharge) may significantly influence the timing of the migration peak. Yellow eels may be collected throughout the year (with exception of the winter months).

Where sampling is completed for analysing non-persistent contaminants in the eel (which may fluctuate over the year and are dependent of the period of application) sampling in autumn may not be appropriate. In this case, the sampling period should be chosen in relation to the period of application of the chemicals.

Note that season, and especially water temperature, may affect disease. Eels may also be injured and stressed through capture as well. Temperature at different times of the year will also influence the detection of parasites and diseases. In the case of viruses, efforts should be made to sample eels when water temperatures are as close to those optimal for specific viruses. The same applies to other parasites and pathogens. Preferably, to be able to compare disease data between years, sampling should be done at similar water temperatures in a country.

In general, for monitoring purposes over several years, sampling season should be kept constant.

2.9 Sampling methods

There are many methods used for sampling eels. For a review on eel fishing gear see e.g. Koops (1980).

In most countries the main fisheries techniques used to catch eels are electrofishing, fyke nets or longlines. In some countries (Germany and The Netherlands) an eel stower with a bottom sieve is also used for capturing silver eels. Additional techniques may include eel traps, baskets, pound nets, and collection of eels may also be possible through recreational or professional fishermen. In each case, aspects of gear selectivity (e.g. in life stage and size) must be considered.

The decision for choosing a particular gear is dictated by the targeted life stage. Gears such as fyke nets, traps and pound nets are more effective for catching silver eels, active during their migration. The most appropriate method for the collection of yellow eel is electrofishing (catching the eel near to the river bank during the diurnal rest phase), fyke netting or baited long lines (targeting yellow eels during nocturnal foraging activities). Local conditions (depth of the water, width of the stream, shape of the river bank, etc.) may restrict the use of specific gear.

National regulations concerning sampling for scientific/monitoring purposes must be considered, and special licenses may be required. All health and safety measures (e.g. regarding electrofishing) also need full consideration.
2.10 Detailed description about sampling methods, sampling sites, habitat characteristics and date

The assessment of the quality of eels should include reporting a detailed description of all relevant parameters, which is important for later interpretation. This description should include methodology and fishing descriptors (date, time), site location and typology, aspects of habitat and water quality and potential environmental pressures. For international assessment purposes this information is crucial to allow pan-European analysis.

Descriptors currently included in the Eel Quality Database:

- Location of the site (with country, county, city, site code (numbered reference), geo-reference (X-Y coordinates, other national grid system, latitude, longitude, watershed, watershed name, watershed area, water body, water body typology, width, salinity, level of anthropogenic pressure (regarding forestry, agriculture, industry, population, fisheries, legal status of fisheries, etc.).
- Survey descriptors (team, owner, contact, sampling method, date and time of survey, framework, reference to sampling protocol, etc.).

2.11 Holding and hygienic measures for eel manipulation

Eels need to be held separately from each sampling site in transport boxes prior to transportation to the laboratory. In the laboratory eels should be held in separate tanks for each site, until sampling, which should be done as soon as possible. This will avoid unnecessary retention of live fish and minimise impact on concentrations of certain contaminants (especially those of water soluble or metabolizable agents). Keeping eels too long in holding tanks may initiate bacterial or parasitic diseases as a result of stress. This may also be triggered by temperature changes. Aerated holding containers should be kept at the water temperature the eels were adapted to, and all water conditions should be optimal. One should be aware that holding facilities with water recirculation might cross-infect tanks. For disease monitoring and assessment of bio-accumulating lipophilic substances, flow-through systems should be used, and the water conditions should be monitored.

Technicians and laboratory personnel responsible for the maintenance and dissection of eels should wear appropriate personal protective clothing. This includes laboratory coats and disposable gloves that ensure good laboratory practice, minimise contamination and help to protect against possible zoonotic bacteria, for example *Vibrio vulnificus* (Haenen et al., 2013). See also chapter 6.2.

2.12 Labelling, storage and preservation of samples and subsamples.

All samples should be assigned a clear ID number, that accompanies all tissues and sample material. This should be used to clearly label all storage containers. This ID number should include reference to individual fish numbers, site reference and a date. The label should be resistant to water, freezing and any solvents used for fixation.

All relevant information, about the samples and subsamples, including workflow processes, data and storage details should be covered in a database, linked to a tissue bank.
After analysis, remaining samples or parts of samples should be archived and preserved, for later examination or future assessments.

Further description of the procedures for contaminant and disease assessments (including storage) is presented in Chapters 4 and 6 respectively.

3 Indices for measuring eel condition and fitness

Apart from measuring body burden of pollutants and assessing infection levels of eel by parasites or other diseases, many other general morphometric characteristics are commonly used as overall indications of eel health. This chapter reviews the different methods and indices used to assess eel condition or fitness.

3.1 Eel condition

Importance of measuring eel condition

Weight-length relationships are used for estimating the weight corresponding to a given length, and condition factors are used for comparing the ‘condition’ or ‘well-being’ of an eel (Tesch, 1968). This is based on the assumption that heavier eels of a given length are in better condition (Froese, 2006). Insufficient fitness (condition and energy resources), amongst other factors, has been reported as a potential restrictive factor, disabling long distance migration and successful reproduction (Belpaire et al., 2009). There are indications that poor quality of the spawners, namely the silver eels migrating to the oceanic spawning grounds, might be a key factor in explaining the decline of the eel stock (Belpaire et al., 2009). In eel a significant negative correlation between pollution load and condition has been reported (Maes et al., 2005). Standard length and weight measurements should be taken as a minimum for any eel examination (whether destructive or non-destructive sampling), as such this data allows an immediate calculation of the condition factor. This is a fundamental analysis in fisheries biology and one that can be ascertained with limited parameters (only length and weight).

How to measure eel condition

Many countries currently use Fulton’s Condition Factor as a means of analysing condition of eels. In Spain, eel condition has also been measured using the Scaled Mass Index (SMI), as described by Peig & Green (2009). However, this approach has only recently been used for fish analysis (Maceda-Veiga et al., 2014) and considered more complex than other measures of condition.

Work by Froese (2006) recommends the use of Le Cren’s ‘relative condition factor’ (1951) when comparing individuals within a sample. Le Cren (1951) reviewed weight-length relationships and condition factor, and introduced the ‘relative condition factor’, which compensates for changes in form or condition with increase in length. It measures “the deviation of an individual from the average weight for length” in the respective sample:

Equation: $K_{rel} = \frac{W}{\hat{L}^b}$.

Where $W$=observed weight and $\hat{L}^b$= expected weight obtained from length weight regression.
Le Cren’s relative condition factor is therefore recommended as the standard for analysing such eel data (Belpaire et al. (2009) and Oliver et al. (2015)). Measurements should be taken in cm and grams. It is of note that if fresh tissues are not used, and analysis is completed on frozen samples, then measurements will need to be corrected for. The length and body mass can be corrected to compensate for shrinkage due to freezing, using factors 1.024 and 1.027, respectively (Wickström, 1986; Clevestam et al., 2011).

### 3.2 Lipids

**Importance of measuring lipids**

Eels accumulate lipids during development from the elver to silver stage (Boëtius & Boëtius, 1985). Accumulation of energy through lipid storage may be affected by environmental factors such as pollution, disease agents, changes in food availability, global changes in the environment, changes in (density-dependent) sex ratios and even life-history characteristics, e.g. restocking (Belpaire et al., 2009). As silver eels fast during their reproductive migration to the Sargasso Sea, the successful completion of their life cycle relies on the quantity of lipids stored beforehand. These lipids are mainly accumulated during the growth phase and provide the energy necessary for migration to the Sargasso Sea, gonad maturation and spawning.

Lipid reserves are essential to cover energetic requirements for silver eel migration and reproduction. Large individuals (females as well as males) with high lipid content are considered to have a higher contribution to the spawning stock. Such knowledge of lipid levels shows a good estimation of whether eels are capable of completing their migration to the Sargasso Sea, and also whether they are able to spawn successfully when they get there. ICES (2012, 2013) carried out preliminary work on this issue and demonstrated with field data the heterogeneity of the reproductive potential in local eel stocks (using parameters such as lipid level, cost of transport, eel size, distance to Sargasso Sea). It was concluded that parameters developed for estimating the condition of escaping silver eels have the potential to be used to calculate the Reproductive Potential of individual female silver eels leaving their catchment. This quantitative approach in estimating eel quality can be integrated into the stock assessments. This has important implications for stock management, although the development of this methodology is hampered by the lack of field verified ‘dose effect’ threshold information and a lack of monitoring data (ICES, 2013).

Clevestam et al. (2011) recommended that analysis of fat content ideally should include the whole eel carcass in order to get the correct estimate of total fat stores. This, however, may not be feasible due to other samples being collected or specific sampling techniques undertaken. As such specific and consistent sections of muscle should be collected.

Larsson et al. (1990) assumed that silver eels only start to migrate once their fat content reached a minimal value (28%), sharing the view of Thurow (1959). It was suggested that, when fat content in the muscle reaches a level of saturation at 28%, lipid levels in the blood start to increase, triggering the production of hormones responsible for metamorphosis and sexual maturation (Larsson et al., 1990). This idea that a critical fat mass must be reached before silvering has been generally accepted as the cue to initiate silvering (Lokman et al., 2003).
Large amounts of work have examined the lipid levels needed for eels to successfully migrate to the Sargasso Sea and varying thresholds (% total body lipid content) have been proposed; 20% (Boëtius & Boëtius 1980), 13.5% (Palstra et al., 2007) and 20.7% (van den Thillart et al., 2007). It has been discussed that at least 13% lipid is necessary for swimming (Belpaire et al., 2009), however additional reserves are required to complete maturation. As such, it has been given that minimal fat levels of 20% are required to allow migration and successful reproduction (van den Thillart et al., 2007).

How to measure lipid levels

Fat content is measured as the lipid concentration in muscle and is expressed in % of muscle wet weight (Belpaire et al., 2009). Where only sections of tissue are collected, it is important to understand that the lipid levels in the muscle are not homogenous across the body length (McCance, 1944; Tesch, 2003). As such, it is vital that consistent areas of the eel are examined to allow better comparison within samples. Currently, different countries take muscle samples from varying parts of the eel body for lipid analysis. Clevestam et al. (2011) used 5 cm long section, taken 5 cm behind the anal cavity, for fat analysis. In Belgium, eels are cut into five equal parts, and the most central part (including tissue of both left and right side), is targeted for lipid analysis. In The Netherlands, fat is analysed in a pool sample consisting of two muscle section from a proximal and a distal part of the body.

Eels should be skinned and filleted, and the same area of the muscle used throughout the analysis. Ideally, a homogenized pool of the whole eel muscle filet would be the most appropriate, however for practical reasons this may not be feasible. A study to assess the variation of lipid levels in individual eel muscle over the body is urgently required.

Procedures for lipid analysis are described under Chapter 4.6.

How to measure lipids with non-destructive techniques

Non-lethal assessment of eels is important where, for instance, eel populations are at low levels or where destructive analysis is not allowable (e.g. for ethical reasons). The use of a fat meter and non-destructive sampling could allow multiple examinations of the same fish to be collected and compared.

Based on work by Klefoth et al. (2013), the use of a fat meter was considered a suitable method to non-lethally estimate energetic status in eel. The sensor is placed on the eel muscle and directly measures water content as the inverse of dry mass (Schoeller, 2000). Due to the size of the sensor of the meter, very small eels cannot be measured efficiently. The fat meter is available in two sensor sizes and the smaller sensor is more suitable if males, as well as females, are planned to be monitored.

The use of fat meters has shown some inconsistencies in analysis and other destructive methods already described are more accurate at providing realistic and reliable lipid values. However, if non-destructive sampling is being completed and an estimate of lipid levels is required, then the use of a fat meter is considered more useful than no data at all.

Note however, that the use of a fat meter on a single eel from a site may not be wholly appropriate due to potential inaccuracies and lack of comparability of data. This lack of comparison to cohorts from the same site means that errors could occur.
3.3 Gross energy

Schreckenbach et al. (2001) demonstrated the possibilities of estimating the gross energy in fish. Gross energy content, expressed as MJ/kg can be analysed in an entire eel. The calculation of the gross energy is based on the analysis of proteins, fat, and nitrogen-free extracts, using 1 g crude protein = 23.9 kJ, 1 g crude fat = 39.8 kJ, and 1 g nitrogen-free extract = 17.6 kJ, following Kleiber (1967). Although this analysis may give a full picture of the energy reserves within an entire eel, and thus may be more accurate in estimating its migration and reproduction potential, the required additional analytical procedures may limit its applicability.

3.4 Gonadosomatic Index (GSI)

The gonadosomatic index is based on the calculation of the gonad mass as a proportion of the total body mass and is used to assess the status of sexual maturation.

The gonad weight increases during silvering. Durif et al. (2009) stated that the GSI rarely goes above 1.5%, but can reach 50% when sexual maturation is hormonally induced.

Recommended equation to use, as per Durif et al. (2009):

\[
\text{GSI} = \frac{\text{mass of gonad}}{\text{body mass}} \times 100
\]

There may be difficulties when collecting gonad samples for GSI analysis. When silvering, eels may have gonad that has embedded into fat within the body cavity, thus making it difficult to analyse efficiently. Also, the delicate nature of the gonad and its ability to stick to organs, makes examination difficult if all of the gonad cannot be removed without damaging. The time consuming nature of gonad removal needs to be considered, especially if other more time-specific testing needs to be completed (such as histology or bacteriology).

The size of the gonad, especially in yellow eels, will also make it difficult to remove the tissues fully and then weigh them to a significant accuracy. In such cases, specific top-pan balance/scales are required. This would rule out any possible examinations in the field. Due to these difficulties, the only life stage to be examined for GSI should be silver eels.

3.5 Hepatosomatic Index (HSI)

The hepatosomatic index assesses the state of the liver by comparing its mass related to body mass. HSI is important as the liver weight is highly correlated with gonad development and vitellogenin levels (Durif et al., 2006). Increased liver weight is also associated with high maturation response. Analysis of HSI should be completed on yellow and silver eels only.

A number of examinations/samples may be collected from the liver of eels. As such, to get a reliable HSI value the liver needs to be weighed early on in any examination prior to sections potentially being removed for histopathology/fat/parasite analysis etc. Care must be given to liver removal from the body cavity, due to its proximity to the gall bladder. Accidental bursting of the gall bladder may contaminate samples or could irritate the skin/eyes.
Recommended equation to use, as per Durif et al. (2009):

\[
\text{HSI}=\frac{\text{mass of liver}}{\text{body mass}} \times 100
\]

### 3.6 Reproduction Index

Durif et al. (2005) listed a number of parameters used to distinguish differences between yellow and silver eels (but not necessarily condition). These include factors that can be used to analyse the reproduction stage of eels, including: growth, fat stores, increase in eye size, increase in pectoral fin and development of liver and gonads.

From these factors, Durif’s study distinguished five stages to determine the progression of female eels from “small, undifferentiated yellow eels to pre-pubertal migratory eels”. Stages corresponded to a growth phase (I and II), a pre-migrant phase (III) and two migrating phases (IV and V).

These five stages give a more realistic picture of the dynamics of eel reproductive development, rather than being just considered as a yellow or silver eel.

Note that also fecundity may be measured in silver eels, see e.g. MacNamara and McCarthy (2012), and MacNamara et al. (2015).

### 4 Sampling and analysis of contaminants

This chapter describes the best available procedures to handle and process eels for eel quality assessment at the laboratory. It covers aspects of killing and anesthetizing the eels, issues regarding subsampling, labelling and storing of tissues, analytical techniques and quality assurance and control.

#### 4.1 General aspects after eel sampling

Guidelines for sampling/subsampling in the laboratory are clearly dependent on the purpose of the study. As such, the targeted substances are crucial for the handling and storage of the respective samples. Contaminants may have quite different behaviour regarding uptake and accumulation in the eels, depending on their chemical properties.

- Strongly lipophilic organic contaminants like PCBs, PBDEs and DDT can bio-accumulate during the life of the eel, especially during its growth. They are taken up slowly through food (equilibrium processes throughout the food-chain) and do not diffuse out of the lipids easily when the eels are in uncontaminated water. As such they are not lost during the migration.

- Other organic lipophilic compounds (including industrial chemicals as well as pharmaceuticals) are less lipophilic and less persistent. New chemicals being brought into production and use are required under the REACH legislation to be less bio-accumulative (nor should they be persistent). Emergent contaminants have been around for many years, but it is only recently that analysis in environmental matrices has been performed. PFOS is a good example: produced since the fifties, persistent, and only analysed in fish since around 2000. Due to the lower lipophilicity, the bio-accumulative properties of these compounds are limited, but the uptake rate is much higher than with PCBs for example. The equilibrium between water concentrations and lipid concentrations
is reached much quicker. While it may take years for very lipophilic compounds to reach equilibrium between the environment and the eel, for these compounds an equilibrium may be reached in days to weeks. Another significant difference is that these compounds enter the fish mainly through the gills. Eels leaving polluted water will eliminate these compounds quickly too, through the same process. The levels of these contaminants in eels are therefore not a reflection of a life-long history, but only of the last weeks or months. As such, factors to take into account while sampling for these types of contaminants are less crucial than for lipophilic compounds. As the uptake is primarily by respiration (gills), any eel, having been exposed to these contaminants, will show similar levels. Levels in eels are therefore less influenced by size, age or lipid content.

- Metals do not only bio-accumulate through the food chain, but exposure also takes place via water (interaction with the gills during respiration). Additionally, few metals readily accumulate in the muscle-lipid and accumulate poorly in the muscle protein. Methyl-mercury is an exception, as it does accumulate well in muscle protein. In most cases size and age of the eel is correlated with the metal level. Most metals are retained by the liver and other organs, at much higher levels than in the muscle. Hence, these organs may also be targeted for analysis.

Generally speaking, sampling for a number of contaminants can be completed in frozen or fresh eels (fresh samples are recommended for several reasons). Samples for biomarkers (see Chapter 7) should always be taken immediately at the site, because most biomarkers are temporarily reactive and fragile. Depending on the biomarkers chosen (e.g. proteomic or transcriptomic), immediate fixation of subsamples (pieces of brain, heart, liver and/or kidney) in liquid nitrogen is strongly recommended before preservation at -80°C.

4.2 Killing and anesthetizing

Methods to kill eels vary a lot from country to country and national ethics and legislations should be taken into account. Different anesthetic methods have been used. For euthanizing eels, some countries use clove oil (C₈H₁₀O₂), Phenoxy-ethanol (C₈H₁₀O₂) or tricaine methanesulfonate „MS-222“ (C₁₀H₁₅NO₅S). Clove oil (or eugenol/iso-eugenol) 30% (30 mL eugenol + 100 mL ethanol 95%) is used at the concentration 3 mL/10L salt/brackish water. Phenoxy-ethanol (ethylene glycol monophenyl ether) is used at concentrations 5 mL/10 L water. MS-222 is used at concentrations of 10-15 mg/l for light sedation, and 60 mg/l for anesthesia (http://www.drugs.com/vet/tricaine-s.html). For euthanizing eels, higher concentrations, like > 200 mg/l are required. Note that the activity is faster at higher water temperatures. Anesthetic agents must be pure and clean to avoid contamination, and used according to the manufacturer guidelines. Eels can be killed by an overdose of some aforementioned anesthetics (Aguilar et al., 2005, Outeiral et al., 2001; Sancho et al., 2003; Martinez-Carrasco et al., 2011a, b), methanosulfonate salt of 3-aminobenzoic acid ethyl ester (Ureña et al., 2007) or benzocaine (Esteve and Alcaide 2009)). Other methods for killing include chilling on ice (Costa-Díaz et al., 2010; Mayo-Hernández et al., 2014), with a blow to the head after being chilled (Gallastegui et al., 2002) (not recommended due to animal welfare), by electroshocking (Sánchez et al., 1998;
Linde et al., 1999), by decapitation (Linde et al., 1999, 2001) (not recommended due to animal welfare).

Note that anesthetics may reduce the viability of some external parasites. A skin and gill smear should preferably be made and read for parasitology directly after anesthetic use.

Some of the methods used in practice are not recommended due to animal welfare issues. However, it is difficult to recommend a specific method, as different animal protection regulations exist over the various countries. In general, researchers should choose the less stressful and most rapid killing method, to minimize suffering.

Take a picture of the eels showing clinical signs or other abnormalities, with the unique ID number and subnumber.

Handling fish has to be performed on a dry, clean, surface, preferably made of materials without impact on the targeted compounds. (e.g. Aluminum, (Inox-) Steel, Glas etc.; plastics should not be used when looking into flame-retardants and plasticizers etc.). Always clean the tools in between use (appropriate solvent and ultra pure water) to avoid cross contamination.

4.3 **Tissue sampling**

Different countries have established varying protocols for which part of the fish/muscle is taken for examination (e.g. contaminant/parasites). A filet directly cut from the bone is the most appropriate. Homogenized muscle tissue of the whole fish is preferable, but due to the size of female silver eels not very practicable. Due to potential functional and structural differences of the muscle fat values along the eel’s body, a harmonised way of taking the samples is advised. Since the skin of the eel is very tough and hard to be homogenized, it is crucial to remove the skin prior to subsequent tissue treatment. Since subcutaneous fat is meant to be included in the sample, caution is advised while skinning the eel.

With samples of internal organs (such as liver, heart, spleen, kidney, gills, brain etc.), it is important to carefully remove the entire organ from the eel’s body cavity. While doing so, be aware to not damage the gall bladder, since the bile may contaminate other tissue and make them useless. Due to potential functional and structural differences within the organ, it is most appropriate to take subsamples from the homogenized whole organ. If not possible (e.g. liver is used for multiple tests (e.g. proteonomic/histology/parasites/viruses), it is important to consistently use the same part of the organ (e.g. left distal lobe of the liver) for better comparability (Kammann et al., 2013).

Bile fluid samples are collected by piercing the gall bladder with a sterile needle fitted to a sterile disposable syringe. Approximately 0.1-1 mL should be stored frozen at -20°C until analysis (Kammann et al., 2013, 2014; Szlinder-Richert et al., 2014a,b)

4.4 **Storage of the samples**

Tissues and samples are valuable and should be stored in a sample tissue bank, if not immediately analysed. Data of individual fish should be recorded in a database. Muscle and liver samples are to be kept in clean, appropriate laboratory containers (depending on the purpose of the targeted substance). In order to avoid contamination from the recipients contaminant-free vials should be used for storage. Samples should be labelled
and stored at -20°C or -80°C respectively. Bile fluid should be kept at -20°C, blood samples or other tissues meant for hormone investigations in cryovials in liquid nitrogen, or at -80°C. Freeze drying should be avoided since this is not recommended for all types of contaminant analysis.

4.5 General treatment of samples and subsampling

Samples are homogenized using an inert mixer to avoid contamination. The mixer needs to be cleaned between samples with a fat-dissolving solution (e.g. contaminant-free acetone, acetonitril) followed by cleaning with ultra-pure water (e.g. HPLC-grade water). In all cases, care has to be taken that all used laboratory material is not interfering with the samples, i.e. that no contaminants are leaching out from mixers, spoons, knives and gloves etc.

Minimal two tissues are relevant to analysis on contaminants, i.e. muscle and the liver. However, since the amount of liver of individual eels is rather limited, not all pollutants can be measured in the liver. Minimally the non-lipophilic contaminant groups (e.g. metals, excluding Hg, and perfluorinated compounds) should be measured in both liver and muscle. Levels of metals and perfluorinated compounds are generally higher in the liver compared to the muscle (Bervoets and Blust, 2003; Consoer et al., 2014; Murakami et al., 2011), and might better reflect the risk posed to the fish than the levels measured in the muscle. In addition bile could be analysed for PAH-metabolites (Kamman et al., 2013, 2014, Szlinger-Richert et al., 2014a,b).

The homogenized muscle sample will be divided into different subsamples for analysis of the different contaminants and stored in an appropriate manner until analysis. The number of subsamples depends on the number of pollutant classes that will be measured.

Regardless of the number and nature of measured contaminants, the lipid content of both the liver and muscle homogenate has to be measured as well. A subsample of approximately 1 g of the respective tissues has to be weighed, transferred to a plastic tube for lipid analysis, and stored at -20°C. Subsamples for contaminant analysis should be stored at -20°C or lower before further testing. Sufficient material should be taken in the different subsamples to allow duplicate analysis. Excess material should be labelled, archived and preserved for later control or for additional measurements. The same holds for remaining samples or parts of samples after analysis.

4.6 Subsample treatment/storage and measurements

Water content or wet weight/dry weight ratio

For some contaminants (e.g. metals) levels are expressed in literature either on a dry weight (dw) or wet weight (ww) basis, and as such it is important always to assess the ww/dw ratio. A subsample of 1 to 5 g (depending on the amount of tissue available) is taken. Ideally the fresh weight should be recorded before freezing. To obtain the dry weight, samples should be oven-dried until they have a constant weight and reweighed after cooling down.

Lipid content
Lipid content in the homogenate can be measured in different ways, e.g. gravimetrically or photometrically (Bligh & Dyer, 1959). In any case the lipid content should be expressed in % of tissue wet weight w/w. Alternatively the lipid content could be measured gravimetrically on the extract for contaminant analysis (Voorspoels et al., 2004).

**Contaminants**

Different extraction and analysis methods are applied in literature, all with their strengths and weaknesses. In this guideline no ‘best methods’ are put-forward. However, care should be taken to apply methods that are sensitive enough to detect levels of contaminants that might be harmful to the eels.

*Polychlorinated biphenyls (PCBs)*

A subsample of 2-3 g should be preserved in contamination-free tubes and stored at -20°C or lower (Chu et al., 2002). Not only should the Σ6 PCBs (PCBs 28, 52, 101, 138, 153 and 180, European Commission, 2011c) be reported but also the concentrations of the individual congeners. Levels should be expressed on a ng/g ww or lw (lipid weight) basis.

*Dioxins*

Since measurement of dioxins requires a very clean environment and analysis costs are very high, a good alternative is measuring the dioxin-like PCBs (DL-PCBs). These are a group of 12 PCBs (Geeraerts et al., 2011), which proved to correlate with dioxin levels (Sezmis et al., 2014).

For dioxin analysis a subsample of 5 g should be stored in contamination-free plastic tubes and stored at -20°C or lower. As with the PCBs not only the ΣPCDD/Fs have to be reported but the individual compounds as well. Levels should be expressed as pg/g ww or ng/g ww or per lw or as pg WHO1998 TEQ g⁻¹ ww or pg WHO2005 TEQ g⁻¹ ww.

*Brominated Flame Retardants (BFRs)*

A subsample of 2-3 g should be preserved in contamination-free plastic tubes and stored at -20°C or lower. As with PCBs not only the ΣPBDEs should be reported but the individual congeners as well. Levels should be expressed on a ng/g ww or lw basis.

**Pesticides**

When OCPs have to be measured a subsample of 2-3 g should be stored in contamination-free plastic tubes and stored at -20°C or lower. Another subsample of 2 g should be preserved for other non-OCPs. Levels should be expressed as pg/g ww, ng/g ww or per lw.

**Metals**

For metals approximately 0.5 g of homogenized tissue should be preserved in acid-washed metal-free plastic vials (Poly Ethylene or Poly propylene) and stored at -20°C. Concerning volatile metals such as Hg, samples should not be oven-dried but freeze-dried. Levels should be expressed as µg/g ww or dw.

The most toxic compound is methyl-mercury. In fish, 80-100 % of the total mercury is present as methyl-mercury (Houserova et al., 2006; EFSA, 2012) and so there is no need for measuring CH₃Hg⁺, which is more expensive and time consuming.
Perfluorinated compounds (PFCs)

A subsample of 1-5 g homogenized tissue should be stored in PFC-free tubes (Stahl et al., 2014). When extracting samples for PFC-analysis, a clean environment is required.

Care has to be taken that the lids of the tubes are not sealed with Teflon. In addition when performing the extraction of the samples for analysis care should be taken that the solvents (methanol or acetonitrile) are PFC-free and that PFC-free water (HPLC-grade water) is used for dilutions. Often different batches of methanol or acetonitrile are contaminated with PFOS and other perfluorinated compounds. Samples should be stored at -20°C or lower.

Concentration can be expressed as ng/g or pg/g ww.

Polyaromatic hydrocarbons (PAHs)

PAHs are rapidly metabolized in fish and do not bio-accumulate in lipids. However, metabolites are secreted in the bile (Ruddock et al., 2003) and measuring PAH-metabolites in bile is a good indication of PAH-exposure of the eel. Data are generally normalized to the bile pigment content and expressed as µg/A380 (Szlinder-Richert et al., 2014a,b).

Other compounds

Other compounds that have been recently measured in fish and might give valuable information can be included as well (e.g. new Brominated Flame Retardants: Sühring et al., 2014; pharmaceuticals: Grabicova et al., 2014).

4.7 Detection limits

Often in a number of the samples tested, certain components will be below the limit of detection (LOD) or the limit of quantification (LOQ). However, when calculating average or median concentrations of a pollutant in a certain population, these non-detected components should not be left out of analysis. Different studies deal differently with values < LOQ. Sometimes the non-detects are considered zero, in other cases these values are equaled to LOQ/2 (Custer et al., 2000), or samples with concentrations below LOQ are calculated as i*LOQ with “i” being the fraction of samples above LOQ (Belpaire et al., 2011b). For a matter of standardisation it is advised to apply LOQ/2.

4.8 Quality assurance and quality control

There is a preference to use laboratories with the ability to complete ring testing, quality control or that have an accreditation status.

Quality assurance and quality control should consist of analysis of procedural blancos and, depending on the pollutants, positive well defined samples spiked with labelled or internal standard concentrations of the compound. Laboratories should have determined reproducibility, repeatability, and robustness prior to routine testing. In addition, certified reference material should be measured (if available) for the specific compounds.

Finally it is advised at regular intervals to participate in proficiency tests (inter-calibration studies in which different laboratories measure pollutants (preferably eel samples from the same fish); (Kammann et al., 2013).
5 Interpretation, visualisation and assessment

This chapter describes how data collected during eel quality assessments may be used to calculate eel quality stock indicators for comparison of local stocks, in the context of international legislation or towards inclusion in international stock assessment.

For international assessment of the quality of the eel stock for eel management and restoration, the availability of raw data is crucial. There is also need for an integrated management tool kit including indices, benchmarks or thresholds for easy assessment and comparison within and between local stocks.

The lack of scientific evidence for the quantification of toxicological effects of specific compounds or disease agents on eel health (i.e. in terms of spawner migration and reproduction), hampers the possibility of formulating clear recommendations on how to interpret and visualise the contamination data of eels.

Currently, there are no well-defined threshold concentrations relating to impairment of migration and reproduction of eels. The few available publications concern dioxins and cadmium. Palstra et al. (2006) reported a negative correlation between embryo survival time and 2,3,7,8-tetrachlorodibenzo-p-dioxine (TCDD) toxic equivalents (TEQs). The observed embryonic malformations were characteristic of eggs exposed to DL-PCBs. Disrupting effects occurred at levels below 4 ng TEQ/kg gonad. However, this study has been criticized because of the low sample size. Pierron et al. (2008) reported oocyte atresia and eel mortality due to cadmium concentrations in the liver tissue over 1.7 μg/g dry weight. In absence of more accurate eel specific threshold values, WGEEL has also discussed the use of toxicological thresholds reported in other fish species (e.g. whiting Von Westernhagen et al., 1989) for use in eel assessment (see ICES, 2010).

In most of the countries that recorded contaminants in local eel, data was compared with the maximum levels for human consumption available in the European Union regulations for metals (Hg, Pb, Cd) measured in eel muscle (EC/466/2001); dioxins, PCB-DL and the 6 ICES PCBs indicators in eel muscle (Europen Commission, 2011c), Benzo(a)pyrene in fish muscle (EC/1881/2006), and sometimes pesticides in meat (86/363/EEC). Table 5.1 summarizes the available maximum levels in these regulations. In some cases, the contaminant concentrations are compared to the WHO Tolerable Daily Intake (TDI) recommendations (see e.g. for dioxins Geeraerts et al., 2011; for PCBs Belpaire et al., 2011b), using intake scenario’s (such as presented in Bilau et al., 2007).

In the context of the eel health assessment, instead of comparing eel contaminations with maximum limits for human consumption, it seems more appropriate to compare them with concentrations recently defined for biota. Such limits are available in the WFD (European Commission, 2013a) that defined Environmental Quality Standards (EQSsEIOTA): upper limit concentrations which cannot be exceeded in ‘prey’ tissue of biota, including fish. These limits are available for fish for 9 contaminants: Hg, HCB, HCBD, Dicofol, HBCDD, heptachlor, Sum PCDD/F + PCB-DL, Sum 6 PBDE, PFOS (Table 5.1). However, these EQSsEIOTA thresholds have been determined by focusing on two protection goals: (1) the protection from chemical accumulation in the food chain, specifically of top predators such as birds and mammals, from risks of secondary poisoning through consumption of contaminated prey, and (2) the protection of human health from deleterious effects resulting from the consumption of food contaminated by chemicals. As a result, in some
cases (e.g. in the case of ∑ PBDEs) these limit values may be extremely low and not supposed to be harmful to eel.

Table 5.1. List of the maximum levels of contaminants regarding human health (EU and US regulations) and environment protection (WFD regulation).

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Maximum levels</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Metals</strong></td>
<td></td>
</tr>
<tr>
<td>Hg (EC/466/2001 - in eel)</td>
<td>1 µg/g ww muscle</td>
</tr>
<tr>
<td>Hg (WFD EQS - in fish)</td>
<td>20 ng/g ww muscle</td>
</tr>
<tr>
<td>Pb (EC/466/2001 - in eel)</td>
<td>0.4 µg/g ww muscle</td>
</tr>
<tr>
<td>Cd (EC/466/2001) - in eel</td>
<td>0.1 µg/g ww muscle</td>
</tr>
<tr>
<td><strong>Pesticides</strong></td>
<td></td>
</tr>
<tr>
<td>α-HCH (86/363/EEC - in meat)</td>
<td>200 ng/g ww muscle</td>
</tr>
<tr>
<td>β-HCH (86/363/EEC - in meat)</td>
<td>100 ng/g ww muscle</td>
</tr>
<tr>
<td>γ-HCH (lindane) (86/363/EEC - in meat)</td>
<td>2000 ng/g ww muscle</td>
</tr>
<tr>
<td>∑ DDTs (86/363/EEC - in meat)</td>
<td>1000 ng/g ww muscle</td>
</tr>
<tr>
<td>Hexachlorobenzene HCB (86/363/EEC - in meat)</td>
<td>200 ng/g ww muscle</td>
</tr>
<tr>
<td>Hexachlorobenzene HCB (WFD EQS - in fish)</td>
<td>10 ng/g ww muscle</td>
</tr>
<tr>
<td>Endrin (86/363/EEC - in meat)</td>
<td>50 ng/g ww muscle</td>
</tr>
<tr>
<td>Chlordane (86/363/EEC - in meat)</td>
<td>50 ng/g ww muscle</td>
</tr>
<tr>
<td>Chlordane (US-FDA - in fish)</td>
<td>300 ng/g ww muscle</td>
</tr>
<tr>
<td>Aldrin and dieldrin (86/363/EEC - in meat)</td>
<td>200 ng/g ww muscle</td>
</tr>
<tr>
<td>Aldrin and dieldrin (US-FDA - in fish)</td>
<td>300 ng/g ww muscle</td>
</tr>
<tr>
<td>DDTs (US-FDA - in fish)</td>
<td>5 µg/g ww muscle</td>
</tr>
<tr>
<td>HCBD (Hexachlorobutadiene) (WFD EQS - in fish)</td>
<td>55 ng/g ww muscle</td>
</tr>
<tr>
<td>Dicofol (close to DDT, insecticide) (WFD EQS - in fish)</td>
<td>33 ng/g ww muscle</td>
</tr>
<tr>
<td>Hexabromocyclododecane (HBCDD) (WFD EQS - in fish)</td>
<td>167 ng/g ww muscle</td>
</tr>
<tr>
<td>Heptachlor and heptachlor epoxide (WFD EQS - in fish)</td>
<td>6.7 × 10^{-3} ng/g ww muscle</td>
</tr>
<tr>
<td>Heptachlor and heptachlor epoxide (US-FDA - in fish)</td>
<td>300 ng/g ww muscle</td>
</tr>
<tr>
<td><strong>PCBs</strong></td>
<td></td>
</tr>
<tr>
<td>ICES ∑ 6 PCBs indicators (28,52,101,138,153,180) (EC/1259/2011 – in eel)</td>
<td>300 ng/g ww muscle</td>
</tr>
<tr>
<td><strong>Dioxins and dioxin-like compounds</strong></td>
<td></td>
</tr>
<tr>
<td>Sum of PCDD+PCDF+ PCB-DL – TEQ (WFD EQS - in fish, crustacean and mollusc)</td>
<td>6.5 pg/g TEQ_{2005} ww muscle</td>
</tr>
<tr>
<td>Sum of PCDD+PCDF + PCB-DL -TEQ (EC/1259/2011 - in eel)</td>
<td>10.0 pg/g TEQ_{2005} ww muscle</td>
</tr>
<tr>
<td>Sum of PCDD+PCDF – TEQ (EC/1259/2011 - in eel)</td>
<td>3.5 pg/g TEQ_{2005} ww muscle</td>
</tr>
<tr>
<td><strong>PBDE</strong> (28+47+99+100+153+154) - (WFD EQS - in fish)</td>
<td>0.0085 ng/g ww muscle</td>
</tr>
</tbody>
</table>
Perfluorooctane sulfonic acid and its derivatives (PFOS) - (WFD EQS - in fish) 9.1 ng/g ww muscle

PAH
Benzo(a)prene (EC/1881/2006 - in fish) 2 ng/g ww muscle

There is an urgent need for experimental work on the assessment of the impact of contaminants on the reproductive success of the eel (ICES, 2013) in order to determine concentration thresholds in regard to migration and/or reproduction capability of the eel.

However, despite the restrictions discussed above, some framework and indices have been developed to represent/assess the quality of the eels, in local and in international context. While representing valuable and promising methods, most of them need further development and/or validation.

5.1 Eel Quality Classes

In Flanders, quality classes were developed based on quantitative distribution of an extensive set of data (means per location) for PCBs, OCPs and metals (Belpaire & Goemans, 2007b). Reference values were fixed for each chemical. These reference values were defined as the 5 percentile value of the means of all sites. A common procedure was used to distinguish four quality classes as a measure of deviation from the reference value, and class boundary values were set. Class limits and reference values for each contaminant are listed in Table 5.2. Class boundary calculations were based on the distribution of the relationship between the recorded values and the reference value. Class 1 represents the ‘not deviating’ class (green colour) with ‘unpolluted or low polluted’ eels. Eels with a slight to moderate pollution level are classified as class 2 ‘slightly deviating’ (yellow). The more polluted eels are assigned to class 3 ‘deviating’ (orange) or 4 ‘strongly deviating’ (red). Depending on the objective of the study, the representation of the distribution of contaminants can be completed at the individual level: percentage of eels belonging to each class within one site (Fig. 5.1) or at the catchment or EMU level (Fig. 5.2): percentage of sites per catchment or EMU belonging to each class (calculating the mean concentration for all eels per site over the catchment or EMU).
Table 5.2. Reference values and boundary values of the quality classes for a series of heavy metals, PCB congeners and organochlorine pesticides as defined in Flanders' Eel Pollution Monitoring Network (EPMN). Values are expressed in ng.g\(^{-1}\) wet weight of muscle tissue, unless indicated as 1 in ng.g\(^{-1}\) lipid weight or 2 in μg.g\(^{-1}\) wet weight of muscle tissue. C: concentration; \(\sum\) PCB is indicated for the 7 PCBs indicators (adapted from Belpaire & Goemans 2007b).

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Reference value (RV)</th>
<th>1</th>
<th>Not deviating log C/RV &lt; 0.4</th>
<th>Slightly deviating 0.4 ≤ log C/RV &lt; 0.8</th>
<th>Deviating 0.8 ≤ log C/RV &lt; 1.2</th>
<th>Strongly deviating log C/RV ≥ 1.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercury</td>
<td>40</td>
<td>&lt; 100</td>
<td>100 - &lt; 252</td>
<td>252 - &lt; 634</td>
<td>≥ 634</td>
<td></td>
</tr>
<tr>
<td>Cadmium</td>
<td>2</td>
<td>&lt; 5</td>
<td>5 - &lt; 12.6</td>
<td>12.6 - &lt; 31.7</td>
<td>≥ 31.7</td>
<td></td>
</tr>
<tr>
<td>Lead</td>
<td>10</td>
<td>&lt; 25</td>
<td>25 - &lt; 63</td>
<td>63 - &lt; 158</td>
<td>≥ 158</td>
<td></td>
</tr>
<tr>
<td>Copper(^2)</td>
<td>0.25</td>
<td>&lt; 0.6</td>
<td>0.6 - &lt; 1.6</td>
<td>1.6 - &lt; 4</td>
<td>≥ 4</td>
<td></td>
</tr>
<tr>
<td>Zinc(^2)</td>
<td>14</td>
<td>&lt; 35</td>
<td>35 - &lt; 88</td>
<td>88 - &lt; 222</td>
<td>≥ 222</td>
<td></td>
</tr>
<tr>
<td>Nickel</td>
<td>14</td>
<td>&lt; 35</td>
<td>35 - &lt; 88</td>
<td>88 - &lt; 222</td>
<td>≥ 222</td>
<td></td>
</tr>
<tr>
<td>Chrome</td>
<td>96</td>
<td>&lt; 241</td>
<td>241 - &lt; 606</td>
<td>606 - &lt; 1521</td>
<td>≥ 1521</td>
<td></td>
</tr>
<tr>
<td>Arsenic</td>
<td>41</td>
<td>&lt; 103</td>
<td>103 - &lt; 259</td>
<td>259 - &lt; 650</td>
<td>≥ 650</td>
<td></td>
</tr>
<tr>
<td>Selenium</td>
<td>205</td>
<td>&lt; 515</td>
<td>515 - &lt; 1293</td>
<td>1293 - &lt; 3249</td>
<td>≥ 3249</td>
<td></td>
</tr>
<tr>
<td>PCB 28</td>
<td>0.12</td>
<td>&lt; 0.3</td>
<td>0.3 - &lt; 0.8</td>
<td>0.8 - &lt; 1.9</td>
<td>≥ 1.9</td>
<td></td>
</tr>
<tr>
<td>PCB 31</td>
<td>0.1</td>
<td>&lt; 0.3</td>
<td>0.3 - &lt; 0.6</td>
<td>0.6 - &lt; 1.6</td>
<td>≥ 1.6</td>
<td></td>
</tr>
<tr>
<td>PCB 28+31</td>
<td>0.25</td>
<td>&lt; 0.6</td>
<td>0.6 - &lt; 1.6</td>
<td>1.6 - &lt; 4</td>
<td>≥ 4</td>
<td></td>
</tr>
<tr>
<td>PCB 52</td>
<td>1</td>
<td>&lt; 2.5</td>
<td>2.5 - &lt; 6.3</td>
<td>6.3 - &lt; 15.8</td>
<td>≥ 15.8</td>
<td></td>
</tr>
<tr>
<td>PCB 101</td>
<td>2.5</td>
<td>&lt; 6</td>
<td>6 - &lt; 16</td>
<td>16 - &lt; 40</td>
<td>≥ 40</td>
<td></td>
</tr>
<tr>
<td>PCB 105</td>
<td>1.2</td>
<td>&lt; 3</td>
<td>3 - &lt; 7.6</td>
<td>7.6 - &lt; 19</td>
<td>≥ 19</td>
<td></td>
</tr>
<tr>
<td>PCB 118</td>
<td>3.5</td>
<td>&lt; 9</td>
<td>9 - &lt; 22</td>
<td>22 - &lt; 55</td>
<td>≥ 55</td>
<td></td>
</tr>
<tr>
<td>PCB 138</td>
<td>7.7</td>
<td>&lt; 19</td>
<td>19 - &lt; 49</td>
<td>49 - &lt; 122</td>
<td>≥ 122</td>
<td></td>
</tr>
<tr>
<td>PCB 153</td>
<td>10</td>
<td>&lt; 25</td>
<td>25 - &lt; 63</td>
<td>63 - &lt; 158</td>
<td>≥ 158</td>
<td></td>
</tr>
<tr>
<td>PCB 156</td>
<td>0.6</td>
<td>&lt; 1.5</td>
<td>1.5 - &lt; 3.8</td>
<td>3.8 - &lt; 9.5</td>
<td>≥ 9.5</td>
<td></td>
</tr>
<tr>
<td>PCB 180</td>
<td>4.5</td>
<td>&lt; 11</td>
<td>11 - &lt; 28</td>
<td>28 - &lt; 71</td>
<td>≥ 71</td>
<td></td>
</tr>
<tr>
<td>(\sum) PCBs</td>
<td>29</td>
<td>&lt; 73</td>
<td>73 - &lt; 183</td>
<td>183 - &lt; 460</td>
<td>≥ 460</td>
<td></td>
</tr>
<tr>
<td>(\sum) PCBs'</td>
<td>240</td>
<td>&lt; 603</td>
<td>603 - &lt; 1514</td>
<td>1514 - &lt; 3804</td>
<td>≥ 3804</td>
<td></td>
</tr>
<tr>
<td>α-HCH</td>
<td>0.05</td>
<td>&lt; 0.1</td>
<td>0.1 - &lt; 0.3</td>
<td>0.3 - &lt; 0.8</td>
<td>≥ 0.8</td>
<td></td>
</tr>
<tr>
<td>γ-HCH</td>
<td>1.3</td>
<td>&lt; 3.3</td>
<td>3.3 - &lt; 8.2</td>
<td>8.2 - &lt; 20.6</td>
<td>≥ 20.6</td>
<td></td>
</tr>
<tr>
<td>Dieldrin</td>
<td>1.1</td>
<td>&lt; 2.8</td>
<td>2.8 - &lt; 6.9</td>
<td>6.9 - &lt; 17.4</td>
<td>≥ 17.4</td>
<td></td>
</tr>
<tr>
<td>HCB</td>
<td>0.5</td>
<td>&lt; 1.3</td>
<td>1.3 - &lt; 3.2</td>
<td>3.2 - &lt; 7.9</td>
<td>≥ 7.9</td>
<td></td>
</tr>
<tr>
<td>p,p'-DDD</td>
<td>2.5</td>
<td>&lt; 6</td>
<td>6 - &lt; 16</td>
<td>16 - &lt; 40</td>
<td>≥ 40</td>
<td></td>
</tr>
<tr>
<td>p,p'-DDT</td>
<td>0.005</td>
<td>&lt; 0.01</td>
<td>0.01 - &lt; 0.03</td>
<td>0.03 - &lt; 0.08</td>
<td>≥ 0.08</td>
<td></td>
</tr>
<tr>
<td>p,p'-DDE</td>
<td>13</td>
<td>&lt; 33</td>
<td>33 - &lt; 82</td>
<td>82 - &lt; 206</td>
<td>≥ 206</td>
<td></td>
</tr>
<tr>
<td>(\sum) DDTs</td>
<td>16</td>
<td>&lt; 40</td>
<td>40 - &lt; 101</td>
<td>101 - &lt; 254</td>
<td>≥ 254</td>
<td></td>
</tr>
</tbody>
</table>
5.1 Eel Quality Classes distribution in Canet (C), Salses (S) and Bages (B) lagoons and La Berre river (R), France. The number under the site code refers to the number of samples for each site. Five contaminants: ∑ 7 PCBs, ∑ DDTs, Cadmium, Copper and Zinc, and 2 pathogens: virus EVEX and the swimbladder index for the parasite Anguillicoloides crassus are considered (Amilhat et al., 2014).

5.2 Eel Quality Index (EQI), derived from the Eel Quality Classes

The WGEEL report 2013 (ICES, 2013) gives some specific advice for planning future reporting of local eel stock assessment for each EMU. In the framework of international assessments and advice, ICES (2010, 2011, 2012) developed an Eel Quality Index for Contaminants. This method defines what are the more important/harmful contaminants and pathogens to consider and which best represent the quality of the eels (in respect of the ability to migrate and reproduce). In particular, the following information was regarded as the minimum requirements for assessing the quality of yellow eels and Eel Quality
Index by EMU): mean size (mm), total wet weight of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 ($\sum$ 6 PCBs), p,p’-DDD, p,p’-DDT, p,p’-DDE ($\sum$ DDTs), cadmium, lead and mercury (ng/g wet weight), and prevalence (%), abundance (n) and intensity of A. crassus (ICES, 2013).

The Eel Quality Index for Contaminants (EQICONT) initially developed in ICES (2010, 2011) was further developed by ICES (2012) and included important contaminants such as Hg, Pb, dioxins and brominated flame retardants (Table 5.3). Threshold values for classifying contamination levels of Hg and Pb were derived from Belpaire & Goemans (2007b). However, within the time limit of WGEEL it was not yet possible to deduce threshold values for dioxins and brominated flame retardants. ICES (2012b) adapted the threshold values of the quality classes as defined by ICES (2010) for the $\sum$ ICES 7 PCBs to values for the $\sum$ ICES 6 PCBs (SUM of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180). Indeed, since the new Dioxin Regulation (European Commission 2011c), the EU set those new harmonised $\sum$ ICES 6 PCB maximum levels, and this will be used in further assessments. PCB 118 contributed to between 8 and 23 % of the $\sum$ ICES 7 PCBs (average 15%) (Belpaire et al., 2011b). So the threshold values were adapted for $\sum$ ICES 6 PCBs by lowering the $\sum$ 7 PCBs by 15% to account for PCB 118 (ICES, 2012b).

Table 5.3. Boundary values in ng.g$^{-1}$ wet weight of muscle tissue of each quality classe for a series of selected contaminants for the calculation of EQICONT (adapted from Belpaire & Goemans, 2007b and ICES, 2012).

<table>
<thead>
<tr>
<th>Class</th>
<th>Not polluted</th>
<th>Slightly polluted</th>
<th>Polluted</th>
<th>Strongly polluted</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\sum$ 6 PCBs</td>
<td>&lt; 62</td>
<td>62 - 155</td>
<td>155 - 391</td>
<td>&gt; 391</td>
</tr>
<tr>
<td>$\sum$ DDTs</td>
<td>&lt; 40</td>
<td>40 - 101</td>
<td>101 - 254</td>
<td>&gt; 254</td>
</tr>
<tr>
<td>Cd</td>
<td>&lt; 5</td>
<td>5 - 12,6</td>
<td>12,6 - 31,7</td>
<td>&gt; 31,7</td>
</tr>
<tr>
<td>Hg</td>
<td>&lt; 100</td>
<td>100 - 252</td>
<td>252 - 634</td>
<td>&gt; 634</td>
</tr>
<tr>
<td>Pb</td>
<td>&lt; 25</td>
<td>25 - 63</td>
<td>63 - 158</td>
<td>&gt; 158</td>
</tr>
<tr>
<td>BFRs</td>
<td>To be identified</td>
<td>To be identified</td>
<td>To be identified</td>
<td>To be identified</td>
</tr>
<tr>
<td>Dioxines</td>
<td>To be identified</td>
<td>To be identified</td>
<td>To be identified</td>
<td>To be identified</td>
</tr>
</tbody>
</table>

Using these quality classes it is possible to calculate EQICONT for individual yellow and silver stage eels. EQICONT is defined as the average value of the quality classes for the measured contaminants.

$$EQI_{CONT} = \frac{\sum_{i=1}^{n} \text{contaminant classes}}{n}$$

with n = the number of measured contaminants

$$1 \leq EQI_{CONT} \leq 4$$

Boundary values of the EQICONT are suggested in Table 5.4 in order to visualize and highlight eels with very low and high load of contaminants.
Table 5.4. Boundary values of the Eel Quality Index.

<table>
<thead>
<tr>
<th>EQI Classes</th>
<th>Not/low polluted</th>
<th>Slightly polluted</th>
<th>Polluted</th>
<th>Strongly polluted</th>
</tr>
</thead>
<tbody>
<tr>
<td>EQI&lt;sub&gt;cont&lt;/sub&gt;</td>
<td>≤1.50</td>
<td>&gt;1.50-2.50</td>
<td>&gt;2.50-3.50</td>
<td>&gt;3.50-4</td>
</tr>
</tbody>
</table>

One has to be aware that in this classification system eels which are heavily infected by one chemical (which may be at a level with toxic effect) but not polluted by other compounds, may be evaluated as **slightly polluted**, thus underestimating potential risks. Alternatively, eels may be assigned to the highest score calculated (**one out, all out**). The highest ranking determines the final value. For example, if one of the contaminants is class 4 and all others are class 1, the final value will be 4. This allows identification of eels that are much polluted for at least one compound.

To determine the health quality of eels in regard to their capability to migrate and reproduce, the integration (synergetic effects) of both types of stressors, contaminants and pathogens, seems appropriate. EQI can also be calculated for diseases (EQI<sub>dis</sub>). There is little literature available on the effect of pathogens on the migration and/or reproduction of eels. Palstra et al. (2007) demonstrated (using swim tunnels) that the swimbladder parasite, *A. crassus* significantly reduced the swimming performance of the silver eels, which may indicate, that they would have insufficient energy for the transoceanic migration. Lefebvre et al. (2002a) suggested that eels with severely damaged swimbladders (Swimbladder Degenerative Index ≥ 4) would not be able to migrate and reproduce successfully. It has also been shown, both in naturally and experimentally infected eels, that this parasite has an effect on the silvering process (Fazio et al., 2012). Finally, according to van Ginneken et al. (2005), the rhabdovirus Eel Virus European X (EVEX) was thought to be responsible for 100% impairment of the migration in swimming tunnels, with development of severe haemorrhages after 1500 km. However, in a later study of Haenen et al. (2010), no EVEX was detected at all in 92 silver eels from the river Rhine and IJsselmeer catchments, but up to to 44% of the eels were infected with AngHV-1 (eel herpesvirus). Although AngHV-1 is considered to be latently present (Van Beurden et al., 2012a,b; Bandin et al., 2014), when only weak positive by Real Time qPCR, it may be lethal to eels at a higher viral load. Based on this information, we consider eels that are positive for EVEX virus or AngHV-1 in cell culture (or which have a low Ct value in the respective Real Time qPCRs, see chapter 6.2) as highly infected (class 4) and the eels with Swimbladder Degenerative Index ≥4 as highly infected (class 4).

Boundary values of the EQI<sub>dis</sub> are suggested in Table 5.5 in order to visualize and highlight eels with very low and high load of pathogens.
Table 5.5. Boundary values of the quality classes for *A. crassus* (using the Swimbladder Degenerative Index) and EVEX and AngHV-1 virus infections.

<table>
<thead>
<tr>
<th>Quality Classes</th>
<th>Not infected</th>
<th>Slightly infected</th>
<th>Moderately Infected</th>
<th>Strongly infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDI</td>
<td>0</td>
<td>1-2</td>
<td>3</td>
<td>≥ 4</td>
</tr>
<tr>
<td>EVEX virus</td>
<td>not present</td>
<td>Present</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AngHV-1</td>
<td>not present</td>
<td>Present with low Ct value (&lt;25) and/or cpe in 1st passage in cell culture</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ EQI_{DIS} = \left[ \sum_{i=1}^{n} \text{pathogen classes} \right] / n \]

with \( n \) = the number of measured pathogens

An integrative index EQI_{TOT} can then be calculated (Fig. 5.3):

\[ EQI_{TOT} = (EQI_{CON} + EQI_{DIS}) / 2 \]

EQI_{TOT} can then be interpreted as the EQI_{CON} (table 2)

Figure 5.3. Example of representation of EQI_{TOT} in 3 French Mediterranean lagoons (Canet : C; Salses-Leucate : S and Bages-Sigean : B). Quality classes are designed for 3 contaminants : PCBs, DDTs and Cd and 1 pathogen : *A. crassus* (using the SDI index) (unpublished results from Amilhat et al., 2014)
Although this method is not ideal as it considers all the contaminants and some pathogens as equally important in the assessment of eel quality, it may be appropriate in order to visualise/identify the percentage of eels particularly affected by contaminants and/or pathogens. Conversely, those with no/few infections can also be identified.

The primary aim of Tables 2 and 4 is to include only stressors which have been reported or are thought most likely to impact eel migration and/or reproduction. Future work should include calculating quality classes (thresholds) for missing contaminants and pathogens, which are believed to have harmful effects on eel. However, to determine those threshold values, more research should be carried out on those contaminants and pathogens and their effect on eel health.

5.3 IMBI: individual mean (multi-metal) bioaccumulation index

In some cases it might be useful to calculate integrated indices by contaminant group, where the contaminant load of an individual is expressed as a single value, for assessment purposes.

This has been elaborated by e.g. Maes et al. (2005, 2013) who used an integrated index for metals. They calculated a relative bioaccumulation index by dividing (standardising) the individual concentration of heavy metal $i$ ($C_i$) by the maximum observed concentration ($C_{i,\text{max}}$) and averaging over all metals, to relate heavy metal bioaccumulation to condition and genetic variability. In these studies, the **individual mean (multi-metal) bioaccumulation index** (IMBI) was defined as:

$$IMBI = \frac{\sum_{i=1}^{n} (C_i / C_{i,\text{max}})}{n}$$

with $n$ the total number of metals, $C_i$ the individual concentration of heavy metal $i$, $C_{i,\text{max}}$ the maximum observed concentration of heavy metal $i$ and $0 < IMBI < 1$.

Note, that IMBI data may be compared between individuals within a specific study, but may not be used for inter study comparison, as this method is sensitive to the $C_{i,\text{max}}$. If in one site $C_{i,\text{max}}$ is very high, it may introduce a bias in the results as the IMBI depends on the most contaminated eels, even if lower concentrations could be considered already as impacted. There is opportunity to adapt and use this approach on a wider basis, defining a $C_{i,\text{max}}$ value over the European population. This method might easily be adapted to other contaminants/contaminant groups (such as POPs).

However, in order to generate quality classes and a $C_{i,\text{max}}$ representative for all European habitats, it seems important to recalculate/define $C_{i,\text{max}}$ from a larger set of data, taking into account the contaminant concentrations variability across the whole of Europe, at least for the most important contaminants considered as indicators of spawner quality (see Table 5.3).

5.4 The Eel Patho–Index (EPI), a Health Status Classification System

Within an EFF (European Fisheries Fund) project monitoring restocking (2012-2015) in North Rhine Westphalia (Germany), many data on eel diseases and parasites have been collected. The visualization of these data was difficult. To easily judge the suitability of different eel habitats for restocking and to get a quick overview of how many healthy eels
would possibly make it to the Sargasso Sea, a management tool was developed. Using this tool the data can be visualized quickly, and one gets a good and quick overview of where the “best” eels are and which rivers are most appropriate for restocking. The management tool, a “Health Status Classification System” is called “Eel Patho-Index (EPI)”. The different disease related characteristics of the eels are being assessed and classified into different classes (blue = 1 = very good health, green = 2 = good health, yellow = 3 = moderate health, orange = 4 = unsatisfactory health, red = 5 = poor health) (Table 5). Different symptoms/parameters get a different rating depending on how much they may influence eel health. The rating of the different parameters is still to be developed and cannot be shown here. After all symptoms of an analysed eel are classified and rated, the final score is calculated by the average value of the scores for the symptoms identified. The eel can then roughly be assigned to its class relating to its health status.

The Eel Patho-Index is an attempt of developing a simple and quick tool to visualize many data. It is still draft version and needs to be discussed and developed further. For more details, see the German country report in Annex 6.

5.5 Reproductive potential in silver eels

In order to develop a common approach to quantifying the effects of silver eel quality on Reproductive Potential and integrating these into stock assessments, ICES (2012 and 2013) made progress in the development of an international index. This index quantifies the status of silver eel fitness (in terms of size and lipid reserves) on reproductive potential of eels. Several authors have proposed that the lipid content of silver eels is crucial for their successful migration and reproduction.

The reproductive potential of a female silver eel (RP) is dependent on several parameters. Apart from other condition parameters (such as physiological state, occurrence of parasites, etc.), RP will be a function of body size, muscle lipid content, and the migration distance to the Sargasso Sea (DSS) (see ICES, 2012b).

The developed approach estimates the potential fecundity of female silver eels in terms of the quantity of egg production.

The net energy of silver eels starting their migration can be roughly estimated using a simplified model (net fat content was calculated assuming all fat is muscle fat, assumptions see Belpaire et al., 2009).

\[ \text{Net fat content at start of migration} = \text{Body mass} \times \% \text{ Lipids/100} \]

The energy requirements (cost of transport, COT) for a silver eel to reach its spawning ground increases with the DSS. Energy expenditure of female silver eels during swimming has been estimated through experiments in swimming tunnels, and is also related to their size (relative energy expenditure decreases with increasing body size). Measurements of COT, derived from swim tunnel experiments, have indicated costs of 11.5 and 17.5mg fat/kg/km, dependent of two different methods used (Palstra & Van den Thillart, 2010). Here we present the range of values, and adopt an intermediate value of this range, 14.5mg fat/kg/km, as a midpoint/mean for graphical presentation. In WGEEL 2012 (ICES, 2012b) a fixed value for COT was taken regardless of the length/body mass of the
eel. This was recognized as a significant weakness in the model, and ICES (2013) addressed this by incorporating a direct relationship between body mass and expenditure. Mean cost of transport is calculated as

\[ \text{Mean COT (g fat)} = \text{Body mass (kg)} \times 14.5 \times (\text{mg fat/kg/km}) \times \text{DSS (km)} \]

DSS being the distance from the sampling site to the spawning location in the Sargasso Sea at 61°00’W and 26°30’N (i.e. the centre of the area described in van Ginneken and Maes, 2005).

From this, the energy remaining for reproduction in female eels by arrival at their spawning ground (ERind) can be deduced:

\[ \text{ERind} = \text{Net fat content at start of migration} - \text{COT} \]

or

\[ \text{ERind} = (\text{Body mass} \times \% \text{Lipids/100}) - (\text{Body mass (g)} \times 0.0000145 \times \text{DSS (km)}) \]

RP was calculated as the mass of eggs which could be produced after using all ERind, based on a conversion factor of 1.72 g eggs/g fat (as used in van Ginneken and van den Thillart, 2000):

\[ \text{RPind} = \text{ERind (g fat)} \times 1.72 \]

If data are available from a representative sample of female silver eels, from a given catchment or EMU, it should be possible to infer the reproduction potential of female silver eel escapement from the catchment or EMU (RPEMU). Individuals with a negative or zero ERind will not contribute to the spawning stock as they will not have energy reserves necessary to reach the spawning ground or for egg production, respectively. From the ERind, the RPEMU can be calculated using the following equation:

\[ \text{RPEMU} = \sum \text{RPind ER > 0} \times \text{Nind ER > 0} \times \text{NEMU ER > 0} \]

\[ \text{NEMU ER > 0} = \text{number of female silver eels with ERind > 0 leaving the catchment} \]

\[ \text{Nind ER > 0} = \text{number of female silver eels with data on lipids and body mass and with a calculated ERind > 0} \]

For a full description and discussion of this index (including examples with field data), we refer to ICES (2013, 2014).

At present the Reproductive Potential Model should perhaps be regarded as heuristic only, because there are necessarily a range of simplifications and shortcomings. We refer to ICES (2014) for a full discussion on these assumptions.

5.6 Effective Spawner Escapement as evaluated in Irish rivers

This percentage was calculated for the Irish Rivers (McCarthy et al., 2011) in order to determine the proportion of “effective spawner escapement”. In order for a silver eel to qualify as a ‘potential effective spawner’ it must meet the four criteria:

1. According to Pankhurst (1982), silver eels must have an eye index of 6.5 or greater (Pankhurst’s Eye Index) in order to be considered mature
2. The minimum pectoral fin index for mature silver eels is 4.3 for females and 4.7 for males (Durif et al., 2005)
3. According to Van den Thillart et al. (2004) the minimum percentage total body lipid content for the successful migration and subsequent spawning of silver eels is 13%.
4. Silver eels must be uninfected by *A. crassus*.

5.7 Conclusion
The methods of reporting the concentration of contaminants and the occurrence of diseases vary a lot between European studies. Some of the integrative methods presented here are promising and may be used, but some need further development and validation. We recommend to report in sufficient detail not only giving the mean value ± standard deviation but also the median with the minimum and maximum values, and refer to the number of eels sampled. In order to represent contamination levels, graphics such as box-plot (median, 1st and 3rd quartile, minimum and maximum) should be preferred to a simple average value with errors. In addition, make the raw data (w.w. and d.w.) accessible via the EQD.

It is also important to analyse and interpret the results in regards to the gender and stage of the eels, as these parameters are, in most of cases, crucial in contamination and disease processes (see Sections 2.3-2.4).

6 Diseases

6.1 Parasitic diseases
Parasitological examinations are only conducted routinely as part of targeted monitoring activities in a small number of European countries. More often, examinations are part of specific research projects carried out by government laboratories, universities or other fish health professionals. Some countries use internal protocols developed to standardise necropsy procedures for eel health assessments, such as the Netherlands and Poland, while a national protocol is being developed in the UK (Lewin et al., 2014; in prep). As such, the sampling and approaches used differ greatly between countries, even within local areas, depending on the questions posed, parasites targeted and resources available.

Parasitological examinations are usually conducted on live eel samples. Despite not being suitable for parasitological examination, dead and frozen eels have been used in some studies targeted to specific parasites, like practised in Spain (Diaz et al., 2006; Costa-Dias et al., 2010; Martinez-Carrasco et al., 2011a, b; Mayo-Hernández et al., 2014, 2015).

6.1.1 *Anguillicoloides crassus*
WKPGMEQ is aware of the recent work of Laetsch et al. (2012) showing that molecular analysis does not support the recent division of Anguillicoloida into two genera, and thus advocating return to the genus name of the parasite to *Anguillicola*. However, after discussion WKPGMEQ will use the name "*Anguillicoloides crassus*" in this report, following the current nomenclature in the World Register of Marine Species and an expert opinion.
Anguillicoloides Moravec and Taraschewski, 1988 are nematode parasites of the swimbladder of eels. This genus contains four species, but only two have been reported from Europe, namely Anguillicoloides crassus (Kuwahara, Niimi and Itagaki, 1974) Moravec and Taraschewski, 1988 and Anguillicoloides novaezelandiae Moravec and Taraschewski, 1988 (Moravec, 2013). The distinction between the two species is mainly done by the shape of the head, the buccal capsule size and the size and number of teeth (Moravec, 2013).

It is assumed that eels can be infected as soon as they start eating invertebrate intermediate hosts, mostly copepods (Moravec, 2013), as such eels of all sizes could be infected and should be screened for this parasite (De Charleroy et al., 1990). To detect this parasite, it is necessary to 1) dissect the eel 2) remove carefully the swimbladder and 3) examine it macroscopically under a stereo microscope. Adults and pre-adults should be collected and counted. If required for further study, parasites can be washed in physiological saline, fixed and stored in 70% ethanol. Distinction between females and males is not usually conducted but is important to be reported. Examination of the swimbladder wall under low magnification stereomicroscope is required to find and count the various larval stages of the parasite (Székely et al., 2009; Lewin et al., 2014. in prep.). Caution should be taken as larvae within the swimbladder wall can be easily missed if this organ is not examined thoroughly.

Severe pathological symptoms may be present in all sizes of eels. The effect of the parasite on its host is related to the number of matured worms and migrating larvae (Székely et al., 2009). Several methodologies have been used to quantify the swimbladder damage (Van Banning & Haenen, 1990; Hartmann, 1994; Mólnar, 1994; Molnár et al., 1993; Haenen et al., 1996; Beregi et al., 1998; Schabuss et al., 2005; Knopf and Lucius 2008; Wysujack et al., 2014). The easiest method to use seems to be the SDI (Swimbladder Degenerative Index) proposed by Lefebvre et al. (2002a). Briefly, this evaluation is based on 3 criteria: transparency/opacity; pigmentation/exudates; and thickness, with each one being scored 0, 1 or 2, and the cumulative index range from 0 (no pathological signs of infection) to 6 (extremely damaged swimbladder). It should be noted that the SDI score could vary dependent on the observer and standardisation is required. Taxonomic distinction between the 2 species of Anguillicoloides is not required for routine monitoring, but depending on the study taxonomic characterization of the two Anguillicoloides species may be envisaged.

6.1.2 Other parasites

Wild eels can host a diverse range of parasites (Molnár, 1983; Borgsteede et al., 1999; Jakob et al., 2009). These parasite infections may cause marked pathological changes which may potentially compromise eel health and fitness, and the role of co-infections in host health cannot be neglected. Where possible comprehensive examination of eels should take account of all parasite infections, which can vary considerably between individuals, environments and populations. Nevertheless, in routine monitoring, efforts should be focused on parasites considered as harmful for eel health (e.g. gill parasites like Pseudodactylogyrus spp. (Buchmann et al., 1987; Buchmann 1993; Saraiva 1995; Aguilar et al., 2005; Kennedy 2007) and blood parasites such as trypanosomes (Zintl et al., 1997; Aguilar et al., 2005) respectively.
To assess infections of trypanosomes, blood samples are required. They may be taken during both destructive and non-destructive examinations (observation under a microscope of a fresh blood droplet between slide and cover-slide). In both cases, blood sampling should take place immediately following anesthesia and before any dissection.

Skin or mucus wet mounts can be taken for routine eel sampling. Skin smears and fresh gill preparates should be taken and read promptly for parasites. The four gill arches of one side should be dissected and examined under a stereomicroscope in order to detect parasites, including *Pseudodactylogyrus* spp. as some reports refer to heavy losses in cultured eel stocks due to these monogeneans (Buchmann et al., 1987; Buchmann 1993; Saraiva 1995; Kennedy 2007).

Where other parasites are found they should be identified to taxonomic groups and numbers/prevalence/intensity recorded. They may also be preserved in 100% ethanol for further molecular identification. Endoparasites in general should be fixed differently depending on the species. Those fixations could be formalin (6-8%), alcohol (75%) or a suitable fixative, like Bouins. Ectoparasites like crustacean, leeches may also be fixed with different solutions (Kroghmann & Holstein, 2010).

Tissues for histopathology are not routinely taken. However, we encourage obtaining fixed tissues for histological studies when abnormalities (or infections of interest) are observed. Ten percent neutral buffered formalin is the most common fixative used, although other fixatives have been used such as Bouin’s or Davidson’s (Haenen et al., 1989; Cadoret et al., 2013; Lewin et al., 2014 in prep.). For histology, a small piece of tissue should be placed in the fixative in a ratio 1:10 volume/volume. It is recommended to take pictures of infections and of the eel parasites.

### 6.1.3 Reporting results

Results are usually reported using parasitological indices (prevalence, intensity, mean intensity, abundance and mean abundance, see Glossary) following Bush et al. (1997). In the case of protozoan or Myxosporidian parasites no attempt to estimate intensity or abundance is usually conducted. However, an indication of infection level (low, moderate or heavy) should be included.

Visualisation of data can be achieved through graphical representation, such as parasite distribution across maps and utilisation of pie chart/bar diagrams to express the prevalence (%) or other infection descriptors (see e.g. Audenaert et al., 2003). Annual trends may be discussed/represented per parasite, per site, per country, or on a European level.

### 6.2 Viral and bacterial diseases

In this section, detailed methods of sampling and testing of eels for viruses and/or bacteria are described.

#### 6.2.1 General introduction

Sampling of eels for virology and bacteriology can be completed in the field, but preferably requires a necropsy room with tables and sterile dissection equipment, a flame and the required plastic tubes/swabs etc. Testing of the eel samples for viruses and bacteria is a specialization of fish disease laboratories.
6.2.2 Viral diseases

According to published literature, there are three primary viral diseases of eel (Wolf, 1988; Van Beurden et al., 2012a,b; Bandin et al., 2014):

1) Anguillid herpesvirus 1 (AngHV-1)
2) Eel Virus European (EVE), and other aquabirnaviruses (IPNV)
3) Eel Virus European X (EVEX)

Other eel viruses, like betanodavirus (Bandin et al., 2014) and picornavirus (Fichtner et al., 2013) may also be present, but as their significance is still unclear they are not currently considered.

Sampling (see also Chapter 2), and subsampling per disease (including numbers):

Sampling

Number of eels to be sampled:

To obtain 95% probability at a 2% disease incidence that a certain population is free of a pathogen (e.g. a virus), you would need a maximum of 150 eels to sample, depending on the population size according to Poisson (Simon & Schill, 1984). However, in practice this is generally only used for notifiable fish diseases. This is not conducted for routine quality assessments for eels and, considering their endangered status may not be appropriate to sample that many fish. The presence and prevalence of viruses may be confirmed from much smaller sample sizes, most typically 30 eels. The numbers of eels to sample for disease diagnosis can be very low if single eels showing clinical signs are targeted (Van Ginneken et al., 2005; Haenen et al., 2009). This includes sampling of ulcerated tissues and the internal organs for disease testing. To minimise the use of eels, it would be advisable, for disease diagnosis, to use sampled eels both for disease diagnosis and contamination analysis. Similarly, tissues (e.g. gills) could be split: one side for parasitology and one side for virology analysis.

Precautions:

Use a laboratory coat and disposable gloves at necropsy. Eels might carry bacteria, which may be harmful to humans. Furthermore, in case of individual sampling, cross contamination of pathogens between eels should be prevented. For every examined eel a new pair of gloves, a cleaned work area and cleaned dissection equipment should be used. All dissection equipment should be disinfected with a chlorine solution, like Virkon S according to manufacturer guidelines. Ethanol should not be used as disinfectant when sampling for PCR, because it does not destroy DNA. Always wash your hands with disinfecting soap after contact with eel and eel water, and after laboratory work.

Take pictures of clinically diseased eels with unique ID number.

Organs sampling for virology:

Preferably, use small tubes with fixative (see below), depending on which virus is tested for. If fixatives are not available, sample the organs (see below) in sterile tubes and submit to a fish disease laboratory. Alternatively for virus isolation, freeze the tubes quickly in liquid nitrogen, and store at -80°C until processing. PCR samples should be stored at -20°C until analysis. Samples may also be stored and transported in a maintenance media
(Armitage et al., 2011). At the fish diseases laboratory, these samples are further processed after thawing, for DNA isolation and subsequent PCR, or virus isolation.

Prepare tubes with fixative:

- one small tube with RNA later (for EVEX virus and for EVE virus, both RNA viruses), and
- one small tube with >70% ethanol/isopropanol (possibly with metal beads for grinding, like IDEXX tubes), for AngHV-1 detection (a DNA virus).

Eel tissues to sample for PCR:

1) Gills from one side of the eel are sampled and pooled (max. 10 eels/pool), into the 2 tubes
2) Brains, heart, kidney and spleen of the eel are sampled and pooled per eel, into the 2 tubes

For virus isolation from small eels (< 10 cm), these may be sampled without fixatives, and pooled in groups of 10 eels/sample. In these small eels the tail is cut off and not used, and the rest of the eel is cut into pieces and subsequently ground (see below), into the 2 tubes.

Note that with virus isolation, titres decrease easily after sampling, especially for the RNA viruses like EVE and EVEX. This means that samples for virus isolation should be immediately processed or stored in -80°C, until further processing.

Note: To increase the chance of detecting viruses, it is important that eels are sampled at appropriate times of the year, when the approximate optimum temperature of each eel virus is reached:

- Anguillid herpesvirus 1 (AngHV-1): 26°C (Haenen et al., 2002, 2010; Van Beurden, 2012a,b)
- Eel Virus European (EVE): 20°C
- Eel Virus European X (EVEX): 15°C

Depending on the purpose of the research and viruses being tested, decisions have to be made on when to sample, what eel stage to sample and how many eels to sample. This allows assessment of the absence/presence or the prevalence of a virus, and/or sample individual eels for testing a disease outbreak.

If PCRs are not the chosen method, organ tissues (without added fixatives like RNA-later or ethanol/isopropanol) may be ground and put into cell culture (Eel-Kidney-1 for instance) for virus isolation.

Subsampling

Preferably, individual eels are tested, as above. In general, pooling of samples can decrease the virus detection.

Test method and existing protocols

- Anguillid Herpes Virus (AngHV-1): qPCR : Van Beurden et al., 2015 or conventional PCR (Rijsewijk et al., 2005)
- Eel Virus European (EVE): qPCR: Orpetveit et al., 2010; other birnaviruses (Cu-trin et al., 2005).
• Eel Virus European X (EVEX): qPCR: Van Beurden et al., 2011 or Meistertheim & Faliex, 2015 (patent publication numbers EP2821507 and WO/2015/000906), or Meistertheim et al., 2015, in prep.

Alternative methods that are proven and valid may be used: PCR is relatively efficient and cheap, but virus isolation (e.g. Eel Kidney-1 (EK-1) cells (Chen et al., 1982)) and subsequent immunological methods, like IPMA (Davidse et al., 1999) or IFAT, may be used. For betanodavirus detection, the RT-PCR method of Olveira et al. (2008) may be used, and for picornavirus detection, the paper of Fichtner et al., 2013 provides sequences.

Reporting results

Apart from specifications listed under Chapter 2 characterizing sampling site, date and methods, the following information should be included in the reports: life stage, number of eels sampled, number of eels showing clinical signs, technique used (PCR or virus isolation, other), name of the virus, number of positive samples from total number of samples.

Visualisation of the data can be made through tables with numbers and prevalences (Haenen et al., 2010), or graphic presentation of virus spread in country charts, and making use of pie chart or bar diagrams to express the prevalence (%) of the virus related to the total number of eel batches tested. Annual trends may be discussed/represented per virus, per site, per country, or across Europe.

6.2.3 Bacterial diseases

Most important pathogenic bacteria isolated from eels (Austin and Austin, 1989; Haenen et al., unpublished):

• Vibrio vulnificus (especially in eels from brackish or marine water) aggressive ulcerative skin lesions, abscesses, congested spleen, ascites, haemorrhages in organs and skin (may be zoonotic = harmful to man, see Dijkstra et al., 2009); occurs especially at water temp of approximately 25°C. Often found in wild eel in Spain (Esteve & Alcaide, 2009). Recorded in various eel farms in the Netherlands (Haenen et al., 2014), including a zoonotic strain.

• Edwardsiella tarda: bloody gut content, granuloma-like view of organs, congested spleen, a.o.

• Pseudomonas anguilliseptica: occurs especially in brackish water (Haenen & Davidse, 2001): glass eels may show a congested kidney, and a whitish layer at their skin. The bacterium is isolated from all organs. The disease is strongly temperature dependent.

• Aeromonas salmonicida atypical: congested eels, ulcers, may also be isolated from internal organs, showing inflammation.

• Aeromonas hydrophila/sobria: congested eels, haemorrhages, isolated from internal organs, showing inflammation.

• Flavobacterium columnare: whitish lesions start at the dorsal fin and proceed towards head and tail, destroying the slime layer and causing superficial whitish inflammations of the skin.
- *Mycobacterium marinum*: lethargy, granulomas in the spleen, liver and/or kidney. Scarcely found.

Note, that *Vibrio vulnificus* and *Edwardsiella tarda* may be seen in conjunction with a virus infection in eel.

**Sampling and subsampling for bacterial disease**

For bacteriology, only eels with clinical signs of bacterial disease will be sampled individually:

Important signs of clinical disease include:
- Ulcers
- Swollen belly
- Haemorrhagic fins and/or skin,
- Red anus, a.o.

**Precautions:**

Use a laboratory coat and disposable gloves at necropsy. Eels might carry bacteria, which may be harmful to humans. Furthermore, in case of individual sampling, cross contamination of pathogens between eels should be prevented. For every eel a new pair of gloves, a cleaned work area and a cleaned set of dissection equipment should be used. Disinfect all dissection equipment with a chlorine solution, like *Virkon S* according to manufacturer guidelines. Ethanol should not be used as disinfectant when sampling for PCR, because it does not destroy DNA. Always wash your hands with disinfecting soap after contact with eel and eel water, and after laboratory work.

Take pictures of clinically diseased eels with unique ID number.

**Numbers of eels to be sampled:**

No strict numbers of eels. Diseased eels should be sampled individually.

**Test method and existing protocols**

- Ulcers, and spleen, liver, kidney are sampled for bacteria, with disposable inoculation needles/sterile scalpel and swabs.
- In case of hemorrhages in the gut this can also be sampled with disposable inoculation needles/sterile scalpels and swabs (e.g. for *Edwardsiella tarda* isolation).

Use the following media, which should be kept at room temperature during necropsy:
- (BHI) Sheep blood agar,
- TCBS agar (for brackish salt water eels, to isolate *Vibrio* spp.),
- Cytophaga agar or Shieh medium (to isolate *Flavobacterium* spp) (Decostere et al., 1997)

Incubate the agar plates for approximately 2–7 days at 18–22°C.
• **Fish TBC testing:** If internal organs (such as the liver, spleen, or kidney) show signs of granuloma, make a fresh smear of the affected area on a glass slide. Let the samples air dry and fixate by passing through a Bunsen flame three times. Stain according to Ziehl Neelsen (Sheehan & Hrapchak, 1980). Examine under a light microscope at high magnification (>400x) with oil immersion, and search for pink rods. A well-covered TBC positive control slide should be used to confirm this. Store the used eel organs at -20°C, allowing testing for *Mycobacterium* spp.

• **Bacterial identification:** Following significant bacterial growth on the agar plate(s), a colony of the most prevalent bacteria is sampled, and sub cultured (pure) at 22°C. It is identified using the following methods:
  
  o MALDI-TOF (Carbonnelle et al., 2011)
  o 16S rRNA typing (Janda & Abbott, 2007) (more expensive), or
  o Gram staining and biochemical tests (time consuming and expensive, e.g. API or Biolog) (Barrow & Feltham, 1993)

**Reporting results**

Apart from specifications listed under Section 2.10 characterizing sampling site, date and methods, following information should be included in the reports: life stage, number of eels sampled, number of eels showing clinical signs, technique used for bacterial identification, name of the bacterium.

Visualisation of the data can be made through graphical representation of individual clinical cases per bacterium in country charts. Annual trends may be discussed/represented per bacterial disease in eel, per site, per country, or across Europe.

### 7 Perspectives of using biomarkers of effects to assess eel health

WKPGMEQ discussed and acknowledged the value and perspectives of using biomarkers in future assessments of the quality of eel stocks. However, currently, biomarker techniques are not sufficiently developed to assess the potential of the eel to realize successful migration and reproduction. Hence, defining the best available methods or strategies is currently difficult to justify. Nevertheless, this chapter aims to review a number of recent and promising biomarker studies in the eel, and further discusses future perspectives and research needs.

In ecotoxicology, a biomarker can be defined as a change that can be observed and/or measured at a molecular, biochemical, cellular, physiological or behavioural level, and reveals a past or present exposure of organisms to environmental chemicals (Lagadic, 2002). Biomarkers monitored at lower biological levels, for example at the cellular and molecular levels, constitute early warning signals as they are more rapidly influenced in response to stress compared to others that are often only visible when adverse effects are irreversible (McCarthy and Shugart, 1990).

A wide range of biomarkers have been developed at various biological integration levels, ranging from molecules to individuals and populations. A list of recent biomarker stud-
ies targeting the eel has been presented in Table 7.1. Biomarkers provide valuable information about the quality/stress level of eels, but it remains difficult to determine which ones are the most useful to investigate the health of eels exposed to contaminants. Information on the organ choice and the target biological level to best illustrate the eel’s health is still missing. It is now crucial to test various biomarkers in the same biological material, but at different biological levels to evaluate the overall response to pollutants. The role of omics technologies (genomics, transcriptomics, proteomics and metabolomics) using global and without a priori (candidate biomarker) approach is important in the discovery of new biomarkers of exposure and effects. However, the number of studies remains scarce for eels.

As a lot of ecotoxicological data were obtained under laboratory conditions, accurate predictions of effects are really difficult to translate to field conditions. Additional difficulties were experienced when trying to discriminate pollution-related changes from natural variations (Cajaraville et al., 2003; Gorbi et al., 2005; Bourdineaud et al., 2006). An incorrect application or interpretation of biomarker responses may lead to erroneous conclusions about pollutant stress or environment quality (van der Oost et al., 2003). Moreover, the various biomarkers frequently used and developed under controlled conditions, are mainly applied in response to single stressors, except for few studies that combined two or more stressing agents (Sures and Knopf 2004; Lorin-Nebel et al., 2013). However, in the field a lot of contaminants (abiotic and biotic) can be found and these stressors could have antagonistic or synergic effects on the biological response. As such, we recommend the rapid evaluation and application of available (and novel) biomarkers developed under laboratory conditions to wild populations, and emphasize the need to evaluate biomarkers in response to multiple stresses (combined abiotic and biotic) and at concentrations measured in the field (e.g. relevant for an organism’s fitness).

On a cautionary note, for various biomarkers used over recent years, the inter-individual variability was sometimes higher than the difference measured between contaminated and reference sites, leading to unreliable use of the relevant biomarker. Additionally, environmental variability and life-stage specific biomarkers expression can play a role in reducing the signal/noise ratio. Whatever the biomarker used, we recommend keeping an individual approach to emphasize the inter-individual variability, taking into account that biomarker variability may differ in function of the biological level analysed (e.g. molecular, tissue or individual level). To obtain the most relevant biomarker, we recommend applying several biomarkers in upcoming studies to reveal the impact of stress on physiology and not merely the endogenous effect (linked to life history of the individual). Physiology is related to gender / life stage and reproductive stage, hence it is crucial to integrate this information when using biomarkers.

Overall, information on biomarkers cannot yet be extrapolated to reliably evaluate the potential of eels to successfully migrate and reproduce in their marine spawning habitat. Few studies have focused on the effect of stress on migration potential through experiments in swim tunnels; or on the threshold-limits of biomarkers corresponding to migration success (Edeline et al., 2006). Understanding the real and long-term effect of contaminants on the reproductive potential of eels is the fundamental goal of biomarker development for eel.
To focus on biomarkers adapted to international surveys, an international coordinated investigation is needed, allowing the definition of a suitable set of biomarkers able to assess eel health (e.g. in terms of survival, migration and reproduction capacity).

Table 7.1. Recent biomarker studies on the European eel at different biological levels.

<table>
<thead>
<tr>
<th>Biological level</th>
<th>Biomarkers</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Behaviour</strong></td>
<td><strong>Exciting phase, ataxia and death</strong></td>
<td>Brusle 1991</td>
</tr>
<tr>
<td></td>
<td><strong>Swimming performance</strong></td>
<td>Palstra et al. 2007a; Palstra et al. 2007b</td>
</tr>
<tr>
<td><strong>Fecundity</strong></td>
<td><strong>Eggs production/Gonad development</strong></td>
<td>Palstra et al. 2006; Palstra et al. 2007a; MacNamara and McCarthy 2012; MacNamara et al. 2015</td>
</tr>
<tr>
<td></td>
<td><strong>Embryo development</strong></td>
<td>Palstra et al. 2007a</td>
</tr>
<tr>
<td></td>
<td><strong>Vitellogenin</strong></td>
<td>Livingstone et al. 2000; Versonnen et al. 2004</td>
</tr>
<tr>
<td><strong>Tissue</strong></td>
<td><strong>Gill structure</strong></td>
<td>Gony 1990; Santos et al. 1990; Pacheco and Santos 2002; Lorin-Nebel et al. 2013</td>
</tr>
<tr>
<td></td>
<td><strong>Lipid reserve/ Fatness</strong></td>
<td>Szlinder-Richert et al. 2014; Sancho et al. 1998b; Ribeiro et al. 2005; Pierron et al. 2007a; Guimarães et al. 2009; Gravato et al. 2010; Clevestam et al. 2011</td>
</tr>
<tr>
<td></td>
<td><strong>Skin disruption/renal tubule alterations</strong></td>
<td>Santos et al. 1990; Pacheco and Santos 2002</td>
</tr>
<tr>
<td><strong>Cellular</strong></td>
<td><strong>DNA integrity/apoptosis</strong></td>
<td>Maria et al. 2002; Maria et al. 2003; Maria et al. 2004a; Ahmad et al. 2006; Gravato et al. 2006; Maria et al. 2006; Ahmad et al. 2008; Nogueira et al. 2009; Guilherme et al. 2010; Guilherme et al. 2012</td>
</tr>
<tr>
<td></td>
<td><strong>Blood component / cell structure</strong></td>
<td>Santos et al. 1990; Santos and Hall 1990; Pacheco and Santos 2001; Maria et al. 2003; Oliveira et al. 2003; Maria et al. 2004a; Teles et al. 2005; Caruso et al. 2010</td>
</tr>
<tr>
<td><strong>Biochemical</strong></td>
<td><strong>Glycemia/ acid lactic/ proteins levels</strong></td>
<td>Sancho et al. 1997; Fernández-Vega et al. 2002a; Teles et al. 2004; Teles et al. 2005</td>
</tr>
<tr>
<td></td>
<td><strong>Hormonal concentrations (E2, cortisol etc.)</strong></td>
<td>Teles et al. 2004; Teles et al. 2005; Teles et al. 2007; Oliveira et al. 2008</td>
</tr>
<tr>
<td><strong>Enzyme activity</strong></td>
<td><strong>glutathione S-transferase GST</strong></td>
<td>Maria et al. 2003; Maria et al. 2004a; Maria et al. 2004b; Ahmad et al. 2006; Guimarães et al. 2009; Gravato et al. 2010; Kammann et al. 2014; Nunes et al. 2014</td>
</tr>
</tbody>
</table>
superoxide dismutase (SOD), catalase
selenium-dependant glutathione per-
oxidases (GPx and Se-GPx).

Ahmad et al. 2006; Buet et al. 2006; Gravato et al. 2006;
Guimarães et al. 2009; Guilherme et al. 2010

acetylcholinesterase (AChE) Sancho et al. 1998a; Fernández-Vega et al. 1999; Sancho et al. 2000; Fernández-Vega et al. 2002b; Buet et al. 2006; Gravato et al. 2006; Guimarães et al. 2009; Gravato et al. 2010; Nunes et al. 2014

uridine-5’-diphospho-
glucuronyltransferase (UDPGT) Buet et al. 2006

Cytochrome P450 CYP1A (7-
ethoxyres-orufin O-deethylase
(EROD)), Pacheco and Santos 1999; Pacheco and Santos 2001; Maria et al. 2002; Maria et al. 2003; Regoli et al. 2003; Maria et al. 2004a; Maria et al. 2004b; Maria et al. 2005; Buet et al. 2006; Gravato et al. 2006; Teles et al. 2007; Guimarães et al. 2009; Kammann et al. 2014; Lyssimachou et al. 2014

ATPase activity Lionetto 1998; Sancho et al. 2003; Buet et al. 2006; Guimarães et al. 2009; Lorin-Nebel et al. 2013

Lactate Dehydrogenase LDH Oliveira et al. 2008; Guimarães et al. 2009; Gravato et al. 2010

Proteomic

Global approach 2D-DIGE Roland et al. 2013; Roland et al. 2014

Targeted approach Metallothioneins Linde 1999; Ureña et al. 2007; Oliveira et al. 2008

Transcriptomic

Global approach Suppressive Sub-
tractive Hybridization, Micro-arrays,
Next Generation Sequencing (cDNA
and RNAseq) Kalujnaia et al. 2007a; Pierron et al. 2007b; Kalujnaia et al. 2007b; Nogueira et al. 2009; Pujolar et al. 2012; Pujolar et al. 2013; Bailey et al. 2015; Churcher et al. 2015

Targeted approach Cytochrome p450,
Metallothioneins Aubry et al. 2007; Pierron et al. 2007b; Maroh et al. 2008; Maes et al. 2013; Giuliani and Regoli 2014

8 International context and perspectives

This chapter describes and discusses the current international legal, and extralegal, framework, as well as recommendations and advice concerning the need for measuring eel quality in Europe (both contaminants and diseases). It also contains a review of what is reported in the Country Reports (Annex 6), national results availability to the international community and the need of an international database.

8.1 Eel Regulation

Council Regulation (EC) N. 1100/2007, Article 8 of 18 September 2007, which establishes measures for the recovery of the European eel stock, cites several measures to ensure protection and sustainable use of the stock but not “quality related” factors. Moreover, the same article mentions that Eel Management Plans may contain other measures, not re-
stricted to those mentioned in the Regulation. Since “anthropogenic mortality factors” can limit the number of migrating silver eels escaping to the sea, contaminant and disease could be included among them (see reviews by Geeraerts et al., 2011; Elie and Girard, 2009, and Palstra et al., 2007, ICES 2008, 2009a, 2010, 2011, 2012).

In the guidance document for the preparation of the Eel Management Plans there is a requirement to “Indicate the proportion of eel of each life stage affected by contaminants, pathogens and parasites. Indicate the degree of contamination, infection and/or parasitic infestation in each EMU.” Unfortunately no indications regarding eel quality are included in the guidance for reporting on the progress of the EMPs.

8.2 Data Collection Framework (DCF)

Since 2000, an EU framework for the collection and management of fisheries data has been in place. This framework was reformed in 2008 resulting in the Data Collection Framework (DCF). Under this framework MS annually collect, manage and make available a wide range of fisheries data needed for scientific advice and assessment of fish stocks (European Commission, 2008a, 2008b, 2010).

For the periods 2009/2010 (Reg. 199/2008 and 665/2008, Decision 2008/949/EC), 2011-13 (Decision 2010/93/EU) and 2014-2016 (C (2013) 5568) this framework doesn’t give any indications about the assessment of contaminants and/or diseases in European eel (as well as in other fish species).

In 2012 an ICES workshop about eel and salmon data collection (WKESDCF; ICES, 2012a) recommended within the new EU-Data Collection Multi Annual Programme (EU-MAP) to include the data requirements on diadromous species information. This included information about contamination and diseases, estimated at EMU level and at appropriate temporal frequencies. Such data should include: infection, intensity and abundance of *Anguilla crassus* and other parasites and diseases, and tissue concentrations of contaminants as recognized by ICES as having a potential impact on effective spawner stock biomass. Looking at the new implementation of the EU MAP covered in the new European Maritime and Fisheries Fund (EMFF), the future strategy of the DCF-sampling is under discussion and it is not clear yet how these new challenges will be dealt with. The EU STECF (2014); (Scientific, Technical and Economic Committee for Fisheries), however, suggested several additions to Data Collection with regard to eel (and salmon) (http://stecf.jrc.ec.europa.eu/documents/43805/674708/2014-04_STECF+14-07+-+Review+of+DCF+part+4_IRC89788.pdf). A proposal for a revised DCF is expected to be presented by the EC in April/May 2015.

8.3 Water Framework Directive (WFD)

The Directive 2000/60/EC of 23 October 2000 is a European Union directive which commits European Union member states to achieve good qualitative and quantitative status of all water bodies by 2015. It is a framework in the sense that it prescribes steps to reach the common goal rather than adopting the more traditional limit value approach. The WFD requires the assessment of the ecological and chemical status of surface waters. Ecological quality is monitored through, among others, the assessment of fish assemblages.

For the chemical status, apart from classical measurements of contaminants in the water phase, the WFD recently decided for some specific contaminants known to bio-
accumulate in fish, to use fish as bio-monitors. The Directive 2013/39/EU dealing with Priority Substances under the WFD amends Dir. 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy and Environmental Quality Standards Directive (EQSD) (European Commission, 2008c). EQS is the concentration of a particular pollutant or group of pollutants in aquatic organisms such as fishes (but also water and sediment) of a given area. Defining EQSbiota has two protection goals: (1) the protection from chemical accumulation in the food chain, specifically of top predators such as birds and mammals, from risks of secondary poisoning through consumption of contaminated prey, and (2) the protection of human health from deleterious effects resulting from the consumption of food contaminated by chemicals. These EQSbiota values are presented in Table 5.1.

The WFD describes how and when EQS for pollutants in biota should be developed. It also states that pollutants presenting a significant risk to water ecosystems should be identified by the European Commission and classified as priority substances, with the most hazardous of these classified as priority hazardous substances.

The Directive requires a minimum sampling frequency of the substance in the relevant matrix at least once every year (Art. 3.4) and MS shall determine the frequency of monitoring in sediment and/or biota so as to provide sufficient data for a reliable long-term trend analysis. As a guideline, monitoring should take place every three years, unless technical knowledge and expert judgment justify another interval (Art. 3.6).

Although contaminant analyses in eel, carried out by several MS under the WFD, are an important source of information about the quality assessment of this species, some restrictions exist in the Guidance Document n. 25, for example “Because of their protected status, eels should only be used for existing trend monitoring (to continue existing monitoring programs) and for this species the principle of conservation has to be respected.” (European Commission, 2010).

8.4 Marine Strategy Framework Directive


The aim of the MSFD is to achieve Good Environmental Status (GES) of the EU’s marine waters by 2020 and to protect the resource-base upon which marine-related economic and social activities depend. The Directive enshrines in a legislative framework the ecosystem approach to the management of human activities having an impact on the marine environment, integrating the concepts of environmental protection and sustainable use. To achieve Good Environmental Status (GES), the MSFD prescribes that the concentration of all contaminants should be assessed and be below the maximum level set for human consumption established by the Community (see section 8.1.6).

Since the ongoing process of establishing fish target species to assess contaminant concentration, the MSFD could be an opportunity, especially for those countries where marine eel populations are present (e.g. Baltic sea region), to collect data on eel quality.
8.5 Human health issues (Food safety)

Regulation (EC) No 315/93 of 8 February 1993 established the principle that maximum levels should be set for contaminants in foodstuffs in order to protect public health. Food containing a contaminant to an amount unacceptable from the public health viewpoint and in particular at a toxicological level, shall not be placed on the market. Maximum levels have been set for certain contaminants. Maximum levels in marine or freshwater food are set in Commission Regulation (EC) No 1881/2006 (then amended by Commission Regulations, 420/2011, 835/2011, 1259/2011). Contaminants that are currently covered under these European food regulations that are relevant to fish, shellfish and fish-related products (such as fish oils) include mercury, lead, cadmium, PCBs, dioxins and dioxin-like PCBs, and PAHs. These maximum levels are presented in Table 5.1.

These European Regulations and related Guidelines give specific indications on maximum levels of contaminants in “Muscle meat of wild caught eel (Anguilla anguilla)”, for dioxins, dioxin-like PCBs, PCBs, Cd, Hg and Pb (metals in “muscle meat of eel” EC 466/2001).

However it should be recognised that for the aims of the assessment of the quality of the eel stock (and more specifically the potential spawners) such thresholds, set up for the protection of human health, are inappropriate. Nevertheless, raw data from contaminant analysis under these regulations could be extremely useful for international assessment and inclusion in an international database.

8.6 Reports of the Joint EIFAAC/ICES Working Group on Eels (WGEEL)

In the last decade WGEEL has discussed the risks of reduced biological quality of (silver) eels and presented an overview and summaries of a variety of reports and data on eel quality (ICES, 2006-2011). ICES (2011, 2012) also recommended to the EU MS that regional eel management should take into account eel quality aspects like contamination and diseases, i.e. including observed or measured impairments of eels in reports.

In 2012 the WGEEL Report (ICES, 2012b) specifically recommended:

**Nr 10**: Where eel quality is poor, silver eels leaving catchments may not be able to contribute to the overall spawning and recruitment of the European stock. There is need to quantify the effects of eel quality on stock dynamics and integrating these into stock assessments. As a useful medium-term solution, we recommend a regular monitoring of the lipid content of escaping silver eels. As a key factor for reproduction and spawner quality, this information allows an approximate quantification of the number of potentially successful female spawners leaving each river basin and their reproductive potential in terms of eggs produced. (To EU Countries)

**Nr 11**: We recommend taking up an obligation of the Member States for the realization of routine monitoring of lipid levels, contamination and diseases in the Eel Regulation (to ICES Secretariat and EU)

WGEEL (ICES, 2013) also gives some specific advice for planning future reporting of local eel stock assessments and for calculating the Reproductive Potential of silver eels for each EMUs (see Ch. 12.7.4). In particular the following information is regarded as the minimum requirements:
For silver eels (Basic requirements for assessing the Reproduction Potential by EMU): mean size (mm), percentage lipid and the sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (Σ 6 PCBs) (ng/g wet weight), and prevalence (%) and abundance (n) of *Anguillicoloides crassus*, providing details of sample, site and date.

For yellow eels (Basic requirements for assessing the quality of the yellow eels and Eel Quality Index by EMU): mean size (mm), total wet weight of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (Σ 6 PCBs), p,p’-DDD, p,p’-DDT, p,p’-DDE (Σ DDTs), cadmium, lead and mercury (ng/g wet weight), and prevalence (%) and abundance (n) of *Anguillicoloides crassus*, providing details of sample, site and date.

### 8.7 OSPAR

The Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) Convention is the current legislative instrument regulating international cooperation on environmental protection in the North-East Atlantic.

In 2010 OSPAR drafted a background document with actions for the European eel (OSPAR Commission, 2010). Background Document for European eel *Anguilla anguilla*.

The following recommendations to the European Commission were made:

1. encourage eel specific indicators to be included as part of the implementation of the Water Framework Directive (WFD) as an indicator of river connectivity and ecological and chemical status;

2. encourage the further development and support of the European Eel Quality Database.

OSPAR should recommend that relevant Contracting Parties:

3. identify areas producing high quality spawners (large sized females, low contaminant and parasite burdens, not impacted by hydropower stations) in order to maximize protection for these areas;

OSPAR proposed also a monitoring system summarized by the following points:

- Silver eel escapement
- Production vs. escapement
- Contaminants, pathogens

### 8.8 Results availability and need of an international database

Five out of eleven countries reported in their Country Reports (Annex 6) the availability of disease and contamination data in local eel stocks to the international community.

All of them made available the results of national eel quality assessments through publications in scientific journals. However, raw data are usually neither included nor accessible.
In most countries such data is included only in institutional or regional reports. Unfortunately these documents are often very difficult to find and may only be written in the country language.

In some cases, eel quality data are reported in the official report to the EU, detailing progress within eel national management plans, under the Eel Regulation (Art. 9 Reg. 1100/2007).

The situation described by the CRs highlights the necessity of making national data on eel quality assessments (ideally by EMU) available, by means of a simple and reliable tool such as an international database.

The availability of an international up-to-date database, compiling eel quality parameters over the distribution area of the European eel is an essential instrument for international stock assessment. It will allow updates of local and stock wide eel quality indices, and will facilitate the integration of eel quality indicators with quantitative data for stock assessment.

As such, the EQD (Eel Quality Database) has been initiated by ICES WGEEL (Belpaire et al., 2011) and further developed by Belgium (INBO). It allows the compilation of contaminant and disease data in anguillids over the world, combined with relevant habitat parameters. The database is constructed with Local Microsoft Access 2010 (FormBuilder Model PTQ7.15), on a SQL-Server 2008. Its general structure is visualized in Annex 6.

However, the long-term management of the EQD needs a structural basis and is currently hampered by insufficient resources. ICES (2009a) suggested that the EQD should be managed at an international level (e.g. by ICES Data Center) or some European agency, with long-term funding options and database management expertise.

## 9 Conclusions

- Methods to estimate eel quality differ between Member States dependent of the objectives. Moreover, eel quality assessment methods between countries have only been harmonised in exceptional cases, primarily in the context of European research projects.
- Most data on eel quality (with respect to contaminants) have been collected for human health considerations and the assessment of habitat quality. These have largely focused on yellow eels. Sampling of silver eel and the assessment of their quality is considered a priority for eel stock restoration. However, there is no comprehensive and long term monitoring of eel quality in silver eels under the framework for eel population recovery.
- Disease monitoring is still only carried out in a few countries and is focused on mainly parasites, such as *Anguillicoloides crassus*, and in some cases also eel viruses, such as EVEX and AngHV-1.
- International assessment of the quality of eel stocks would only be possible if raw data was accessible. Even if data are scarce, most of them are not available and cannot be included in the European database, and hence are not available for international assessment.
The workshop made good progress in elaborating common best practice for evaluating eel quality.

- Methods were compared and progress was made to develop guidelines. Field sampling, procedures and laboratory practices have been described for testing of contaminants and diseases. Methods for the estimation of the condition of eel have been reviewed and some recommendations were made.

- Possible ways to integrate data and to implement them into eel quality indices have been suggested.

The workshop reviewed recent work using biomarkers to assess eel health, and acknowledges the value and perspectives of these techniques in future assessments of the quality of eel stocks. Currently however, biomarker techniques are not sufficiently developed to assess the potential of eel to realize successful migration and reproduction. WKPGMEQ recognizes the need to support and initiate international coordinated research to allow development of suitable set of biomarkers to assess eel health (e.g. in terms of survival, migration and reproduction capacity).

The workshop provided requirement and recommendations for assessing eel quality under existing international frameworks and agreed the need of an international database.
## Recommendations

<table>
<thead>
<tr>
<th><strong>RECOMMENDATION</strong></th>
<th><strong>ADRESSED TO</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Harmonised methods for eel quality assessments and reporting should be implemented by the Member States.</td>
<td>ICES Member States</td>
</tr>
<tr>
<td>2. WKPGMEQ reiterates the recommendation issued by WGEEL (ICES, 2012b) to take up an obligation of the Member States for the realization of routine monitoring by Member States, of lipid levels, contamination and diseases in the Eel Regulation. More specifically WGEEL 2013 (ICES, 2013) defined a set of basic requirements for assessing the quality of the silver eels (the mean size (mm), percentage lipid and the sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (Σ 6 PCBs) (ng/g wet weight)) and for the yellow eels (the mean size (mm), total wet weight of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (Σ 6 PCBs), p,p’-DDT, p,p’-DDE (Σ DDTs), cadmium, lead and mercury), and for both life stages the prevalence (%) and abundance (n) of Anguillicoloides crassus.</td>
<td>ICES Secretariat -&gt; EU</td>
</tr>
<tr>
<td>3. Considering the current gaps in knowledge to fully assess the effects of current levels of contaminants on eel, monitoring efforts should be coupled to other research actions. WGEEL 2013 recommended the initiation of an internationally coordinated research project, in order to improve the understanding and quantification of the effects of contaminants on the reproductive success of the European eel, for integration in stock wide assessments.</td>
<td>EU, SCICOM</td>
</tr>
<tr>
<td>4. Raw data should be made available to the international community through WGEel for inclusion in the Eel Quality Database. This could be facilitated through an ICES Data Call.</td>
<td>ICES Member States, WGEEL, ICES Secretariat</td>
</tr>
<tr>
<td>5. However, the long-term management of the Eel Quality Database needs a structural basis and is currently hampered by insufficient resources. WGEEL (ICES, 2009a) already suggested that the eel quality database should be managed at an international level (e.g. by ICES (ICES Data Centre) or a European agency, with long-term funding options and database management expertise.</td>
<td>ICES Data Centre</td>
</tr>
</tbody>
</table>
Annex 1: References


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Simon R.C., Schill W.B. 1984. Tables of sample size requirements for detection of fish infected by pathogens: three confidence levels for different infection prevalence and various population sizes. Journal of Fish Diseases 7: 515-520.


### Annex 2: Participants list

<table>
<thead>
<tr>
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<th>Address</th>
<th>Email</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
<td>Name</td>
<td>Institution</td>
<td>Location</td>
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<td>Institution</td>
<td>Email</td>
</tr>
<tr>
<td>-----------------------</td>
<td>------------------------------------------------------------------------------</td>
<td>--------------------------------------------</td>
</tr>
</tbody>
</table>
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<th>Organization</th>
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<tbody>
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</tr>
</tbody>
</table>
Tuesday 20 January 2015

9.30-10.00  P  Arrival, getting organized

10.00-10.15  P  Welcome (Prof Maurice Hoffmann, Division Head INBO) + Practical Issues Presentations Agenda

10.15-10.40  P  Introduction and objectives (including ICES work on eel quality), ToR

11.00-12.30  P  Presentations by country, with focus on framework, method, problems (contaminants and diseases)

Poland
Germany
United Kingdom
The Netherlands
Belgium
France
Spain
Portugal
Italy

Presentation on Eel Quality Database
Presentation network eel diseases

12.30-13.30  Lunch

13.30-14.30  P  Introduction to working groups (by task leaders) - Presentations on tasks

- Framework national cases and national guidelines (on contaminants and health)
- General issues on eel sampling for contaminants and/or diseases
- General issues on measuring eel condition (fitness, lipid levels)
- Extra: Patho-index
- Contaminants: sampling and analysis
- Contaminants: interpretation, visualisation and assessment
- Anguillicoloides and other parasitic diseases of the eel
- Viral and bacterial diseases
- Perspectives of using biomarkers of effects to assess eel health
- International needs and future perspectives

14.30-15.30 SG  Subgroups split up.
  Working in subgroups. Discussion on tasks.

16.00-17.30 SG  Working in subgroups. Discussion.

19.00  WKPGMEQ Dinner

**Wednesday 21 January**

9.00-10.30 SG  Working in subgroups. Drafting text.

11.00-12.30 SG  Working in subgroups. Drafting text.

12.30-14.00  Lunch

14.00-16.00 SG  Working in subgroups. Drafting text.

16.15-20.00 SG  Working in subgroups. Drafting text.

**Thursday 22 January 2015**

9.00-10.30 SG  Working in subgroups. Last edits on subgroup chapters.

10.45  Draft report available for review

10.45-11.45 I  Reading on through report, individually

11.45-12.30  Lunch

12.30-14.45 P  In plenary. Reading/commenting on report, finalizing report.
  Large meeting room 4th floor

15.00-16.30 P  In plenary. Reading/commenting on report, finalizing report.
  Large meeting room 4th floor

16.30  End of meeting,
Annex 4: WKPGMEQ terms of reference

Workshop of a Planning Group on the Monitoring of Eel Quality under the subject “Development of standardised and harmonised protocols for the estimation of eel quality” (WKPGMEQ)

2013/2/SSGEF08The Workshop of a Planning Group on the Monitoring of Eel Quality under the subject “Development of standardised and harmonised protocols for the estimation of eel quality” (WKPGMEQ), chaired by Claude Belpaire*, Belgium, and Olga Haenen*, The Netherlands, on contaminants, and on eel diseases respectively, will meet in Brussels, Belgium, 20–22 January 2015 to:

a ) Design standardised and harmonised protocols for the estimation of eel quality with regard to the bioaccumulation of contaminants (including sampling, analysis and reporting).

b ) Design standardised and harmonised protocols for the estimation of eel quality with regard to diseases (including sampling, analysis and reporting).

WKPGMEQ will report by 28 February 2015 (via SSGEF) for the attention of the ICES WGEEL, WGRECORDS and SCICOM.

Supporting information

<p>| Priority | WGEEL 2012 stated that to improve the assessment of the impact of contaminants and diseases on effective spawner biomass and reproductive success, national routine monitoring programmes are urgently required. The Eel Regulation does not refer to the health status of the population of European eel or possible impacts on the population due to contamination and diseases. Hence, regular monitoring programmes for eel are neither run nor reported to the EU. WGEEL 2012 recommended that Member States implement routine monitoring of lipid levels, contamination and diseases, but also identified the need to develop standardised and harmonised protocols for the estimation of eel quality, so that national data would be comparable and could be reliably incorporated in international stock assessments. In 2015 WGEEL will organize a Workshop of a Planning Group on the Monitoring of Eel Quality, in order design standardised and harmonised monitoring protocols, to facilitate the integration of eel quality parameters in quantitative assessment of the reproductive potential of the stock. |
| Scientific justification | Reliable assessment of the eel stock quality and its quantitative effect on the reproductive stock is currently not possible, due to insufficient spatial and temporal coverage. WGEEL(2009) emphasized the need to establish a comprehensive overview with improved spatial coverage of the quality of the eel population across Europe as an essential and urgent requirement. Many countries have started compiling data on the health status of eels in their water bodies. Objectives for these monitoring actions are diverse and there is a large amount of information collected by EU member countries. However, procedures with respect to sampling, analysis and reporting are not harmonised, jeopardising stock wide assessments and risking inefficient deployment of resources. Understanding of the reproductive potential of the international spawning stock is a key component to predicting the effects on stock recovery of changes to silver eel escapement arising from management actions implemented within Eel Management Plans. |</p>
<table>
<thead>
<tr>
<th>Resource requirements</th>
<th>None other than financial, see below. The host institution will provide meeting facilities.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants</td>
<td>WGEEL Participants, other experts/representatives from member states</td>
</tr>
<tr>
<td>Secretariat facilities</td>
<td>Sharepoint</td>
</tr>
<tr>
<td>Financial</td>
<td>Support is requested to cover the travel and subsistence costs of those attending the workshop.</td>
</tr>
<tr>
<td>Linkages to advisory committees</td>
<td>WGEEL and ACOM</td>
</tr>
<tr>
<td>Linkages to other committees or groups</td>
<td>WGRECORS, SCICOM, Eel diseases group EAFP</td>
</tr>
<tr>
<td>Linkages to other organizations</td>
<td>FAO EIFAAC, GFCM, EU DG MARE, EU DG ENV</td>
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### Annex 5: Glossary

#### Eel life history terms and terms related to eel quality

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Bioaccumulation and biomagnification</td>
<td>Bioaccumulation is the process through which fishes may take up, store and accumulate contaminants from the water in which they live. This uptake can happen through different routes: by taking chemicals in through their gills (bioconcentration) or through their diet and the ingestion of contaminated prey (biomagnification).</td>
</tr>
<tr>
<td>Bootlace, fingerling</td>
<td>Intermediate sized eels, approx. 10–25 cm in length. These terms are most often used in relation to stocking. The exact size of the eels may vary considerably. Thus, it is a confusing term.</td>
</tr>
<tr>
<td>Eel River Basin or Eel Management Unit</td>
<td>“Member States shall identify and define the individual river basins lying within their national territory that constitute natural habitats for the European eel (eel river basins) which may include maritime waters. If appropriate justification is provided, a Member State may designate the whole of its national territory or an existing regional administrative unit as one eel river basin. In defining eel river basins, Member States shall have the maximum possible regard for the administrative arrangements referred to in Article 3 of Directive 2000/60/EC [i.e. River Basin Districts of the Water Framework Directive].” EC No. 1100/2007.</td>
</tr>
<tr>
<td>Elver</td>
<td>Young eel, in its first year following recruitment from the ocean. The elver stage is sometimes considered to exclude the glass eel stage, but not by everyone. To avoid confusion, pigmented 0+cohort age eel are included in the glass eel term.</td>
</tr>
<tr>
<td>Environmental Quality Standard</td>
<td>A term most often used in reference to an annual average. A legally binding limit value under the Water Framework Directive, either internationally or nationally.</td>
</tr>
<tr>
<td>Glass eel</td>
<td>Young, unpigmented eel, recruiting from the sea into continental waters. WGEEL consider the glass eel term to include all recruits of the 0+ cohort age.</td>
</tr>
<tr>
<td>Intensity (of infection)</td>
<td>Number of individuals of a particular parasite species in a single infected host.</td>
</tr>
<tr>
<td>Mean Abundance</td>
<td>Total number of individuals of a particular parasite species in a sample of a particular host species divided by the total number of hosts of that species examined (including both infected and uninfected hosts).</td>
</tr>
<tr>
<td>Mean Intensity</td>
<td>Average intensity of a particular species of parasite among the infected members of a particular host species.</td>
</tr>
<tr>
<td>Prevalence</td>
<td>Number of hosts infected with one or more individuals of a particular parasite species (or taxonomic group) divided by the number of hosts examined for that parasite species.</td>
</tr>
<tr>
<td>Quality of spawners</td>
<td>WGEEL defined this term as “the capacity of silver eels to reach spawning areas and to produce viable offspring” (ICES, 2006).</td>
</tr>
<tr>
<td>River Basin District</td>
<td>The area of land and sea, made up of one or more neighbouring river basins together with their associated surface and groundwaters, transitional and coastal waters, which is identified under Article 3(1) of the Water Framework Directive as the main unit for management of river basins. The term is used in relation to the EU Water Framework Directive.</td>
</tr>
<tr>
<td>Silver eel</td>
<td>Migratory phase following the yellow eel phase. Eel in this phase are characterized by darkened back, silvery belly with a clearly contrasting black lateral line, enlarged eyes. Silver eel undertake downstream migration towards the sea, and subsequently westwards. This phase mainly occurs in the second half of calendar years, although some are observed throughout winter and following spring.</td>
</tr>
</tbody>
</table>
Stocking (formerly called restocking) is the practice of adding fish [eels] to a waterbody from another source, to supplement existing populations or to create a population where none exists.

Silvering is a requirement for downstream migration and reproduction. It marks the end of the growth phase and the onset of sexual maturation. This true metamorphosis involves a number of different physiological functions (osmoregulatory, reproductive), which prepare the eel for the long return trip to the Sargasso Sea.

Unlike smoltification in salmonids, silvering of eels is largely unpredictable. It occurs at various ages (females: 4–20 years; males 2–15 years) and sizes (body length of females: 50–100 cm; males: 35–46 cm) (Tesch 2003).

Yellow eel (Brown eel) is a life-stage resident in continental waters. Often defined as a sedentary phase, but migration within and between rivers, and to and from coastal waters occurs and therefore includes young pigmented eels (‘elvers’ and bootlace).

Zoonosis is any disease or infection that is naturally transmissible from vertebrate animals to humans and vice-versa (WHO).

<table>
<thead>
<tr>
<th>Acronyms</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ACOM (ICES)</td>
<td>Advisory Committee on Fishery Management</td>
</tr>
<tr>
<td>AngHV-1</td>
<td>Herpesvirus of eel, also named HVA (herpesvirus anguillae)</td>
</tr>
<tr>
<td>API</td>
<td>Analytical Profile Index (biochemical method for identification of bacteria)</td>
</tr>
<tr>
<td>BFR</td>
<td>Brominated Flame Retardant</td>
</tr>
<tr>
<td>BHI</td>
<td>Brain Heart Infusion</td>
</tr>
<tr>
<td>Cd</td>
<td>Cadmium</td>
</tr>
<tr>
<td>COT</td>
<td>Cost of Transport, energetic requirements of eels to complete their journey to the Sargasso Sea (expressed as kJ.km⁻¹.kg⁻¹)</td>
</tr>
<tr>
<td>CR</td>
<td>Country Report</td>
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<tr>
<td>DCAL</td>
<td>Department of Culture, Arts &amp; Leisure, N. Ireland</td>
</tr>
<tr>
<td>DCF</td>
<td>Data Collection Framework</td>
</tr>
<tr>
<td>DDT</td>
<td>Dichlorodiphenyltrichloroethane</td>
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<tr>
<td>DL-PCB</td>
<td>Dioxin-like Polychlorinated Biphenyl</td>
</tr>
<tr>
<td>EAFP</td>
<td>European Association of Fish Pathologists</td>
</tr>
<tr>
<td>EFF</td>
<td>European Fisheries Fund</td>
</tr>
<tr>
<td>EIFAAC</td>
<td>European Inland Fisheries &amp; Aquaculture Advisory Commission</td>
</tr>
<tr>
<td>EK-1</td>
<td>Eel Kidney -1 cells (Chen et al., 1982), a cell line</td>
</tr>
<tr>
<td>EMFFF</td>
<td>European Maritime and Fisheries Fund</td>
</tr>
<tr>
<td>EMP</td>
<td>Eel Management Plan</td>
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<tr>
<td>EMU</td>
<td>Eel Management Unit</td>
</tr>
<tr>
<td>EPI</td>
<td>Eel Patho-Index</td>
</tr>
<tr>
<td>EPMN</td>
<td>Eel Pollution Monitoring Network (Flanders, Belgium)</td>
</tr>
<tr>
<td>EQD</td>
<td>Eel Quality Database</td>
</tr>
<tr>
<td>EQI</td>
<td>Eel Quality Index</td>
</tr>
<tr>
<td>EQS</td>
<td>Environmental Quality Standard</td>
</tr>
<tr>
<td>EQSBIO7A</td>
<td>Environmental Quality Standard derived in biota</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>EQSD</td>
<td>Environmental Quality Standards Directive</td>
</tr>
<tr>
<td>ER</td>
<td>Energy remaining for reproduction in female eels by arrival at their spawning ground (ERind)</td>
</tr>
<tr>
<td>EU - MAP</td>
<td>EU-Data Collection Multi Annual Programme</td>
</tr>
<tr>
<td>EU DG ENV</td>
<td>Directorate-General for the Environment of the European Commission</td>
</tr>
<tr>
<td>EU DG MARE</td>
<td>Directorate-General for Maritime Affairs and Fisheries of the European Commission</td>
</tr>
<tr>
<td>EVE</td>
<td>Eel Virus European, a bi-RNA virus of eel</td>
</tr>
<tr>
<td>EVEX</td>
<td>Eel Virus European X, a rhabdovirus of eel</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organisation</td>
</tr>
<tr>
<td>GFCM</td>
<td>General Fisheries Commission of the Mediterranean</td>
</tr>
<tr>
<td>GSI</td>
<td>Gonadosomatic Index</td>
</tr>
<tr>
<td>HBCDD</td>
<td>Hexabromocyclododecane</td>
</tr>
<tr>
<td>HCB</td>
<td>Hexachlorobenzene</td>
</tr>
<tr>
<td>HCBD</td>
<td>Hexachlorobutadiene</td>
</tr>
<tr>
<td>Hg</td>
<td>Mercury</td>
</tr>
<tr>
<td>HIS</td>
<td>Hepatosomatic Index</td>
</tr>
<tr>
<td>ICES</td>
<td>International Council for the Exploration of the Sea</td>
</tr>
<tr>
<td>IFAT</td>
<td>Immuno fluorescence test</td>
</tr>
<tr>
<td>IMBI</td>
<td>Individual Mean (multi-metal) Bioaccumulation Index</td>
</tr>
<tr>
<td>INBO</td>
<td>Research Institute Nature and Forest, Belgium</td>
</tr>
<tr>
<td>IPMA</td>
<td>Immuno Peroxidase Monolayer Asssay</td>
</tr>
<tr>
<td>K</td>
<td>Condition Factor</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of Detection</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of Quantification.</td>
</tr>
<tr>
<td>MALDI-TOF</td>
<td>Matrix Assisted Laser Desorption Ionisation – Time Of Flight (method of bacterial identification)</td>
</tr>
<tr>
<td>MS</td>
<td>Member States</td>
</tr>
<tr>
<td>NDL-PCB</td>
<td>Non-Dioxin-like Polychlorinated Biphenyl</td>
</tr>
<tr>
<td>OSPAR</td>
<td>Convention for the Protection of the Marine Environment of the North-East Atlantic</td>
</tr>
<tr>
<td>PAH</td>
<td>Polycyclic Aromatic Hydrocarbon</td>
</tr>
<tr>
<td>Pb</td>
<td>Lead</td>
</tr>
<tr>
<td>PBDEs</td>
<td>Polychlorinated Biphenyl Ethers</td>
</tr>
<tr>
<td>PCDD/F</td>
<td>Polychlorinated Dibenzo-p-Dioxin and Polychlorinated Dibenzofuran</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>PFAAs</td>
<td>Perfluoroalkyl Acids</td>
</tr>
<tr>
<td>PFCs</td>
<td>Perfluorinated compounds</td>
</tr>
<tr>
<td>PFOS</td>
<td>Perfluorooctanesulfonic acid or perfluorooctane sulfonate</td>
</tr>
<tr>
<td>POPs</td>
<td>Persistent Organic Pollutants</td>
</tr>
<tr>
<td>qPCR</td>
<td>RealTime (quantitative) Polymerase Chain Reaction</td>
</tr>
<tr>
<td>RBD</td>
<td>River Basin District</td>
</tr>
<tr>
<td>RP</td>
<td>Reproduction Potential</td>
</tr>
</tbody>
</table>
The reproduction potential of female silver eels escaping from the catchment or EMU (RP is calculated as the mass of eggs which could be produced)

rRNA Reverse Ribo Nucleic Acid

SCICOM ICES’ Science Committee

SDI Swimbladder Degenerative Index.

SHIEH Medium for bacterial culture according to Decostere et al. (1997)

SQL Structured Query Language

SSB Spawning Stock Biomass

SSGEF ICES’ SCICOM Steering Group on Ecosystems Functions

STECF Scientific, technical and economic committee for fisheries

TBC Tuberculosis, caused by Mycobacterium spp.

TCBS Thiosulfate Citrate Bile Salts Sucrose Agar

TCDD 2,3,7,8-tetrachlorodibenzo-p-dioxine

TDI Tolerable Daily Intake

TEQ 2005 Toxic Equivalence

ToR Terms of Reference

US FDA US Food and Drug Administration

WFD Water Framework directive

WG Working group

WGEEL Joint EIFAAC/ICES Working Group on Eel

WGRECORDS ICES’ Working Group on the Science Requirements to Support Conservation, Restoration and Management of Diadromous Species

WKESDCF ICES workshop about eel and salmon data collection

WKPGMEQ Workshop of a Planning Group on the Monitoring of Eel Quality under the subject “Development of standardized and harmonized protocols for the estimation of eel quality”

HCH Hexachlorocyclohexane is a six chlorine substituted cyclohexane, HCH has several isomers (α -HCH, β –HCH, γ - HCH (lindane) ), some of them used as pesticides.
Annex 6: Eel Quality Database – general structure
Annex 7: Country Reports: Report on the methods and assessment of eel quality (contaminants and diseases)

In preparation for the Working Group, participants of each country have prepared a Country Report, in which the most recent information on eel contaminants and diseases are presented. These Country Reports aim at presenting the best information which does not necessarily coincide with the official status.

Participants from the following countries provided a Country Report to WKPGMEQ.

- Belgium
- France
- Germany
- Ireland
- Italy
- Netherlands
- Poland
- Portugal
- Spain
- Sweden
- The United Kingdom of Great Britain and Northern Ireland
Report on the methods and assessment of eel quality (contaminants and diseases) in:

Belgium

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Introduction
This country report has been drafted in preparation of the ICES Workshop of a Planning Group on the Monitoring of Eel Quality: “Development of standardized and harmonized protocols for the estimation of eel quality” (WKPGMEQ), held at INBO Brussels from 20 to 22 January 2015.

This report aims to document the ways eel quality is assessed, measured, reported, with the aim to make a guidance document with better standardisation of methods. With “Methods” we mean everything connected with Sampling – Identification – Treatment – Subsampling – Analytical Procedures – Reporting – Visualisation – Database issues. Consequently, the results of the assessments are not relevant here, but the way the results were collected, visualized, reported, etc. is of utmost importance. Results of these monitoring and scientific work are described in the cited references.

In Belgium the competences in issues such as environmental management and freshwater fisheries are divided over the Flemish and Walloon communities, and scientific and routine monitoring are seldom aligned between both regions. Hence the subheadings of this report are divided in two parts ‘Flanders’ and ‘Wallonia’.

2. Framework national cases and national guidelines (on contaminants and health)

2.1 Contaminants
Flanders

Flemish Eel Pollution Monitoring Network: a routine monitoring network measuring contaminants in eel
A research program using eel to track PCBs, BFRs, pesticides, metals and other compounds in Flemish water bodies was initiated by INBO and run from 1994-2009, initially on demand of the Ministries of Environment and Human Health, to indicate potential risks for the human consumption of freshwater fish. However, the Flemish eel pollution network also follows the presence and temporal trend of these compounds in the environment which gave substantial contribution to Flanders’ environmental management. Moreover the network contributed to the assessment of the potential impact of these compounds on fish species on individual and population level. An overview of the combined use of measuring contaminants in eel has been illustrated in following Fig. 1 (From Belpaire and Goemans, 2008). This monitoring network generated a significant amount of results published in various papers, dealing with the methodology, the spatial and temporal trends of diverse contaminants in eel, effects on the eel and the ecological quality, and risk assessments of consuming freshwater fish. See the reference list below for more details. One report (Maes et al., 2008) is summarizing the methods, and results in terms of spatial and temporal trends, for the period 1994-2005. In short, for the period 1994-2005, the network consisted of individual analysis of PCBs, pesticides and heavy metals from 2839 individual eels. Eel were always caught between March and October. Total annual catch varied between 25 eel in 1996 and 732 in 2000. On average, 237 eel were captured each year. Yellow eel were sampled at 365 different stations using fyke nets or an electro-fisher. Stations (see e.g. Figs 5 and 9) were characterised as rivers or brooks, canals, polder water courses or closed water bodies such as old meanders, ponds or lakes. Stations were situated in all 11 river basins. Between 1994 and 2005, 91 stations were visited twice for sampling; 16 stations were sampled three times, 6 stations were sampled four times and 2 stations were sampled five times. One station (Lake Weerde, a man-made water body) was sampled eight times between 1997 and 2005.

The Network was financed by the Flemish authorities and several regional and federal agencies were informed about the results (Flemish Environmental Agency, Fisheries service (ANB) and Fisheries Commissions, Water managers, Federal Health Services (FAVV), ...). Data are accessible to international community through the publication of the reports, through the VIS database (INBO), through participation to the ICES WGEEL and implementation of the data in the EQD.
Framework | Focused substances | Measures
--- | --- | ---
EEL POLLUTION BIOINDICATOR | Sampling & Analysing | NATIONAL EEL MANAGEMENT PLAN (Data Collection Regulation)
 | | Focus on lipid content, PCBs, Anguillicola, viruses or other factors affecting spawner quality
 | | WATER FRAMEWORK DIRECTIVE (and other environmental issues)
 | | Achieving good chemical status for 33 substances (CEC 2006a)
 | | HUMAN HEALTH PROTECTION (SANITARY CONTROL OF FISHERIES PRODUCTS)
 | | EU Regulations: Dioxins and PCBs, Cd, Pb, Hg
 | | National legislation, e.g. Belgium Sum PCB,
 | | FISHERIES AND TRADE RESTRICTIONS

Figure 1. Possibilities of combined use of monitoring contaminants in the eel (Belpaire and Goemans, 2008).

Other initiatives to measuring contaminants in eel

Studies measuring other compounds.

Other studies measuring contaminants in eel have been undertaken in order to measure other compounds than the aforementioned PCBs, pesticides and heavy metals, such as Volatile Organic Compounds (Roose et al., 2003), Dioxins (Geeraerts et al., 2011), Brominated Flame Retardants (Roosens et al., 2008, 2010, Malarvannan et al., 2014), PFOS (Hoff et al., 2005, Roland et al., 2014), Dyes, Organophosphorus Flame Retardants and PAHs (work in progress).

Studies relating contaminants to effects on the eel.

Several studies have been performed to assess the impact of (particular) contaminants on the eel.
In eel, Versonnen et al. (2004) investigated potential effects of xenoestrogens, and measured plasma vitellogenin (VTG) content in 142 eels sampled at 20 different locations of variable pollution levels. The plasma VTG content of eels was very low, despite a very high internal load of endocrine disrupters. Therefore, no indications were found for estrogenic effects to occur in natural freshwater eel populations in Flanders. These results suggest that immature yellow European eel might not be the best sentinel species to study the effects of estrogenic compounds on VTG levels of wild fish populations. Most probably, endocrine disrupting effects of pollutants related with reproduction, will only become apparent during the maturing silver eel stage.

Several studies assesses the genotoxic effects of contaminants in Flemish eels at the targeted (candidate detox genes) and transcriptome-wide gene expression level (Maes et al., 2005; 2013; Pujolar et al., 2012; 2013). See under Chapter 7 for more details.

Van Campenhout et al. (2008) studied the effect of metal exposure on the accumulation and cytosolic speciation of metals in livers of European eel by measuring metallothioneins (MT) induction. This research was carried out in four sampling sites in Flanders showing different degrees of heavy metal contamination (Cd, Cu, Ni, Pb and Zn). It was concluded that the metals, rather than other stress factors, are the major factor determining MT induction.

The effects of perfluorooctane sulfonic acids (PFOS) in Flemish eels were studied by Hoff et al. (2005), indicating that PFOS induces liver damage, and by Roland et al. (2013) evaluating the toxicological effects in eel peripheral blood mononuclear cells (PBMCs) at the protein expression level.

Geeraerts et al. (2007) analysed our extensive dataset of contaminants by statistical modelling and concluded that PCBs, especially the higher chlorinated ones, and DDTs, have a negative impact on lipid content of the eel. We further demonstrated that fat stores and condition decreased significantly during the last 15 years in eels in Flanders (Geeraerts et al., 2007) and in The Netherlands (Belpaire et al., 2009), jeopardizing a normal migration and successful reproduction of this endangered species.

**Studies relating contaminants in eel with effects on biodiversity.**

To better understand the relationship between the presence of pollutants in biota and the ecological water quality, Van Ael et al. (2014) analysed datasets of bioaccumulated chemicals in European eel (N=1156) and the Ecological Quality Ratio (EQR) based on the assessment of fish assemblages at 185 locations. For most pollutants, EQR scores were lower when pollutant levels were higher. Higher chlorinated PCBs, and to a lesser extent also the lower chlorinated ones, significantly influenced the ecological water quality. The study showed that empirical data are important for refining threshold levels of contaminants to better protect the aquatic ecosystems.

**Studies relating contaminants in the eel, with human health risks.**

Bilau et al. (2007) used a probabilistic approach to assess the intake and exposure of recreational fishermen by PCBs, based on several consumption scenario’s. The approach has
also been used in other assessments of human exposure risks (e.g. Malarvannan et al., 2014, Belpaire et al., 2011, Roosens et al., 2010).

**EELIAD, an international research including the monitoring of the quality of silver eels leaving continental waters.**

In the context of an FP7 research consortium, silver eels from 14 catchments over 8 European countries were sampled for PCBs, OCPs, BFRs and heavy metals (Work in progress). A sampling protocol has been developed and described (EELIAD sampling protocol).

**A recent study in the context of the Water Framework Directive**

In order to meet the requirements of the European Commission to measure contaminants in aquatic biota (Water Framework Directive, WFD), a feasibility study was carried out in 2013-2014 and bioaccumulation of hydrophobic micropollutants was measured in muscle tissue of eel (*Anguilla anguilla*) and perch (*Perca fluviatilis*) from Flemish waterbodies (De Jonge et al., 2014). Quantified pollutants included the WFD biota substances mercury (Hg), hexachlorobenzene (HCB), hexachlorobutadiene (HCBd), Polybrominated diphenyl ethers (PBDE), Hexabromocyclododecane (HBCDD), perfluorooctane sulfonate (PFOS) and its derivates, dicofol, heptachlor and heptachlorepoxide. Measured Hg and HCB concentrations were compared between species and in time, based on historical data of eel pollutant monitoring in Flanders. The study was carried out to assess how Flanders could implement the requirements of the WFD. The study recommended eel as the most suitable species to monitor bioaccumulation of hydrophobic micropollutants in Flanders. This conclusion was based on both practical aspects (spatial distribution and amount of biomass) and species-specific advantages of the immature eel related to biomonitoring (sedentary, no gender issues, no reproduction). This study was carried out by University Antwerp and INBO, on demand of the Flemish Environmental Agency, who is the competent authority for regional/national reporting for WFD. Data are reported in the report (De Jonge et al., 2014), which is public.

**Wallonia**

### 2.2 Diseases

**Flanders**

Apart from two region-wide assessments in 1986 and 2000, there is currently no routine monitoring of eel diseases in Flanders.

However, substantial work on eel diseases has been carried out as scientific surveys and experimental work, mostly, but not exclusively, on *Anguillicoloides*. The University of Leuven was one of the pioneers in this research area and published several reports in the period 1980-2000. In Belgium the parasite was first observed in 1985 (Belpaire and De Charleroy, 1985). The papers described the initial spreading in Flanders and reported
about the status of the parasite over 3 monitoring assessments (1986, 1996 and 2000)(Belpaire et al., 1989 a, b; Audenaert et al.2003). Other work focused on life cycle, population biology, and larval biology (De Charleroy et al.1989; De Charleroy et al.1990; Thomas and Ollevier 1992, 1993; Thomas, 1993). Latest work was a contribution to the EELIAD program assessing the status of Anguillicoloides in silver eels from a few Flemish waters (work in progress).

Work on other parasites included the report and description of a new mite (Histostoma anguillarum) parasitizing the gills of eels under aquaculture conditions (Fain and Belpaire, 1985).

In another report (Schabuss et al., 1997) the endoparasitic helminth communities in eel from four cut off river meanders from Flemish rivers were investigated.

Some bacterial work on eel was carried out during the 1980s, mostly on aquaculture or restocking populations (Belpaire and De Charleroy 1985; Noterdaeme et al., 1991; Noterdaeme et al., 1996).

The best available documents describing the methods to assess the status of Anguillicoloides is probably the M& M section published in Audenaert et al. (2003), and the protocol used in EELIAD.

Wallonia

As part of the European FEP project 32-1202-005, the detection of the presence of Anguillid herpesvirus (HVA) will be performed in each eel captured in the Lesse basin between October 2013 and September 2015. The protocols used to detect HVA in serum samples are entirely described in the paper of Rijsewijk et al. (2005). This work is performed by the scientists of the Unit of Research in Environmental and Evolutionary Biology (URBE) (University of Namur) and is co-funded by the European Commission and the Service Public de Wallonie (SPW).

3. General issues on eel sampling for contaminants and/or diseases in your country. Treatment of the sampled eels on site and in the laboratory

3.1 Ethics and legislation

Flanders

The European eel is currently classified as Critically Endangered by the IUCN, as well as in Flanders (Red List), however in Flanders no specific protection measures are currently in force (other than a minimum legal size limit for recreational fishermen).

The sites studied are all part of public water bodies managed by the water authorities of the Flemish government and as a public and governmental research institute INBO is authorized in sampling activities for research purposes. Permit to use electrofishing and fykenet fishing has been issued by the fisheries authority (Agency for Nature and Forests).
Authorisation is required to carry out animal experiments by the federal public Animal health care service of Health, Food chain safety and Environment in accordance to article 11 § 4 of Royal Decree of 6 April 2010 on the protection of laboratory animals. All efforts were made to minimize suffering.

A number of data and variables are collected to characterize the sampling site. Also the EQD requires the collection of variables.

Georeferences used in Flanders are the Lambert coordinates (reference 0-0 is Paris, Notre-Dame), which can be transformed into latitude and longitude.

Wallonia

In the European FEP project 32-1202-005, fifty sites have been selected for eel samplings. For each sampling station, many water details are recorded: water temperature, oxygen saturation, pH and conductivity. Water transparency and coloration are also observed. Moreover, wind speed, cloud cover, hydrology, pollution level and livestock accessibility are mentioned in the sampling site reports.

3.2 Sampling procedures

Flanders

In the framework of the Eel Pollution Monitoring Network, the objective was to sample 10 yellow eels in the length class 45-55 cm (females). Fisheries technique was mainly electrofishing and fykenet fishing. Five individuals from each site were analysed individually. This holds the advantage to estimate individual variability, and to assess effects on individual level.

In some other studies, mostly due to budgetary reasons, samples from individual eels were pooled.

In a specific site, eels were sampled on a distance of max 250 m. Using electrofishing, both river banks were fished over a distance of max 250 m. See Belpaire et al. (2008) for variability in eels within a river.

Yellow eels were fished between March and November.

In the case of silver eel studies, those are fished in the period September-December, through fyke fishing.

Wallonia

In the 50 sampling stations selected in the Lesse sub-basin (FEP project 32-1202-005), eels are captured using two fishing methods: electrofishing and fyke nets. Each station is sampled twice. The first day, an electrofishing is performed and followed by the placement of fyke nets. Their number depends on the station size. Forty-eight hours later, an electrofishing is performed again and the fyke nets are removed. Each eel is anesthetized, tagged with an integrative passive transponder (PitTag) and blood is taken directly in the caudal vein. After many morphological observations and measurements, the fish are re-
leased into the river. Eels are captured between the months of April and November. Few days during the period from July to February, at the confluence between the Lesse and the Meuse rivers, the width of the Lesse river is covered by a large fyke net, particularly the flooding days, in order to capture migrating silver eels. These fish get the same treatment as the others: PitTagging, blood sampling and morphological measurements before their release.

3.3 Stage, gender, morphometrics

Flanders

There is no formal identification of the species. We assume all individuals are *A. anguilla.* Both fresh weight and length are recorded twice, once after sampling (alive), but also after preservation in the deepfreezer. Both data are inserted in the database.

L is recorded in cm, W in g.

For yellow eel, gender is not recorded.

Eel stage (yellow/stage) is distinguished macroscopically. In the framework of the Eel Pollution Monitoring Network only yellow eels are targeted. For some specific studies silver eels are targeted, in this case gender is identified, and additional morphometrics are recorded (eye width and height, length pectoral fins, on both sides), to allow the calculation of the silvering index.

Wallonia

In the FEP project 32-1202-005, the stage of eels captured is determined using the silvering index developed by Durif (2003). For that, many morphological measurements are made on each fish: the weight (in g; precision of 1 mg), the total length (in mm; measured with a tape; precision of 1 mm), the length of the left pectoral fin (in mm; measured with a caliper; precision of 0.01 mm) and the horizontal and vertical diameters of the left eye (in mm; measured with a caliper; precision of 0.01 mm). The presence of neuromasts on the lateral line and of a color contrast between the dorsal and ventral sides of the eels are also noted but are not involved in the index calculation. In parallel, physiological parameters (growth hormone and thyroid hormones levels) are measured in eel serum or plasma in order to compare the results with the stage calculated by the silvering index defined by Durif (2003). No gender distinction is possible in this project since animals are not sacrificed. However, we assumed that eels with a total length of more than 45 cm are females.

3.4 Treatment

Flanders

Eels sampled in the field are batch labelled during transport and brought to the lab of INBO where 8 batches can be stored separately in tanks within a partial recirculation system. They are kept for maximum 3 days before dissection. In other cases, eels are frozen directly after sampling, for later process.
Eels are euthanized using clove oil, or by chilling them down directly in deepfreezer.

INBO uses a standardized labelling combining unique numbers of sampling site, individual eel and individual tissue.

All tissue samples are stored in deepfreezer at -24° and archived in this tissue bank, samples are described in a database.

Wallonia

In the Lesse FEP project, no eels were sacrificed or stored in the lab. The eels captured were maintained in cages directly in the river before being anesthetized in freshwater containing 0.1 mL clove oil/L. All the manipulations are made with gloves, even in the field. Blood is aseptically sampled. Serum and plasma are isolated using a mobile centrifuge, kept on ice before being stored in the lab at -80°C. In order to collect plasma, blood is taken with an heparinized syringe (0.1 ml of heparine for 2.5 mL of blood), kept on ice during 1 hour and centrifuged 15 minutes at 4 000 rpm. Serum is isolated from blood sampled with a non-heparinized syringe, kept 1 hour at outside temperature and centrifuged 5 minutes at 9 000 rpm. Each fish is sampled with a new sterile syringe and the samples are kept in separated sterile tubes in order to avoid cross contamination between the samples.

4. General issues on eel condition (fitness, lipid levels)

4.1 Condition

How is eel condition (in terms of length weight relation) in your country usually measured, expressed (condition index, condition factor, ...), what equation is used?

Flanders

*Condition and length/weight relationship*

The availability of robust length–weight relationship might be valuable for assessing condition. Verreycken *et al.* (2011) describe the length–weight relationship \( W = aL^b \) in eel (and other species) from Flanders. 17 586 individual eel length–weight \( (L/W) \) data, collected during 2839 fish stock assessments between 1992 and 2009, were used (Fig. 2). Those data were collected by INBO in the framework of the Flemish Freshwater Fish Monitoring Network. The study area includes 1426 sampling locations characterized as lacustrine as well as riverine habitats, including head streams, tributaries, canals, disconnected river meanders, water retaining basins, ponds and lakes.

Following equation was found:

\[
W = 0.0011 \ L^{3.130}
\]

\[
r^2 = 0.98
\]
Figure 2. Length–weight relation of European eel (n = 17,586) sampled over Flanders in the period 1992–2009.

In order to ascertain to what extent the log10a and b values calculated for the Flemish populations fell within the range available from other studies, we compared the Flemish values with the values available in FishBase (Froese and Pauly, 2010) from other countries. Flemish a and b values both fell within the 95% CL of the mean European a and b values (Fig. 3).

Our data originate from over almost two decades, irrespective of sampling sites, dates and seasons. Because of the dense sampling network in a small geographic area over a long sampling period, extremes are balanced out. Therefore and through the fact that Flanders is situated centrally in Europe, our a and b values may be applicable as reference marks for an European L/W relation for eel. Moreover, our TL range covered the whole range between minimum and maximum length in sufficient numbers, making a and b values valid as mean values for all length ranges (Verreycken et al., 2011).
Figure 3. Estimated intercepts (log10a; Y-axis) versus estimated slope (b; X-axis) for the log10 transformed L/W regression and regression line for European eel from European datasets, as available in Fishbase (Froese and Pauly, 2010), compared to the Flemish populations (■; 1992–2009). Linear regression equation and $r^2$ are given (n = number of L/W relationships, including Flanders); (Verreycken et al., 2011).

Several papers (among others Froese (2006)) present critical assessments of the use of the various condition indices in fish.

To assess the relative condition of individuals under pollutant stress, Maes et al. (2005) used Ricker’s (1975) condition index (CI). CI was calculated as 1000 ($\frac{WB}{L^b}$), where respectively $L$ and $W$ relate to standard length in millimetres and body weight in milligrams (King, 1995). The coefficient $b$ was calculated as the slope from the log$WB$ –log $L$ regression analysis for all three basins, as allometric growth was detected. The relative condition of each individual from each basin was then assessed using the formula. ANOVA tests were performed to compare the mean relative condition between basins.

Belpaire et al. (2009) calculated the condition factor in eel, following Le Cren’s relative condition factor (Le Cren 1951), as recommended by Froese (2006). The relative condition factor ($k = \frac{W}{W'}$) compares the observed ($W$) and expected ($W'$) weight of each individual, where expected weight is obtained using the length–weight regression ($W = aL^b$) of each individual.

Also Hoff et al. (2005) used Le Cren’s condition factor.

VersonnEN et al. (2004) used the condition factor (CI) calculated as total weight (g)/total length$^3$ (cm). Byer et al. (2013) used Fulton’s K condition factor. In Pujolar et al. (2013) condition was measured as Fulton’s condition index was calculated as CI=$W/L^3$. Length and weight was measured respectively in mm and g.
Wallonia

4.2 HSI

Flanders
Since the liver is the major detoxification and lipid storage reserve organ, changes in weight of this organ will relate to detoxification and energy storage.

In Maes et al. (2005) and Maes et al. (2013) the hepatosomatic index (HSI) was calculated as

\[ \text{HSI} = \left( \frac{W_l}{W_b} \right) \times 100 \]

where \( W_l \) and \( W_b \) represent wet liver weight and wet body weight, respectively.

Weight effects on HSI were removed, followed by an ANOVA on the residuals of the weight-HSI regression for group comparison.

Wallonia

4.3 GSI

Flanders
In Flanders the GSI in eels has only been assessed during EELIAD work on female silver eels, using the EELIAD protocol.

Wallonia

4.4 Lipid levels

Flanders
Lipid levels have been analysed in each eel which was analysed individually for contaminants. From ca 4000 individual eels, data on muscle lipid levels are available. Eels were skinned and filleted, and the same part of the muscle was used for analysis throughout the full period (mid-part of the body, see Fig. 4). Lipid was extracted from the muscle tissue and quantified using the Bligh & Dyer (1959) method. Quality was assured by participation in QUASIMEME interlaboratory proficiency testing schemes (http://www.quasimeme.org). Z-scores rarely exceeded 0.6 in absolute value, whereas Z-scores below 2 are satisfactory. The fat content is measured as the lipid concentration in muscle and is expressed in % of muscle wet weight (w/w). A discussion about the measurements of lipid levels in eel is presented in Belpaire et al., 2009. As fat levels vary dependent of a.o. size, stage and gender, fat level data should always be presented in combination with data on size, stage and gender.
During a preliminary study (results not published) lipid levels of 45 silver eels most of them above 60 cm, were measured both with a fatmeter and with analytical method. The relationship between both methods equals

\[
\%\text{Fat}_{\text{Analytical}} = 0.7262 \%\text{Fat}_{\text{Fatmeter}} + 1.0786
\]

\[
R^2 = 0.2649
\]

During this experiment, – on average – the fatmeter was measuring higher values compared to the analytical method. \(R^2\) values were quite low.

Wallonia

4.5 Other condition related issues

Flanders

Wallonia

5. Contaminants

5.1 Sampling and analysis

5.1.1 PCBs

Flanders

Maes et al. (2008) described the methods for PCB analysis in the regular Flemish Eel Pollution Monitoring Network.

The measurements were made on individual eels. Fish tissue was extracted using the Bligh and Dyer method (1959). The extract was evaporated (Rotavapor) and a minimum of 100 mg lipid was dissolved in hexane and applied on an aluminum oxide chromatography column. After elution with hexane, the lipid free eluate was evaporated and applied on a silica gel chromatography column. PCB congeners, p,p'-DDE and HCB were isolated after elution with hexane. After elution with diethylether/hexane (10/90) the remaining organochlorine pesticides were isolated. Both fractions were evaporated to 1 ml, after addition of an internal standard (tetrachloronaphtalene) and separated by gas chromatography using a 224 J. Rtx-5ms capillary column (60 m, 0.25 mm, 0.25 mm), with helium as a carrier gas and an electron capture detector (ECD). The detection limit for both PCBs and pesticides was 0.5 ng g\(^{-1}\) lipid weight.

QA/QC: PCB analysis was carried out at DVZ, the Sea Fisheries Department, Ostend. Quality assurance consisted of the analysis of procedural blanks, reproducibility and repeatability tests, injection of standard solutions as unknowns, and analysis of certified
reference material. The institute routinely analyse samples in the framework of the international proficiency testing scheme QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) for organochlorines in biological samples and participate in intercalibration studies organized by the IAEA (International Atomic Energy Agency). Internal quality assurance at DVZ was realised by monthly analyses using certified reference materials. Gas chromatography equipment was calibrated every 60 samples and every 20 samples, two standards were analysed.

In Belpaire et al., 2011 PCB measurements were made on pooled samples. The following PCB congeners (IUPAC numbering) were targeted for analysis: 18, 28, 31, 44, 49, 52, 74, 87, 95, 99, 101, 105, 110, 118, 128, 138, 149, 151, 153, 156, 170, 177, 180, 183, 187, 194, 195, 199, 206 and 209.

Samples with concentrations below LOQ were calculated as f*LOQ with “f” being the fraction of samples above LOQ (or the detection frequency). All results were expressed as ng/g wet weight (ww). Total PCB level is indicated as Sum 30 PCBs and totals the 30 PCB congeners previously mentioned. Seven congeners are considered as indicator PCBs (28, 52, 101, 118, 138, 153, 180) and their sum is commonly used in European countries to report PCB contamination. This is further abbreviated as Sum 7 PCBs.

Full method is described here: CB 143 was used as the internal standard for the quantification of PCBs. All individual PCB standards were obtained from Dr. Ehrenstorfer Laboratories (Augsburg, Germany). All solvents used for the analysis (acetone, dichloromethane, iso-octane, n-hexane, methanol) were of SupraSolv® grade (Merck, Darmstadt, Germany). Sodium sulphate (Merck) and silica gel (0.063–0.200 mm, Merck) were pre-washed with n-hexane and heated overnight at 150 °C before use. Extraction thimbles (25×100 mm, Whatman®, England) were pre-extracted for 1 h with hexane/acetone (3:1; v/v) and dried at 100 °C for 1 h. Empty polypropylene columns for clean-up (25 ml) were purchased from Alltech (Lokeren, Belgium). The method used for sample extraction and clean-up has been previously described and validated (Voorspoels et al., 2004). Briefly, a homogenised sample of approximately 1 g pooled eel muscle was weighed, mixed with anhydrous Na2SO4 and spiked with 40 ng of internal standard (CB 143). Further, the samples were extracted for 2 h by hot Soxhlet (Büchi, Flawil, Switzerland) with 100 ml hexane/acetone (3:1, v/v). The lipid content was determined gravimetrically on an aliquot of the extract (105 °C, 1 h), while the rest of the extract was cleaned on ~8 g acidified silica and successively eluted with 20 ml hexane and 15 ml dichloromethane. The cleaned extract was concentrated to approximately 2 ml using a rotary-evaporator and further to near dryness under a gentle nitrogen stream. The dried extract was reconstituted in 100 μl iso-octane and analysed for PCBs using gas chromatography–mass spectrometry (GC–MS) with electron impact ionization (EI). An Agilent 6890 GC–5973 MS system operated in EI mode was equipped with a 25 m×0.22 mm×0.25 μm HT-8 capillary column (SGE, Zulte, Belgium). The ion source, quadrupole and interface temperatures were set at 230, 150 and 300 °C, respectively. One μl of the cleaned extract was injected in cold pulsed splitless mode (injector temperature 90 °C (0.03 min) rising to 300 °C with 700 °C/min), pressure pulse 25 psi and pulse time 1.50 min. The splitless time was 1.50 min. Helium was used as the carrier gas at constant flow (1.0 ml/min). The temperature of the HT-8 column was kept at 90 °C for 1.50 min, then increased to 180 °C at a rate of 15 °C/min (kept for 2.0 min), further increased to 280 °C at a rate of 5 °C/min and finally raised to 300 °C at a rate of 40 °C/min, and kept for 20 min. The MS was used in
the selected ion-monitoring (SIM) mode with 2 ions monitored for each PCB homologue group. Dwell times were set to 30 ms (Belpaire et al., 2011).

Fig. 4. Location of the muscle tissues targeted for analysis by INBO.

Wallonia

5.1.2 Pesticides

Flanders

The pesticides which are targetted for analysis in the Flemish Eel Pollutant network are listed in Maes et al., 2008. This paper reports on alfa-HCH and gamma-HCH (lindane), which were banned in Belgium in 2002. Cyclodienes in this study include dieldrin, endrin and chlordane. The use of dieldrin has been prohibited since 1974 while the use of endrin has never been authorised at all. Chlordane is a mixture of different components of which only transnonachlor was assessed. The use of the latter substance by agriculture has been prohibited since 1981 but non-agricultural use was allowed until 1998. Hexachlorobenzene (HCB) was formerly used as an fungicide and was banned in 1974. Concentrations of three chloroethanes (p,p'-DDD, p,p'-DDT, p,p'-DDE) were measured and their sum used as a proxy of total DDT (SumDDT). DDTs have been banned since 1974 in case of agricultural application and since 1976 for all other uses.

See under PCBs for analytical procedures and QA/QC.

Wallonia
5.1.3 Brominated Flame Retardants

Flanders

Roosens et al. (2010) described the measurements of BFRs in Flemish eels. Between 3 and 10 eels were caught per location, an amount ranging between 1 and 4 g of their muscle tissue was pooled and analysed for PBDE congeners (28, 47, 49, 66, 99, 100, 154, 153, 183 and 209) and HBCD (α-, β- and γ-) isomers. The muscle samples were taken from the mid part of the body (See Fig.4, same as for PCBs and OCPs). PBDEs reference standards were bought from Wellington Laboratories (Guelph, ON, Canada) and Accustandard (New Haven, CT, USA). Standards of individual 12C-HBCD and 13C-HBCD isomers were purchased from Wellington Laboratories. All solvents used for the analysis (acetone, dichloromethane, iso-octane, n-hexane, and methanol) were of SupraSolv® grade (Merck, Darmstadt, Germany). Sodium sulphate (Merck) and silica gel (0.063–0.200 mm, Merck) were prewashed with n-hexane and heated overnight at 150 °C before use. Extraction thimbles (25×100 mm, Whatman®, England) were preextracted for 1 h with hexane/acetone (3/1; v/v) and dried at 100 °C for 1 h. Empty polypropylene columns for clean-up (25 mL) were purchased from Alltech (Lokeren, Belgium).

About 1 g pooled eel muscle sample was weighed, homogenized with Na2SO4 and spiked with internal standards (BDE 77, BDE 128, 13C-BDE 209, 13C-α-HBCD, 13C-β-HBCD and 13C-γ-HBCD), hot Soxhlet extracted during 2 h with hexane:acetone (3:1) and cleaned-up on acidified silica (Voorspoels et al., 2004). Prior to the clean-up, a fraction of the extract was taken to determine the lipid content gravimetrically. Minor adaptations were required as PBDEs were analysed with GC–ECNI/MS and HBCDs with LC–MS/MS. The cleaned extract was evaporated to dryness, redissolved in 0.5 mL hexane and eluted from pre-packed silica cartridges (Varian) with 6 mL hexane (for GC analysis) and 6 mL DCM (for LC analysis). Both fractions were evaporated to incipient dryness and redissolved in 100 μL iso-octane and 100 μL methanol, respectively.

The determination of PBDEs was performed with an Agilent 6890GC-5973MS equipped with a 15 m×0.25 mm×0.10 μm DB-5 capillary column and operated in electron capture negative ionization (ECNI) mode. The ion source, quadrupole and interface temperatures were 250, 150 and 300 °C, respectively. Helium was used as carrier gas at constant flow (1.0 mL/min) and with methane as moderating gas. The MS was operated in SIM mode (m/z 79 and 81 were monitored for the entire run, m/z 487 and 495 were monitored for BDE 209 and 13C-BDE 209, respectively). Dwell times were set to 40 ms. One μl of the extract was injected in solvent vent mode and the splitless time was 1.50 min. The oven temperature was programmed from 90 °C, kept for 1.5 min, then increased with 15 °C/min to 295 °C, kept for 15 min. The determination of ΣHBCDs and separation of α-, β-, and γ-HBCD was achieved using a dual pump Agilent 1100 Series liquid chromatograph equipped with autosampler and vacuum degasser. An Agilent Zorbax Extended-C18 reversed phase analytical column (50 mm× 2.1 mmi.d., 3.5 μm particle size) was used. A mobile phase of (a) water and (b) methanol at a flow rate of 200 μL/min was applied for elution of HBCD isomers; starting at 75% (b) then increased linearly to 100% (b) over 7 min; this was held for 12 min followed by a linear decrease to 75% (b) over 0.5 min and held for 10 min. The target analytes were baseline separated on the LC column with retention times of 7.0, 7.5, 7.8 min for α-, β- and γ-HBCD respectively. Mass spectrometric analysis was performed using an Agilent 6410 triple quadrupole mass spectrometer operated in the ES negative ion mode. MS/MS detection operated in the MRM (multiple
reaction monitoring) mode was used for quantitative determination of the HBCD isomers based on m/z 640.6 to 79 and m/z 652.6 to 79 for the native and 13C-labelled diastereomers, respectively. The analytical procedures were validated through analysis of procedural blanks, duplicate samples, and certified material SRM 1945 (PBDEs in whale blubber, which has also indicative values for HBCDs). Obtained values were not deviating with more than 10% from the certified values and all samples were blank-corrected. Recoveries of internal standards were all above 80%. Method quantification limits (LOQs) for individual PBDE congeners and individual HBCD diastereomers were based on procedural blanks (10×SD) and the amount of sample taken for analysis (typically 1 g eel muscle).

LOQs for tri-hepta PBDEs range between 1 and 2 ng/g lipid weight (lw), for BDE 209 LOQ was 10 ng/g lw, while LOQs were 1, 2 and 2 ng/g lw for α-, β- and γ-HBCD, respectively. Samples with concentrations below LOQ were calculated as f×LOQ with f being the fraction of samples above LOQ.

Wallonia

5.1.4 Dioxins

Flanders

Geeraerts et al., 2011 assessed dioxin levels in eels from 38 Flemish locations. To avoid effects of possible variation in body burden of individual eel from a particular site due to variation in size, sex or age, the authors aimed to analyse pooled samples from 10 individuals per site. But, this objective could not be met at all sites due to low abundances. On each locality 4–10 yellow eel were captured and live transported to the laboratory. Again low abundances did not allow sampling for a standardised eel length, so eel were of variable length (range 33.9–64.1 cm) and weight range (59.7–566.4 g).

At the lab, fish were measured, weighed, skinned and samples of muscle tissue (10 g fresh weight each) were removed, labelled and stored at −20 °C. Due to budgetary reasons, measurements were made on pooled samples. From each sampling location, tissues from individual eel were pooled prior to homogenisation and analysis (5.0 g).

Analysis were carried out via gas chromatography, full methods and QC-QA are described in Geeraerts et al., 2011.

Results are expressed as pg g⁻¹ fresh weight for spatial analyses. To estimate health risks from human consumption and effects on eel, results are expressed in pg WHO1998 TEQ g⁻¹ (lower bound) on fresh weight basis. Measurements below limits of quantification (LOQ) were treated as half the LOQ value of the congener considered.

For a list of the compounds, see Geeraerts et al., 2011.

Maximum consumption levels of dioxins, dioxin-like PCBs and non dioxin-like PCBs in foodstuffs are regulated in the Dioxin regulation, which applies since 1 January 2012 (Com Reg EU No 1259/2011).
Wallonia

5.1.5 Metals
Subsampling in the lab. What part of the body for which analysis? Which tissues?
Which compounds?
How to measure, analytical procedures?
QC-QA?
How to report? Body weight basis, lipid weight basis, dry weight basis, body burden?
What units to use?

Flanders
Maes et al. (2008) described the methods for measuring the heavy metals within Flanders’ Eel Pollution Monitoring Network. Those measurements were made on individual muscle tissue samples. The part of the body used for analysis is illustrated in Fig. 4. Nine heavy metals (cadmium, lead, mercury, chromium, nickel, copper, zinc, arsenic, selenium) were determined.

Fish muscle tissue (between 3 and 5 g) was placed in an oven for 12 h at 50 °C. Once cooled, 100 ml HNO3 was added and the analyte was dried again at 450 °C for 1 h. Subsequently, 1 ml HNO3 was added to the ash and diluted using distilled water. Trace elements of Cr, Ni, Cu, Zn, Cd and Pb in solution were analysed using ICP-OES (Spectra AA-400 with Zeeman correction, Varian). The detection limits for each of these metals varied: 2 ng g⁻¹ wet weight for Cd and Pb, 10 ng g⁻¹ wet weight for Ni, 35 ng g⁻¹ wet weight for Cr and 100 ng g⁻¹ wet weight for Cu and Zn. As and Se were determined using GF-AAS. Prior to analysis, fish tissue was heated in a medium of 5 ml HNO3 and 3 ml H2O2 and afterwards diluted in distilled water. The detection limits for As and Se were 10 and 35 ng g⁻¹ wet weight, respectively. Hg was quantified using AAS (AMA 254 mercury analyser, Altec). Hg was detected if the concentration was higher than 10 ng g⁻¹ wet weight.

QA/QC: metal analysis was carried out at CODA, the Veterinary and Agrochemical Research Centre, Tervuren). Quality assurance consisted of the analysis of procedural blanks, reproducibility and repeatability tests, injection of standard solutions as unknowns, and analysis of certified reference material. The institute routinely analyse samples in the framework of the international proficiency testing scheme and participate in intercalibration studies organized by the IAEA (International Atomic Energy Agency). Quality of heavy metal analyses performed at CODA was assured using reference materials and blanks every 12 samples (daily for Hg). ICP-OES and GF-AAS equipment was calibrated every 15 samples.

Maes et al. (2005) and Maes et al. (2013) used an integrated index for metals. They calculated a relative bioaccumulation index by dividing (standardizing) the individual concentration of heavy metal i (Ci) by the maximum observed concentration (Cimax) and averaging over all metals, to relate heavy metal bioaccumulation to condition and genetic
variability. Thus, the \textbf{individual mean (multi-metal) bioaccumulation index} (IMBI) was defined as:

$$\text{IMBI} = \left[ \frac{\sum_{i=1}^{n} \left( C_i / C_{i,\text{max}} \right)}{n} \right]$$

with $N$ the total number of metals, $C_i$ the individual concentration of heavy metal $i$, $C_{i,\text{max}}$ the maximal observed concentration of heavy metal $i$ and $0 < \text{IMBI} < 1$. To allow a broader comparison of bioaccumulation levels, this index should be recalculated over the entire datasets, to allow a relative bioaccumulation comparison of IMBI levels.

Wallonia

5.1.6 PAHs

Flanders

Some preliminary work has been done on the analysis of PAHs in eel muscle, in the framework of the study of De Jonge et al., 2014. This work is in progress.

Wallonia

5.1.7 Others?

Flanders

Perfluorooctane sulfonate (PFOS) and its derivates were analysed in eel in the framework of a preliminary study for reporting the presence of some chemicals in biota as required by the Water Framework Directive (2013/39/EU (EC, 2013)). Biota threshold standard for PFOS is 9.1 μg kg$^{-1}$ fresh weight (EC, 2013).

The methods are described in De Jonge et al. (2014, in Dutch). In short, methods are as follows:

The measurements were performed on pools. Eels were skinned and gutted, and the analysis was performed on the muscle tissue taken from the mid part of the body.

To the homogenized tissue was added to 10 ml of acetonitrile and labelled internal standards. The samples were sonicated and shaken overnight. After centrifugation, the supernatant was evaporated to 1 ml and then further purified with activated carbon (ENV1-carb). The obtained extract was diluted with water and filtered. Each sample was extracted in duplicate. The perfluoroalkyl chemicals were measured using a Ultra Performance Liquid Chromatograpy (UPLC), which is connected to a tandem quadrupole mass spectrometer (MS / MS). The analyzes were performed with a ACQUITY BEH C18 column. The mobile phase consisted of acetonitrile and water (both with 0.1% formic acid).
D’Hollander et al. (2010, unpublished data) analyzed 8 different perfluoralkyl substances (PFAAs) in eel muscle (i.e. PFOS, PFOA, PFNA, PFBS, PFHxS, PFBA, PFHxA and PFDA) on the same individuals as measured by Roosens et al. (2010) on BFRs. Between 3 and 10 eels were caught per location and muscle tissue was pooled. The extraction method to determine the PFAAs in the muscle tissue is primarily based on the procedure developed by Powley et al. (2005). Pooled muscle tissues (1g, ww) were homogenized with an Ultra-Turrax dispersing tool and placed within individual 50ml polypropylene (PP) tubes. The samples were then each spiked with 80 μl of internal standard mixture (MPFAS = 125 pg/μl concentration consisting of 13C6-PFOS, 13O2-PFHxS, 13C3-PFBA, 13C2-PFHxA, 13C-PFNA, 13C-PFNA, 13C-PFDA) as well as 10mL of acetonitrile (ACN) followed by proper mixing via vortex. The samples were placed in Ultrasonic bath for 10 minutes repeated three times, with vortex mixing ensuing between the first two 10 minute periods. Afterwards samples were left collectively on a shaker to be mixed at 230 rpm uninterrupted overnight (≥16 hours). Samples were then centrifuged at 2400 rpm for 10 minutes with subsequent transfer of the supernatant (no pellet) into new pre weighted 15 ml PP tubes. The acquired extract is evaporated to 0.5 mL using a Speedvac and once required volume is obtained the tubes are weighed with the sample weigh effectively calculated.

For sample purification, eppendorf tubes were firstly prepared each with 25mg Envi-Carb and 50 μl glacial acetic acid (100%) followed by the transfer of the evaporated extract. Rinsing of the empty 15mL tubes proceeded twice, through adding 250 μl ACN each time, vortex and transferring the flushed liquid into the already filled eppendorf tube. Properly closed and turned upside down few times, the eppendorf tubes are vortexed for 1 minute and centrifuge at 10 000rpm for 10 minutes. Pipette of cleaned supernatant into new reaction tubes preventing Envi-Carb into the mix. If however the extract is not colourless the cleanup process is repeated, otherwise extract is ready for filtration.

For filtration 195 μl HPLC grade water, 2mM ammonium acetate and 105 μl of extract (note to first shake it first well) was added to an empty eppendorf tube and vortexed thoroughly. The resultant 300 μl was filtered (0.2 μm, syringe filter, OASIS Medical Inc. USA) using a syringe into polypropylene injection vials after which the vial was capped with septa-less polyethylene screw caps (pre slit). Vial with extract is ready for UPLC analysis.

Determination of analytes was quantified using an AQUITY Ultra Performance Liquid Chromatography (UPLC) coupled to a tandem quadrupole mass spectrometer (AQUITY, TQD, Waters, USA) with electrospray interface operating in negative ion mode (ES-MS/MS). Separation was performed on an ACQUITY BEH C18 column (1.7μm particle size; 50 x 2.1 mm, Waters, USA). The injection rate proceeded with a volume of 10 μL with a flow rate of 450 μL/min. Mobile phases was conveyed by a gradient program consisting of ACN with 0.1 % formic acid and water with 0.1% formic acid. The following analytes and internal standards with mass transition were monitored and used for detection were [precursor ion (m/z) → product ion (m/z)]: 213 → 169 (PFBA), 313 → 269 (PFHxA), 217 → 172 (13C3-PFBA), 315 → 270 (13C2-PFHxA), 413 → 369 (PFOA), 463 → 419 (PFNA), 513 → 469 (PFDA), 563 → 519 & 269 (PFUdA), 663 → 619 (PFTrA), 713→ 669 (PFTrA), 299 → 99 (PFBS), 403 → 84 (13O2-PFHxS), 399 → 99 (PFHxS), 499 → 80,99 (PFOS), 599 → 80 (PFDA), 313→296 (PFHxA), 421→376 (13C2PFOA), 472→427 (13C2PFNA), 519→474 & 270 (13C2PFDA), 570→525 (13C2PFuA), 507→80 (13C2PFOS). For quantification an external calibration curve was used. Non-labelled standards of PFBS, PFHxS,
PFOS, PFDS, PFBA, PFHxA, PFOA, PFNA, PFDA, PFUdA, PFDoA, PFTra and PTFeA were used to construct ten-level calibration curves ($r^2>0.9$). The internal standards (MPFAS) were added to the samples prior to extraction and the same amount of these standards was added to each calibration point. The calibration curves were created preceding the analysis and was analysed in the same run. The results are corrected for matrix effects and recovery was based on the response area of the internal standards. Concentrations of sulfonates are based on the ion, not on the salt. Note at least two calibration curves were analysed for each run and also that pure ACN was injected for every ten injections to avoid carry over effects.

**Quality Assurance and Quality Control (QA/QC)**

Duplicates for each 1g of wet tissue were incorporated and each sample was injected twice. Moreover for each lot of approximately ten samples a routine procedural blank of HPLC grade water was spiked with the internal standard mixture and analyzed simultaneously. These measures were taken to assure correctness and quality of the routine method in case anomalies were to arise. The response of the internal standard in the samples provides information on possible matrix effects, suppression or enhancement of the signal. The analytes limit of quantification (LOQ) is based on peak with signal-to-noise ratio of 10:1 which was 100 pg/g for PFOA, 120 pg/g for PFOS and 65 pg/g for PFNA. Imperatively, to avoid contamination of the analytes samples never came into contact with polytetrafluorethylene (PTFE) products during the process starting with sample collecting up until analysis of the respective samples. Thus the usage of PP products is of utmost importance. Also before the use of solvents they were checked for potential contamination and the resultant consistent usage from the same bottle needs emphasis.

Samples with concentrations below LOQ were calculated as $f \times $LOQ with $f$ being the fraction of samples above LOQ.

**Wallonia**

**5.2 Interpretation, visualisation and assessment**

**Flanders**

Following text refers to Belpaire and Goemans 2008.

Analyses of a series of chemicals generates a database of quantitative data which have to be interpreted. There is a strong need for a normative framework with clear benchmarks to which the data should be compared. This framework can consist of various types of benchmarks. The WFD (CEC, 2006a) proposes ‘Environmental quality standards’ (EQS), limit concentrations (e.g. in hexachlorobenzene, hexachlorobutadiene and methylmercury) which cannot be exceeded in ‘prey’ tissue of biota. No Observed Effect Concentrations (NOEC) have been described for specific chemicals for certain organisms, including eel (see PAN Pesticides Database, 2007). For some compounds (e.g. Hg, Pb, Cd, dioxins, furans and dioxin-like PCBs (WHO-PCDD/F-PCB-TEQ), …) human health safety standards for fish have been set by the European Commission (CEC, 2001; 2006e) or by
additional national legislation (e.g. consumption limit for indicator-PCBs for fisheries products in Belgium; Belgisch Staatsblad, 2002), some with special values for eel. (Note the 2002 Belgian consumption limit (75 ng.g⁻¹ wet weight), has changed from 1/1/2012 on 300 ng.g⁻¹ wet weight following the European legislation, see below).

In Flanders, quality classes were developed based on quantitative distribution of the data (means per location) for PCBs, OCPs and heavy metals (Goemans et al., 2003). Reference values were fixed for each chemical. These reference values were defined as the 5 percentile value of the means of all sites. A common procedure was used to distinguish four quality classes as a measure of deviation from the reference value, and class boundary values were set. Class limits and reference values for each contaminant are listed in Table 13.3. Class boundary calculations were based on the distribution of the relationship between the recorded values and the reference value. Class 1 represents the 'not deviating' class (blue colour) with ‘unpolluted or low polluted’ sites. Sites with a slight to moderate pollution level are classified as class 2 ‘slightly deviating’ (green). The more polluted sites are assigned to class 3 ‘deviating’ (yellow) or 4 ‘strongly deviating’ (red).

In Figures 5 and 6, an example is given of a cartographic and graphic representation of the distribution of Sum PCBs in eel. Figure 6 indicates that, of a total of 351 sites, only 21% of the sites are relatively clean, while 57% of the sites are polluted and assigned to classes 3 or 4 (deviating or strongly deviating from the reference value). The map shows that most of the unpolluted or low polluted sites are located in the Yser basin, which is mainly characterized by agricultural land use.

In order to allow general status reports, more condensed reporting can be achieved by representing a combination of various chemicals e.g. within a region or as a function of time. This has been done in the annual state of the environment and the nature reports of Flanders. An example is given in Fig. 7 (Peeters et al., 2006). These representations are useful for showing temporal changes or spatial variation in environmental and biotic quality.

For Sum PCBs, possible management objectives and benchmarks have been proposed by Belpaire and Goemans (2004) and are illustrated in Fig. 8. Action and target threshold values are proposed at 460 and 183 ng/g wet weight respectively. The action threshold can be seen as a limit which never may be exceeded; sites above this limit should be sanitized. The target threshold is the objective to attain within a planned timeframe.
Table 1. Reference values and boundary values of the quality classes for a series of heavy metals, PCB congeners and organochlorine pesticides as defined in the EPMN. Values are expressed in ng.g\(^{-1}\) wet weight of muscle tissue, unless indicated as \(^{i}\) in ng.g\(^{-1}\) lipid weight or \(^{2}\) in µg.g\(^{-1}\) wet weight of muscle tissue. C: concentration (Belpaire and Goemans 2008).

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Reference value (RV)</th>
<th>Not deviating (\log \frac{C}{RV} &lt; 0.4)</th>
<th>Slightly deviating (0.4 \leq \log \frac{C}{RV} &lt; 0.8)</th>
<th>Deviating (0.8 \leq \log \frac{C}{RV} &lt; 1.2)</th>
<th>Strongly deviating (\log \frac{C}{RV} \geq 1.2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercury</td>
<td>40</td>
<td>&lt; 100</td>
<td>100 - &lt; 252</td>
<td>252 - &lt; 634</td>
<td>≥ 634</td>
</tr>
<tr>
<td>Cadmium</td>
<td>2</td>
<td>&lt; 5</td>
<td>5 - &lt; 12.6</td>
<td>12.6 - &lt; 31.7</td>
<td>≥ 31.7</td>
</tr>
<tr>
<td>Lead</td>
<td>10</td>
<td>&lt; 25</td>
<td>25 - &lt; 63</td>
<td>63 - &lt; 158</td>
<td>≥ 158</td>
</tr>
<tr>
<td>Copper(^2)</td>
<td>0.25</td>
<td>&lt; 0.6</td>
<td>0.6 - &lt; 1.6</td>
<td>1.6 - &lt; 4</td>
<td>≥ 4</td>
</tr>
<tr>
<td>Zinc(^3)</td>
<td>14</td>
<td>&lt; 35</td>
<td>35 - &lt; 88</td>
<td>88 - &lt; 222</td>
<td>≥ 222</td>
</tr>
<tr>
<td>Nickel</td>
<td>14</td>
<td>&lt; 35</td>
<td>35 - &lt; 88</td>
<td>88 - &lt; 222</td>
<td>≥ 222</td>
</tr>
<tr>
<td>Chrome</td>
<td>96</td>
<td>&lt; 241</td>
<td>241 - &lt; 606</td>
<td>606 - &lt; 1521</td>
<td>≥ 1521</td>
</tr>
<tr>
<td>Arsenic</td>
<td>41</td>
<td>&lt; 103</td>
<td>103 - &lt; 259</td>
<td>259 - &lt; 650</td>
<td>≥ 650</td>
</tr>
<tr>
<td>Selenium</td>
<td>205</td>
<td>&lt; 515</td>
<td>515 - &lt; 1293</td>
<td>1293 - &lt; 3249</td>
<td>≥ 3249</td>
</tr>
<tr>
<td>PCB 28</td>
<td>0.12</td>
<td>&lt; 0.3</td>
<td>0.3 - &lt; 0.8</td>
<td>0.8 - &lt; 1.9</td>
<td>≥ 1.9</td>
</tr>
<tr>
<td>PCB 31</td>
<td>0.1</td>
<td>&lt; 0.3</td>
<td>0.3 - &lt; 0.6</td>
<td>0.6 - &lt; 1.6</td>
<td>≥ 1.6</td>
</tr>
<tr>
<td>PCB 28+31</td>
<td>0.25</td>
<td>&lt; 0.6</td>
<td>0.6 - &lt; 1.6</td>
<td>1.6 - &lt; 4</td>
<td>≥ 4</td>
</tr>
<tr>
<td>PCB 52</td>
<td>1</td>
<td>&lt; 2.5</td>
<td>2.5 - &lt; 6.3</td>
<td>6.3 - &lt; 15.8</td>
<td>≥ 15.8</td>
</tr>
<tr>
<td>PCB 101</td>
<td>2.5</td>
<td>&lt; 6</td>
<td>6 - &lt; 16</td>
<td>16 - &lt; 40</td>
<td>≥ 40</td>
</tr>
<tr>
<td>PCB 105</td>
<td>1.2</td>
<td>&lt; 3</td>
<td>3 - &lt; 7.6</td>
<td>7.6 - &lt; 19</td>
<td>≥ 19</td>
</tr>
<tr>
<td>PCB 118</td>
<td>3.5</td>
<td>&lt; 9</td>
<td>9 - &lt; 22</td>
<td>22 - &lt; 55</td>
<td>≥ 55</td>
</tr>
<tr>
<td>PCB 138</td>
<td>7.7</td>
<td>&lt; 19</td>
<td>19 - &lt; 49</td>
<td>49 - &lt; 122</td>
<td>≥ 122</td>
</tr>
<tr>
<td>PCB 153</td>
<td>10</td>
<td>&lt; 25</td>
<td>25 - &lt; 63</td>
<td>63 - &lt; 158</td>
<td>≥ 158</td>
</tr>
<tr>
<td>PCB 156</td>
<td>0.6</td>
<td>&lt; 1.5</td>
<td>1.5 - &lt; 3.8</td>
<td>3.8 - &lt; 9.5</td>
<td>≥ 9.5</td>
</tr>
<tr>
<td>PCB 180</td>
<td>4.5</td>
<td>&lt; 11</td>
<td>11 - &lt; 28</td>
<td>28 - &lt; 71</td>
<td>≥ 71</td>
</tr>
<tr>
<td>Sum PCBs</td>
<td>29</td>
<td>&lt; 73</td>
<td>73 - &lt; 183</td>
<td>183 - &lt; 460</td>
<td>≥ 460</td>
</tr>
<tr>
<td>Sum PCBs(^1)</td>
<td>240</td>
<td>&lt; 603</td>
<td>603 - &lt; 1514</td>
<td>1514 - &lt; 3804</td>
<td>≥ 3804</td>
</tr>
<tr>
<td>α-HCH</td>
<td>0.05</td>
<td>&lt; 0.1</td>
<td>0.1 - &lt; 0.3</td>
<td>0.3 - &lt; 0.8</td>
<td>≥ 0.8</td>
</tr>
<tr>
<td>γ-HCH</td>
<td>1.3</td>
<td>&lt; 3.3</td>
<td>3.3 - &lt; 8.2</td>
<td>8.2 - &lt; 20.6</td>
<td>≥ 20.6</td>
</tr>
<tr>
<td>Pollutant</td>
<td>Reference Value</td>
<td>Lower Bound</td>
<td>Upper Bound</td>
<td>Value Range</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>----------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>Dieldrin</td>
<td>1.1</td>
<td>&lt; 2.8</td>
<td>2.8 - &lt; 6.9</td>
<td>6.9 - &lt; 17.4</td>
<td>≥ 17.4</td>
</tr>
<tr>
<td>HCB</td>
<td>0.5</td>
<td>&lt; 1.3</td>
<td>1.3 - &lt; 3.2</td>
<td>3.2 - &lt; 7.9</td>
<td>≥ 7.9</td>
</tr>
<tr>
<td>p,p'-DDD</td>
<td>2.5</td>
<td>&lt; 6</td>
<td>6 - &lt; 16</td>
<td>16 - &lt; 40</td>
<td>≥ 40</td>
</tr>
<tr>
<td>p,p'-DDT</td>
<td>0.005</td>
<td>&lt; 0.01</td>
<td>0.01 - &lt; 0.03</td>
<td>0.03 - &lt; 0.08</td>
<td>≥ 0.08</td>
</tr>
<tr>
<td>p,p'-DDE</td>
<td>13</td>
<td>&lt; 33</td>
<td>33 - &lt; 82</td>
<td>82 - &lt; 206</td>
<td>≥ 206</td>
</tr>
<tr>
<td>Sum DDTs</td>
<td>16</td>
<td>&lt; 40</td>
<td>40 - &lt; 101</td>
<td>101 - &lt; 254</td>
<td>≥ 254</td>
</tr>
</tbody>
</table>

Figure 5. Sampling sites of the Eel Pollutants Monitoring Network in Flanders and geographical distribution of quality classes in Flemish eels for Sum PCBs (N = 351 sites, 1994–2005). Reference value and quality class boundaries are given. Sum PCBs equals the sum of the 7 indicator congeners (CB 28, 52, 101, 118, 138, 153 and 180) (Belpaire and Goemans 2008).
Figure 6. Distribution of Sum PCB quality classes in Flemish eels (N = 351 sites, 1994-2005). See Table 1 for reference values and boundary values of the quality classes. At 57.2% of the sites, PCB levels in eels are deviating or strongly deviating from the reference value (Belpaire and Goemans 2008).

Figure 7. Status of heavy metals in eel in Flanders (after Peeters et al., 2006 in Flanders environmental report 2006). Data distribution is based on the means per site sampled between 1994 and 2005; the number of sites is indicated. See Table 1 for reference values and boundary values of the quality classes of the heavy metals (Belpaire and Goemans 2008).
Figure 8. Mean Sum PCB values in eel from 351 sites in Flanders (1994-2005): distribution between quality classes and comparison with threshold values for action or target values as proposed by Belpaire and Goemans (2004). Detection limit (2 ng.g\(^{-1}\) wet weight), reference value (29 ng.g\(^{-1}\) wet weight) and the Belgian consumption limit (75 ng.g\(^{-1}\) wet weight) are included in the figure (Belpaire and Goemans 2008). (Note in this figure the Belgian consumption limit is on 75 ng.g\(^{-1}\) wet weight, but this has changed from 1/1/2012 on 300 ng.g\(^{-1}\) wet weight following the European legislation).

Using quality classes allows for example for graphical presentation of the frequency of occurrence of a compound over a region, or can be used for illustrative cartographic representation of the distribution of a compound over a network of selected sites. The maps below show a selection of examples for several compounds in eel in Flanders (from Belpaire, 2008).
Examples of distribution maps for contaminants in eel.

For scientific purposes, Maes et al. (2005) and Maes et al. (2013) used an integrated index for metals. They calculated a relative bioaccumulation index by dividing (standardizing) the individual concentration of heavy metal $i$ ($C_i$) by the maximum observed concentration ($C_{i\text{max}}$) and averaging over all metals, to relate heavy metal bioaccumulation to condition and genetic variability. Thus, the individual mean (multi-metal) bioaccumulation index (IMBI) was defined as:

$$\text{IMBI} = \frac{\sum_{i=1}^{n} (C_i/C_{i\text{max}})}{n}$$

with $N$ the total number of metals, $C_i$ the individual concentration of heavy metal $i$, $C_{i\text{max}}$ the maximal observed concentration of heavy metal $i$ and $0 < \text{IMBI} < 1$.

In the framework of international assessments and advice ICES (2010, 2011, 2012) developed an Eel Quality Index for Contaminants. The text and examples below refer to the ICES (2012) report.

The Eel Quality Index for Contaminants (EQL\text{CONT}) initially developed in ICES (2010, 2011) was further developed including important contaminants such as Hg, Pb, dioxins and brominated flame retardants. Threshold values for classifying contamination levels of Hg and Pb were derived from (Belpaire and Goemans, 2007). However within the time
limit of this WG session it was not yet possible to deduce threshold values for dioxins and brominated flame retardants.

ICES (2012) adapted the threshold values of the quality classes as defined by WG Eel 2010 for the Sum ICES 7 PCBs to values for the Sum ICES 6 PCBs (SUM of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180). Indeed since the new Dioxin Regulation (Com Reg EU No 1259/2011), the EU set those new harmonised Sum 6 ICES PCB maximum levels, and this will be used in further assessments. PCB 118 contributed to between 8 and 23 % of the Sum ICES 7 PCBs (average 15%) (Belpaire et al., 2011). So the threshold values were adapted for Sum ICES 6 PCBs by lowering the Sum 7 PCBs by 15% to account for PCB 118.

Using this quality classes it is now possible to calculate EQICONT for individual yellow and silver staged eels. EQICONT is defined as the average value of the quality classes for the measured contaminants, resulting in a one, two, three or four star eel.

Using a dataset compiled by Belpaire et al. (2012, unpublished) of 1010 yellow or silver eels from > 313 sites over 7 countries which were collected from various sources including both research and surveillance and/or targetted environmental and food safety programmes. The rationale of the collection of the national data may differ considerably between countries or reports. Efforts to monitor the health status of eel in a certain country are not always designed to be representative for the whole country or area. Therefore the results presented per country cannot be regarded as an overview of the environmental quality for specific countries (ICES, 2012).

Specifications on the origin of the data used are presented in Table 2.
Table 2. Origin of the data presented in Fig. 10: Nsites Number of sites, Nan Number of eels analysed individually (I) or number of analysed aggregate samples of several eels (A), DFs dioxins and furans, DL PCBs Dioxin-like PCBs, NDL-PCBs non dioxin-like PCBs, FW freshwater, BW brackish water, SW sea water, RNW Random National Network, NWP Network with sites chosen because of known or presumed pollution (Belpaire et al., 2012, unpublished) (ICES, 2012).

<table>
<thead>
<tr>
<th>Country</th>
<th>Rationale</th>
<th>Nsites</th>
<th>Nan</th>
<th>Period</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norway</td>
<td>Fjords, sea or estuaries, BW and SW, RNW</td>
<td>29</td>
<td>144 (I)</td>
<td>2010</td>
<td>Duinker, Durif et al., in prep.</td>
</tr>
<tr>
<td>UK (Scotland)</td>
<td>Small and large rivers, FW, RNW</td>
<td>31</td>
<td>146 (I)</td>
<td>2004-2009</td>
<td>Macgregor et al., 2010</td>
</tr>
<tr>
<td>Ireland</td>
<td>Sampling for eel screening, Estuary and lakes</td>
<td>9</td>
<td>9</td>
<td>2005-2009</td>
<td>McHugh et al., 2010</td>
</tr>
<tr>
<td>Germany</td>
<td>Lake Constance and River Rhine, FW</td>
<td>4</td>
<td>20 (I)</td>
<td>2008</td>
<td>Wahl et al., 2010</td>
</tr>
<tr>
<td>Poland</td>
<td>Two BW lagoons</td>
<td>2</td>
<td>24 (I and A)</td>
<td>2000-2008</td>
<td>Szlinder-Richert et al., 2010</td>
</tr>
<tr>
<td>Belgium</td>
<td>Rivers, canals and lakes, RNW</td>
<td>48</td>
<td>48 (A)</td>
<td>2000-2007</td>
<td>Belpaire et al., 2011</td>
</tr>
<tr>
<td>Belgium</td>
<td>Rivers, canals and lakes, RNW</td>
<td>38</td>
<td>38 (A)</td>
<td>2000-2007</td>
<td>Geeraerts et al., 2011</td>
</tr>
<tr>
<td>Belgium</td>
<td>Small rivers in Wallonia, FW, NWP</td>
<td>36</td>
<td>36 (A)</td>
<td>2001-2004</td>
<td>Thomé et al., 2004</td>
</tr>
<tr>
<td>France</td>
<td>Large rivers, NWP</td>
<td>116</td>
<td>604</td>
<td>2008-2010</td>
<td>ANSES</td>
</tr>
</tbody>
</table>

Using these data results in following figure showing the frequency distribution of eels of different quality classes in these catchments. Due to time constraints and shortness of data available this assessment of EQI<sub>CONT</sub> is based only on the Sum ICES 6 PCBs. This figure clearly shows to what extend quality status of eels vary between catchments and countries. In central European countries eel quality status by contaminants is considerably lower compared to e.g. countries from more northerly latitudes. It must be noted that factors including site selection (industrial/rural), habitat type, number of sites, and differences between sampling program objectives may all contribute to the results as reported (ICES, 2012).
Figure 10. Demonstration of the Eel Quality Index of Contaminants (EQICOM) based on ICES 6 PCBs of 1010 yellow or silver eels from 313 sites over 7 countries from Belpaire et al. (2012, unpublished).
Warning: This graph is based on preliminary data; the intention is to show the technique, but specific outcomes will certainly change in future assessments (ICES, 2012).

Table 3. Boundary values of the quality classes for a series of selected contaminants for the calculation of EQICONT. EQICONT is defined as the average value of the quality classes for the measured contaminants, resulting in a one, two, three or four star eel (ICES, 2012).

<table>
<thead>
<tr>
<th>Class</th>
<th>Not impacted</th>
<th>Slightly impacted</th>
<th>Impacted</th>
<th>Strongly impacted</th>
</tr>
</thead>
<tbody>
<tr>
<td>EQI value</td>
<td>****</td>
<td>***</td>
<td>**</td>
<td>*</td>
</tr>
<tr>
<td>Sum 6 PCBs</td>
<td>&lt; 62</td>
<td>62 - 155</td>
<td>155 - 391</td>
<td>&gt; 391</td>
</tr>
<tr>
<td>Sum DDTs</td>
<td>&lt; 40</td>
<td>40 - 101</td>
<td>101 - 254</td>
<td>&gt; 254</td>
</tr>
<tr>
<td>Cd</td>
<td>&lt; 5</td>
<td>5 - 12.6</td>
<td>12.6 - 31.7</td>
<td>&gt; 31.7</td>
</tr>
<tr>
<td>Hg</td>
<td>&lt; 100</td>
<td>100 - 252</td>
<td>- 634</td>
<td>&gt; 634</td>
</tr>
<tr>
<td>Pb</td>
<td>&lt; 25</td>
<td>25 - 63</td>
<td>- 158</td>
<td>&gt; 158</td>
</tr>
<tr>
<td>BFRs</td>
<td>To be identified</td>
<td>To be identified</td>
<td>To be identified</td>
<td>To be identified</td>
</tr>
<tr>
<td>Dioxines</td>
<td>To be identified</td>
<td>To be identified</td>
<td>To be identified</td>
<td>To be identified</td>
</tr>
</tbody>
</table>

Following text is taken from ICES (2012)

As reported earlier (ICES 2011), dioxin and PCB levels are measured in several countries in order to compare the levels with the EU consumption limits, and to protect the health of eel consumers. These EU limits were recently adapted and a new EU Dioxin Regulation (Com Reg EU No 1259/2011) came into force on 1 January 2012, which set maximum levels of dioxins, dioxin-like PCBs and non dioxin-like PCBs in foodstuffs. This Regulation sets specific threshold values for wild caught European eel (Table 1) (ICES 2012).

The new maximum levels for dioxins and dioxin-like PCBs are an update of the existing 2006 levels. They are now based on the 2005 WHO-Toxic Equivalence Factors. Those lev-
els are at a similar level of stringency as former ones. Some countries had national PCB maximum levels, some not. Now the EU set those new harmonised Sum 6 ICES PCB maximum levels. For comparison maximum levels for most sea fish are set on 75 ng/g wet weight (ICES 2012).

Table 4. Maximum levels in the Dioxin regulation as regards to dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs, which applies since 1 January 2012 (Com Reg EU No 1259/2011).

<table>
<thead>
<tr>
<th>Foodstuffs</th>
<th>SUM OF DIOXINS (WHO-PCDD/F-TEQ)</th>
<th>SUM OF DIOXINS AND DIOXIN-LIKE PCBs (WHO-PCDD/F-PCB-TEQ)</th>
<th>SUM OF PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle meat of wild caught eel (Anguilla anguilla) and products thereof</td>
<td>3.5 pg/g wet weight</td>
<td>10.0 pg/g wet weight</td>
<td>300 ng/g wet weight</td>
</tr>
</tbody>
</table>

The primary objective of this Dioxin regulation is to protect human health. However, considering the high levels of repro-toxic compounds often reported in the eel, this Regulation may have implications on professional and/or recreational eel fisheries, and hence also on the stock of the European eel.

WG Eel (2011) reported that maximum consumption levels for PCDD/Fs and DL-PCBs were exceeded in a significant proportion of the cases.

Belpaire et al. (2012) collated information of Sum 6 PCB levels from 986 yellow or silver eels from 314 sites over 8 countries (see Fig. 11). These data were taken from various sources including both research and surveillance and/or targeted environmental and food safety programs. The rationale of the collection of the national data may differ considerably between countries or reports. Efforts to monitor the health status of eel in a certain country are not always designed to be representative for the whole country or area. This analysis showed that 38.2% of the eels were not compliant with the Dioxin Regulation with regard to non-dioxin-like PCBs (N= 986 analyses) (Range 0 – 13 223 ng/g ww, Mean = 503 ng/g ww, Std Dev. = 960 ng/g ww) (ICES 2012).
6. Diseases

6.1 Sampling and analysis of eels for parasitology (*Anguillicoloides* and others)

Flanders

Apart from two region-wide assessments in 1986 and 2000, there is currently no routine monitoring of eel diseases in Flanders, but see under 6.1.1 and 6.1.2.

Wallonia

6.1.1 *Anguillicoloides crassus*

Flanders

Substantial work on eel diseases has been carried out as scientific surveys and experimental work, mostly, but not exclusively, on *Anguillicoloides*. The University of Leuven was one of the pioneers in this research area and published several reports in the period 1980-2000. In Belgium the parasite was first observed in 1985 (Belpaire and De Charleroy, 1985). The papers described the initial spreading in Flanders and reported about the sta-
tus of the parasite over 3 monitoring assessments (1986, 1996 and 2000) (Belpaire et al., 1989a, b; Audenaert et al., 2003). Other work focused on life cycle, population biology, and larval biology (De Charleroy et al., 1989; De Charleroy et al., 1990; Thomas and Ollevier 1992, 1993; Thomas, 1993). Schabusss et al., (1997) assessed Anguillicoloides communities in eel from four cut off river meanders from Flemish rivers. Latest work was a contribution to the EELIAD program assessing the status of Anguillicoloides in silver eels from a few Flemish waters (work in progress).

The best available documents describing the methods to assess the status of Anguillicoloides in Flanders is probably the M&M section published in Audenaert et al., (2003). In this study, the sampling aim was the collection of 10 eels in the range of 35 to 45 cm length from each site, within the framework of a survey of bioaccumulation of contaminants in eels throughout Flanders. However, it was not always possible to sample the required number from the targeted length class. The swimbladders were stored in 4% formalin and transferred to 70% ethanol. The adult nematodes were sexed and counted macroscopically. Larvae were identified and counted using a binocular with transmitted light by flattening the swimbladder wall between 2 glass slides. Larvae surrounded by a more dense and opaque tissue, often partially resorbed, were identified as capsules, and the thickness as well as rupture of the swimbadder wall were registered. The swimbladders were divided into 3 categories according to the thickness of the wall. In the absence of parasitic stages, swimbladders with thin walls (<1 mm) were assumed to be uninfect ed, whereas those with thick walls (1 to 3 mm) were assumed to be infected in the presence of large amounts of connective tissue and tissue proliferation, and not infected when no histopathological reaction had occurred. Swimbladders with very thick walls (>3 mm), combined with brown fluids in the swimbladder lumen as a result of the disintegration of adult nematodes, pointed towards a former infection. Prevalence, mean intensity of infection, and abundance of Anguillicola crassus were estimated at all sites. Prevalence was calculated as the number of infected eels divided by the total number of eels investigated at each site, while mean prevalence was the average prevalence for each basin or for all sites combined. Mean infection intensity represents the total number of nematodes divided by the total number of infected eels. Parasite abundance was calculated as the total number of nematodes per eel, including uninfected specimens. For these calculations, the cut-off value was arbitrarily set at 7 eels per site to avoid loss of information. Data of former studies on these infection parameters in Flanders in 1986, when 424 eels were sampled from 19 sites (Belpaire et al., 1989), and 1990–1991, when 345 eels were collected from a single site over a period of 13 months (Thomas & Ollevier 1992b), were used for comparative purposes. The mean prevalence and mean infection intensity in 1996 and 1997 and in 2000 were also calculated for adults only to allow a comparison with the results of a survey in 1986 in which no larvae were counted.
Figure 12: Example of presentation of trend in infection of Anguillicoloides in eel in Flanders (taken from Audenaert et al., 2003). Anguilla anguilla infected by Anguillicola crassus. Relative proportions of parasite development stages in the swimbladder of European eels in Flanders. Inner circle: 1990 and 1991 data (Thomas & Ollevier 1992b); middle circle: 1996 and 1997 data; outer circle: 2000 data (Audenaert et al., 2003).

Wallonia

6.1.2 Other, like trematodes?

Flanders

Work on other parasites included the report and description of a new mite (Histioptoma anguillarum) parasitizing the gills of eels under aquaculture conditions (Fain and Belpaire, 1985). The parasite, which may not be obligate, has been reported from other eel culture facilities, but has never been reported in wild eels.

Schabuss et al., (1997) assessed the endoparasitic helminth communities in eel from four cut off river meanders from Flemish rivers. For this swimbladder, intestine tract, and eyes of the fish were examined using the conventional parasitological techniques (Mora‐vec et al., 1992). See Schabuss et al. (1997) for the treatment of specific parasites. The study gave data on six parasites: Proteocephalus macrocephalus and Botriocephalus claviceps (Cestoda), Anguillicola crassus and Camallanus lacustris (Nematoda), Acanthocephalus lucii and Acanthocephalus anguillae (Acanthocephala). Prevalence, intensity, mean intensity and abundance were calculated using the definitions of Margolis et al. (1982). Also the Simpson Index and the Shannon Diversity Index were calculated (following Begon et al., 1986 and Kennedy, 1995).

Wallonia
6.2 Sampling and analysis of viral and bacterial diseases

6.2.1 Viral diseases

Flanders

Flanders has no experience with the assessment/monitoring/diagnosis of viral diseases in eel.

Wallonia

As mentioned before, the detection of the presence of Anguillid herpesvirus (HVA) is performed in each eel captured in the Lesse basin. The detection is made in serum samples, with a minimum volume needed of 50 μL and following the protocols of Rijsewijk et al. (2005). Briefly, DNA is extracted using DNeasy Blood and Tissue Kits (Qiagen), the concentration is evaluated with a Nanodrop and DNA is amplified by Polymerase Chain Reaction (PCR). The use of this technique allows the detection of the virus in blood but not the extent of the contamination.

6.2.2 Bacterial diseases

Flanders

Some bacterial work on eel was carried out during the 1980s, mostly on aquaculture or restocking populations. These assessments included bacterial culture, diagnosis, and screening for antibiotic resistance (in the context of treatment of diseases in eel culture conditions. Most disease agents were related to Aeromonas, Pseudomonas or Vibrio species. Reports were ad hoc internal reports (see e.g. Belpaire and De Charleroy 1985). Two technical papers on bacterial strains were published (Noterdaeme et al., 1991; Noterdaeme et al., 1996). Since then, bacterial work on eel (and by extension) on fish) in Flanders has ceased.

Wallonia
7. Perspectives of using biomarkers of effects to assess eel health

Flanders

Genetic biomarkers

Several toxicogenomic studies have been carried to assess the relationship between pollution and genetic biomarkers in Flemish eels (Maes et al., 2005; 2013; Pujolar et al., 2012; 2013). The main findings of these studies are presented below. We refer to the material and method section of these papers for details about sampling and analytical procedures.

Maes et al., (2005) studied the relationship between heavy metal bioaccumulation, fitness (condition) and genetic variability in the European eel. The muscle tissues of 78 sub-adult eels, originating from three Belgian river basins (Scheldt, Meuse and Yser), were examined for nine heavy metal pollutants (Hg, Cd, Pb, Cu, Zn, Ni, Cr, As and Se), while in total 123 individuals were genotyped at 12 allozyme and 8 microsatellite loci. A significant negative correlation between heavy metal pollution load and condition was observed, suggesting an impact of pollution on the health of sub-adult eels. In general, a reduced genetic variability in strongly polluted eels was observed, as well as a negative correlation between level of bioaccumulation and allozymatic multi-locus heterozygosity (MLH). Microsatellite genetic variability did not show any pollution related differences, suggesting a differential response at metabolic enzymes and possibly direct overdominance of heterozygous individuals.

Maes et al. (2013) investigated the relationship between muscular bioaccumulation levels of metals (Hg, Cd, Pb, Cu, Zn, Ni, Cr, As and Se), PCBs and organochlorine pesticides (DDTs), the health status (condition factor and lipid reserves) and the associated transcriptional response in liver and gill tissues for genes involved in metal detoxification (metallothionein, MT) and oxidative metabolism (cytochrome P4501A, CYP1A) of xenobiotic compounds. In total 84 resident eels originating from three Belgian river basins (Scheldt, Meuse and Yzer) were analysed along with five unpolluted aquaculture samples as control group. There was a large spatial variation in individual contaminant intensity and profile, while tissue pollution levels were strongly and negatively associated with condition indices, suggesting an important impact of pollution on the health of sub-adult resident eels. Gene transcription patterns revealed a complex response mechanism to a cocktail of pollutants, with a high variation at low pollution levels, but strongly down-regulated hepatic and gill gene transcription in highly polluted eels. Resident eels clearly experience a high pollution burden and seem to show a dysfunctional gene transcription regulation of detoxification genes at higher pollutant levels, correlated with low energy reserves and condition. To fully understand the evolutionary implications of pollutants on eel reproductive fitness, analyses of mature migrating eels and the characterization of their transcriptome-wide gene transcription response would be appropriate to unveil the complex responses associated with multiple interacting stressors and the long-term consequences at the entire species level. In the meanwhile, jointly monitoring environmental and tissue pollution levels at a European scale should be initiated, while preserving high quality habitats to increase the recovery chance of European eel in the future.
Pujolar et al. (2012) explored the transcriptomic dynamics between individuals from high (river Tiber, Italy) and low pollution (lake Bolsena, Italy) environments, which were measured for 36 PCBs, several organochlorine pesticides and brominated flame retardants and nine metals. To this end, the authors first (i) updated the European eel transcriptome using deep sequencing data with a total of 640,040 reads assembled into 44,896 contigs (Eeelbase release 2.0), and (ii) developed a transcriptomic platform for global gene expression profiling in the critically endangered European eel of about 15,000 annotated contigs, which was applied to detect differentially expressed genes between polluted sites. Several detoxification genes related to metabolism of pollutants were upregulated in the highly polluted site, including genes that take part in phase I of the xenobiotic metabolism (CYP3A), phase II (glutathione-S-transferase) and oxidative stress (glutathione peroxidase). In addition, key genes in the mitochondrial respiratory chain and oxidative phosphorylation were down-regulated at the Tiber site relative to the Bolsena site. Together with the induced high expression of detoxification genes, the suggested lowered expression of genes supposedly involved in metabolism suggests that pollution may also be associated with decreased respiratory and energy production.

The objective of the study of Pujolar et al. (2013) was to better understand the transcriptional response of European eels chronically exposed to pollutants in their natural environment. A total of 42 pre-migrating (silver) female eels from lowly, highly and extremely polluted environments in Belgium and, for comparative purposes, a lowly polluted habitat in Italy were measured for polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and brominated flame retardants (BFRs). Multipollutant level of bioaccumulation was linked to their genome-wide gene transcription using an eel-specific array of 14,913 annotated cDNAs. Shared responses to pollutant exposure were observed when comparing the highly polluted site in Belgium with the relatively clean sites in Belgium and Italy. First, an altered pattern of transcription of genes was associated with detoxification, with a novel European eel CYP3A gene and glutathione S-transferase transcriptionally up-regulated. Second, an altered pattern of transcription of genes associated with the oxidative phosphorylation pathway, with the following genes involved in the generation of ATP being transcriptionally down-regulated in individuals from the highly polluted site: NADH dehydrogenase, succinate dehydrogenase, ubiquinol-cytochrome c reductase, cytochrome c oxidase and ATP synthase. Although the authors did not measure metabolism directly, seeing that the transcription level of many genes encoding enzymes involved in the mitochondrial respiratory chain and oxidative phosphorylation were down-regulated in the highly polluted site suggests that pollutants may have a significant effect on energy metabolism in these fish.

**Metallothioneins**

Van Campenhout et al. (2008) studied the effect of metal exposure on the accumulation and cytosolic speciation of metals in livers of European eel by measuring metallothioneins (MT) induction. This research was carried out in four sampling sites in Flanders showing different degrees of heavy metal contamination (Cd, Cu, Ni, Pb and Zn). It was concluded that the metals, rather than other stress factors, are the major factor determining MT induction.
Vitellogenin

As part of a large-scale monitoring program of bioaccumulating contaminants in the yellow phased European eel (*Anguilla anguilla*) in Flanders (Belgium), Versonen et al. (2004) investigated potential effects of xenoestrogens in these fish. The study assessed plasma vitellogenin (VTG) content, measured in 142 eels sampled at 20 different locations, in relation to the internal pollution levels. To validate the blood VTG assays, a small number of eels (n = 8) was exposed to 10 µg ethinylestradiol / L (EE2) for 9 days. In this experiment, VTG was detected as a protein with a molecular weight of 214 kDa and confirmed by Western blotting. Compared to the solvent controls, significantly higher concentrations of VTG were measured in EE2 exposed eel. However, the VTG content was relatively low compared to other fish species exposed to high concentrations of estrogens. The plasma VTG content of eels from the field study was very low, despite a very high internal load of endocrine disrupters. These results, together with previously published studies, suggest that immature yellow European eel might not be the best sentinel species to study the effects of estrogenic compounds on VTG levels of wild fish populations.

Wallonia

Because the New European Chemicals Legislation (REACH) is asking for alternatives to animal testing and reduction of animals sacrificed in ecotoxicology and in accordance with conservation biology considerations, the development of an appropriate and reproducible methodology to obtain a post-nuclear fraction of isolated European eel peripheral blood mononuclear cells (PBMC) have been developed during the PhD thesis of Roland Kathleen (Pierrard et al., 2012). These protocols have been used to evaluate the toxicity of xenobiotics using a subproteomic approach. In a first study, we have studied the in vitro toxicity of perfluorooctane sulfonate (PFOS) in eel PBMC before performing a proteomic analysis using 2D-DIGE technique (Roland et al., 2013). Besides providing clues on the cellular pathways mainly affected by PFOS, results allowed the identification of proteins rarely found in other ecotoxicological proteomic studies. These proteins could constitute potential biomarkers of exposure to PFOS in fish. In order to determine the specificity of the proteomic pattern observed after in vitro PFOS contaminations, we have completed the set of data with in vitro exposures to two other xenobiotics, dichlorodiphenyltrichloroethane (DDT) and cadmium, using exactly the same methodologies as for the PFOS experiments (unpublished data). Lastly, the in vivo toxicological effects of PFOS on the whole animal have been investigated. For that purpose, the protein expression profiles in PBMC of yellow eels exposed in vivo to environmental PFOS concentrations, as well as after in situ samplings of fish from Belgian rivers displaying different levels of PFOS contamination, have been studied (Roland et al., 2014). The comparison of the in vitro, in vivo and in situ results conducted to the identification of two proteins that might be potentially used as biomarkers of PFOS exposure in fish species. Moreover, the recurrence of the main functional classes of proteins affected by PFOS exposure lead us to think that in vitro exposure of cells to pollutants might be useful in the prediction of the in vivo toxicity of these compounds.

Most often, proteomics is still largely tributary of the 2-DE technique. Even if 2-DE is a powerful technique, it has several drawbacks to the discovery of more interesting proteins. The relatively low- (< 10 kDa) and high-molecular weight (> 200 kDa), hydropho-
bic, extreme acidic (pH<4) or alkaline proteins (pH>10) are not present or underrepresented in gels. This explains trends that can be observed from the literature, and can be summarized rather easily as “the lower the complexity, the better the performance” (Rabilloud et al., 2010). One of the best solutions to counteract the disadvantages of proteomic techniques and to increase the sensitivity and the opportunity to discover potential specific protein biomarkers is to reduce sample complexity for instance by fractionation of the proteome (Patterson, 2004). That’s why we decided to work on the post-nuclear fraction of cells. Although the 2DE technology has many advantages such as robustness, the ability to run gels in parallel, and the unique ability to analyze the all proteome at high resolution, it has its intrinsic limitations (Rabilloud et al., 2010). The comparison of proteomic results remains quite difficult as technical variations were induced between in vitro, in vivo and in situ experiments. Differences in gel polymerisation or Cydye labelling could limit a good comparison of gels among the experiments. Even if we have chosen to work on the post-nuclear fraction of PBMC, multiple spots (spots containing several protein identifications) were still observed and had to be discarded from the analysis, leading to an important loss of information. These multiple spots may also be the result of mass spectrometry techniques which are more and more powerful, enhancing the ability to detect peptides even at very low concentrations. Moreover, the main remaining factor for variability lies in the biological sample itself. In eukaryotic organisms, the genetic heterogeneity and the poor experimental control of the physiological states are the major sources of variability (Rabilloud et al., 2010). Considering these elements, it was unexpected to discover proteins in common between in vitro, in vivo and in situ experiments.

The interest of protein biomarkers in ecological risk assessment is recognized because such measures provide early warning indicators of ecologically relevant effects on biological systems. In biomarker research, external factors as well as those inherent to the individual itself should not affect the response of the protein biomarker candidates. The response of a biomarker to a pollutant or a class of pollutants has to be the most specific as possible. As for biochemical biomarkers, it is becoming increasingly clear that the predictive utility of individual biomarker proteins may be limited. To develop more specific and sensitive biomarkers, the use of a set of proteins is required and the identification of protein expression signatures (PES) or exposure “fingerprints” may reflect the exposure to specific classes of pollutants (Feron & Groten, 2002; Hook, 2010; Sanchez et al., 2011) in a robust and unbiased way (Bradley et al., 2002). A set of proteins could potentially achieve higher accuracy and specificity than any individual proteins (Sanchez et al., 2011) and not fluctuate as much with environmental factors (Hook, 2010). Validation in the reproducibility, specificity and sensitivity of a proteomic signature is required before its application in the field (Nilsen et al., 2011). The identification of the proteins of interest has to be confirmed by Western-blot analysis. Moreover, as said in Forbes et al., (2006), biomarker responses have a better chance of being interpreted correctly in site-specific assessments with knowledge regarding the species exposed/impacted and the toxicant that are present. Field populations are generally exposed to complex mixtures of pollutants that may lead to a biomarker response different from the one predicted from the single-chemical exposure; so to be specific, the biomarker response has to indicate the exposure to the tested compound (or class of compounds) even in the presence or absence of the other pollutants present in the mixture (Forbes et al., 2006). In consequence, the selected biomarkers have to be validated in mixtures experiments. The xenobiotics
are not the only stressors in field studies. The salinity, which may differ between our sampling sites, as well as the water temperature, the pH or other abiotic factors, may be responsible of the proteomic changes observed in our in situ study. Biomarkers have also to be validated on fish showing different sizes, ages, physiological or reproductive status. Once the biomarker PES selected and validated, the hopeful future applications of this specific signature should be the development of an ELISA test or a protein array that would be easily and rapidly performed in organisms sampled in the field.

8. International needs (making your results available, and international database)

Flanders

In order to make the results of eel quality assessments in Flanders available to the international community, results are published through publications in scientific journals, and through institutional reports (in Dutch). We always strive to include the raw data at annexed tables, also when published in scientific papers, when feasible, we upload the data as Supplementary Information.

Data are archived on the INBO database, and some data are available via VIS, a web-based Fish database including Eel Contaminant Data, as there was a strong need to compile all contaminant data and make them available for internal and external use. ‘VIS’ is a database compiling data on freshwater fish in Flanders, currently comprising raw data of fish stock assessments, but also derived data like fish-based ecological integrity evaluations and fish contamination data. This database is available on the net since 2007, and is accessible to all external users, like policy makers, river basin managers, fish stock managers, water quality managers, inspection services, recreational fishermen.

The database allows queries to report on contamination status for a certain species in a catchment, river or site. Data can be downloaded and trends can be visualised by figures, an example is presented in Fig. 13. Since February 2008, a site-based consumption advice based on the most recent results, has been added in the reports.

The contaminant database has been the initial model for the European Eel Quality Database.

Further work on VIS will include implementation of more flexible tools for data storage, input and treatment as well as customer oriented communication (including an English version)(From Belpaire, 2008).
Figure 13. Example of a query for contamination data in eel from a site on River Maas (VIS, 2008). For more information about units and abbreviations we refer to the VIS website.

Moreover, data are made available through the ICES WG EEL group and the yearly eel country reports, and through reporting to the ICES WGEEL Eel Quality Database.

A recent preliminary feasibility study (De Jonge et al., 2014) has assessed the possibility for reporting eel contaminant data in the framework of the WFD requirements for reporting several contaminants in biota.

Some data have been reported to EU in the eel management plans in the framework of the Eel Regulation.

Eel contaminant data are not reported in the framework of the CFP.

Wallonia

9. Other issues, remarks

Flanders

Wallonia

10. References


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Report on the methods and assessment of eel quality (contaminants and diseases) in:

FRANCE

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Introduction

This country report has been drafted in preparation of the ICES Workshop of a Planning Group on the Monitoring of Eel Quality: “Development of standardized and harmonized protocols for the estimation of eel quality.” (WKPGMEQ), held at INBO Brussels from 20 to 22 January 2015.

In France many programmes are realized to collect data on eel quality (viruses, diseases, contaminants…). These programmes such as glass eel restocking programme, PCB plan… involve many actors. The text below may not include all information about each programme but, for more details, the attached table will allow you to contact the referral persons.

1) Glass eels restocking programme

In France, since 2010, the Ministry in charge of Ecology publishes each year a call for projects entitled "restocking of Eels in France". This call for proposals aims to clarify the
funding arrangements but also the administrative and technical elements for restocking activities conducted by Eel Management Units (EMU).

The french eel restocking association (ARA France) is particularly involved in the financing and organization of the restocking actions and six EMU are now involved in restoring eel stocks (Artois-Picardie, Seine-Normandy, Brittany, Loire Vendée coastal Sèvre-Niortaise, Garonne-Dordogne-Charente-Seudre-Leyre and Adour coastal currents).

The main tasks of ARA France are:
• to provide technical support to Eel restocking project developers in France, and, where appropriate, ensure project porting;
• to coordinate restocking action in France and ensure that the specifications established in the French restocking program are followed;
• to promote eel restocking actions in France and promote the French sector of Eel production at national and community level;
• to contribute to the annual synthesis of stocking operations at the community level.

In addition to recommendations of the call for projects, a good practice guide for the glass eels industry exists (http://www.repeuplementanguille.fr/IMG/pdf/gbpcivelle_vf_cle828e56.pdf). It gives important details about the fishing procedures, sanitary controls and restocking actions.

The global procedure of restocking is as follow:
- selection of areas suitable for restocking (with a low rate of mortality factors);
- checking of the sanitary quality of glass eels (absence of lesions, parasites, viruses, ...);
- marking of otoliths to follow the glass eels during many years;
- packaging and transportation;
- acclimatization and releasing;
- scientific monitoring (at 6 months, 1 year and 3 years after the spill) to evaluate the effectiveness of actions.

The National Museum of Natural History (MNHN) and the National Office for Water and Aquatic Environments (ONEMA) contributed to the definition of the sampling conditions, storage, transportation, released and scientific monitoring of this French restocking program.

In 2014, about 18 million glass eels (about 5700 kg) were released in areas favorable to the development of the species in France (see figure 1).
2. Framework national cases and national guidelines (on contaminants and health)

2.1 Contaminants

Describe if there is/was/shall be a routine monitoring network measuring contaminants in eel in your country. Mention the objectives and the framework (human health, WFD, monitoring environmental quality, eel regulation, …). Who is involved, who is reporting, and to who, who is paying? How are the data accessible to international community. Specify period and regional scale. Include references.

PCB national program: http://www.pollutions.eaufrance.fr/pcb/. Monitor (1) PCB, PCDD/F and Hg since 2008, (2) HCB, HCBD since 2009.

Describe if there are/were/shall be other initiatives to measuring contaminants in eel in your country. Mention the objectives and the framework (human health, WFD, monitoring environmental quality, eel regulation, research, …). Who is involved, who is reporting, and to who, who is paying? How are the data accessible to international community. Specify period and regional scale. Include references.

Are there documents describing national guidelines to assess contamination in eel (please give short description + reference)?

In the context of the restocking program, no analysis of contaminants is done. Contaminants (PCBs, pesticides, metals, …) are considered only for the qualification of restocking sites.
Otherwise the national health consumer authority (DGAL) is monitoring contaminants in a multiannual sampling plan (PCB, pesticides, metal and so on…) performed at retailers level.

Concerning the PCB issue the national office for aquatic environment (ONEMA) has been collected numerous data monitoring eel and environmental contamination for about 15 years.

Those data are theoretically available for public consultation one year after the analysis has been completed.

### 2.2 Diseases

Describe if there is/was/shall be a routine monitoring network measuring disease agents in eel in your country. Mention the objectives and the framework (human health, eel regulation, …). Who is involved, who is reporting, and to who, who is paying? How are the data accessible to international community? Specify period and regional scale. Include references.

Describe if there are/were/shall be other initiatives to measuring disease agents in eel in your country. Mention the objectives and the framework (human health, eel regulation, research, …). Who is involved, who is reporting, and to who, who is paying? How are the data accessible to international community? Specify period and regional scale. Include references.

Are there documents describing national guidelines to assess contamination in eel (please give short description + reference)?

The call for projects “restocking of eels in France” give details concerning the assessment of the quality of glass eels used for restoring actions (http://www.repeuplementanguille.fr/IMG/pdf/appel_a_projets_repeuplement_2014-2015_cle8341d5.pdf). Quality controls are done i) after the fishing to evaluate the impact of this operation on glass eels and ii) during the storage prior to the release to check the sanitary status of the animals.

To evaluate the impact of the fishing, a labeling with indigo carmine (0.5 g.l⁻¹) is realized on a sample of 50 eels. This coloration allows to detect lesion areas using a binocular microscope. The number of glass eels impacted must be count to establish a proportion of impacted fish.

To define the sanitary status, contamination with Anguillicoloides crassus, Pseudodactylogyrus sp., Ichthyophthirius multifiliis is analyzed on 6 batches of 10 glass eels for each pouring operation. Detection of Eel European Virus X (EVEX) must be done on 1 batch of 10 glass eels from each estuary of fishing. For estuary where the yearly analysis was positive, another analysis on 6 batches of 10 glass eels must be done before each releasing operation. If the release is carried out in areas free of VHSV and IHNV, a virological control must be done on 6 batches of 10 glass eels.

**Two recommendations could be done concerning EVEX:**
- the necessity to realize a large epidemiological study about the prevalence of EVEX in wild Eels population;
- the necessity to better define the impact (pathogenicity ?) of EVEX on wild Eels.
3. General issues on eel sampling for contaminants and/or diseases in your country. Treatment of the sampled eels on site and in the laboratory.

3.1 Ethics and legislation

Legislation: General issues on eel sampling including EU and national legislation (sampling is experimental work with animals – eels are protected species – etc.); Ethical issues. Required permits.

How do you present/describe sampling sites?

Fishing activity/ anthropic pressures/ Surface/river basin surface/ salinity: annual mean (min-max)/ water temperature: annual mean (min-max) /depth: annual mean (min-max):

Which data are recorded characterizing the sampled sites? GIS data, season, air temp, water temp, water quality (pH, salt content etc....)

GIS data, sampling period, water temperature, water quality (when possible: salinity, pH, O2, contaminants), when possible: sediment quality (metals, PCB, PBDE)

If geo references are collected, which system is used?

WGS84

Glass eels must be collected only in the downstream parts of estuaries (salinity between 15 and 18 per thousand) and at a pigmentary stage lower than VIA2.

3.2 Sampling procedures

How many eels are sampled per site? How to sample? Which fisheries techniques? Advantages and disadvantages.

When to sample? Impact on stages.

Fishing procedures must limit the trauma of individuals that will be used for restocking. Are advocated a small speed of the ship (between 3 and 4 knots) and a short duration of the fishing (10 minutes).

To evaluate the impact of the fishing, a labeling with indigo carmine (0.5 g l⁻¹) must be realized on a sample of 50 eels. This coloration allows to detect lesion areas using under a binocular microscope. The number of glass eels impacted must be count to establish a proportion.

<table>
<thead>
<tr>
<th>How many eels are sampled per site?</th>
<th>It depends of the study.</th>
</tr>
</thead>
<tbody>
<tr>
<td>For silver eels release protocol: 60 for biometry and lipids content measures (kept alive, using eugenol for anesthesia) and 15 (half females and half males, killed) for contaminants and diseases analysis.</td>
<td></td>
</tr>
</tbody>
</table>
How to sample? | Randomly from the capture
---|---
Which fisheries techniques? | We usually work in collaboration with professional fishermen. They use traditional passive nets: capeciades, an assemblage of 3 fyke nets.
Advantages and disadvantages. | Eels size captured depend of the mesh size used by the fishermen.
When to sample? Impact on stages. | Depending of the targeted stage. In the context of the eel management plan, would be interesting to focus on silver eels to document their health status at their departure time for the reproduction site. Silver eels are captured during the escapement season (October to January in the French Mediterranean lagoons).

### 3.3 Stage, gender, morphometrics

Do you distinguish eel species? Which protocols do you use?

No, we consider only one species, the European eel.

Only European glass eels from french estuaries are used for french restocking actions (pigmentary stage lower than VIA2).

Do you distinguish eel stage? Which method to distinguish yellow from silver eels?

Yes, with the presence of black dots on the lateral line, dark pectoral fins, contrasted black back and white belly, eyes width. We also use Pankhurst ocular index (1982) and Durif index when possible (needs measures of the pectoral fins and eyes)

The pigmentary stage is determined using the classification of Elie et al. (1982) on a sample of 50 individuals.

Do you distinguish gender? How?

No.

Yes when possible.

For living yellow eels : > 45cm = Female, the other are considered as indeterminate

For living silver eels : > 45cm = Female and ≤45 cm= Male

For dead eels we use macroscopic examination of the gonads under a stereomicroscope. The determination is possible for specimen > 30-35 cm.

How do you measure and express morphometrics (weight, length, accuracy issues, units)? Living animals? Or after fixation, freezing?

The individual weights and sizes are measured on a sample of 50 glass eels.
The pigmentary stage is determined using the classification of Elie et al. (1982) on a sample of 50 individuals.

Weight in g, length in cm. Better on living animals but if not possible, we have a good correlation between frozen and fresh wet.

3.4 Treatment:
Live storage. How, how long, which water system?
We avoid storage (may change lipid contents, K). If needed, short period, in oxygenated tank, with the corresponding salinity/water of their natural habitat to avoid stress.

How do you euthanize/anaesthetize eels (= animal experiment)? Ethics considerations.

Euthanasia: we keep the eel a long time in anesthetic + freezing. Or decapitation after anesthesia.

Anaesthesia: we use eugenol or iso-eugenol 30% (30 mL eugenol + 100 mL ethanol 95°) at the concentration 3ml/10L salt/brackish water.

Hygiene measures, what measures do you use not to cross contaminate samples from various sites, protective clothing (gloves, etc.), human health considerations.

We sample different sites at different time or we keep them in different containers.

Labelling issues/techniques.
We store eels individually in plastic bags with the label in the bag, in a freezer. The label must record the site/date/individual code. The label must be written on a special paper resistant to water.

When chemical contaminants are measured we avoid trash bags as they may contain contaminants.

Pectoral fin clips in alcool 70° for about 30 silver eels over 8 sites (same year)

How and where to store samples? Prior to, and after analysis.

Do you have a tissue database and a tissue bank. Please describe.

On ship: storage in a 100 to 500-liter tank with oxygenation and continuous or regular change of water.

In hatchery: maintaining individuals in tanks for at least 6 days. Ideally, glass eels can be maintained between 1 to 3 weeks in water at less than 10 °C without food as long as they are not pigmented. Storage do not exceed 2 months to reduce the sexual determination process and infections by biological agents (phenomenon facilitated by high densities). To limit contamination, soft water must be used in closed circuit.

Scientific programme: When capturing restocked eels 6 months, one year and 3 years after the release, all eels are anesthetized with eugenol to measure it. A sample of 50 eels in the class size searched are frozen to take otoliths.
4. General issues on eel condition (fitness, lipid levels):

Do you incorporate eel condition parameters in your assessments? Which?

Yes, condition factors and lipid levels (fatmeter or/and chemical analysis)

4.1 Condition

How is eel condition (in terms of length weight relation) in your country usually measured, expressed (condition index, condition factor, ...), what equation is used?

Assessment of glass eels quality with biometrics: In each restocking actions, the restocked population are measured and weighed (a sample of 50 glass eels by project). The protocol does not set rules for calculating the factor or condition index.

Fulton condition factor: \( K=100 \frac{W}{LT^3} \) (with the weight W in g and the total length LT in cm).

Le cren condition factor: \( Kn = \frac{W}{We} \) (\( We=aL^b \); a and b are determined from the whole sample)

4.2 HSI

Hepatosomatic index HSI, do you use it, perspectives and limitations?

Yes in some studies but should be done on eviscerated weight

4.3 GSI

Gonadosomatic index GSI, do you use it, perspectives and limitations?

Yes when possible but should be done on eviscerated weight. Difficult for males as the gonads are very thin. Difficult when we work with frozen animals as some part of the eels containing gonads is used for contaminants analysis.

4.4 Lipid levels

Is analysis of lipid levels included in your assessments?

Do you distinguish between eel stages?

Yes, when possible and we distinguish between eel stages.

How are your lipid levels data expressed?

In % fat in fresh muscle.

What part of the body?

When chemical analysis: we use always the same part of muscle (the skin is removed) (Belpaire/eeliad protocol), the analysis is done together with PoPs analysis.
When we use the fatmeter we follow the fatmeter protocol (Distell ©)

Methods? QA/QC?

**Gas chromatography (Dr. Adrian Covaci  and Dr. Malarvannan Govindan from Toxicological Center / University of Antwerp/ Universiteitsplein 1/ 2610 Wilrijk / Belgium )**

Experiences with fat meter device?

Yes but not very good correlation between chemical analysis results and fatmeter results but may come from the unadapted large sensor for the males, or the fact that the chemical analysis is made on a small piece of muscle, whereas the fatmeter is measuring lipids on the whole body

4.5 Other condition related issues

Include/discuss any other issue you consider relevant here

5. Contaminants :

5.1 Sampling and analysis

*Per contaminant group: PCBs, pesticides, BFRs, Dioxines, Metals, PAHs, others*

*Describe procedures in your country.*

We did not studied dioxines

<table>
<thead>
<tr>
<th>PCBs</th>
<th>Pesticides</th>
<th>BFRs</th>
<th>Metals</th>
<th>PAHs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Which PCB to group as e.g. Sum PCBs (ICES 7, ICES 6)</td>
<td>EU 6 ICES (there is a high correlation bwt 7/6 PCBs)</td>
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</tr>
<tr>
<td>Do you target some specific congeners as of special concern?</td>
<td></td>
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</tbody>
</table>

Subsampling in the lab. What part of the body for which analysis? Which tissues?

<p>| Muscle only – cause liver too small (see figure below) | Muscle and liver (see figure below) | Muscle |
| Which compounds? | | |
| 30/ 209 compounds , including EU DDTs + HCB + HCHs; trans-chlordane, cis-chlordane, oxychlordane, cis- | 7/ 209 PBDEs : 28,47,99 ; 100 ;153,1 | Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se | 11 PAH |</p>
<table>
<thead>
<tr>
<th>6PCBs</th>
<th>nonachlore, trans-nonachlore</th>
<th>54,185</th>
<th>V, Zn</th>
</tr>
</thead>
</table>

**How to measure, analytical procedures? QC-QA?**

| How to report? Body weight basis, lipid weight basis, dry weight basis, body burden? |
| In order to compare with other studies, results should be indicated in dry weight, wet weight (for human health limits) and/or %humidity, lipid weight. |

**What units to use?**

<table>
<thead>
<tr>
<th>ng/g wet weight</th>
<th>ng/g wet weight</th>
<th>ng/g wet weight</th>
<th>μg/g wet weight</th>
<th>ng/g wet weight</th>
</tr>
</thead>
</table>

### 5.1.1 PCBs

Subsampling in the lab. What part of the body for which analysis? Which tissues?

How to measure, analytical procedures, where to measure, what to record (e.g. bile volume, colour, proteins)

QC-QA?

Which PCB to group as e.g. Sum PCBs (ICES 7, ICES 6)

Do you target some specific congeners as of special concern?

How to report? Body weight basis, lipid weight basis, dry weight basis, body burden?

What units to use (can make life easier)?

### 5.1.2 Pesticides

Subsampling in the lab. What part of the body for which analysis? Which tissues?

Which compounds?

How to measure, analytical procedures?

QC-QA?

How to report? Body weight basis, lipid weight basis, dry weight basis, body burden?

What units to use?
5.1.3 Brominated Flame Retardants
Subsampling in the lab. What part of the body for which analysis? Which tissues?
Which compounds?
How to measure, analytical procedures?
QC-QA?
How to report? Body weight basis, lipid weight basis, dry weight basis, body burden?
What units to use?

5.1.4 Dioxins
Subsampling in the lab. What part of the body for which analysis? Which tissues?
Which compounds?
How to measure, analytical procedures?
QC-QA?
How to report? Body weight basis, lipid weight basis, dry weight basis, body burden?
What units to use?

5.1.5 Metals
Subsampling in the lab. What part of the body for which analysis? Which tissues?
Which compounds?
How to measure, analytical procedures?
QC-QA?
How to report? Body weight basis, lipid weight basis, dry weight basis, body burden?
What units to use?

5.1.6 PAHs
Subsampling in the lab. What part of the body for which analysis? Which tissues?
Which compounds?
How to measure, analytical procedures, where to measure, what to record (e.g. bile volume, colour, proteins)
QC-QA?
How to report? Body weight basis, lipid weight basis, dry weight basis, body burden?
What units to use (can make life easier)
PAHs: Need to and how to normalize? See e.g. Ruddock et al. 2003 for the monitoring of those contaminants please refers the sampling and analysis procedure of the DGAL and the ONEMA.
5.1.7 Others?

5.2 Interpretation, visualisation and assessment

Which threshold values to use? Maximum allowable levels? EU WFD levels in biota. Ecotoxicological thresholds. Human health limits. Give a list of contaminant threshold values (legal or indicative ones) used in your country.

There are no threshold values for the eels. We use human health limits:

<table>
<thead>
<tr>
<th>Pollutant</th>
<th>Concentration limite dans le muscle frais en ng/g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Metals</strong></td>
<td></td>
</tr>
<tr>
<td>Mercury Hg (EC/466/2001 – in eel)</td>
<td>1000</td>
</tr>
<tr>
<td>Lead Pb (EC/466/2001 – in eel)</td>
<td>400</td>
</tr>
<tr>
<td>Cadmium Cd (EC/466/2001 – in eel)</td>
<td>100</td>
</tr>
<tr>
<td><strong>Pesticides</strong></td>
<td></td>
</tr>
<tr>
<td>α-HCH (86/363/EEC: in meat)</td>
<td>200</td>
</tr>
<tr>
<td>β-HCH (86/363/EEC: in meat)</td>
<td>100</td>
</tr>
<tr>
<td>γ-HCH (lindane) (86/363/EEC: in meat)</td>
<td>2000</td>
</tr>
<tr>
<td>ΣDDTs(86/363/EEC: in meat)</td>
<td>1000</td>
</tr>
<tr>
<td>DDTs (USFDA1- in fish )</td>
<td>5000</td>
</tr>
<tr>
<td>HCB(86/363/EEC: in meat)</td>
<td>200</td>
</tr>
<tr>
<td><strong>PCBs</strong></td>
<td></td>
</tr>
<tr>
<td>Σ6 PCB indicateurs(EC/1259/2011- anguille)</td>
<td>300</td>
</tr>
</tbody>
</table>

Do you use contaminant quality classes for interpretation, visualisation and assessment.

We used EQI index from ICES 2012, calculated for individual eels. This index is based on quality classes from a monitoring made on eels in Flanders (cf Belpaire).

1http://www.fda.gov/iceci/compliancemanuals/compliancepolicyguidancemanual/ucm123236.htm
How do you visualise your results? Give examples?

How do you visualise your results? Give examples?

![Frequency distribution chart]

Fig. 2. Quality classes distribution based on Belpaire and Goemans (2007), in Canet (C), Salses (S) and Bages (B) lagoons and La Berre river (R). The number under the site code refers to the number of samples for each site. Class 1 corresponds to the not impacted eels, class 2: the slightly impacted, class 3: the impacted and class 4 the strongly impacted. Five contaminants: Sum7PCBs, SumDDTs, Cadmium, Copper and Zinc, and 2 pathogens: parasite Anguillicoloides crassus (AC) and virus EVEX are considered. Reference: Amilhat et al. (2014).

6. Diseases

6.1 Sampling and analysis of eels for parasitology.

<table>
<thead>
<tr>
<th>A. crassus</th>
<th>Pseudodactylyorus spp</th>
<th>Intestinal parasite</th>
<th>Myxidium giardidi Protozoa_Cnidospora Myxosporidae</th>
</tr>
</thead>
<tbody>
<tr>
<td>What part of the body for which analysis, per stage of the eel? Which tissues? Sampling and subsampling per parasite (including numbers).</td>
<td>Same method for all stages. Observation (wall and lumen) of the swimbladder under a stereomicroscope.</td>
<td>Observation of each gill rakers under a stereomicroscope for yellow and silver eels. Use of microscope for species identification.</td>
<td>Observation of each gill rakers under a stereomicroscope for yellow and silver eels.</td>
</tr>
<tr>
<td>Eggs; L2; L3; L4; Adults (Males and females). Count of the necrotic individuals (Count of individual number of each stage from L3 to adult, and differentiate male and female. SDI (Lefebvre index) + our own calibration protocol for transparent-</td>
<td>Number of parasite per side (left and right gill) and when possible, individual identification of each species: Pseudodactylyorus bini and P. anguillae in most of the case.</td>
<td>(*)Number of parasite of each species.</td>
<td>Class of intensity: 0: 0 myxidium / 1: &lt;10 myxidium / 2: 10-50 myxidium / 3: &gt;50 myxidium</td>
</tr>
<tr>
<td>Question</td>
<td>Method 1</td>
<td>Method 2</td>
<td>Method 3</td>
</tr>
<tr>
<td>-------------------------------------------------------------------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>Which test method, existing protocols (larvae or not …)</td>
<td>Dissection</td>
<td>Dissection</td>
<td>Dissection</td>
</tr>
<tr>
<td></td>
<td>When fresh and possible, total weight of the parasite load, length of the swimbladder.</td>
<td>No distinction between larva and adult</td>
<td>No distinction between larva and adult</td>
</tr>
<tr>
<td>Identification issues of the parasite. Is the parasite identified on species level. How? Which reference/technique is used?</td>
<td>L3 in the wall without trophic uptake (nothing in the gut) and L4 in the wall (with trophic uptake, often greenish) or in the lumen. Adult is when the copulatory organs are visible.</td>
<td>Identification made by observation of hamuli morphology. When not possible it is reported as <em>Pseudodactlogyrus spp.</em></td>
<td>No species identification</td>
</tr>
<tr>
<td>QC-QA status for these techniques at the lab?</td>
<td>Double blind observation followed by discussion for consensus.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>How to report? Number of (stages) of which parasites? (e.g. clinics, morbidity, prevalence, intensity (no. of parasites per eel), pathology (fibrosis etc.))</td>
<td>Depending on the aim of the publication: results per stages + SDI or usually, for quality: living L3+L4+Adults + SDI</td>
<td>Depending on the aim of the publication: results are presented per species, per gill side per site and time.</td>
<td>Prevalence and class of intensity</td>
</tr>
<tr>
<td></td>
<td>For special and/or temporal comparison: use of the epidemiological parameters (Bush <em>et al.</em> 1997). We often select only prevalence and intensity.</td>
<td>For special and/or temporal comparison: use of the epidemiological parameters (Bush <em>et al.</em> 1997). We often select only prevalence and intensity.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>%SDI±4 (4 considered as severely damage with a risk of no migration/reproduction)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>What units to use (can make life easier)? (absolute numbers, %, scores?)</td>
<td>Mean intensity ± SD (min-max)</td>
<td>Mean intensity ± SD (min-max)</td>
<td>Mean intensity ± SD (min-max)</td>
</tr>
<tr>
<td></td>
<td>Prevalence (%)</td>
<td>Class of intensity</td>
<td></td>
</tr>
<tr>
<td>How are results visualized, provide examples.</td>
<td>For prevalence: pies on maps or bars and for the other parameters: mean ± SD; or bars.</td>
<td>For prevalence: pies on maps or bars and for the other parameters: mean ± SD; or bars.</td>
<td>% contribution bars of each intensity classes</td>
</tr>
</tbody>
</table>
For SDI: % contribution bars.
If needed, we use class of intensity (example in eelid class: 0 / 1-10/ > 10) to evaluate the impact of parasitism on lipid content, condition factor…

<table>
<thead>
<tr>
<th>Impact assessment. Which method is used?</th>
<th>We tried to compare intensity with condition factor, lipids content.</th>
</tr>
</thead>
</table>

(*) At least 9 species of intestinal parasites have been reported: 1 species of Acanthocephala (*Pomphorhunchus laevis*) 5 species of digenean trematoda (*Bucephalus anguillae, Deropristis inflata, Helicometra fasciata, Lasiotocus longicystis* et *Lecithochirium gravidum*), 2 species of cestoda (*Bothriocephalus claviceps, Proteocephalus macrocephalus*) and 1 species of nematoda (*Hysterothylacium aduncum*). Larvae have not been taken into account in that list.

When relevant, we analyze the parasites species richness and diversity with classical indices.

Three parasites are identified if present in the samples: *Anguillicoloides crassus, Pseudodactylogyrus* sp., *Ichthyophthirius multifiliis* is analyzed on 6 batches of 10 glass eels for each pouring operation.

- What part of the body for which analysis, per stage of the eel? Which tissues? Sampling and subsampling per parasite (including numbers).
- What is recorded, which stages, numbers, swimbladder damage, etc?
  - Stage < VIA2
- Which test method, existing protocols (larvae or not …)
- Identification issues of the parasite. Is the parasite identified on species level. How? Which reference/technique is used?
- QC-QA status for these techniques at the lab?
- How to report? Number of (stages) of which parasites? (e.g. clinics, morbidity, prevalence, intensity (no. of parasites per eel), pathology (fibrosis etc.)).
- What units to use (can make life easier)? (absolute numbers, %, scores?)
- How are results visualized, provide examples.
- Impact assessment. Which method is used?

For the monitoring of those pathogens please refers the sampling and analysis procedure of the regional aquaculture veterinary body locating SW France: GDSA, LASAT and LVD 40 where part of the national parasitological,
bacteriological and virological status of the different batches from restocking eel program are performed.

6.1.1 *Anguillilicolaoides crassus*

6.1.2 Other, like trematodes?

6.1.3 Other....

6.2 Sampling and analysis of viral and bacterial diseases

Three rhabdoviruses are identified if present in the samples: Viral Haemorrhagic Septicemia (VHS) virus, Infectious Haematopoietic Necrosis (IHN) virus, Eel European Virus X (EVEX)

In an experimental program for restocking in Aquitaine, VHS is researched by PCR (contact GDSAA/LVD 40).

6.2.1 Viral diseases : EVEX (for Perpignan)

- What is recorded in the necropsy room, clinical pathology etc.?
  Only viewable anomalies.
  nothing

- What part of the body for which analysis, per stage of eel? Which tissues? Sampling and subsampling per group of pathogens (including numbers).
  The tail part is eliminated and the analysis is performed on all the rest of the animal (head and abdominal cavity). All analyses are done on a sample of 10 glass eels (mix). The geographical origin are recorded.
  We sample a small piece of brain, heart, spleen and kidney from fresh or frozen yellow and/or silver eel. The samples are put together in a separate tube (one tube per eel) and kept frozen (if fresh tissues: the tube is quickly immersed in liquid nitrogen before being stored in -80 or -20 °C, if tissues originate from frozen eels: quickly restore in –20°C)

- Which isolation and/or test method for identification, existing protocols with references per virus
  Glass eels samples were prepared as described in the Commission Decision of 22 February 2001. Samples are analyzed on different cell lines: EPC, BF2, RTG and/or KF1. In the case of apparition of cytopathic effects (CPE), identification of the virus is done. Two French standards were used for the identification of VHSV and IHNV: NF U 47-220 and NF U 47-221 (identification of the virus after cell culture using IF). EVEX is identified by IF using homemade sera.
  NB: CPE induced by other viruses like IPN can be observable but in that case, the mention "no VHSV, no IHNV, no EVEX" is put in the analysis report.
  Molecular tool. This method is presently under an international patent pending

- QC-QA status for these techniques at the lab?
NF U 47-220 and 221 are realised under accreditation (referential ISO 17025). Research of EVEX is not integrated in the scope of accreditation but the analyses are done under the same rules of quality. Positive and negative controls were added for each analysis, cell lines and reagents are regularly controlled, all the equipments used are under metrological control and all steps from receiving the samples to sending the analytical results are recorded.

The identification is validated by virological test: cellular culture followed by IF performed by ANSES (as described previously).

- How to report? Clinical pathology, name of the virus, subtype, prevalence, pathology?

On the analysis report, we put the result of the culture step (presence or absence of CPE after one or two (if negative at the end of the first passage) passages in cell culture) and, if a CPE was observed, the results of the identification step: presence or absence of VHSV, IHNV or EVEX.

Prevalence (%)

- What units to use (can make life easier)?

Results are only qualitative: presence or absence of CPE in cell culture and, if the virus present is identified as VHSV, IHNV or EVEX, the name and the method of identification used.

Number and/or %

- How are results visualized, provide examples.

In cell culture: presence of CPE under inverse microscope. IF: presence of fluorescent cells (infected by the virus) under epifluorescence microscope.

% in a text

- Impact assessment. Which method is used?

Not enough positive cases to assess impact.

6.2.2 Bacterial diseases:

In an experimental program for restocking in Aquitaine, flavobacterium is researched (contact GDSAA/LVD 40).

- What is recorded in the necropsy room, clinical pathology etc.?

- What part of the body for which analysis, per stage of eel? Which tissues? Sampling and subsampling per group of pathogens (including numbers).

- Which isolation and/or test method for identification, existing protocols with references per bacterium

- QC-QA status for these techniques at the lab?

- How to report? Clinical pathology, name of the bacterium, subtype?, antibiotic resistance?, prevalence, pathology?
7. Perspectives of using biomarkers of effects to assess eel health

In the Anses lab, different biomarkers are used but only for rainbow trout and sea bass at this time (see the slide below). Some of them could probably be used for Eels without a lot of development and could give interesting information. Others, particularly molecular tools, need more work but allow to have a closer monitoring.

We analysed gene expression involved in oxidative stress (catalase (CAT), Glutathion-S-transferase (GST and GSTPi) ) and in detoxification activity (metallothioneine 1 (MET))

RTqPCR method
Multi-pollution, many different processes involved
Need more research and development

8. International needs (making your results available, and international database)

It will be great to have at the European level regular elements related to restocking actions and quality of wild eels.

A recurrent problem is the delay for virological analyses done using classical methods of cell cultures (about 15 days if samples are negatives). Validated classical or real time PCR
could strongly improve the procedure of sanitary control and allow to release more rapidly batches. Increasing the time the glass eels are staying in tanks can lead to injuries and health issues that may jeopardise the restocking efficiency and so on the future eel population.

Are the results of eel quality assessments in your country made available to the international community? Describe mechanisms e.g.

- through publications in scientific journals: yes
- through institutional reports: yes
- through the national contact point of the ICES WG EEL group and the yearly eel country reports: yes
- through reporting to the ICES WGEEL Eel Quality Database: no
- through reporting to EU in the framework of the WFD: no
- through reporting to EU the progress in the eel management plans in the framework of the Eel Regulation: yes
- through reporting in the framework of the CFP: no

Are raw data included, accessible? no

When published in scientific papers, are your raw data uploaded as Supplementary Information? no

9. Other issues, remarks

A work related to the evolutionary dynamics and genetic diversity from three genes of Anguillid Rhabdovirus was recently published by the Anses lab (Bellec et al., 2014; Journal of General Virology, 65:2390-2401).

- It is important to record when doing contaminants and parasites analyses: the stage of the eels (glass eel, yellow, silver), the length, the age, the sampling site, the season (when the sampling have been done). Bioaccumulation and presence of parasites will depend on these parameters.

- Issues concerning the certification of the analyses. The requested amount of muscle is too high (not enough tissue when analyse small eels and when several contaminants analyses are needed).

- Localisation of the analysed tissue is an issue: if taken in the tail or next to the body cavity, is there a difference as we know that there is a lipid content difference.

- Issue concerning the cost of the monitoring, the cost of the analysis, example PCB DL and NDL.

- Issue related to the consequences of the results for the fishermen (closure of the fishery)

- When samples are taken to analyse contaminants, the otoliths should be stored
- Issue on pooling several eels together. There is such an inter individual variation in concentration of contaminants and/or intensity of parasites and/or virus presence among the same site, we should avoid pooling, this may mask the real situation.

- Also avoid considering one site as representative of the region/country, we should work at river basin level.

- Issue concerning the results coming from freshwaters and brackish/marine waters. They should not be assimilated.

- Issue concerning freezing impact on the weight, the time of freezing, the water type (brackish of freshwater), and the size of the frozen eel.

- In order to compare contaminants results between locations, the results should be indicated in dry wet +% humidity

- The method should be clearly informed for parasite examination (not the same to consider adults A. crassus or adults and larvae).

- Issue on result report: should we use mean + error or median (box plot) as there is an important inter individual variability

- Issue on the property of the data in the database (published and not published data/raw data).

- Issue on detection limits. In some case, the method used is not suitable to detect low concentrations of some contaminants in eels. The DL should be clearly indicated. How to mean the data with <DL data?

- Other compounds that could affect the eel health: drugs, oestrogens…. 

- More research should be done on the impact of the pollutant of the health of the eel. Issues on (1) multipollution: multi-contaminants/diseases and interactions; (2) pollution peak versus continuous low pollution: what effect on the health of the eel. How to identify the more impacting compounds affecting the eel’s health without this information. Issue on the use of appropriate physiological biomarkers.

- Use other index than EQI to represent the multi contamination/diseases: IMBI multipollutant index from Maes (2013)? ....

10 References


Call for projects entitled “restocking of eels in France”.

Good practice guide for the glass eels industry.
The management plan for Eel in France. 


Reports:


<table>
<thead>
<tr>
<th>Which stage</th>
<th>What</th>
<th>Where</th>
<th>When</th>
<th>Who involved</th>
<th>What lab or organization</th>
<th>How</th>
<th>For more information, please contact</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size of the glass eel, yellow eels and/or silver eels</td>
<td>Parameters followed</td>
<td>Geographical area of sampling</td>
<td>Date of sampling</td>
<td>Coordinator, participants and program</td>
<td>Laboratory, name and location</td>
<td>Method of analysis</td>
<td>Name, E-mail</td>
<td>Realized within the national protocol concerning restocking eel program</td>
</tr>
<tr>
<td>Glass eels</td>
<td>Parasitology, anatomo-pathological examination</td>
<td>Somme</td>
<td>Spring 2012 and 2013</td>
<td>ARA-France, repeuplement anguille (restocking program)</td>
<td>Consulting firms</td>
<td>Visual-Macro and Microscope</td>
<td>Patrick GIRARD, <a href="mailto:patagir@club-internet.fr">patagir@club-internet.fr</a></td>
<td></td>
</tr>
<tr>
<td>Glass eels</td>
<td>Parasitology</td>
<td>Most important glass eel fishing estuaries in France</td>
<td></td>
<td>ARA-France, repeuplement anguille</td>
<td>LVD 40, Mont de Marsan, LASAT</td>
<td>Visual-Macro and Microscope</td>
<td>Jérémie Souben, <a href="mailto:repeuplementanguille.france@gmail.com">repeuplementanguille.france@gmail.com</a>, Patrick Daniel, <a href="mailto:p.daniel@labopl.com">p.daniel@labopl.com</a>, <a href="mailto:contactmdm@labopl.com">contactmdm@labopl.com</a>; <a href="mailto:lasat@lasat.fr">lasat@lasat.fr</a></td>
<td></td>
</tr>
<tr>
<td>Glass eels</td>
<td>virology SHV, NHI and EVEX</td>
<td>Most important glass eel fishing estuaries</td>
<td>Fishing period (November to May) every year</td>
<td>ARA-France, repeuplement anguille</td>
<td>LVD 40, Mont de Marsan</td>
<td>Cell culture (CC)</td>
<td>Jérémie Souben, <a href="mailto:repeuplementanguille.france@gmail.com">repeuplementanguille.france@gmail.com</a>, Patrick Daniel, <a href="mailto:p.daniel@labopl.com">p.daniel@labopl.com</a>, <a href="mailto:contactmdm@labopl.com">contactmdm@labopl.com</a></td>
<td></td>
</tr>
<tr>
<td>Glass eels</td>
<td>virology SHV, NHI and EVEX</td>
<td>Most important glass eel fishing estuaries</td>
<td></td>
<td></td>
<td>ANSES-Brest</td>
<td>Cell culture (CC)</td>
<td>Jérémie Souben, <a href="mailto:repeuplementanguille.france@gmail.com">repeuplementanguille.france@gmail.com</a>, Thierry Morin, thier-</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>Techniques</td>
<td>Area of releasing</td>
<td>Period</td>
<td>Consulting Firms</td>
<td>Realized within the national protocol concerning restocking eel program in the Aquitaine region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>-------------------------------------</td>
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<td>-------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elvers/ yellow eels</td>
<td>Parasitology; anatomo-pathological examination</td>
<td>Area of releasing</td>
<td>Autumn and spring</td>
<td>Consulting firms</td>
<td>Health status evaluation of the released eel during 3 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adulte</td>
<td>Parasitology; anatomo-pathological examination</td>
<td>Somme</td>
<td>Autumn 2012</td>
<td>ARA-France, repeuplementanguille</td>
<td>Realized within the national protocol concerning restocking eel program</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow eels</td>
<td>Parasitology; anatomo-pathology; virology</td>
<td>Dordogne</td>
<td>Summer 2002</td>
<td>Consulting firms, LDA 34, Visual-Macro and Microscope, cell cultures</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adulte</td>
<td>Trace metallic elements, dioxins, PCB, pesticides, HAP, brominated compounds, perfluoralkyls</td>
<td>official sampling planned at retail level</td>
<td>Every year</td>
<td>Ministère de l'agriculture, DGAL. Annual monitoring and control plan for contaminants in foodstuffs</td>
<td>Authorized laboratories with COFRAC certification, Ministère de l'écologie et de l'agriculture, ONEMA, DGAL, Agences de l'eau Artois-Picardie / Seine-Normandie et Loire-Bretagne, Plan national PCB</td>
<td>yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adulte</td>
<td>Dioxines, PCB-DL and PCB-NDL</td>
<td>sampling planned at national level</td>
<td>2008 to 2014</td>
<td>Laberca, Oniris, Nantes</td>
<td>Olivier perceval, <a href="mailto:olivier.perceval@onema.fr">olivier.perceval@onema.fr</a>, Charlotte Grastilleur, <a href="mailto:charlotte.grastilleur@agriculture.gouv.fr">charlotte.grastilleur@agriculture.gouv.fr</a>, <a href="mailto:bpmed.sdssa.dgal@agriculture.gouv.fr">bpmed.sdssa.dgal@agriculture.gouv.fr</a></td>
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<td>Silver eels</td>
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<td>Loire</td>
<td>AAIPPBLB, monitoring of silver eels</td>
<td>Mathieu Bodin <a href="mailto:aaippblb@laposte.net">aaippblb@laposte.net</a>, Philippe Boisneau <a href="mailto:philippe.boisneau@wanadoo.fr">philippe.boisneau@wanadoo.fr</a></td>
<td>European program aimed to evaluate the quality of the future breeders</td>
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<td>Yellow and silver eels</td>
<td>Parasitology; anatomo-pathology; virology; swimming aptitude (hyperbaric chamber)</td>
<td>Few river basins: Rhin, Loire, Adour-Garonne</td>
<td>2001 to 2003</td>
<td>LDA 34, Université de Brest, MNHN, consulting firms</td>
<td>Visual-Macro and Microscope, cell culture, Anatomomorphology, internal and external parasitology</td>
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<td>Yellow and silver eels</td>
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<td>Garonne, Dordogne, Gironde</td>
<td>2011 to 2013</td>
<td>ANR, Université Bordeaux 1, CNRS, Cemagref</td>
<td>Toxicological analysis ; Anatomomorphology, Anatomo-pathology</td>
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<td><a href="mailto:elsamilhat@univ-perp.fr">elsamilhat@univ-perp.fr</a>/faliex@univ-perp.fr/gsimon@univ-perp.fr</td>
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<td>Eel Type</td>
<td>Metals/PCBs/Ocs/PBDE</td>
<td>Site</td>
<td>Year</td>
<td>University</td>
<td>Analysis Method</td>
<td>Collaborators</td>
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<td><strong>Silver eels</strong></td>
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<td>French Mediterranean lagoons (11 sites) in Languedoc Roussillon</td>
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<td>GC-MS (Gas chromatography–mass spectrometry), LC-MS/MS (Liquid chromatography–tandem mass spectrometry) / ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry) and Atomic Absorption Spectrometry (AAS)</td>
<td><a href="mailto:elsamphilhat@univ-perp.fr">elsamphilhat@univ-perp.fr</a>/faliex@univ-perp.fr/gsimon@univ-perp.fr</td>
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<td>2009 et 2010 (3 samples/year)</td>
<td>University of Reims (laboratoire Eco-toxicologie)</td>
<td>Graphite furnace atomic absorption spectrophotometry (AAS) and flame AAS</td>
<td>Séverine Paris (<a href="mailto:severine.paris@univ-reims.fr">severine.paris@univ-reims.fr</a>)</td>
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<td>Gas chromatography and mass spectrometry</td>
<td>Marc Chevreuil (<a href="mailto:marc.chevreuil@upmc.fr">marc.chevreuil@upmc.fr</a>)</td>
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<td>Parasites (A. crassus; Pseudodactylogyrus spp.)/intestinal parasites</td>
<td>University of Perpignan (BETM/CEFREM)</td>
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<td>Elisabeth Faliex (<a href="mailto:faliex@univ-perp.fr">faliex@univ-perp.fr</a>)</td>
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<td>University of Reims (laboratoire Eco-toxicologie) and Perpignan (BETM/CEFREM)</td>
<td>enzymatic activities and genes expression</td>
<td>Séréine Paris (<a href="mailto:severine.paris@univ-reims.fr">severine.paris@univ-reims.fr</a>) et Elisabeth Faliex (<a href="mailto:faliex@univ-perp.fr">faliex@univ-perp.fr</a>)</td>
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| Silver eels | PCBs et Ocs/metals (Cu, Cd, Zn)/ lipids | 3 lagoons (Canet, Bages-Sigean and Leucate) and 2007 and 2008, during the migration season | University of Perpignan and IFREMER Sète | LDA PO | Gas chromatography and mass spectrometry | els.amilhat@univ-perp.fr/ faliex@univ-perp.fr/ gsimon@univ-perp.fr | Elsa Amilhat, Geraldine Fazio, Gael Simon, Marc Manetti, Severine Paris, Laurence Delahaut, Henri Farrugio, Ray-
<table>
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<tr>
<th>Parasitology: A. crassus</th>
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<th>pignan</th>
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<td>EVEX virus (50 eels)</td>
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<td>Silver eels</td>
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<td>macro and microscopic examination</td>
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<td>Health status assessment: pathological codes</td>
<td>All area</td>
<td>Realized between 2010 and 2014</td>
<td>Association Santé Poissons Sauvages (ASPS)</td>
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<td>All stage; glass eel, yellow and silver eels</td>
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<td>Pathological codes are used for eel and other species on various places but reviews are incompletely realized that could be useful. A generalization of the implementation of the pathological</td>
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<td>Silver eel</td>
<td>Organic persistent contaminants (7 PCBs, HCB, HCH, Transnonachlor, 4 metabolites of DDT)</td>
<td>Estuary of Gironde, Dordogne, Garonne, Bassin d’Arcachon</td>
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<td>Silver eel</td>
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<td>Indicators of metabolic capacity</td>
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<td>Parasitology; anatomo-pathological and macroscopical examination</td>
<td>CDC Ile de Ré et CREAA (Cédric Hennache) : Monitoring of eels before and after transformation of the Suivi anguilles avant/après réhabilitation des ouvrages d'entrée d'eau</td>
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<td>Directly done in the field</td>
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<td>Heavy metals (Arsenic, Lead, Cadmium, Mercury, Nickel)</td>
<td>Extensive ponds and lagoons on saltmarshes of Charente-Maritime (French Atlantic Coast) (Seudre, Ré)</td>
<td>Ifremer Nantes France (Mireille Cardinal) and CCMAR Faro</td>
<td>See also CCMAR Faro Portugal (Luisa Valente)</td>
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<td>Taxa</td>
<td>Procedure</td>
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<td>Yellow eels and or Silver eels</td>
<td>Parasitology; anatomopathological, macroscopical examination</td>
<td>Extensive ponds and lagoons on saltmarshes of Charente-Maritime (French Atlantic Coast) (Seudre, Ré island and Arcachon)</td>
<td>Eric Buard et Philippe Blachier - CREAA - SEACASE European Program (2007-2009). Partenaires = IRSTEA, IFREMER</td>
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<td>Yellow eels</td>
<td>Parasitology; anatomopathological, macroscopical examination</td>
<td>Extensive ponds on saltmarshes of Charente-Maritime (French Atlantic Coast) Seudre</td>
<td>Cellule Migrateurs Charente Seudre (Eric Buard, Audrey Postic-Puivif, François Albert) Monitoring of the yellow eel population in the dyked marine marsh of Seudre</td>
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<td>glass eels and yellow eels</td>
<td>Parasitology anatomo-pathological, macroscopic examination</td>
<td>River basin Charente and Seudre</td>
<td>June or July of 2009, 2010, 2011, 2013</td>
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Report on the methods and assessment of eel quality (contaminants and diseases) in:

Germany

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Introduction
This country report has been drafted in preparation of the ICES Workshop of a Planning Group on the Monitoring of Eel Quality: “Development of standardized and harmonized protocols for the estimation of eel quality.” (WKPGMEQ), held at INBO Brussels from 20 to 22 January 2015.

This country report aims to give an overview of methodologies and assessments concerning eel quality, contaminants and pathogens done on a broader scale in Germany. It does not intend to claim for completeness of all available assessed methods in all German research facilities and/or institutes.

Germany is a federal state with a three-tiered system of government: the federation (national level), the Bundesländer (federal states, provinces, or regional level), and municipalities (local level). Fisheries laws are executed by the federal states / Bundesländer as in principle, according to the constitution, the federal laws and regulations are executed by the administration of the states. In terms of the legislative power at the federal level, the country can enact laws on sea and coastal fisheries within the so-called "concurrent legislation", whereas the federal states are exclusively responsible for national inland water fisheries.

Eel management in Germany is based on National Eel Management Plans (EMP) for 9 German River Basin Districts. The relevant German river systems belong to the ICES Ecoregions North Sea (Rhine, Elbe, Weser, Ems, Eider) and Baltic Sea (Oder, Warnow/Peene, Schlei/Trave). The Danube, which drains into the Black Sea, is not con-
sidered to constitute natural eel habitats at a relevant level and hence, no stock indicators have been calculated for the Danube and no EMP has been established for this system.

![Map of River Basins in Germany](image)

Fig. 1: River Basin Districts (RBD) in the Federal Republic of Germany: Eider, Schlei/Trave, Elbe, Warnow/Peene, Oder, Weser, Ems, Rhine, Meuse and Danube.

**Eel data collection in the EU-Data Collection Framework (2008/199/EC) in German freshwater habitats (From the technical report 2013):**

Sampling of European eel data in freshwaters is mandatory under the Data Collection Framework (DCF). Data collection was coordinated and performed by the Thünen Institute of Fisheries, the Federal Research Institute for Rural Areas, Forestry and Fisheries. In Germany, sampling has started in spring 2009. The recent years of sampling have been considered as a “pilot” phase.

During the ongoing sampling period from 2011 to 2013 the proposal for the German national program intended the gathering of 600 eels from the Baltic Sea and 300 eels from the North Sea, including the respective discharging river basin districts (RBD; according to WFD, see figure 1). However, recent sampling aimed at 600 eels from North Sea and 300 eels from the Baltic Sea, which has proven to be a more practical approach. If possible, 100 silver and 100 yellow eels per RBD were sampled annually, according to Council Decision (EC) No 949/2008.

So far, sampling focused on gathering biological parameters of eel in commercial catches of inland fisheries. Analyses include length, weight, age, sex and maturity. The results of the biological sampling of eels in the freshwaters are regularly included as an Annex to the WGEEL German country report. Additional parameters such as infestation with the invasive swim bladder nematode *Anguillicoloides crassus*, fitness indicators (HSI, GSI and fat content) as well as contamination status with several pollutants (e.g. Heavy metals, BFR’s, PCB’s & PAH’s) have been analyzed in a number of eels from different origins, partly in cooperation with other institutions. However, these additional investigations are not mandatory under the DCF but were recommended in the respective German annual reports. Since 2011 sampling scheme within the German DCF has slightly changed,
still focussing on biological parameters but now including further, additional standard sampling procedures aiming towards eel quality questions.

Regarding the comparably high numbers of sacrificed fish from different sampling locations in Germany solely for the collection of (yet) basic biological data, it seems necessary and logical that fish sampled in line with the DCF are made accessible and in put to best use in terms of further scientific approaches. Looking at the new implementation of the EU-Data Collection Multi Annual Programme (previously referred to as DCR, DCF, and now DCMAP) covered in the new European Maritime and Fisheries Fund proposal (EMFF) the future strategy of the DCF-sampling is under discussion and possibly may change (e.g. eventually inclusion of detailed data about fishing effort in direct relation to catches and even inclusion of eel quality data).

2. Framework national cases and national guidelines (on contaminants and health)

2.1 Contaminants

Up to date, no official routine monitoring network measuring contaminants in eels is set up in Germany. No national guidelines are available.

In line with routine collection of biological data within the above-mentioned “pilot phase” of the German DCF, some studies focusing on contaminants in eels have already been conducted. While the acquisition of these eels from commercial fisheries was covered by the German DCF, further analytical investigations were financed by research projects or budgetary funds of the involved institutions. The objectives of these studies mostly are / were basic research. Results of some of these works were made accessible to the international community due to their publication in scientific journals, others are already submitted or still in preparation.

Nevertheless, among EMUs, federal states may monitor or investigate certain contaminants in fish (among them eels) for different reasons in case studies, in connection with analyses of biota within the water framework directive or investigations connected with human consumption (usually rather low sample numbers). Sampling-plans of fish in context with food laws are made spontaneously by the respective organs and therefore do not follow routine regularities. Results of some of these works are made accessible to international community by scientific publications or reports accessible on the regional administrations web pages, which may impair (international) accessibility.

2.2 Diseases

No official routine monitoring network measuring diseases or disease agents in eel is set up in Germany at present. No national guideline documents exist.

Within the pilot phase of the German Data Collection Framework histological sections of liver, and spleen as well as blood film / blood smear samples were taken from individuals from most relevant river basin districts. Final results have yet to be published.

Also, some further studies regarding this aspect may have been carried out by individual research institutions or in the framework of EMPs on some regional level. Results of some of these works are accessible to international community due to their inclusion in
scientific reports, national reports regarding the Eel Management Plans or to their publication in scientific journals.

3. General issues on eel sampling for contaminants and/or diseases in your country. Treatment of the sampled eels on site and in the laboratory.

3.1 Ethics and legislation

Some of the studies mentioned in this report are conducted with wild eels purchased from commercial fishermen or are caught within studies planned and conducted by regional authorities and administrations. In that case, usually no additional permissions are required. For sampling of eels that are underneath the keeping size limits or within closed seasons (in Germany these are defined by the authorities of the federal states) special permissions are needed.

Depending on the purpose and the initiator of the respective approach, further permissions from regional administrations may be required. As for example it is necessary to sample fish in certain areas where no commercial fisheries is present and electro-fishing is necessary. Animal ethics permissions are generally required for research projects involving live animals.

Sampling site descriptions differs greatly among studies. While season and exact sampling dates are usually given, sampling locations are often provided in form of stream-kilometer and or the place of which fisherman is operating from.

Within Samplings done by the LANUV in North-Rhine-Westphalia dates and locations are recorded and marked in GIS. (Geo reference : ETRS89/UT)

3.2 Sampling procedures


Sampling of eels differ greatly among federal states. While in some states no own sampling is done at all, others do have certain sampling strategies, depending on the respective study. Glass eels and farmed eels for restocking purposes for example are regularly screened for parasites (Anguillicolaoides crassus & and gastrointestinal parasites) in Berlin, Sachsen-Anhalt, Brandenburg and Mecklenburg Vorpommern. In North Rhine Westphalia a study for the up to 20 eels per site (farmed eels for stocking; up to 40 eels per batch) are sampled, using different fishery techniques: Electric fishing, fyke nets, stownets / schokker (silver eels).

Distinguishing eel species is not done within any kind of routine monitoring. Nevertheless, in 2 publications by Marohn et al. (2014a, 2014b) eel from the Schwentine-river system in Germany were distinguished on species level following the protocol by Frankowski & Bastrop (2010). In some states eels for restocking are/were also regularly checked on species level using the protocol by Frankowski & Bastrop.

Regarding samplings done within the DCF:
Due to the limited number of commercial fishermen and better comparability, sampling was restricted to only few locations. To optimize comparability, eels were preferably collected downstream in the system close to the estuaries. If necessary, exceptions from this general approach were made. Within the Data Collection Framework, it is aimed to sample each 100 yellow and 100 silver eels per RBD annually. For eel quality approaches, additional samples of at least each 15 yellow- and 15 silver eels per EMU were taken and stored if not directly analyzed.

These samples include: muscle samples excised from the filet right at the level of the anus, bile samples, liver samples and sometimes histological sections of liver, and spleen as well as smear samples. Swim bladders are sampled of every single fish obtained within the DCF.

Yellow eels were mostly collected in spring/summer and silver eels in autumn. To our impression, sampled eels derived from commercial fisheries differ greatly in their stage-composition. While with fyke nets commercial inland fishermen in Germany mostly target yellow eels in their feeding phase, stownets are usually used during the migration peaks to target mainly silver eels.

3.3 Stage, gender, morphometric

Eel stages are usually and preferably distinguished by the staging system introduced by Durif (2005) or Pankhurst (1982), also (in the DCF) an additional macroscopic rating is done by general appearance into yellow and silver eels before the additional parameters like eye diameter or fin-length are collected. Gender is determined either via staging by Durif (2005) or via macroscopic assessment of the gonads.

Total length is determined either immediately after catch (to the nearest 0.5 cm) or after thawing. In the second case the values are corrected by assuming a reduction of 2.5% according to Wickström et al. (1986). Total weight is also determined either immediately after catch or after thawing. In the second case the values are corrected by assuming a reduction of 2.8% according to Wickström et al. (1986)

3.4 Treatment

Eels sampled in line with the German DCF

If necessary to store fish, they were kept in flow-through systems and held not longer than 10 days until sampling was done. Fish were euthanized using ethylene glycol monophenyl ether or clove oil and decapitated / otoliths were excluded before further sampling of tissue / organs.

Fish and samples were excised and handled with nitril laboratory gloves only on a cleansed aluminum working surface. Nothing but aluminum and glassmade tools and devices were used while handling the fish. Muscle and liver (tissue-) samples are / were wrapped in sheets of new, clean aluminum foil, labelled with an id number stored inside disposable aluminum pads with lids at -20°C. Bile fluid was also kept at -20°C while blood samples were kept in cryovials at -80°C.

Tissues and samples which were not put directly to use for actual scientific approaches are kept and stored in form of a sample tissue bank, data are maintained in form of a database kept in connection with biological parameters raised for the German DCF.
4. **General issues on eel condition (fitness, lipid levels)**

Eel condition parameters in the assessments made in line with the DCF are modified Fultons Condition factor, lipid levels, HSI and GSI.

Other condition factors used in Germany are gross energy (GE) values for Farm eels and allover health status for yellow eels. (Study by Institut für Binnenfischerei e.V. in Potsdam, Brandenburg, ) see report (link).

### 4.1 Condition

Since no standardized function of the length-weight relationship for eel is available, defining condition in absolute terms makes little sense. Thus, condition for eels sampled in line with the DCF is only described relative to the average length-weight relationship (either of all sampled eel, or eel from a specific RBD), for males and females separately, as follows:

\[ C = \frac{W}{a^L} \]

With C: Condition Factor, W: Mass (g), a: linear factor, b: exponent from length weight relationship

Based on all available data from German RBD’s the constants are \( a = 0.00025 / b = 2.9 \) for males and \( a = 0.0003 / b = 3.44 \) for females. Since sampling is restricted to commercial catches with a Minimum Landing Size between 35cm and 50cm data for males, however, only cover a very small percentage of the size range.

### 4.2 HSI

Hepatosomatic index is not part of the standardized sampling procedure in the DCF. Liver weight and consequently the hepatosomatic index were recorded for most sampled eel anyways.

### 4.3 GSI

Gonadosomatic Index is also not part of the standard sampling procedure in the DCF. It was, however, collected for a large subsample of eel from 2012 and 2013. We consider the GSI important information for further investigations on spawner quality, but thorough preparation of the gonads is extremely time consuming and thus, mostly limited by the availability of time/personnel.

### 4.4 Lipid levels

Most of the eel sampled in the DCF (routinely staged after Durif (2005) and Pankhurst (1982)) were analysed for their whole muscle fat content with the fatmeter device (FM-692, Distell, UK). Lipid levels are expressed as % of total muscle wet mass. Besides that, lipid levels were gravimetrically determined for all eel which were analysed for contaminants, using a piece of filet (approx. 15-20g) cut from the left half of the body, distal of the anus.

In terms of quality control, lipid levels were analysed gravimetrically for a small subsample of eel for a quick internal verification. While the study is not yet finished, preliminary results suggest that on average (i.e. average fat content of several eels) the fatmeter
provides good estimates of the fat content, while values for individual fish are not always in complete agreement with the gravimetrically determined reference value. Furthermore, our subjective impression is that precision (i.e. reproducibility of fatmeter results) is rather high.

Outside the DCF, a study was conducted by Klefoth et al. 2013 who came to the conclusion that the fatmeter provides generally good estimates of the fat content.

5. Contaminants

5.1 Sampling and analysis

5.1.1 PCBs

Subsampling in the lab, Extraction and Clean-up

1 – 3 g homogenized muscle tissue of yellow and silver eels was excised from the skeletal muscle just behind the level of the anus. 3 g gonad tissue was used in case of silver eels and as much as available from yellow eels. Glass eels and elvers were pooled into samples of 3 g. For egg samples 3-5 g were used. All subsamples were stored at -20°C until analysis.

The frozen subsamples were homogenised with anhydrous Sodium sulfate (Na2SO4) (Merck) using a stainless steel/glass 1 L laboratory blender (neoLab Rotorblender). Prior to extraction all samples were spiked with mass labelled surrogate standards analog for each analyzed compound (WHO PCB+PCB-170+PCB-180 CLEAN-UP STANDARD (13C12, 99%), CIL, USA).

Extraction and clean-up were performed in accordance with the method described in Sühring et al. (2013), using accelerated solvent extraction with subsequent gel permeation chromatography and silica gel clean-up. 500 pg (absolute) 13C-PCB-141 and 13C-PCB-208 was added as an injection standard to each sample.

The lipid content of samples was determined gravimetrically from separate aliquots following a method described in Sühring et al. (2013).

Instrumental Analysis

The instrumental analyses were performed on a GC/MS-system (Agilent 6890 GC/5973 MSD) fitted with a HP-5MS column (30 m x 0.25 mm i.d. x 0.25 μm film thickness, J&W Scientific) in negative chemical ionization mode (NCI) using methane as ionization gas. The instrument was operated in selected ion monitoring mode. Samples were analyzed for dl-PCBs (IUPAC numbering) 77, -81, -105, -114, -118, -126, -156, -157, -157, -167, -169, -189 as well as PCB 170 and -180. PCB 170 and PCB 180 were included because of their role as active inducers of EROD activity and their quantitative significant presence in environmental samples.

QA/QC

Extraction and clean-up were conducted in a clean lab (class 10000).
Surrogate recoveries were determined for every eel sample and all concentrations were recovery corrected.

A blank test, using Na2SO4 treated similar to real samples, was conducted with every extraction batch (five samples). All blanks were either below MQL or otherwise 1-2 magnitudes lower than lowest sample concentrations. Average blank values were subtracted from concentration found in the samples.

The limit of detection (LOD) was calculated from a signal to noise ratio of three or by using the blank standard deviation method (where applicable). The limit of quantification (LOQ) was calculated from a signal-to-noise ratio of ten or using the blank standard deviation method (where applicable). The LOD ranged from 0.003 ng/g wet weight (ww) for PCB 189 to 0.256 ng/g ww for PCB 180. The LOQ ranged from 0.004 ng/g ww for PCB 189 to 0.724 ng/g ww for PCB 180. For further quality control, a twofold measurement was conducted for samples from areas with low sample numbers and a threefold measurement was done for PCB 126 from randomly selected samples from remaining areas. Results for PCB 123 were excluded from our results due to incomplete chromatographic separation.

**Reporting**

All concentrations were calculated on a wet weight as well as lipid weight basis. pg/g or ng/g were used as units for reporting.

### 5.1.2 Pesticides

N/A

### 5.1.3 Brominated Flame Retardants

*(Method described was used in Sühring et al. 2013 and Sühring et al. 2014)*

**Subsampling in the lab, Extraction and Clean-up**

1 – 3 g Muscle tissue of yellow and silver eels was excised from the skeletal muscle just behind the level of the anus. 3 g gonad tissue was used in case of silver eels and as much as available from yellow eels. Glass eels and elvers were pooled into samples of 3 g. For egg samples 3-5 g were used. All subsamples were stored at -20°C until analysis.

The frozen subsamples were homogenised with anhydrous Sodium sulfate (Na2SO4) (Merck) using a stainless steel/glass 1 L laboratory blender (neoLab Rotorblender). Prior to extraction all samples were spiked with mass labelled surrogate standards 13C-BDE-28, 13C-BDE-47, 13C-BDE-99, 13C-BDE-153, 13C-BDE-183, 13C-MeOBDE-47, 13C-MeOBDE-100, 13C-HBB, 13C-synDP and 13C-PBBz (Wellington Laboratories, Cambridge Isotopes).

Extraction and clean-up were performed in accordance with the method described in Sühring et al. (2013), using accelerated solvent extraction with subsequent gel permeation chromatography and silica gel clean-up. 500 pg (absolute) 13C-PCB-141 and 13C-PCB-208 was added as an injection standard to each sample.

The lipid content of samples was determined gravimetrically from separate aliquots following a method described in Sühring et al. (2013).
Instrumental Analysis

In order to obtain maximum sensitivity as well as selectivity extracts were analysed by gas chromatography/mass spectrometry (Agilent QQQ 7000). In electron capture negative ionisation mode (ECNI) with single MS (GC-MS) and in electron ionisation mode (EI) with tandem-mass spectrometry GC-MS/MS.

For analysis in EI mode a Restek 1614 column (15m x 0.25 mm i.d. x 0.10 μm film thickness, Restek) and a restriction capillary (0.8m x 0.1 mm i.d., deactivated) was used with Helium (purity 99,999%) as carrier gas and a constant column flow of 2.5 mL/min. The injector was operated in pulsed-splitless mode (injection pulse 25 psi for 2 min) with an inlet temperature program: 60 °C for 0.3 min, 300 °C min-1 until 280 °C and held for a final 10 min. The GC oven program was as follows: initial 60 °C for 1 min, 10 °C min-1 until 280 °C and held for 10 min, 40 °C min-1 until 300 °C and held for 2 min. A 5 min backflush was conducted as post-run at 300°C with a flow of 5.1446 mL/min to reduce analysis time and increase working life of the column.

The instrument was operated in multiple reactions monitoring mode (MRM) at 70 eV. The mass range was scanned from 70 to 900 m/z at 1 s/scan for the full-scan mode. General parameters for MRM were as follows: Gain factor 50, filament current 35 μA, dwell time 50 ms. The MS transfer line was held at 280°C, the ion source temperature was 230 °C and quadrupole temperatures were 150°C. In the collision cell Nitrogen was used as collision gas at a flow of 2.25 mL/min and Helium as quench gas at 1.5 mL/min.

Samples were analysed for nine PBDEs (BDE–28, –47, –68, –85, –99, –100, –103, –119, –133, –153, –154, –183), eight methoxyated PBDEs (5MeOBDE-47, 6MeOBDE-47, MeOBDE-49, -68, -99, -100, -101, -103), twenty four alternate BFRs (2,4,6-tribromophenol (2,4,6-TBP), 2,4,6-tribromophenyl allylether (TBP-AE), 2-bromoallyl 2,4,6-tribromophenyl ether (BATE), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), Decabromodiphenylethane (DBDPE), 2,3-dibromopropyl-2,4,6-tribromophenyl ether (TBP-DBPE), 2-ethyl-1-hexyl 2,3,4,5-tetabromobenzoate (EH-TBB), Hexabromobenzene (HBB), Hexachlorocyclopentadiene (HCCPD), Hexachlorocyclopentadienyl-dibromomyclooctane (DBHCTD), Pentabromo- benzyl acrylate (PBBa), Pentabromobenzyl bromide, 1-bromoethy-2,3,4,5,6-pentabromobenzenes (PBBB), Pentabromobenzene (PBZ), Pentabromoethybenzene (PBE), Pentabromotoluene (PBT), Tetabromo-p-xylene (TXB), 2,4,6-tetrabromoisocyanurate (TBA), Tris(2,3-dibromopropyl) isocyanurate (TBC), Tetabromo-o-chlorotoluene (TBCT), Tetrabromophthalic anhydride (TBP-Anh), Bis(2-ethyl-1-hexyl)tetabromophthalate (TBP), α/β-tetabromoethylyclohexane (α/β-DBE-DBCH), α/β-1,2,5,6-tetabromocyclooctane (α/β-TBCO)), Dechlorane Plus (DP), the one- and two-fold dechlorinared DP species (aCl11DP [-1Cl+1H], aCl10DP [2Cl+2H], DPMA, and Dechlo rane 602, 603 and 604 (see Table 1 for MS/MS parameters and instrumental detection limits).

NCI analysis was based on a method developed by Möller et al. (Möller et al. 2010). The method was extended to include further analytes and a backflush system. The instrument operated in single ion monitoring mode (SIM) with methane as reactant gas. It was fitted with a HP-5MS column (30m x 0.25mm i.d. x 0.25 μm film thickness, J&W Scientific) and a restriction capillary (0.8m x 0.1 mm i.d., deactivated).The injector was operated in pulsed-splitless mode (injection pulse 20 psi for 2 min) with an inlet temperature program: 60 °C for 1 min, 500°C/min until 28 °C and held for a final 20 min. The GC oven
program was as follows: initial 60°C for 2 min, 30°C/min until 180°C, 2 °C/min until 280°C, 30 °C/min until 300°C and held for 5 min. A 15 min backflush was conducted as post-run at 300°C with a flow of 5.1446 mL/min. General parameters for SIM were as follows: Gain factor 50, filament current 35 μA, dwell time 50 ms. The MS transfer line was held at 280°C, the ion source temperature and quadrupole temperatures were 150°C. In NCI eels were analysed for 14 alternate BFRs (TBP-AE, PBB, TBCT, BATE, PBEB, PBT, HBB, TBP-DBPE, PBBA, DBHCTD, EH-TBB, BTBPE, TBC and TBFH), syn and anti-DP, aCl11DP, aCl10DP, 1,5-DPMA, Dechlorane 602, 603 and 604, as well BDE-66, BDE-100 and BDE-154.

QA/QC
Extraction and clean-up were conducted in a clean lab (class 10000). Materials containing FRs were avoided during sample preparation and analysis.

Surrogate recoveries were determined for every eel sample and all concentrations were recovery corrected.

A blank test, using Na2SO4 treated similar to real samples, was conducted with every extraction batch (five samples). General concentrations of FR in blanks were between 1 pg absolute for HBB and 140 pg absolute for TBP. Average blank values were subtracted from concentration found in the samples.

The limit of detection (LOD) was calculated from a signal to noise ratio of three or by using the blank standard deviation method (where applicable). The limit of quantification (LOQ) was calculated from a signal-to-noise ratio of ten or using the blank standard deviation method (where applicable). LODs in ECNI ranged from 0.2 pg absolute for DDC-DBF to 190 pg absolute for HCTBPH. In EI LODs ranged from 0.3 pg absolute for DPMA to 25 ng absolute for BDE-183.

Twofold measurements were conducted for all samples.

Reporting
All concentrations were calculated on a wet weight as well as lipid weight basis. pg/g or ng/g were used as units for reporting.

5.1.4 Dioxins
A number of studies targeting Dioxins along with other contaminants in eels from different German water-bodies are available in publications in peer-reviewed journals. Extraction usually is done by soxhlet extraction with toluene and analyses for Dioxins were performed by high-resolution Gas chromatography / high-resolution mass spectrometry.

Listed are some of the recent German studies targeting dioxins in eels:
Wiesmüller, T., Schlatterer, B. (1999) investigated PCDDs, PCDS and coplanar PCBs in eel filet tissue from the Havel and Oder river. Stachel et al. (2004) investigated PCDDs and Furans as well as dl-PCBs in muscle samples of 24 eels in River Elbe. Stachel et al. (2007) investigated PCDD and Fs in individual and composite samples of eel muscle in different locations along the river Elbe. Guhl et al. (2014) investigated muscle tissue of a total of 119 eels in 13 sampling sites of North Rhine-Westphalian rivers for PCDD/PCDF, PCBs and PBDEs as well as for PFOS, HCB, perfluorooctane and mercury.
For Quality Control blank measurements and spiked internal standards were used in most studies.

5.1.5 Metals
A number of studies targeting metals in eels from different German water-bodies are available in international publications in peer-reviewed journals.

Also, a number of eels deriving from the DCF samplings were analysed for lead, cadmium and mercury in muscle and liver tissue. Results have not been published yet.

5.1.6 PAHs
Background
PAH are rapidly metabolized in fish so that their metabolites accumulated in the bile fluid are widely accepted as measures for PAH exposure. Further on PAH metabolites in fish are recommended as core monitoring parameter in European Seas (HELCOM 2012a, OSPAR 2008). PAH metabolites in bile samples can be determined by different methods reviewed in Ariese et al. (2005). For single metabolite identification HPLC or GC methods should be preferred which have been shown to produce comparable results (e.g. Kammann et al., 2013). PAH metabolites have been successfully measured in eel (Ruddock et al. 2003, Nagel et al. 2012a, Kammann et al., 2014, Szlinder-Riechert et al., 2014.)

Sampling
As samples bile fluid is collected by piercing the gall bladder with a needle fitted to a disposable syringe. Bile samples of approximately 0.1-0.5 mL should be stored frozen until analysis.

Method
In Germany the HPLC-method is used as described in Kammann et al. (2014). In brief 25μL fish bile were mixed with 95μL water and 5μL β-glucuronidase/arylsulfatase solution (30-60 U/ml). The mixture was subsequently incubated for 2 h at 37°C on a heated shaker. The enzymatic reaction was stopped by the addition of 125μL of ethanol. After centrifugation the supernatants were used for HPLC analysis immediately. PAH metabolites 1-hydroxypyrene and 1-hydroxynaphthene were separated by HPLC (Lachrom System; Merck Hitachi). Samples were chromatographed on a Nucleosil 100-3 C18 (3 x 125 mm) reversed phase column at a flow of 0.55 mL/min. The initial mobile phase was acetonitrile/0.1% trifluoroacetic acid 50/50 (v/v). After 10 min the solvent composition progressively changed to 60 % acetonitrile over 4 min and reached afterwards 100% acetonitrile within 2 min. A fluorimetric detector was attached to the HPLC system. The excitation/emission wavelength pairs for 1-hydroxypyrene and 1-hydroxynaphthene were 346/384 nm and 256/380 nm, respectively.

For the quantification of bile pigments 25μL of bile or appropriate dilution was added to 475μL of water and the absorbance was recorded at 380 nm in microplates and recalculated for a standard cuvette.
Recorded parameters

- 1-hydroxypyrene and 1-hydroxphenantrene were recorded in ng/ml bile.
- Absorbance at 380 nm (A380) were recorded in arbitrary units (a.u.) /ml bile. This is a relating parameter.
- information on the developmental stage
- Optional: results related to A380 nm are given in ng/a.u.380nm

QA/QC

The limit of detection (LOD) and the limit of quantification (LOQ) depend on the analytical instrumentation and matrix complexity. As an example the LOD and the LOQ for 1-hydroxypyrene (1-hydroxphenantrene) was 3.4 (0.5) and 22.5 (1.7) ng mL⁻¹ bile, respectively for eel analysed with the HPLC method described above.

Quality assurance was performed by an internal laboratory standard added to each sample batch and by participation in an international intercalibration trial of PAH metabolites in fish bile samples taken place in 2010 under the lead of Thünen Institute (Kammann et al. 2013). No standard reference materials are available at the moment on the market.

Need for normalisation

PAH metabolites as well as bile pigments tend to concentrate in the fish bile when the fish is starving. Therefore it is not surprising that the concentration of PAH metabolites in eel bile is influenced by the developmental stage (Nagel et al., 2012b). A normalization of PAH metabolites to bile colour (A380) is requested, when fish of different nutrition stages and bile colours (e.g. yellow eel and silver eel) shall be compared. However, every relating parameter adds new errors to the data set and should be only used if advantageous for the interpretation. This can be the case when a correlation between A380 and PAH metabolites is present in the data set.

It should be taken into account that no accepted standard substance exists yet for A380 which represents an unknown mixture of different aromatic bile components. As a consequence the arbitrary absorption units may differ with the measuring device.

For an international database we suggest to use ng/ml or ng/g data of single PAH metabolites because they have been proven to be comparable from lab to lab. A380 should be an optional information as relating parameter.

Assessment criteria

Some threshold values exist for PAH metabolites in fish bile indicating the level of contamination for “unacceptable effects” to the organism (ICES, 2012). Even if these values are related to marine fish species and not to the eel, they can act as a rough guidance for the contaminant levels.
5.1.7 Bile color

Background

The bile color or bile pigments is a simple measure for the nutrition status of the eel. Measured as absorbance at 380 nm (A380) it can indicate if a fish is starving. This information alone or together with the maturation stage and the fat content may lead to a better identification of eel in the premigratory stage.

Kammann et al. 2013 could show for eel caught in German rivers that when bile pigments were used for the identification of starving/migrating eel the biological characteristics (maturation stage, fat, length, age, eye diameter, etc.) of these fish fitted well to migratory eel identified in previous investigations. The authors hypothesized that bile pigments can be new indicators contributing to identify the proportion of migratory eel, which is crucial for a fresh water habitat in the light of the European eel management.

Sampling

As samples bile fluid is collected by piercing the gall bladder with a needle fitted to a disposable syringe. Bile samples of approximately 0.1 mL should be stored frozen until analysis.

Method

For the quantification of bile pigments 25μL of bile or appropriate dilution was added to 475μL of water and the absorbance was recorded at 380 nm in microplates and recalculated for a standard 1cm cuvette.

(The bile colour assessed as A380 is a relating parameter for PAH metabolites in bile.)

Recorded parameters

- Absorbance at 380 nm (A380) were recorded in arbitrary units (a.u.) /ml bile.

QA/QC

None yet

There is no problem with this parameter when comparisons are done in one laboratory only. If results shall be compared on a broader basis some standardisation is recommended in advance.

5.2 Interpretation, visualisation and assessment

The Eel Patho-Index, a Health Status Classification System

In the context of a scientific monitoring of restocking, within an EFF project (2012-2015) in North Rhine Westphalia, a lot of eel health data have been collected. The prompt visualization of these data is a quite difficult task. To easily examine the suitability of different eel habitats for stocking and to get a quick overview of how many healthy eels
will possibly make it to the Sargasso Sea, a management tool has been developed. With the help of this tool data can be visualized quickly and one can get a good and quick overview of where the “best” eels are and which rivers are most appropriate for stocking. The management tool, a “Health Status Classification System (HSCS)”, is called “Eel Patho-Index (EPI)”. The different symptoms of the eels are being assessed and classified into different classes (blue = 1=very good health, green = 2=good health, yellow = 3 = moderate health, orange = 4= unsatisfactory health, red = 5= poor health) (Tab. 1). Different symptoms/parameters need to get a different rating depending on how much they influence the eel quality. The rating of the different parameters is still to be developed and cannot be shown here, yet.
When all symptoms of an analyzed eel are classified and rated, the final score of an eel is the mean value of the classes for the symptoms identified. The eel can then be assigned to its class relating to its health status.
The Patho-Index is an attempt of developing a simple and quick tool to visualize a lot of data. It is still in a working process and needs to be discussed and developed further.

Table 1: Eel Patho-Index (EPI) Classes.

<table>
<thead>
<tr>
<th>External diagnosis</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>No detectable inflammations, ulcers or lesions of gills and skin/fins</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No severe inflammations, ulcers or lesions of gills and skin/fins</td>
<td></td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slight or distinct external lesions (inflammations, ulcers, pock-marked skin/fins)</td>
<td></td>
<td></td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>External lesions (inflammations, ulcers, pock-marked skin/fins), not in correlation to a diagnosis of a disease and/or a change in clinical data (like hematological parameters)</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Internal diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No detectable pathological symptoms of the inner organs (including swim bladder)</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Distinct (macroscopic) pathological change of one or several inner organs (including intestinal tract), exudate in the coelom</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Viral diseases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viral disease(s): negative</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Viral disease(s) positive in the PCR but no “typical” clinical signs and cell cultures negative</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Viral disease(s) positive in cell-cultures and in PCR (Ang-HVI or/and EVE, EVEX).</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Bacterial diseases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial disease(s): negative</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Bacterial disease(s) by relevant bacteria not seldom, but more or less often isolation of “unspecific agents” (blood and/or inner organs), whose pathogenicity is unclear.</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Bacterial culture positive, clinical signs negative, or divergent</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Bacterial culture of relevant bacteria positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Hematological data</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemogram: normal values;</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td></td>
</tr>
</tbody>
</table>
Hemogram shows lightly abnormal values;  
Hemogram shows pathological changes;  
Hemogram pathological changed, no relevant clinical signs;  
Hemogram typical pathological changed, especially strong anaemia, relevant clinical signs;  

<table>
<thead>
<tr>
<th>Physiological data</th>
</tr>
</thead>
</table>
| Physiological data (Lactat, Glucose, Lipids, etc.): normal values | x  
| Physiological data (Lactat, Glucose, Lipids, etc.): lightly abnormal values | x  
| Physiological data (Lactat, Glucose, Lipids, etc.): abnormal values | x  

<table>
<thead>
<tr>
<th>Parasites</th>
</tr>
</thead>
</table>
| No detectable relevant ecto- and/or endoparasites | x  
| Relevant parasites, especially *Anguillicoloides crassus*, could be present, but only in a small rate of infestation (swim bladder wall not distinctly thickened, not black). | x  
| Relevant parasites often found. (*Trypanosoma granulosum*, *Myxidium*, *Pseudodactylogyrus*) a special aspect: Parasitation by the Nematode *Anguillicoloides crassus*, which is linked to distinct pathological change of the swim bladder-wall. | x  
| Relevant parasites found. --> Untypical laboratory or clinical data by classical diseases | x  
| Very high infestation by *Anguillicoloides crassus* and a strong pathological change of the swim bladder | x  

<table>
<thead>
<tr>
<th>Mycosis</th>
</tr>
</thead>
</table>
| No relevant mycosis | x  
| Systemic mycosis of *Ichthyosporidium hoferi* and *Branchiomycetes* of gills | x  

<table>
<thead>
<tr>
<th>Level</th>
<th>Eel health status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>very good</td>
</tr>
<tr>
<td>2</td>
<td>good</td>
</tr>
<tr>
<td>3</td>
<td>moderate</td>
</tr>
<tr>
<td>4</td>
<td>unsatisfactory</td>
</tr>
<tr>
<td>5</td>
<td>poor</td>
</tr>
</tbody>
</table>

### 6. Diseases

#### 6.1 Sampling and analysis of eels for parasitology (*Anguillicoloides* and others)

For general assessments of parasites in eel see Jakob *et al.* 2009

In North Rhine Westphalia the major parasites analyzed are *Pseudodactylogyrus*, *Anguillicoloides crassus*, *Sporozoa* (*Myxidium*) and *Trypanosoma*. For the analysis of parasites Jakob *et al.* (2009) is used. Gills, gut, mucous membrane and especially the swim bladder are examined.
6.1.1 Anguillicoloides crassus

Anguillicoloides crassus is generally monitored in eels meant for restocking purposes in a number of German federal states (e.g. Mecklenburg Vorpommern, Brandenburg, Berlin, Sachsen-Anhalt). Also, a number of studies have been carried out concerning the infestation rates and damage (Hartman classes, Hartman 1994) of eels in different water bodies. Some of these studies are available to the international community due to their publication in peer-reviewed journals others are made available online in form of (regional or project-based) reports.

For Anguillicoloides crassus sampling and analysis of fish sampled within the DCF:

The whole swim bladder of each individual eel is excluded from the dead eels body and stored frozen at -20°C (individually packed) in aluminum foil. Swim bladders are examined after thawing and general signs of damage (Hartman classes, Hartman 1994), presence of larvae in the swim bladder wall as well as intensity (count of A.c. in swim bladder) and size (<1, <2, <3, <4, >4 cm – length classes) of A.c infestation are recorded. Results are yet unpublished.

Recent German studies concerning A. crassus (from the appendix of the German report for the WGEEL)

The infestation of eels with the swim bladder parasite Anguillicoloides crassus in north German inland and coastal waters was studied by Wysujack et al. (2014). Leuner (2013) studied the infestation with A. crassus in eels in Lake Starnberger See. Kullmann (2014) studied the infestation with A. crassus in eels from the river Elbe estuary, the Kiel Canal and the Elbe-Lübeck-Canal. Information on infestation of eels with A. crassus is also given by Marohn et al. (2014a) for the Schwentine system.

Scientists from University Duisburg/Essen made following remark regarding this topic:

The swim bladder wall of eels should always be searched for larval stages of Anguillicoloides sp. for a lack of adult stages inside the swim bladder does not equal an uninfecteed eel (see Dangel & Sures, 2013).

Please note that, even though Moravec (2006) split the genus Anguillicola into the two genera Anguillicola and Anguillicoloides due to morphological differences, the phylogenetic study of Laetsch et al. (2012) on Anguillicolidae, however, found no support for the maintenance of these two genera, therefore all species of the family should be referred to as Anguillicola.

6.1.2 Other, like trematodes?

The parasite Trypanosoma is found in fresh or fixed blood smears of wild eels. It could be detected in over 90 % of eels examined from the Rhine in North Rhine Westpalia (Lehmann et al., 2007).

6.2 Sampling and analysis of viral and bacterial diseases

In DCF: Only rough skin condition and obvious disease patterns are recorded.
6.2.1 Viral diseases

- What is recorded in the necropsy room, clinical pathology etc.?
  Inner and external symptoms of viral disease as there are an intense reddening of the skin, anemia and/or exsudates.

- What part of the body for which analysis, per stage of eel? Which tissues?
  Sampling and subsampling per group of pathogens (including numbers).
  Spleen: HVA; Gills; Heart, liver and spleen.

- Which isolation and/or test method for identification, existing protocols with references per virus
  The tissues are inoculated onto an EK-1 Cell-line. If the Cytopathic effect (CPE) is positive a PCR is performed to determine a HVA (Rijsewijk et al. 2004).

6.2.2 Bacterial diseases

- What is recorded in the necropsy room, clinical pathology etc.?
  Inner and external symptoms of bacterial disease as there are an intensive reddening of the skin, ulcers, exudates or internal bleeding. The major bacteria found are *Aeromonas*/*Pseudomonas* and *Vibrio*.

- What part of the body for which analysis, per stage of eel? Which tissues?
  Sampling and subsampling per group of pathogens (including numbers).
  There are subcultures taken from the liver, spleen and kidney.

- Which isolation and/or test method for identification, existing protocols with references per bacterium
  TS agar, CA agar, TCBS agar, Mac Conkey agar, sheep blood agar. Biochemical differentiation with Testkits (API).

7. Perspectives of using biomarkers of effects to assess eel health

The activity of the metabolism enzymes EROD and GST have been determined in eel caught in German rivers (Kammann et al. 2014). The authors concluded that EROD activity in the liver of European eel can be used to describe the habitat quality in German rivers. However, EROD is a parameter which is influenced by many biological parameters. It has been discussed controversially for a long time for marine monitoring and the method is well described e.g. in Hylland et al. 2012.

Metallothioneins (MT) in livers of eel from German rivers have been investigated in 2014 by the Thünen Institute. The results are still unpublished.

We think that biological effect assessments like EROD and MT have the potential to support habitat quality assessment since contamination levels in eel are often fairly high, so that biological effects are more likely to occur. However, assessment criteria for eel are missing and available data are scarce.
8. International needs (making your results available, and international database)

Depending on the institution and the purpose of each scientific approach, results of some of the studies concerning eel quality, contaminants and diseases are made available through publications in peer reviewed journals, through institutional reports on the respective websites, or as reports mentioned in the appendices of the WG EEL country reports. Unfortunately some reports are also only made accessible in German language on regional institute / administrations web pages, which most likely impairs (international) accessibility.

Raw data usually are neither included nor accessible. Sometimes supplementary information is uploaded with publications in peer-reviewed journals.

9. Other issues, remarks

The Thünen Institute is active in environmental monitoring of marine fish. Eels could fit in the existing monitoring program of contaminants and their effects in the future. However, as money is limited, contaminants in eel were measured by third parties on project bases only, or closely linked to the Framework of eel sampling done within the DCF.

At the moment the quality data on eel are not reported to national or international data bases. The performed chemical analyses have been paid by research budgets of the respective research institutions Thünen Institute, Helmholtz Zentrum Geesthacht (HZG), University Duisburg-Essen or RWTH Aachen respectively.

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Additional information from Ireland

This note was prepared in preparation of the ICES Workshop of a Planning Group on the Monitoring of Eel Quality: “Development of standardized and harmonized protocols for the estimation of eel quality” (WKPGMEQ), held at INBO Brussels from 20 to 22 January 2015.

Eel Quality in Ireland: Some initial observations

T K McCarthy (National University of Ireland, Galway) and Russell Poole (Marine Institute, Ireland)

In the Republic of Ireland (RoI) various government agencies are involved in monitoring water quality especially The Environmental Protection Agency (EPA); The Marine Institute (MI) and Inland Fisheries Ireland (IFI). These organizations produce regular local and national reports, that are available on the internet and as hard copies, and they participate in various international research programmes and undertake monitoring required by EU legislation. University based research teams are also actively engaged in a wide variety of projects on aquatic environmental topics. However, as in many areas of applied research, lots of the results of work undertaken by the various agencies and by university students remains in the grey literature or in unpublished reports. Limitations in resources and the pressure to deal with present day problems make it difficult to collate and interpret results of some earlier studies. It seems likely that the situation in Northern Ireland (NI) parallels that in RoI, though different government agencies are involved.

Fish kills in the RoI that are reported to IFI and MI are logged and annual summaries are published but, though these regularly involve eels, the focus until recently was mainly on salmonid fish populations and any loss of eels has not been quantified. Eel population monitoring, as required by the EU Regulation for Restoration of the European Eel Stock, relies in part on the results of multi-species fish surveys (electrofishing, fyke-netting etc.) on fish surveys undertaken in respect of the EU Water Framework Directive. Fish kills in RoI have generally been associated with release of organic waste (agricultural, sewage and industrial sources) to streams and rivers. Point sources involving toxic chemicals are relatively rare though out most of the country, reflecting the pattern of industrial development. However, direct monitoring of local or once-off contamination of eel habitats may be inadequate and ideally there should be more comprehensive monitoring by examination of representative eel samples.

Few studies of contaminant levels have been undertaken in Ireland. Earlier results were made available in studies of a series of European countries. Santillo et al. (2005) reported on contaminants (Brominated Flame Retardants and PCBs) from 20 samples in 10 countries and the levels found in two Irish samples were among the lowest of all the samples they examined. Further international collaborative work involving Irish eel samples may be anticipated from large scale EU funded projects (e.g. Eelad). Nationally funded work is limited because eel is not routinely sampled in monitoring contaminants in food supplies. Mc Hugh et al. (2010) who found that levels of various contaminants present in Irish eel samples were similar to those reported in many parts of Europe but that levels of
POP (persistent organic pollutants) in Irish eels were lower in comparison to those studied elsewhere. White et al. (2014) described subsequent research that involved application of congener based multi-matrix profiling techniques for identification of potential PCDD/F sources in the Burrishoole catchment – how representative this is of other catchments in Ireland is currently unknown. A MI Report (McGovern et al. 2011) provides details of protocols used in laboratory analyses as well as summary results for a wide range of marine food contaminants. The only use of eels in this programme involves tracking an historical release of PCB’s, following an industrial fire, into the Breagagh tributary of the River Nore.

There has been a lot of research undertaken on parasites of eels in Ireland (in both RoI and NI). A general review of the earlier literature (McCarthy et al. 2009) illustrated the biodiversity of the parasite assemblages in Irish eel populations and, as noted earlier (Conneely & McCarthy 1986) it was pointed out that indigenous fish hosts generally have more species rich assemblages than those of introduced fish species. The latter make up over half the listed species in Ireland. Irish eel parasite species assemblages reflect environmental conditions (water flow, eutrophication etc.) and presence of other host fish species. Many common parasites of Irish eels were anthropogenically introduced along with cyprinids and other recent fish introductions (McCarthy et al. 2009). However, non-indigenous host-specific eel parasites have been progressively introduced in the past few decades including: Pseudodactylogyrus anguilae (1st recorded by McCarthy & Rita 1991), Anguillicola crassus (1st recorded by McCarthy et al. 1999) (Pseudodactylogyrus bini (1st recorded by Copley & McCarthy 2001). The biogeographical status of the nematode Daniconema anguilae another species that was recently added to the list of Irish eel parasites by Morressey and McCarthy (2008) remains to be established. However, there are now many records and eel population surveys of the other three recent eel specific introductions and range expansion by A crassus is well documented (Evans and Matthews, 1999, Evans et al. 2001, Morressey & McCarthy 2007, McCarthy et al. 2009, Beccera‐Jurado, et al. 2014 and McCarthy unpublished). Further work on A. crassus has been undertaken in a variety of research projects (undergraduate and post‐graduate levels) at NUI Galway and monitoring of eel populations of hydropower impacted rivers in which silver eel escapement is being estimated (e.g. McNamara & Mc Carthy 2012 and McCarthy et al. 2014) and during eel surveys undertaken by state agencies (IFI, MI) in support of Irelands Eel Management Plan (EMP). Reports on EMP related research are compiled annually and reported as Country Reports to the Working Group on European eel (WGEEL). The protocols used for analysis of parasite infection parameters vary (e.g. some agencies do not record larval A. crassus infections) but are generally well described in the publications and reports referenced above. A major gap in our knowledge of eel pathogens in Ireland relates to the almost total lack of information on viral infections, though the risk of introductions associated with importation of glass eels from Britain and mainland Europe has been highlighted.

Fat levels in Irish eels have been monitored in the Rivers Shannon, Erne and Lee by the National University of Ireland (unpublished) using both the laboratory Soxhlet method and field measurements using Distell Fat meters (calibrated vs Soxhlet method). The recorded fat levels have been used, together with morphometric data and parasite burdens, to calculate an Index of Effective Spawner Escapement. It was considered that a silver eel may not be an effective spawner if:
1. It fails to meet certain published criteria (Eye Index and Pectoral Fin index) for definition as a silver eel (Durif et al. 2005)
2. It had low fat levels (Van den Thillart et al. 2004)
3. It was infected by A. crassus.

Eels were excluded if they fail to meet any of these three sets of criteria, though it was mostly due to presence of A. crassus, (which has high prevalence in the Rivers Shannon and Erne but is absent from the River Lee). Though it is appreciated that not all eel researchers will agree with this approach to monitoring eel quality, the analyses have drawn attention for the need for a more comprehensive approach to eel quality monitoring in Ireland. The results suggested that, though spawner escapement from rivers such as the Shannon and Erne appear to be high and that they meet EMP targets, eel quality greatly reduces the potential contribution of silvers eels from major Irish rivers to the recovery of the European eel stock. Fat levels were recorded in Irish eels tagged during the Eliaid project and are also being monitored in some other Irish rivers e.g. by MI at Burrenhouse (R. Poole, unpublished) using calibrated fat meters (Distell).

Fecundity of Irish eels has been investigated in the River Shannon (MacNamara & McCarthy 2012) and provisional estimates of egg production from some Irish rivers have been calculated. However, further research may be needed, both in Ireland and elsewhere, to allow for adoption of this parameter in routine eel quality monitoring.

A comprehensive review, of the available data on eel quality and the monitoring protocols used in Ireland, has not been possible in advance of the WKPMEQ meeting in Brussels. However, these preliminary observations may be helpful at this stage and perhaps over the next few days or after the meeting it may be possible to contribute in other ways.

In the event of there being a recognition at the WKPMEQ of the need for a funded project to advance our knowledge of the consequences of poor eel quality, we in NUI Galway and in the Marine Institute will be keen to participate and to share our experiences and research resources. We expect other Irish eel researchers will also be well disposed to such a development.

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Report on the methods and assessment of eel quality (contaminants and diseases) in:

Italy

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Introduction

This country report has been drafted in preparation of the ICES Workshop of a Planning Group on the Monitoring of Eel Quality: “Development of standardized and harmonized protocols for the estimation of eel quality.” (WKPGMEQ), held at INBO Brussels from 20 to 22 January 2015.

In September 2009 the final version of the Italian Eel Management Plan was presented, prepared by the Directorate General of Maritime Fisheries and Aquaculture (Ministry of Agriculture, Food and Forestry, MIPAAF), with the support of experts from the scientific community and representatives Regions. The Italian National Plan in its final version, approved in 2011, takes into account the complex reality of the national territory.

Member States then, every three years, and starting in 2012, are required to report to the Commission on the implementation of management plans, and on the effectiveness of the measures. In these periodic reports, each Member State should explicitly evaluate and quantify the state of the stock based on the assessments presented in the Management Plan, in terms of escaping biomass, and to determine the reduction of mortality by short, medium and long term measures. The purpose of these periodic reports is to provide data for a stock assessment on a global level (stock-wide assessment).

The need to prepare management plans first, required Member States, an impressive job of collecting data on both the various activities of exploitation of eel both individual stock locally, at the level of Management Units (catchment areas or individual systems, or management units at the administrative level, as the regions in Italy). In Italy only 9 regions (there are 20 regions in this country) decided to produce a Regional management plan.
2. Framework national cases and national guidelines (on contaminants and health)

2.1 Contaminants

There is not currently a routine monitoring network measuring contaminants in eel in Italy. As described in the previous section, EMUs in Italy correspond to the Administrative regions and none of the 9 which contribute to the National Management plan have foreseen measures for controlling contamination in eel local stocks (MIPAAF, 2014).

In 2006 the Italian Ministry of University and Scientific Research founded a research project (PRIN Project 2006 n. 2006054928, “An Integrated Approach to the Conservation and Management of the European Eel in the Mediterranean Region”) that dealt for the first time with the assessment of European eel contamination and diseases in two coastal lagoons and in a river. The project was carried out by University of Parma, University of Politecnico di Milano, University of Padova and University of Rome “Tor Vergata”.

In 2015 a research project of the Agricultural Research Council (CRA), a National Research Organization, granted by the Italian Ministry of Agriculture Food and forestry (MIPAAF) is going to start with the aim of assessing silver eel spawner quality in two coastal lagoons in central Italy (Tyrrhenian coast). This project will highlight the importance of considering contaminants and diseases in eel management plans and conservation measures of each Italian EMUs by trying to set up common analytical protocols.

From 1997 to 2013 some independent scientific studies (n. 16) have been carried out in different sites describing contamination and diseases in European eels in Italy (pdf are available in the SharePoint).

2.2 Diseases

No routine monitoring network measuring disease agents in eel exist in Italy

3. General issues on eel sampling for contaminants and/or diseases in your country. Treatment of the sampled eels on site and in the laboratory.

3.1 Ethics and legislation

Legislation: General issues on eel sampling including EU and national legislation (sampling is experimental work with animals – eels are protected species – etc.); Ethical issues. Required permits.


3.2 Sampling procedures

In Italy no references are found concerning national standardization of methodology on eel scientific surveys. The most usual method to sample eels are fyke nets since coastal lagoons are the most important ecosystems for eels in Italy. In most of the cases eels were obtained from fisherman, who caught them using also longlines.
3.3 Stage, gender, morphometrics
In most of the studies weight and length are measured in anesthetized or frozen eels. Eel stage is generally reported. In some scientific studies a combination of methods for stage determination has been used: eye index (Punkhurst, 1982) and both macroscopic and histological analysis of gonads (Grandi & Colombo, 1996).

3.4 Treatment
In some scientific studies eels were euthanized by submerging them in a solution of water and 2-phenoxyethanol: \((\text{C}_8\text{H}_{10}\text{O}_2)\) (2%) till death.

4. General issues on eel condition (fitness, lipid levels)

4.1 Condition
Pujolar et al. (2009; 2013) determined the eel condition factor \((K)\) as \(K = (\text{TW} \times \text{TL}^{-3})\) in which \(\text{TW}\) was the total weight (in gram), and \(\text{TL}\) was the total length (in millimetre).

4.2 HSI
NA

4.3 GSI
NA

4.4 Lipid levels
In all scientific studies taken in account lipid levels were analyzed as % in muscle tissue.

4.5 Other condition related issues
NA

5. Contaminants
European eels from several site were analyzed for contamination in independent studies in Italy. The locations studied were listed below:
Rivers: Po (Emilia Romagna, Delta), Tiber (Latium, low course), Garigliano (Campania, estuary),
Coastal lagoons: Orbetello (Tuscany), Santa Giusta (Sardinia), Lesina and Varano (Apulia), Caprolace (Latium)
Lakes: Bacciano and Bolsena (Latium, volcanic lakes)
5.1 Sampling and analysis

5.1.1 PCBs

All scientific studies taken into account analyzed PCB levels in muscle tissue (mainly dorsal part just behind the head) of eel (only one also in liver, Renzi et al., 2013). The number of PCBs analysed varied from 5 to 33 but all papers showed the Sum PCBs values. Unit of measured were also different such as ng/g or μg/g in both lipid and wet weight basis. The most used method to asses PCB contamination has been the gas chromatography–mass spectrometry (GC-MS).

5.1.2 Pesticides

Bibliographic research of scientific papers dealing with contamination by pesticides in European eels from Italian environments results in ten articles (Bressa et al., 1997; Corsi et al., 2005; Orban et al., 2009; Burroni et al., 2010; Bettinetti et al., 2010; Ferrante et al., 2011; Pujolar et al., 2012, 2013; Quadroni et al., 2013, Renzi et al., 2013). Different compounds were analyzed such as p,p′-DDE, p,p′-DDT, p,p′-DDD, HCB, α,β,γ-HCH. There are also differences in unit of measures (ng/g or μg/g) in both lipid and wet weight basis. The most used analytical procedure was both gas chromatography–mass spectrometry (GC–MS) with electron impact ionization (EI), and by liquid chromatography–mass spectrometry (LC–MS).

5.1.3 Brominated Flame Retardants

Three papers analyzed Brominated Flame Retardants (Burroni et al., 2009, Miniero et al., 2011; Pujolar et al., 2013). They were polychlorodibenzo-p-dioxins (PCDDs), polychlorodibenzo-p-furans (PCDFs) and polybromodiphenylethers (PBDEs: 17, 28, 47, 66, 99, 100, 138, 153, 154, and 183). Different unit of measures were used (ng/g or μg/g) in both lipid and wet weight basis. In all cases muscle tissues were used.

5.1.4 Dioxins

See above under 5.1.3.

5.1.5 Metals

Three recent studies (Storelli et al., 2007; Pujolar et al., 2012; Renzi et al., 2012) analysed the concentration of heavy metals in muscle and liver (only one study) of eels. Metals analysed were Ag, As, Cd, Co, Cu, Cr, Hg, Ni, Pb and Zn. Results obtained with atomic absorption spectrophotometer (Storelli et al., 2007; Renzi et al., 2012) or High Resolution Inductively Coupled Plasma Mass Spectrometer (Pujolar, et al. 2012).

The unit of measure used was ng/g or μg/g of wet weight. Only Renzi et al. (2012) carried out a quality assurance and quality control (QA/QC).

5.1.6 PAHs

Only two studies cope with Polycyclic aromatic hydrocarbons (fluoranthene = Fl, benzo(b)fluoranthene = BbF, benzo(k)fluoranthene = BkF, benzo(a)pyrene = BaP, benzo(g,h,i)perylene = BgP, indeno(1,2,3-c,d)pyrene = InP)(Patrolecco et al., 2010 and Renzi et
analyzed with RP-HPLC with fluorescence detection technique. Unit of measure was ng/g wet wt. Muscle tissue was used.

5.1.7 Others?
NA

5.2 Interpretation, visualisation and assessment
NA

6. Diseases

6.1 Sampling and analysis of eels for parasitology (*Anguillicoloides*)

6.1.1 *Anguillicoloides crassus*
Assessment of *Anguillicoloides crassus* presence is carried out in biological samplings for DCF programme for European eel, but results are not reported. Pujolar et al. (2009) and Quadroni et al. (2013) search for this parasite in different environment (Tiber river, Lesina lagoon and Caprolace lagoon). After dissected the eel, swim bladder was removed carefully and examined macroscopically with the aids of a stereomicroscope. Parasites were weighted and the swim bladders were analyzed in order to detect the presence of encapsulated larvae in the wall. Classical epidemiological parameters—i.e. prevalence (percentage of infected individuals on the total examined eels), abundance (number of parasites in a specimen) and mean intensity (average number of parasites in the infected eels)—were calculated according to Bush et al. (1997). Following Szekely et al. (2005), the infection level was calculated as the ratio between the parasite total weight and the eel weight (Wp/W). The relative swim bladder length or Length Ratio Index (LRI), computed as the ratio between the swim bladder length and the eel length (Ls/L), was used for establishing the degree of the mechanical swim bladder damage: the smaller LRI, the higher the damage (Lefebvre et al. 2011).

6.1.2 Other, like trematodes?
NA

6.1.3 Other....
NA

6.2 Sampling and analysis of viral and bacterial diseases
NA

6.2.1 Viral diseases
NA

6.2.2 Bacterial diseases
NA
7. Perspectives of using biomarkers of effects to assess eel health

NA

8. International needs (making your results available, and international database)

The few and fragmented results of eel quality assessments in Italy are available to the international community only through publications in scientific journals and the national contact point of the ICES WG EEL group and the yearly eel country reports.

The possibility to share results on eel contaminant and diseases throughout an international database would be of great importance for further improvement and knowledge of stock assessment of this species.

9. Other issues, remarks

NA

10. References


Report on the methods and assessment of eel quality (contaminants and diseases) in:

The Netherlands

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Introduction

This country report has been drafted in preparation of the ICES Workshop of a Planning Group on the Monitoring of Eel Quality: “Development of standardized and harmonized protocols for the estimation of eel quality.” (WKPGMEQ), held at INBO Brussels from 20 to 22 January 2015.

2. Framework national cases and national guidelines (on contaminants and health)

2.1 Contaminants

Routine monitoring network measuring contaminants in eel since 1978.

First for environmental reasons and food safety, later (since 2005) only for human safety (Food Safety)

Only IMARES involved initially (back then RIVO), now in cooperation with RIKILT, Institute for Food Safety in order of Ministry of Economical affairs.

IMARES/RIKILT report to the Ministry. Reports are publicly available when released by the Ministry (mostly in the year after monitoring)

Eels are samples in spring, early summer by electro fishing. For the long term trend monitoring, only yellow eels are monitored, between 30-40 cm. Since couple years also eels between 45-70 cm from various locations. Pooled samples of max 25 specimen (in some locations less, due to lack of eel). Sample locations all over The Netherlands, with focuss on main rivers.

Number of sites per year: 20-30

Are there documents describing national guidelines to assess contamination in eel (please give short description + reference)? Protocol by IMARES itself is leading.

2.2 Diseases

Routine monitoring network measuring disease agents in eel
Within the framework for eel regulation, diseases and parasites are also monitored. 30 locations (fished by fisherman) are monitored each year, eels are analysed twice per location (early and late summer), all over the Netherlands.

IMARES performs monitoring, “suspicious eels”; eels with a suspicion for other diseases, are sent to CVI Lelystad (Olga Haenen et al.) for diagnosis: no.s of tested eel per year vary, depending on offer by IMARES and other senders.

Reports by IMARES, to Ministry and ICES.
Report by CVI of each casus to sender, and report of all cases anonymously yearly to Ministry, EU Ref Lab for Fish Diseases, and ICES.

3. General issues on eel sampling for contaminants and/or diseases in your country. Treatment of the sampled eels on site and in the laboratory.

3.1 Ethics and legislation
DEC approved sampling protocol
How do you present/describe sampling sites?
In case of Contaminants; Date and location recorded, on “google earth” sampled locations are marked
For diseases/parasites: from catches of local fisherman, eels are selected. Location of fyke nets is known.

3.2 Sampling procedures
How many eels are sampled per site?
Contaminants: Max 25 eels 30-40 cm, max 25 eels >45 cm (preferably 50-65 cm) All yellow eels
Electric fishing, can not be used in deep canals nor in brackish waters.
End of May, June, when water temperatures rise and eels become active.
For parasites/diseases: 4, 4, 4, 2, 2, 2, 2 and 2 eels are selected in the length class 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90 and 91-100 cm respectively

3.3 Stage, gender, morphometrics
Do you distinguish eel stages? Which method to distinguish yellow from silver eels?
Contaminants: Any eel showing any sign of silvering (eyes, fins, color) are immediately released.
Gender is determined visually after dissection.
Weight and length after freezing (accuracy on 1 gram and 1 mm).
For disease/parasites: Gender and maturity are determined (eye diameter (height, width), fin size)
Weight and length of eel after freezing (accuracy on 1 gram and 1 mm), and liver weight is also determined.

3.4 Treatment
IMARES: Euthanize/anaesthetize eels with clove oil.

Contaminants:
Eels are dissected in the lab with clean knifes and cutting boards
Samples are stored with unique LIMS number in a special freezer (-25C).
With LIMS all data are retrievable. Fillets of specimens back to 1978 are stored.

Diseases
- Parasites (IMARES): Eels are dissected in lab.
- Disease diagnosis at CVI: The sample gets a unique LIMS number. Eels are measured (length), interpreted as being yellow or silver eel (or glass or post glass eels), checked for external abnormalities, euthanized with 2-phenoxy ethanol, and dissected in the lab with clean cutlery. A fresh blood smear (taken from the caudal vein) is screened for blood parasites, and the colour of the blood is also noted (anemic/normal). Eels are decapitated. Skin smears and fresh gill preparations are screened for parasites and bacteria, bacteriology may be done from skin ulcers and internal organs (especially liver, spleen kidney), and pooled gills, and a pool of kidney, spleen, heart of max 10 eels may be sampled for virology (virus isolation and/or PCR testing for the viruses AngHV-1, EVEX and EVE). Internal abnormalities are noted, and swimbladders are screened for Anguillicoloides. The belly cavity and gut contents are screened for parasites. Other abnormalities are noted. Musculature is checked for haemorrhages. Histopathology may be done as well, after organs are fixed in buffered formaldehyde.

4. General issues on eel condition (fitness, lipid levels)
Do you incorporate eel condition parameters in your assessments? No

4.1 Condition
Fulton index

4.2 HSI
Hepatosomatic index HSI, do you use it. Liver weight is recorded, but index is not used.

4.3 GSI
Gonadosomatic index GSI, do you use it, perspectives and limitations? No

4.4 Lipid levels
Is analysis of lipid levels included in your assessments? Yes, only for contaminants
Do you distinguish between eel stages? Yes
How are your lipid levels data expressed? In % wet weight
What part of the body? Fillet of head AND tail part are mixed equally.
Methods; Bligh and Dyer, total fat. An accredited method.

4.5 Other condition related issues

5. Contaminants

5.1 Sampling and analysis

5.1.1 PCBs
Subsampling in the lab. What part of the body for which analysis? Which tissues?
Fillet, part of head and tail.
How to measure, analytical procedures, where to measure, what to record (e.g. bile
volume, colour, proteins)
Solvent extraction, clean-up and analysis on GC-MS. Accredited methods.
QC-QA? Ringtests, accreditation, use of IRM, CRM, recovery and internal standards
Both ndl-PCBs and dl-PCBs are analysed
Report on Wet weight basis, ng/g for PCBs.

5.1.2 Pesticides
Identical to PCBs.

5.1.3 Brominated Flame Retardants
Identical to PCBs.

5.1.4 Dioxins
Identical to PCBs, but always analysed on GC-HRMS.

5.1.5 Metals
Identical to PCBs,
However, samples are stored in pastic, homogenised with titaniaum steel apparatus.
Analysis on ICP-MS, wet weight basis, mg/kg.

5.1.6 PAHs
Not analysed in eel (fish in general).
5.1.7 Others?

5.2 Interpretation, visualisation and assessment

Levels expressed on wet weight for Maximum allowable levels,
Levels expressed on lipid weight for trend analysis, comparison of locations

No contaminant quality classes for interpretation, visualisation and assessment. Levels are scored below or above limits. By model the percentage of the eel-catch that exceeds the maximum threshold levels (sum-TEQ or sum-ndl-PCB) is calculated.

Reports to Government, including graphs and tables.

Results can be found in Boer et al., 2010, Van den Thillart et al., 2009, and Van Ginneken et al., 2009.

6. Diseases

6.1 Sampling and analysis of eels for parasitology (Anguillicoloides crassus and others):

6.1.1 Anguillicoloides crassus

IMARES:

- Whole eel analysed visually, especially the swim bladder.
- What is recorded: length, weight, sexe, maturaty, thickened swim bladder, number of swim-bladder parasites, “cauliflower disease”. White spot and “red disease (red head)” are also noted.
- Internal protocol
- Swim bladder parasite: yes or no.
- QC-QA status for these techniques at the lab are not described, based on expertise analyst.
- How to report? Number of parasites, yes or no thickened swimbladder, cauliflower disease.
- What units: % of eels analysed
- Results are shown in Tables (example: Graaf, de, and Deerenberg, 2014).
- Furthermore, the following references present results: Banning, van & Haenen, 1990; Haenen, 1995; Haenen et al., 1996; Lefebvre et al., 2011; Palstra et al., 2006, 2007.

6.1.2 Others, like trematodes?

- Not done in IMARES screening, Done at CVI at general diagnosis, facultatively. Most importantly are found:
  - Trypanosomes: found in fresh or fixed blood smears of wild eels. See Rhine study with LANUV (Haenen et al., 2010), high percentage of wild eels was positive.
- *Trichodina* spp.: at skin and gills
- *Chilodonella* spp.: idem
- *Ichthyobodo* spp.: idem
- *Myxidium* spp.: in lateral organ and in head of wild eels, the holes of the lateral line are congested, full of the parasites (found once in NL, years ago)
- *Pseudodactylogyrus* spp.: at necropsy, found at skin, but more at gills
- *Gyrodactylus* spp.: idem

**Reference:** Borgsteede et al. 1999.

### 6.2 Sampling and analysis of viral and bacterial diseases

#### 6.2.1 Viral diseases

- Not done in IMARES screening. Done at CVI in diagnostic testing, on request. In the past also done as surveillance, as research.
- What is recorded in the necropsy room, clinical pathology etc.? Clinical signs of viral disease:
  - EVE: congested skin and operculum, haemorrhages in skin, pale gills, anemia, pale organs
  - EVEX: red head and fins due to haemorrhages, anemia, pale gills, organs, petechial haemorrhages
  - AngHV-1: tiger like haemorrhagic pattern at skin, anemia, pale gills and internal organs
- What part of the body for which analysis, per stage of eel? Which tissues? Sampling and subsampling per group of pathogens (including numbers) (Davidse et al., 1999).
  - For virus isolation from post glas eels, pools of gills and pools of kidney, heart and spleen are taken from max 10 eels per pool.
  - For virus isolation of glass eels, whole fish are taken, and, after the tail has been cut off, ground in pools of 10 glass eels.
- Which isolation and/or test method for identification, existing protocols with references per virus
  - The tissues are directly ground with pestle and mortar in sterile medium containing antibiotics, and inoculated onto Eel Kidney-1 cells, and incubated at 15, 20 and 25°C respectively, in 2 subsequent blind passages (Davidse et al., 1999). EVE replicates best at 20, EVEX at 15 and AngHV-1 at 25°C. After cytopathic effect has occurred, the virus is identified with Immunu-assays, like IPMA or IFAT, but nowadays, qPCR is used or these three viruses:
• Anguillid Herpes Virus (AngHV-1): qPCR: Van Beurden et al., 2015 or conventional PCR (Rijsewijk et al., 2005)
• Eel Virus European (EVE): qPCR: Orpetveit et al., 2010.
• Eel Virus European X (EVEX): qPCR: Van Beurden et al., 2011 or Meistertzheim & Faliex, 2015 (patent publication numbers EP2821507 and WO/2015/000906), or Meistertheim et al., 2015, in prep..
• QC-QA status for these techniques at the lab?
  o Cell culture: ISO 17025; further eel tests: ISO 9001.
• How to report? Clinical pathology, name of the virus, subtype, prevalence, pathology?
  o CVI reports each diagnostic result to the sender, and reports once a year without names of senders to the Ministry of Economic Affairs, in the year report.
  o Furthermore, peer reviewed papers are written on viral infections of eel, like Haenen et al., 2012; Haenen & Wahl, 2007; Van Beurden et al., 2012.
• What units to use (can make life easier)?
  o Virus name (no eel virus is notifiable for EU, OIE or other)
• How are results visualized, provide examples.
  o Peer reviewed papers are written on eel viral diseases. Pictures are made for our own lab, to educate people on eel diseases, like 3rd years students veterinary sciences.

Non specific fin hemorrhages were seen in wild silver eels from the River Rhine and IJsselmeer (Haenen et al., 2010).

• Impact assessment. Which method is used?
  o n.a.

Results NL (health, especially parasites and viruses):
In the 1980s, the swimbladder parasitic nematode Anguillicoloides crassus was introduced in wild European eel. It caused acute and severe swimbladder lesions in eel populations, but this effect became milder in time. In a study on the health of wild spawners (silver eels, A.anguilla) originating from the Dutch River Rhine and Lake IJsselmeer it was concluded, that the silver eels had a proper condition, with nonspecific fin hemorrhages,
and were frequently infected with *Trypanosoma* spp., *A. crassus* and/or *Anguillid herpesvirus 1* (AngHV-1), depending on the season (Haenen et al., 2010). AngHV-1 is the most frequent observed virus in wild eel in the Netherlands. Furthermore, *Eel rhabdovirus European X* (EVEX) has been detected in silver eel (Van Ginneken et al. 2004, 2005). AngHV-1 has recently been molecularly characterized in detail by Van Beurden et al. (2012), who also performed a retrospective study on the prevalence of European eel viruses in the Netherlands. Some further papers on eel viruses are Haenen et al., 2002, 2009; Van Beurden, 2012; Van Beurden et al., 2010, 2011; Galinier et al., 2012; Bellec et al., 2014.

### 6.2.2 Bacterial diseases

- Not done in IMARES screening. Done at CVI as part of general diagnosis, on request. In the past also done as surveillance, as research, see ref. list.
- What is recorded in the necropsy room, clinical pathology etc.?
  - Clinical signs of a bacterial infection, like:
    - *Vibrio vulnificus* (especially in eels from brackish or marine water) agressive ulcerative skin lesions, abcessus, congested spleen, ascites, haemorrhages in organs and skin (may be zoönotic = harmful to man, see Dijkstra et al. 2009); occurs especially at water temp of appr. 25°C. Found some years, in various eel farms in NL (Haenen et al., 2014), including a zoonotic strain, which caused a serious zoonosis in the eel farmer.
    - *Edwardsiella tarda*: bloody gut content, granuloma-like view of organs, congested spleen, a.o.
    - *Pseudomonas anguilliseptica*: occurs especially in brackish water: glass eels may show a congested kidney, and a whitish layer at their skin (Haenen & Davidse, 2001). The bacterium is isolated from all parts. The disease is strongly temperature dependent: 2 weeks > 26°C helps to cure without any antibiotic.
    - *Aeromonas salmonicida* atypical: congested eels, ulcers, may also be isolated from internal organs, showing inflammations
    - *Aeromonas hydrophila/sobria*: congested eels, hæamorrhages, isolated from internal organs, showing inflammations
    - *Mycobacterium marinum*: lethargic eels, internally granuloma in spleen, liver and/or kidney. Scarcely found.
    - Note, that *V.vuln* and *Edw.tarda* may be combined with a virus infection in eel.
- What part of the body for which analysis, per stage of eel? Which tissues? Sampling and subsampling per group of pathogens (including numbers).
- Ulcers and spleen, liver, kidney are taken specimens from, with disposable öses.
- In case of haemorrhages in gut also the gut content may be sampled like above, for *Edwardsiella tarda* testing.

- Which isolation and/or test method for identification, existing protocols with references per bacterium
  - (BHI) Sheep blood agar, Marine agar (for brackish salt water eels), Cytophaga agar (to isolate myxobacteria), are inoculated, and incubated during at least 7 days at 22°C.
  - After a pure bacterial culture has appeared at the agar plates, a colony is taken, and typed by MALDI-TOF (and alternatively, by 16S rRNA typing). Biochemical tests are too expensive for routine identification, but can be used to assist the other methods.

- QC/QA status for these techniques at the lab?
  - ISO 9001.

- How to report? Clinical pathology, name of the bacterium, subtype?, antibiotic resistance?, prevalence, pathology?
  - CVI reports each diagnostic result to the sender, and reports once a year without names of senders to the Ministry of Economic Affairs, in the year report.
  - Furthermore, peer reviewed papers are written on bacteria from eel, like Haenen *et al.*, 2014. For *Vibrio vuln.*, also genotyping has been done. Antibiotic tests are done on request, for the sender, but not in case of wild eel.

- What units to use (can make life easier)?
  - Name of bacterium.

- How are results visualized, provide examples.
  - A picture is taken in the necropsy room. Peer reviewed papers are written. Pictures are used for presentations and publications.

*Vibrio vulnificus* lesions in eels from a Dutch eel farm (Haenen *et al.*, 2014).
• Impact assessment. Which method is used?
  o Not a special method.
  o Results NL (bacteria): Bacteria do not seem to play a major role in disease of wild eels (Haenen et al., 2010). At farms, however, their negative impact may be big (Haenen et al., 2014).

7. Perspectives of using biomarkers of effects to assess eel health
None.

8. International needs (making your results available, and international database)
The results of eel monitoring are made available to the international community
- through publications in scientific journals (in preparation),
- through institutional reports,
- through the national contact point of the ICES WG EEL group and the yearly eel country reports, YES
- through reporting to the ICES WGEEL Eel Quality Database, partly
- through reporting to EU the progress in the eel management plans in the framework of the Eel Regulation, YES

Are raw data included, accessible?
When published in scientific papers, are your raw data uploaded as Supplementary Information? Partly.

9. Other issues, remarks
Concerning diseases, most of the samples CVI gets are from eel farms. This has helped to increase knowledge on eel diseases in general. However, the water temperatures at Dutch (indoor) eel farms are often around 25°C, much higher than the water temp in nature.

Restocking eels might be a risk of introducing disease, and therefore health checks are necessary prior to restocking, especially when eels from eel farms are restocked into the wild. At least parasites, like A.crassus, and viruses should be tested for, like AngHV-1, EVEX, and EVE. It is important to know the status of a certain watershed regarding these pathogens in wild eel to decide what to test for.

Levels of contaminants in eel, both yellow and silver, can be high in eels originating from polluted areas (downstream large rivers). No negative correlation with lipid weight and or condition factor observed have been observed, nor any effect of pollution level on eel density.

In conclusion, although no direct relationship can be made between the decline of the eel population and specific pathogens, a number of pathogens (Predominantly AngHV1 + Trypanosoma + Anguillicoloides crassus) have shown potential to severely hamper the health of eel in the wild, with AngHV-1 and A.crassus also under farmed conditions.
10. References


Report on the methods and assessment of eel quality (contaminants and diseases) in: Poland

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Introduction

This country report has been drafted in preparation of the ICES Workshop of a Planning Group on the Monitoring of Eel Quality: “Development of standardized and harmonized protocols for the estimation of eel quality.” (WKPGMEQ), held at INBO Brussels from 20 to 22 January 2015.

2. Framework national cases and national guidelines (on contaminants and health)

2.1 Contaminants

In Poland, monitoring the quality of eel is carried according to the Polish Eel Management Plan, designated in accordance with Regulation (EC) 1100/2007 of 18 September 2007. Annual projects concerning eel quality are financed by Operational Programme “Sustainable Development of the Fisheries Sector and Coastal Fishing Areas 2007-2013 (OP Fish). Projects are conducted by the Institute of Inland Fisheries in Olsztyn (IFO). Analysis of chemical contaminants are subcontracted to the National Marine Fisheries Research Institute.

Monitoring of chemical contaminants is aimed at examining the eel quality in terms of human health (contaminants are measured in muscles tissue) but results are also evaluated in terms of environmental quality of eel habitat, according to the environmental quality standards (EQS) designated within the WFD.

Approximately 60 eel samples are analysed each year. Results are reported during meetings of ICES working group WGEEL and part of them is published in scientific papers (Szlinder-Richert et al. 2014).

Sampling is conducted by IFO while the analysis of contaminants are performed in NMFRI. Fish samples are collected in the north Poland, from sites in various geographic regions and catchments with differing land use pressures. So far, sampling was conducted in the Vistula and Szczecin lagoons, in the Puck Buy, the Mazurian Lakes and in Lake Jamno.
Fig. 1 Sampling sites

The Szczecin Lagoon is a shallow, inland water basin situated in the southwestern part of the Baltic Sea at the mouth of the Oder River. The total area of the lagoon is 912 km$^2$, of which 457 km$^2$ lie within Polish borders, and the remainder is in Germany. Increasing pollution from industrial wastewater was noted in the lagoon in the beginning of the 1960s, since the Oder River collects substantial amount of effluents from the chemical and other heavy industries in southern Poland. The Vistula Lagoon is the largest brackish water basin in the southern Baltic. It is shared by Russia and Poland, and 328 km$^2$ of the total area of 838 km$^2$ is inside Polish borders. This highly eutrophic lagoon is connected to the Gulf of Gdansk by the Pilawska Strait. The mouths of a few branches of the Vistula
and Pasleka rivers flow into the lagoon. The Gulf of Gdańsk is a partially enclosed basin which is exposed to high anthropogenic pressure from the three large coastal cities of Gdynia, Sopot, and Gdańsk. The Bay of Puck is the shallow, western part of the Gulf of Gdansk, and is separated from the Baltic Sea by the Hel Peninsula Sandbar.

The Mazurian lakes, located in northeastern Poland, comprise the largest complex of lakes connected by canals in the country and occupy approximately 20% of the surface area of the Mazurian Lake District. Lake Jamno is located in northwest Poland and is connected to the Baltic Sea by the Jamierski Nurt canal. The lake is supplied by two rivers.

2.2 Diseases

3. General issues on eel sampling for contaminants and/or diseases in your country. Treatment of the sampled eels on site and in the laboratory.

3.1 Ethics and legislation

3.2 Sampling procedures

3.3 Stage, gender, morphometrics

Fish are caught using fyke nets in the period from May to September. Number of samples per sampling site is various depending on the year. Usually chemical contaminants are measured in 8-20 eel samples per site. Sampling sites are described by the geographical name of the water body. Data of sampling is recorded. Eel life stage is determined using the method described in Durif et al. (2005). Most of eel sampled collected are female. Morphometrics are measured after freezing, weight in grams (with an accuracy to 1 g), length in centimeters (with an accuracy to 1 cm). In the majority of cases the sample comprise of one individual, only in case of small individuals (weight<300g) pooled samples are prepared.

After transferring eels to the laboratory, they are anesthetized with Propiscin (supplied by Inland Fisheries Institute in Olsztyn, Poland) (Kazuń and Siwicki, 2012), and then killed by decapitation. They are frozen in polyethylene bags and stored at-18°C until the analyses are performed.

Lipid content and chemical contaminants are measured in a sample consisted of the homogenized muscle tissue form the hole fish. The samples are prepared in Class 10,000 clean room laboratories. Clean room garments, masks, and gloves are worn by all personnel who followed clean room procedures strictly during all of the critical treatment and sample analysis phases.

After analysis, freeze-dried samples are stored in refrigerator for a period of about 6 month.
4. General issues on eel condition (fitness, lipid levels)

4.1 Condition

4.2 HSI

Hepatosomatic index is not reported in monitoring, because the liver is not sampled, however there is a possibility to measure the HIS index.

4.3 GSI

GSI is not reported in Polish monitoring.

4.4 Lipid levels

Lipid content (%) is determined gravimetrically after Soxhlet extraction with diethyl ether in accordance with AOAC (1990) procedures. Each sample is measured in triplicate and the mean value is calculated. Lipid content is expressed in % (gram of lipids in 100 g of wet muscle tissue).

The extractable lipid percentages in the muscle tissues of the eel collected so far ranged from 4.89% to 30.90%. Lipid levels above 20% were observed in 37% of the samples from the Vistula Lagoon, 43% of the samples from the Szczecin Lagoon, 56% of the samples from the Puck Bay, and 26% of the samples from lakes.

4.5 Other condition related issues

5. Contaminants

5.1 Sampling and analysis

5.1.1 PCBs

In all samples six ndl-PCBs (28, 52, 101, 153, 138, 180) are measured. Each compound is determined but only the sum of ndl-PCBs is reported. Contaminants are determined in ng/g lipid and subsequently concentrations in ng/g wet muscles tissue are calculated. Measurements are conducted in NMFRI. Ndl-PCBs are measured together with OC pesticides in the same extracts.

OCPs and marker PCBs are analyzed according to the method described previously by Szlinder-Richert et al. (2008, 2009), with the exception of the extraction method. Briefly, samples are freeze-dried and extracted using accelerated solvent extraction (ASE). The ASE conditions are as follows: a mobile phase hexane: dichloromethane 1:1 v/v; system pressure 1500 psi, oven temperature 125°C; static time 5 min; flush volume 60%; 2 cycles per sample. An aliquot of lipid (0.5 g for muscle samples) is dissolved in hexane and treated with a mixture of (1:1 v/v) concentrated sulfuric acid and 30% fuming sulfuric acid for 3 h. After centrifuging and freezing the lower layer, the clean extract are separated, and the lower layer is re-extracted with hexane. The extracts are combined, and the contaminants are assayed using an Agilent Technologies 6890N series gas chromatograph (GC) equipped with a 7683 autosampler and an electron-capture detector (ECD). A Rtx-5 (Restek USA), 60 m_0.25 mm I.D. capillary column with 0.1 mm film thickness was
used. Quantification was conducted based on the area of standard peaks. The concentrations are calculated using six-point standard curves. The calibration curves showed good linear behavior ($r^2 > 0.99$).

The detailed description of chemical analysis, standards and reference materials used as well as the description of quality assurance/control procedures and validation parameters are provided as the Supplementary information in paper by Szlinder-Richert et al. 2014a.

In addition to ndl-PCBs in 30 samples dl-PCBs are measured, but not in each year.

### 5.1.2 Pesticides

Organochlorine pesticides measured are: α, β, γ-HCH, HCB, pp'-DDT, pp'-DDE, pp'-DDD. Concentrations are reported as sum HCH, sum DDT and HCB in ng/g wet muscle tissue.

Analytical protocol used id described in the section 5.1.1.

### 5.1.3 Brominated Flame Retardants

Brominated flame retardants measured are: PBDE (IUPAC nos. 28, 47, 100, 99, 154, 153, 183) and HBCDD. HBCDD was included into the monitoring in 2014.

Freeze-dried samples are weighed and then extracted using accelerated solvent extraction (ASE) as described for OCPs and marker PCBs. After extraction, the solvents is removed and about 5 g of lipids is weighed and then dissolved in n-hexane and quantitatively transferred into a membrane. Aliquots of 0.5 ml of internal standard solution is added. The membrane containing the sample is placed in a glass cylinder filled with dichloromethane:n-hexane 4:6 v/v solvent mixture. Dialysis is performed for 72 h. Every 24 h, the dialysate is removed and a fresh portion is poured into the cylinder. The combined extracts are concentrated using a vacuum evaporator to about 1 ml. The samples are clean further using absorption chromatography. Samples are dissolved in hexane and placed on the top of a glass column filled with 0.5 g of anhydrous Na₂SO₄, 2 g of deactivated silica, 6 g of acidic silica gel, 2 g of deactivated silica, and again with anhydrous Na₂SO₄. The analytes are eluted with n-hexane (PBDEs) following a 50 ml of mixture of dichloromethane: hexane 1:3 v/v (HBCDD). The eluent containing HBCDD and PBDE are concentrated to a volume of 50 μl and then analyzed.

The recovery standard solutions for PBDE determinations is prepared using standard solutions of BDE 77 and BDE 128 in isooctane at concentrations of 50 μg ml⁻¹. The recovery standard for HBCDD measurements is prepared from a solution of C₁₃ α-HBCDD in toluene at a concentration of 50 μg ml⁻¹. The recovery standard mixture added to samples is prepared in n-nonane and contains, BDEs and C₁₃ α-HBCDD at concentrations of 10 ng ml⁻¹ and 20 ng ml⁻¹, respectively. A solution of 4,4'-dibromoocatafluorobiphenyl (DBOFB) in hexane at a concentration of 250 μg ml⁻¹ is used to prepare the syringe standard solution, which contained DBOFB at a concentration of 25 μg ml⁻¹, and is prepared in nonane.

PBDEs and total HBCDD are assayed with an Agilent Technologies 6890N series gas chromatograph with a mass spectrometer (GC-MS). The analyses are performed in SIM mode. PBDEs under the following conditions.
A Rtx-5 (Restek USA) 30 m, 0.25 mm I.D. capillary column with 0.1 mm film thickness was used. A 2 µl aliquot of a given sample was injected splitlessly at 260°C. The column oven temperature was programmed as follows: 90°C (1 min) – 210°C min⁻¹ to 230°C – 5°C min⁻¹ – 300°C (12 min). Helium (purity 99.999%, Messer, Poland) was used as the carrier gas at a constant flow of 1 ml min⁻¹. The electron energy is set at 70 eV, the ion source temperature is 250°C, and the transfer line temperature is 300°C.

Total HBCDD is assayed using an Rxi®-5Sil MS (Restek, USA) 15m, 0.25 mm I.D. capillary column with 0.1 mm film thickness. A 2 µl aliquot of a given sample is injected splitlessly at 250°C. The column oven temperature is programmed as follows: 150°C (1 min) – 20°C min⁻¹ to 330°C (5 min). The electron energy is set at 70 eV, the ion source temperature is 250°C, and the transfer line temperature is 300°C.

The detailed description of chemical analysis, standards and reference materials used as well as the description of quality assurance/control procedures and validation parameters are provided as the Supplementary information in paper by Szlinder-Richert et al. 2014a.

Results are reported as sum of PBDE and sum of HBCDD in ng/g wet muscle tissue.

5.1.4 Dioxins

Analysis of dioxins is subcontracted to the laboratory that is accredited for the analyses of PCDD/F/dl-PCBs. Before extraction, the freeze-dried samples are spiked with a mixture of the isotope labelled PCDD/Fs and dl-PCBs. The samples were extracted with the use of hexane–dichloromethane in a Soxhlet apparatus. Clean-up is performed using a multilayer silica column followed by separation on acid and basic alumina columns. The samples are concentrated under a nitrogen flow to 30µl. Prior to instrumental analysis, syringe standards were added to the samples. Quantification is performed with a GC/HRMS system equipped with a DB-5 MS (J&W Scientific, USA) 60 m, 0.25 mm I.D. capillary column with 0.25 mm film thickness connected to a high resolution magnetic sector spectrometer (VG AutoSpec) operating in EI+ selected ion monitoring mode at a resolution of 10,000.

The calculation of the TEQ values for each sample was done by multiplying the individual congener levels measured in each sample with the appropriate Toxic Equivalency Factor (TEF). The TEFs set by the World Health Organization (WHO) for humans in 2005 are applied. The data represent upperbound concentrations are reported. Results are expressed in TEQ pg/g wet muscle tissue.

5.1.5 Metals

The muscle tissues is excised from the eels and homogenized. About 2 g of fish tissue is weighed out for analysis. The material is mineralized with 65% nitric acid and 30% hydrogen peroxide in MARS-5 microwave ovens (CEM, United States).

Metal concentrations are determined with atomic absorption spectrometry. A spectrometer Perkin-Elmer 4100 (GmbH, Bodenseewerk, Germany) equipped with a graphite furnace is used to determine concentrations of Cd, and Pb in fish. Hg content is assayed with the cold vapor atomic absorption method in an AMA 254 mercury analyzer. The analyses are conducted according to the following procedure. Tissue samples of about 100 mg is placed in the combustion chamber of the analyzer where they are dried and
then burned at a temperature of 600ºC in an oxygen atmosphere. The measurements are conducted as follows programs: fish muscle tissue – drying time 70 s, decomposition time 120 s, waiting time 50 s; fish liver tissue – drying time 100 s, decomposition time 160 s, waiting time 60 s. Each series of analyses is preceded by measurements of mercury in reference materials of a similar matrix with certified mercury content.

Each sample is analyzed in two replicates. Quality assurance for each series of analyses is provided by parallel analyses of reference materials.

5.1.6 PAHs

Determination of PAH metabolites is planned in the samples collected in 2014. Bile samples are stored at temperature -70ºC.

Contaminants will be determined in fish bile with HPLC/FL method. Results will be normalised into bile pigment content. The PAH metabolites (1-OH Phe and 1-OH Pyr) will be analyzed using the modified method of Ariese et al., 1993. Aliquots of 50 µl of bile, 50 µl of water, 20 µl of β-glucuronidase and arylsulfatase enzyme solutions (30 and 60 Uml⁻¹ respectively, Merck) will be added to a microcentrifuge tube and incubated at 40ºC. After four hours, the reaction will be terminated by adding 250 µl of cooled methanol. The samples will be centrifuged at 5000 rpm for 10 minutes. The supernatant will be transferred to a glass vial calibrated to 0.5 ml, and 250 µl of methanol will be added to the remaining sediment, mixed on a vortex-type shaker and centrifuged. The supernatants will be combined, concentrated under a gentle stream of nitrogen and adjusted to a volume of 0.5 ml.

The concentrations will be calculated with external standard method using six-point calibration curves determined from prepared standard solutions.

5.1.7 Others?

5.2 Interpretation, visualisation and assessment

The results are evaluated in terms of environmental quality and consumer health. Reference values used are shown in a Table

<table>
<thead>
<tr>
<th>Compound/group of compounds</th>
<th>Reference value</th>
<th>Source of the reference value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCB</td>
<td>100 ng/g ww</td>
<td>Council Directive 86/363/EEC (maximum permissible limit for food)</td>
</tr>
<tr>
<td></td>
<td>10 ng/gww</td>
<td>WFD: EQS</td>
</tr>
<tr>
<td>Sum DDT</td>
<td>1000 ng/g ww</td>
<td>Council Directive 86/363/EEC (maximum permissible limit for food)</td>
</tr>
<tr>
<td></td>
<td>33ng/ww</td>
<td>WFD: EQS</td>
</tr>
<tr>
<td>Sum PCDD/F</td>
<td>3.5 ng TEQ/kg ww</td>
<td>Commission Regulation 1259/2011/EC</td>
</tr>
<tr>
<td>Sum PCDD/F/dl-</td>
<td>10 ng TEQ/kg ww for eel</td>
<td>Commission Regulation 1259/2011/EC</td>
</tr>
</tbody>
</table>
**PCB** | 6.5 ng TEQ/kg ww for other fish species  
---|---  
**Marker PCBs** | 300 ng/g ww for eel and 75 ng/g ww for other fish species  
**HBCDD** | 167 ng/g ww  
**Sum PBDEs*** | 0.0085 µg kg⁻¹ ww  
**Sum PBDE** | 4 ng/g ww  
**Sum PBDEs** | 0.7 µg/kg bw/week  
**Sum PCDD/F/dl-PCBs** | 14 pg TEQ kg⁻¹ bw week⁻¹  

*sum of six indicator congeners in fish: triBDE 28, tetraBDE 47, pentaBDE 99 and 100, hexaBDE 153 and 154*

### 6. Diseases

#### 6.1/ 6.2 Sampling and analysis of eels for parasitology (*Anguillicoloides* and others) and analysis of viral and bacterial diseases

Studies to evaluate the health of eel from different fisheries enterprises and from different environmental conditions in various aquatic basins are performed as part of the monitoring project. A special protocol for monitoring eel health was developed and applied in the studies, and the eel from each of the enterprises are subjected to the same diagnostic procedures. Before the examinations, the eel are anesthetized with Propiscin (IFI Ol-sztyn). Each of the fish is examined individually for clinical and anatomopathological changes on the skin or in the gills and internal organs that would indicate the presence of disease. Blood samples are collected for further hematological, biochemical, and immunological test. In addition samples are collected from particular parts of the fish and from the organs for virological, bacteriological, and immunological tests. The immunological tests include determining the activity of non-specific cellular and humoral immune defense mechanisms and resistance to infections. Full parasitological tests are also performed in order to confirm parasitic infection of skin, gills, and internal organs (swimbladder, digestive tract). Bacteriological tests included isolating and identifying pathogenic bacteria that threatened the health and life of the fish. The virological tests focused on isolating and identifying two viruses that are highly pathogenic to eel: EVEX, which is required by the European Union, and anguilid herpesvirus (AnHV). Simultaneously, tests are performed to determine if other viruses that are pathogenic for fish are present (VHSV, IHNV, IPNV, SVCV).

The analysis of the test results indicated that no significant differences were observed in the health of the fish that were subjected to clinical, anatomopathological, biochemical, or immunological tests. No pathology that would indicate disease was noted on the skin or in the gills of the tested fish, and anatomopathological examinations confirmed this evaluation as no pathology was noted in any of the internal organs (liver, kidneys, spleen, digestive tract). Bacteriological tests on the skin, gills, and internal organs did not indi-
cate the presence of any pathogenic bacteria that could threaten health, and only saprophytic bacterial flora that occurs permanently in waters was isolated. Simultaneously, neither the EVEX nor the AnHV viruses, which are both pathogenic to eel, were detected among the fish tested. A significant element of the test was that no viruses that are pathogenic to other fish species were isolated among the eel tested which indicates that they are not carriers of pathogenic viruses of other fish species cultured in Poland. However, the parasitological tests focused on the eel swimbladder indicated a very high infection prevalence with the nematode Anguillicoloides crassus among the fish tested. The analysis of the test results of individual eel permit concluding that the degree of infection with the parasitic nematode A. crassus has an impact on the activity of non-specific cellular and humoral immune defense mechanisms that provide resistance to infections and on levels of total protein and glucose, which are fundamental parameters used to evaluate fish condition. A strict dependence between the degree of parasitic infection and fish condition was noted. In conclusion, the eel tested did not exhibit pathological changes, and microbiological and immunological tests confirmed the good health of the fish.

7. Perspectives of using biomarkers of effects to assess eel health
In Poland, there are currently no plans to incorporate biomarkers into monitoring of eel quality.

8. International needs (making your results available, and international database)

9. Other issues, remarks

10. References


Report on the methods and assessment of eel quality (contaminants and diseases) in: Portugal

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Introduction
This country report has been drafted in preparation of the ICES Workshop of a Planning Group on the Monitoring of Eel Quality: “Development of standardized and harmonized protocols for the estimation of eel quality.” (WKPGMEQ), held at INBO Brussels from 20 to 22 January 2015.

In Portugal an interagency working group to prepare the Eel Management Plan (EMP) was created. The working group is coordinated by the National Forest Authority (NFA), including representatives of the Directorate-General for Fisheries and Aquaculture (DGPA), the Nature Conservation and Biodiversity Institute, IP (ICNB), the Water Institute, I.P. (INAG) and the National Institute of Biological Resources (INRB / IPIMAR). To join the working group, EDP - Energias de Portugal, SA, and the Faculty of Science, University of Lisbon - Institute of Oceanography were also invited (Plano de gestão da enguia 2009-2012; Resposta do Estado Português ao Regulamento (CE) nº 1100/2007. Dezembro de 2008, Revisão – Novembro 2010)

2. Framework national cases and national guidelines (on contaminants and health)

2.1 Contaminants
No routine monitoring network measuring contaminants in eel exist in Portugal

2.2 Diseases
No routine monitoring network measuring disease agents in eel exist in Portugal

3. General issues on eel sampling for contaminants and/or diseases in your country. Treatment of the sampled eels on site and in the laboratory

3.1 Ethics and legislation
In Portugal eel fishery is regulated under the 2007 European recovery plan (council regulation EC 1100/2007 of 18 September) that establishes measures for the recovery of the stocks of the European eel. The catch, holding, transport and marketing of eel is generally not allowed in the months of October, November and December (article 1, Ordinance 180/2012, from 6 June of Minister of Agriculture, Sea, Environment and Territory Planning). Since January 2014 recreational eel fishing is not allowed (decree 14/2014, 23 January of the Presidency of the Council of Ministries and Ministers of Finances, National
Defense, Economy, Environment, Territory and Energy Planning, and Agriculture and Sea).

For scientific purposes an annually electro-fishing license is needed. Fishing is controlled by General Direction of the Maritime Authority (DGAM) and by National Forest Authority (AFN).

The use of animals for scientific purposes are regulated by the Directive 2010/63/EU of the European Parliament and the Council of 22 September 2010 and transposed to Portuguese legislation by the decree 113/2013, 7 August, Minister of the Agriculture, Sea, Environment and Territory Planning.

3.2 Sampling procedures

In Portugal no references are found concerning national standardization of methodology on eel scientific surveys.

Sampling is often localized in maps but site description and water parameters are rarely recorded. Saraiva (1994) referred the sampling river system clime (values of average annual rainfall, atmospheric annual average temperature) and other river system features and determined water temperature, pH, total hardness, alkalinity (as CaCO₃), dissolved oxygen (O₂), ammoniac nitrogen (NH₄), nitrate (NO₃) nitrite (NO₂), water-soluble phosphates (PO₄) and silicates (Si). Macro-invertebrate were also collected for determination of biological quality of the water by the method proposed by De Pauw & Vanhooren (1983). Domingos et al. (2006) referred the river system features and determined flow conditions and depth and water transparency. Neto et al. (2010 and 2011) reported water salinity. Sampling season generally was reported.

The most usual method to sample eels is electro-fishing (Saraiva & Chubb, 1989; Saraiva & Molnár, 1990; Rodrigues & Saraiva, 1996; Saraiva, 1996; Saraiva & Eiras, 1996; Cruz & Eiras, 1997; Cardoso & Saraiva, 1998; Cruz & Davies, 1998; Saraiva & Moravec, 1998; Saraiva et al., 2002a, b; Saraiva et al., 2005; Domingos et al., 2006). In some cases eels were obtained from fisherman, who caught them using fyke nets and longlines (Hermida et al., 2008; Neto et al., 2010, 2011).

3.3 Stage, gender, morphometrics

Most of the studies are conducted in eels longer than 22 cm. Weight and length are measured in anesthetized or frozen eels. Eel stage is generally not reported. Anesthetic used are ethylene glycol monophenyl ether (Saraiva & Molnár, 1990) and benzocaine (Cardoso & Saraiva, 1998; Domingos et al., 2006; Neto et al., 2010; 2014). Neto et al. (2010) determined the total and eviscerated eel weight, liver weight and spleen weight and the length in frozen eels.

Domingos et al. (2006) divided freshwater eels in four classes: class 1 (50–99 mm) specimens newly arrived in freshwater; class 2 (100–149 mm), class 3 (150–249 mm) eels in their first and second years of life in freshwater respectively and class 4 (≥ 250 mm) eels that had spent more than 2 years in freshwater and in which sex differentiation had already occurred for 50% of the specimens.

3.4 Treatment

4. General issues on eel condition (fitness, lipid levels)

4.1 Condition

Domingos et al., (2006) determined the eel condition factor (K) as
\[ K = \left( \frac{TW \times TL}{c} \right) \times 10^c \]
in which TW was the total weight (in gram), TL was the total length (in millimetre) and c = 5 (as TL was expressed in millimetre).

Guimarães et al. (2009) determine the Fulton’s condition factor (CF = 100 x total weight (g)/length^3 (cm)). In our laboratory we also frequently determine the Fulton’s condition factor. In accordance with Lloret et al. (2014) we consider the value “1” as the threshold for healthy fish.

4.2 HSI

Guimarães et al. (2009) determine the hepatic somatic index (HSI = 100 x Liver weight (g)/total weight (cm)). In our laboratory we also frequently determine the hepatic somatic index. We assumed, in accordance with Lloret et al. (2014) that HSI is an accurate measure of fish condition and that lipids usually should constitute more than 50% of the liver wet weight.

4.3 GSI

4.4 Lipid levels

4.5 Other condition related issues

5. Contaminants

Metals

Pérez Cid et al. (2001) determined by electro thermal atomic absorption spectrometry the levels of Cd, Cu, Ni and Pb in muscle.

Neto et al. (2011) analysed the concentration of Cd, Cu, Hg, Pb and Zn in the liver of eels. Samples of freeze-dried homogenised hepatic tissue were processed according to Julshmann et al. 1982. Metal concentration analyses were performed by inductively coupled plasma mass spectrometry (ICP-MS). The assessment of total Hg concentrations was carried out by pyrolysis atomic absorption spectrometry (AAS) with gold amalgamation using an Advanced Mercury Analyser (AMA) according to Válega et al. (2006).

6. Diseases

6.1 Sampling and analysis of eels for parasitology (Anguillicoloides and others)

In parasitological surveys in our laboratory we sample 30 eels. This size of sample is required for 95% confidence in ability to detect at least one infected eel when it is
assumed that the proportion of infected ones is 9.5% and size populations is bigger than 10,000 (Simon and Schill, 1984). Infection levels determinations is used in accordance to the terminology proposed by Bush et al. (1997). According to these authors prevalence is the number of hosts infected with one or more individuals of a particular parasite species (or taxonomic group) divided by the number of hosts examined for that parasite species. Infection intensity is the number of individuals of a particular parasite species in a single infected host and mean intensity is the average intensity of a particular species of parasite among the infected members of a particular host species. Abundance is the number of individuals of a particular parasite in/on a single host regardless of whether or not the host is infected. Mean abundance is the total number of individuals of a particular parasite species in a sample of a particular host species divided by the total number of hosts of that species examined (including both infected and uninfected hosts).

6.1.1 *Anguillicoloides crassus*

*Anguillicoloides* Moravec et Taraschewski, 1988 are nematodes parasites of swimbladder of eels. This genus contains four species but only two have been reported from Europe, *Anguillicoloides crassus* (Kuwahara, Niimi et Itagaki, 1974) Moravec et Taraschewski, 1988 and *Anguillicoloides novaezelandiae* Moravec et Taraschewski, 1988 (Moravec, 2013). The distinction between the two species is mainly done by the head shape, buccal capsule size and size and number of teeth (Moravec, 2013).

It is assumed that eels can be infected as soon as they start eating invertebrate intermediate hosts, mostly copepods. It means that eel from all sizes could be infected and should be assessed for this parasite. Cardoso & Saraiva (1998) assessed this parasite after dissection of the eel, they removed carefully the swimbladder and examined it macroscopically and with the aids of a stereomicroscope. These authors collected, counted, washed in physiological saline, fixed and stored specimens in 70% ethanol, determined the life cycle stage of each parasite and determined the infection levels referred above. Neto et al. (2010 and 2014) examined the eel swimbladder macroscopically and record the number of adults and “sub-adults” (pre-adults) and determined the health status of the swimbladder using the SDI (Swimbladder Degenerative Index) proposed by Lefebvre et al. (2002). Briefly this evaluation is based on 3 criteria; transparency/opacity; pigmentation/exudates, and thickness, with each one being scored 0, 1 or 2 and the cumulative index range from 0 (no pathological signs of infection) to 6 (extremely damaged swimbladder).

6.1.2 Others parasites

Blood parasites (*Trypanosoma granulosum* and *Babesiosoma bettencourtii*) were studied in blood smears staining by May-Grunwald Giensa (Eiras, 1988: Saraiva & Eiras, 1996; Cruz & Eiras, 1997; Cruz & Davies, 1998). Eel Myxozoa *Myxidium giardi* and *Myxobolus portucalensis* morphology were studied by Transmission Electron Microscopy (TEM) and Optic Microscopy (MO) using standard methods (Azevedo et al., 1989; Saraiva & Molnár, 1990). The effects of *Myxidium giardi* in host tissues were analyzed by Ventura & Paperna (1984) using standard histological technique. It is assumed that *Pseudodactylogyrus anguillae* and *Pseudodactylogyrus bini* were introduced in Europe with imported *Anguilla japonica* in last century, more or less at the same time of *A. crassus*. Stereomicroscopic isolation, MO identification in glycerine-gelatine slides, are used to identify both species in Portu-
guese eels (Saraiva, 1995; Rodrigues & Saraiva, 1996). These authors also study the histo-pathological effects of these parasites in gills fixed in 10% neutral buffered formalin and stained in Haematoxylin-Eosin (H&E). The eel specific nematodes *Rhabdochona anguillae* and *Spinitectus inermis* were identified in hot fixed specimens with the aid of MO and Scanning Electron Microscopy (SEM) (Saraiva & Moravec, 1998; Saraiva et al., 2002a, b). Several parasite surveys has been conducted using standardized parasitological techniques; macroscopic examination, MO observation of wet mounts and several processing techniques according to taxonomic parasite group (Varela et al., 1984; Saraiva & Chubb, 1989; Cruz e Silva et al., 1993; Saraiva & Eiras, 1996; Saraiva et al., 2005; Hermida et al., 2008). In these studies many generalist and eel specialist parasites were detected and their seasonality, population and community parameters were determined.

7. Perspectives of using biomarkers of effects to assess eel health

Ahmad et al. (2006) determined in liver, kidney and gill the lipid peroxidation (LPO) by the method of Utley et al. (1967) modified and adapted by Fatima et al. (2000). The same authors determined several antioxidant parameters on the post mitochondriol supernatant (PMS) fraction according to the method of Ahmad et al. (2000). Glutathione peroxidase (GPX) activity was assayed according to the method described by Mohandas et al. (1984) as modified by Athar and Iqbal (1998). Catalase (CAT) activity was assayed by the method of Claiborne (1985) as described by Giri et al. (1996). Glutathione S-transferase (GST) activity was determined by the method of Habig et al. (1974) with some modifications (Raisuddin et al., 1994). Estimation of reduced glutathione (GSH) was determined by the method of Jollow et al. (1974) as described by Iqbal et al. (1999). Total protein contents were determined according to the Biuret method (Gornall et al., 1949).

Gravato et al. (2010) determined in whole body of glass eels frozen in liquid nitrogen several oxidative stress parameters. These authors also determined glass eel gill ATPase and glutathione-S-transferase (GST), head (without eyes) cholinesterase (ChE) and muscle lactase dehydrogenase (LDH).

In yellow eels Guimarães et al. (2009) and Gravato et al. (2010) determined, brain cholinesterase (ChE), from 1st and 2nd branchial arches from the left gill glutathione-S transferase (GST) and ATPase, from a portion of dorsal muscle lactase dehydrogenase (LDH) and from liver several oxidative parameters. These authors’ used the following methods. ChE activity was determined as described by Ellman et al. (1961), adapted to microplate (Guilhermino et al., 1996). Na+/K+-ATPase activity was determined using ouabain as described by McCormick (1993). GST activity was determined as described by Habig et al. (1974). LDH activity was determined as described by Vassault (1983) adapted to microplate (Diamantino et al., 2001). Endogenous lipid peroxidation (LPO) was determined by measuring the thiobarbituric acid reactive substances (TBARS) according to Ohkawa (1979) and Bird and Draper (1984), with the adaptations described by Filho et al. (2001) and Torres et al. (2002). Enzymatic and nonenzymatic antioxidants were determined in the post-mitochondriol supernatant (PMS) of glass eels’ whole body and yellow eels’ liver. Briefly, GPx activity was determined according to Mohandas et al. (1984); GR activity was assayed according to Cribb et al. (1989); CAT activity represents the H2O2 consumption obtained at 240 nm in the presence of H2O2 (Claiborne, 1985); TG content (GSH+GSSG) and oxidized glutathione (GSSG) were determined at 412nm, using a recycling reaction of GSH.
with 5,50-dithiobis (2-nitrobenzoic acid) in the presence of GR excess (Tietze, 1969; Baker et al., 1990). 2-Vinyl-pyridine was used to conjugate GSH for GSSG determination (Griffith, 1980). GSH was calculated by subtracting GSSG from the TG levels. GSH/GSSG ratios were expressed according to Pena-Llopis et al. (2001). Protein concentration was determined according to the Bradford method (Bradford, 1976).

Maria et al. (2006) determined the genotoxicity in yellow eels by DNA strand breaks according to Rao et al. (1996), with minor modifications, in gills, blood, liver and kidney DNA.

Teles et al. (2007) determined the liver ethoxyresorufin-O-deethylase (EROD) and alanine transaminase (ALT) activities, plasma levels of cortisol, 17β-estradiol (E2), thyroid-stimulating-hormone (TSH), free thyroxine (T4), free triiodothyronine (T3), as well as glucose and lactate. The erythrocytic nuclear abnormalities (ENA) frequency was also scored by these authors as a genotoxicity indicator. EROD activity was measured in microsomal fraction as described by Burke & Mayer (1974) and adapted by Pacheco & Santos (1998). Alanine transaminase (ALT) activity was measured in the cytosolic fraction (supernatant resulting from microsomal isolation), according to a colorimetric method based on the measurement of the pyruvate produced by the transamination reaction (Reitman & Frankel, 1957). Microsomal and cytosolic protein concentrations were determined according to the Biuret method (Gornall et al., 1949). Plasma cortisol, TSH, T3, T4 and E2 were performed in plasma, using diagnostic ELISA direct immunoenzymatic kits. Plasma glucose was measured spectrophotometrically (340 nm) according to a method modified from Banauch et al. (1975). Plasma lactate levels were determined spectrophotometrically (340 nm) according to the method modified from Noll (1974). The blood smears were fixed with 100% methanol for 10 min and stained with Giemsa (5%) for 30 min. In order to evaluate genotoxicity, the erythrocytic nuclear abnormalities were scored in 1000 mature erythrocytes sample per fish, according to the criteria of Schmid (1976), Carrasco et al. (1990) and Smith (1990), later adapted by Pacheco & Santos (1996).

8. International needs (making your results available, and international database)

9. Other issues, remarks

10. References


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Report on the methods and assessment of eel quality (contaminants and diseases) in:

Spain

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Introduction
This country report has been drafted in preparation of the ICES Workshop of a Planning Group on the Monitoring of Eel Quality: “Development of standardized and harmonized protocols for the estimation of eel quality.” (WKPGMEQ), held at INBO Brussels from 20 to 22 January 2015.

The eel management plan in Spain is based on a National Eel Management Plan (EMP) and 12 specific EMPs (11 EMPs for the Autonomous Communities with eel populations that can complete their life cycle in these basins, and 1 EMP specific for the Ebro River Basin). Each Autonomous Community, defined as an Eel Management Unit (EMU) has exclusive competences on eel. Methodology used for eel’s quality assessment (contaminants and diseases) from Spanish environments differs greatly among these EMUs while no data are available regarding other ones.

2. Framework national cases and national guidelines (on contaminants and health)

2.1 Contaminants
In Spain there is not a routine monitoring network measuring contaminants in eels. Most of the EMUs did not include routine monitoring of contaminants in their EMPs. Nevertheless, some studies have been conducted in other EMUs in the frameworks of eel regulation plans, in the EU water framework directive, in the framework of Real Decret 60/2011 of 28 January which includes the transposition of European Directives 2008/105/CE and 2009/90/CE into the Spanish legal system or in the framework of research projects. They are mainly funded by regional administrations or by research projects. No national guidelines are available. The results of some of these works are accessible to international community due to their publication in scientific journals or to the EMPs of regional EMUs while other ones are included in scientific reports sometimes accessible in the regional administrations web pages which hinder accessibility.

2.2 Diseases
In Spain there is not a routine monitoring network measuring diseases in eels. In the framework of EMPs, some of the Spanish EMUs proposed to study the eel sanitary status. Nevertheless, the achievement of this goal has been very limited. These studies are mainly funded by research projects as well as regional administrations. No national guidelines are available. The results of these works are accessible to international com-
munity due to their inclusion in scientific reports, in the EMPs or to their publication in scientific journals published between 2001 and 2015.

2.3 Ethics and legislation

Some of the studies are conducted with wild eels purchased from local fishermen and so, no permission is required. Permissions to regional administrations are required when electro-fishing method is used.

Animal ethics permissions are required to regional administration for research projects involving animals. The use of animals for scientific purposes are regulated by the Directive 2010/63/EU of the European Parliament and the Council of 22 September 2010 and transposed to Spanish legislation by the decree 53/2013, 1st February, Minister of Presidency.

Sampling site descriptions differs greatly among studies. GIS data, air and water temperature as well as water quality are usually not recorded. Season is usually reported.

2.4 Sampling procedures

Number of sampled eels differs greatly among studies, being very low (N=3) in some studies conducted in environmental works not specifically targeted to European eel monitoring (Belenguer et al. 2014). Up to 953 eel were studied in a parasitological study (Ottorial et al. 2001) while up to 49 eels were monitored in a contaminant work (Esteve et al. 2012). In some studies, mostly due to budgetary reasons, samples from individual eels were pooled. Fisheries are conducted with electrofishing, fyke nets or longlines.

2.5 Stage, gender, morphometrics

There is no formal identification of the species. We assume all individuals are A. anguilla.

Most of the parasitological studies are conducted with yellow or silver eels while Grano-Maldonado et al. (2011) conducted a parasitological study in a subsample of 12 glass eels from a sample with 12600 specimens. Eel stage is not reported in some studies. Contaminant studies are conducted in yellow or silver eels. When silver eels are being sampled gender is usually reported. For sex determination, silver females were identified macroscopically by the color of the back and belly, as well as developed gonads while others stages were determined on the basis of body length (Esteve et al. 2009; Esteve et al. 2012). Age was estimated based on otolith reading (Mayo-Hernández et al. 2015) as well as ocular index (Díaz et al. 2007).

In most contaminants studies tissues were dissected out (sometimes pooled) and then stored deep-frozen until contaminant extraction (Liné et al. 1999; Linde et al. 2001; Usero et al. 2003; Santillo et al. 2006; Ureña et al. 2007; Parera et al. 2013; Belenguer et al. 2014) while in other ones eels were stored at -20 °C until dissection of selected organs (Bordajandi et al. 2003; Sánchez et al. 1998).

Parasitological studies are conducted immediately after dissection (Sánchez et al. 1992; Gallastegui et al. 2002, Esteve et al. 2009), eels are maintained alive in freshwater tanks with aeration and recirculating flow for up to 4 days before examination (Aguilar et al. 2005) or maintained frozen until analysis (Díaz et al. 2006; Costa-Dias et al. 2010; Martínez-Carrasco et al. 2011a, b; Mayo-Hernández et al. 2014, 2015). On the other hand, bac-
teriological studies are conducted immediately after dissection (Alcaide et al. 2006; Esteve et al. 2009; Bandín et al. 2014) while tissue samples for virological ones are stored frozen (Bandín et al. 2014).

Units used for morphometric data are always centimeters (length) and grams (weight).

2.6 Treatment

Aguilar et al. (2005) maintained wild eels alive in freshwater tanks with aeration and recirculating flow for up to 4 days before parasitological examination. Grano-Maldonado et al. (2011) reported a parasitological study conducted in wild glass eels maintained in a recirculation system with constant aeration (dissolved oxygen 8–9 ppm, 90% saturation) and daily water renewal (40 L/h) for restocking purposes. Andree et al. (2013) maintained wild glass eels in 14 m³ rectangular tanks (initial density= 15 kg/m³) with ambient flow-through fresh water (flow rate = 7300 L/h; approximately 1 renewal of total tank volume/h). These authors reported several peaks in mortality occurring during their captivity period as well as multiple bacterial strains isolated from moribund animals.

Eels were euthanized by chilling in ice (Costa-Días et al. 2010; Mayo-Hernández et al. 2014), with a blow to the head after being chilled (Gallastegui et al. 2002), by electrofishing (Sánchez et al. 1998; Linde et al. 1999), by decapitation (Linde et al. 1999, 2001), by an overdose of different anesthetics (2-phenoxyethanol (Aguilar et al. 2004), tricaine methanesulfonate-MS222 (Outeiral et al. 2001; Sancho et al. 2003; Martínez-Carrasco et al. 2011a, b), methanesulfonate salt of 3-aminobenzoic acid ethyl ester (Ureña et al. 2002) or benzocaine (Esteve and Alcaide 2009)). Some studies did not report the method used to euthanize/anaesthetize eels.

In order to avoid contamination or cross-contamination of the samples, eels were wrapped either individually or as a pooled sample in sheets of new, clean aluminum foil and placed inside transparent polyethylene bags and frozen (Santillo et al. 2005, 2006) or freeze-dried at –80°C and lyophilized (Belenguer et al. 2014). Many studies did not report measures to avoid contamination. The use of gloves and protective clothes is not reported although assumed.

3. General issues on eel condition (fitness, lipid levels)

3.1 Condition

Costa-Días et al. (2010) measured eel condition using condition factor (K) calculated as $K = 10^3 \times \frac{mass}{length^3}$ while Esteve et al. (2012) used condition index (CI) calculated as $100(\frac{W^3}{L^3})$ (Ricker, 1975) and Mayo-Hernández et al. (2015) used Scaled Mass Index (SMI) calculated according to Peig and Green (2009). SMI, as a proxy of body condition, is considered an excellent measure of the energy capital accumulated in the body as a result of feeding and has previously been validated with data on body components such as fat and protein (Peig and Green, 2009). This novel condition index has only been recently used in fish (Maceda-Veiga et al. 2014). The disadvantage is the higher complexity of this index compared to K or CI.
3.2 HSI

Ureña et al. (2009) and Esteve et al. (2012) used hepatosomatic index (HSI) as described by Durif et al. 2005 by in contaminants studies. HSI = 100(WL × WB⁻¹) where WB was body mass in grams, WL was liver mass in grams and the coefficient c was calculated as the slope from the log WL – log WB regression analysis. This index was not included in the parasitological studies conducted in Spain.

3.3 GSI

3.4 Lipid levels

3.5 Other condition related issues

Sancho et al. (2009) used liver somatic index (LSI) and water content (WC) as indicators of general condition of fish after exposure to herbicides. Disturbances in the LSI of exposed animals are indicating possible hepatic injured lesions. This index becomes an easy and rapid biomarker of xenobiotics presence in fish medium (Heath, 1995). LSI was calculated as percentage of wet weight organ (g) per wet weight (Kg) of the individual.

For WC measurement one portion of both muscle tissue and hepatic organ from every individual fish was wet weighted and then dried for 72h at 105 °C until constant weight and then reweighed to determine WC (Sancho et al. 2003).

Mayo-Hernández et al. (2015) used splenic somatic index (SSI) and fluctuating asymmetry (FA) to evaluate the biological cost resulting from parasites in wild eels.

SSI is a simple method for estimating the absolute or relative abundance of immunologically active cells and is widely used as a simple measure in immune response against parasites in fish. If parasites cause an immune reaction, one would expect the spleen to increase in size due to its important haematopoietic function for leucocyte synthesis. SSI was calculated according to Peig and Green (2009).

FA is the most used measurement of developmental instability. It is a macroscopic measure of the difference of development between left and right from perfect bilateral symmetry caused by environmental or biotic stresses. FA is a useful indicator of condition since it has been shown to be extremely sensitive to stress and it has been previously used in eels as a measure of parasite virulence (Fazio et al. 2005 and Sahyoun et al. 2007). Mayo-Hernández et al. (2015) measured the maximum pectoral fin length and the distance between the operculum, labial commissure on each side, maximum otolith length and maximum otolith.

4. Contaminants

4.1 Sampling and analysis

4.1.1 PCBs

Table 1 summarizes the sampling and analysis of PCBs in European eels from Spanish environments.
Table 1. Methodology followed for PCBs analysis in European eels from Spain.

<table>
<thead>
<tr>
<th>Source</th>
<th>Eel origin</th>
<th>No</th>
<th>Sample</th>
<th>Method</th>
<th>Units</th>
<th>Which PCB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bordajandi et al. 2003</td>
<td>River Turia (Valencia EMU)</td>
<td>11</td>
<td>Dorsal</td>
<td>GC-ECD</td>
<td>ng/g ww</td>
<td>Sum PCBs, Ortho-substituted PCBs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>muscle</td>
<td>GC‐ECD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Santillo et al. 2005*</td>
<td>River Miño (Galicia EMU)</td>
<td>4</td>
<td>Muscle</td>
<td>GC-ECD</td>
<td>ng/g ww or ppb</td>
<td>ICES7</td>
</tr>
<tr>
<td></td>
<td>River Ebro (Catalonia EMU)</td>
<td>5</td>
<td>Muscle</td>
<td>GC‐ECD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parera et al. 2013</td>
<td>River Ebro (Catalonia EMU)</td>
<td>P</td>
<td>Muscle</td>
<td>GC-HRMS</td>
<td>pg/g ww</td>
<td>dl-PCB</td>
</tr>
</tbody>
</table>

GC-ECD= gas chromatography with electron capture detection, GC-HRMS= gas chromatography/high-resolution mass spectrometry, dl-PCB= dioxin like PCB, ng/g ww= nanograms per gram of wet weight, ppb=parts per billion, pg/g ww= picograms per gram of wet weight, P= a pooled sample of 1kg of muscle meat tissue from unknown number of eels was analyzed, * QC-QA implemented.

4.1.2 Pesticides

Table 2 summarizes the sampling and analysis of pesticides in European eels from Spanish environments.

Table 2. Methodology followed for pesticides analysis in European eels from Spain.

<table>
<thead>
<tr>
<th>Source</th>
<th>Eel origin</th>
<th>No</th>
<th>Sample</th>
<th>Method</th>
<th>Units</th>
<th>Which compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belenguer et al. 2014*</td>
<td>River Júcar (Valencia EMU)</td>
<td>3</td>
<td>Entire fish</td>
<td>LC-MS/MS QuEChERS</td>
<td>ng/g ww</td>
<td>Several herbicides and insecticides</td>
</tr>
<tr>
<td>Bordajandi et al. 2003</td>
<td>River Turia (Valencia EMU)</td>
<td>11</td>
<td>Dorsal muscle</td>
<td>GC-ECD</td>
<td>ng/g ww</td>
<td>DDT, DDE, TDE</td>
</tr>
</tbody>
</table>

LC-MS/MS= liquid chromatography tandem mass spectrometry, GC-ECD= gas chromatography with electron capture detection, ng/g ww= nanograms per gram of wet weight, * the effect on fish condition was evaluated.

4.1.3 Brominated Flame Retardants

Table 3 summarizes the sampling and analysis of brominated flame retardants in European eels from Spanish environments.

Table 3. Methodology followed for BFR analysis in European eels from Spain.

<table>
<thead>
<tr>
<th>Source</th>
<th>Origin</th>
<th>No</th>
<th>Sample</th>
<th>Method</th>
<th>Units</th>
<th>Which dioxine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Santillo et al. 2005*</td>
<td>River Miño (Galicia EMU)</td>
<td>4</td>
<td>Muscle</td>
<td>LC-MS</td>
<td>ng/g ww or ppb</td>
<td>HBCD, TBBP-A</td>
</tr>
<tr>
<td>River</td>
<td>(Catalonia EMU)</td>
<td>No</td>
<td>Sample</td>
<td>Method</td>
<td>Units</td>
<td>Which dioxine</td>
</tr>
<tr>
<td>-------</td>
<td>-----------------</td>
<td>----</td>
<td>--------</td>
<td>--------</td>
<td>-------</td>
<td>--------------</td>
</tr>
<tr>
<td>River Ebro</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>River Miño (Galicia EMU)</td>
<td>4</td>
<td>Muscle</td>
<td>NICI-GC/MS</td>
<td>ng/g ww or ppb</td>
<td>11 PBDEs</td>
<td></td>
</tr>
<tr>
<td>River Ebro</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Santillo et al. 2006* | River Miño (Galicia EMU) | 4 | Liver | LC-ESI-ITMS | ng/g ww | PFOS, PFHxS, PFOA, PFDA |
| Parera et al. 2013 | River Ebro (Catalonia EMU) | 5 | Muscle | GC-ECNI | ng/g ww | SCCPs |
| Parera et al. 2013 | River Ebro (Catalonia EMU) | P | Muscle | GC-HRMS | pg/g ww | PBDEs |

**4.1.4 Dioxins**

Table 4 summarizes the sampling and analysis of dioxins in European eels from Spanish environments.

**Table 4. Methodology followed for dioxins analysis in European eels from Spain.**

<table>
<thead>
<tr>
<th>Source</th>
<th>origin</th>
<th>No</th>
<th>Sample</th>
<th>Method</th>
<th>Units</th>
<th>Which dioxine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bordajandi et al. 2003</td>
<td>River Turia (Valencia EMU)</td>
<td>11</td>
<td>Dorsal muscle</td>
<td>GC-HRMS-SIM</td>
<td>ng/g ww</td>
<td>PCDDs, PCDFs</td>
</tr>
<tr>
<td>Parera et al. 2013</td>
<td>River Ebro (Catalonia EMU)</td>
<td>-</td>
<td>Muscle</td>
<td>GC-HRMS</td>
<td>pg/g ww</td>
<td>PCDDs, PCDFs</td>
</tr>
</tbody>
</table>

LC-MS= liquid chromatography – mass spectrometry, NICI-GC/MS=negative ion chemical ionisation mass spectrometry, liquid chromatography-electrospray ion trap mass spectrometry (LC-ESI-ITMS) with perfluorinated sulphonates being detected in MS mode and perfluorinated acids in MS/MS, GC-ECNI= Gas chromatography in combination with electron capture negative ion mass spectrometry, GC-HRMS= gas chromatography/high-resolution mass spectrometry, HBCD=hexabromocyclododecane, TBBP-A= tetrabromobisphenol-A, PBDEs= polybrominated diphenyl ethers, PFOs= perfluorooctane sulphonate, PFHxS=perfluorohexane sulphonate, PFOA=perfluorooctanoic acid, PFDA= perfluorodecanoic acid, SCCPs= short chain chlorinated paraffins, ng/g ww= nanograms per gram of wet weight, pg/g ww= picograms per gram of wet weight, ppb=parts per billion, P= a pooled sample of 1kg of muscle meat tissue from unknown number of eels was analyzed, * QC-QA implemented.
dibenzofurans, ng/g ww= nanograms per gram of wet weight, pg/g ww= picograms per gram of wet weight

4.1.5 Metals

Table 5 summarizes the sampling and analysis of metals in European eels from Spanish environments.

Table 5. Methodology followed for metal analysis in European eels from Spain.

<table>
<thead>
<tr>
<th>Source</th>
<th>Origin</th>
<th>No</th>
<th>Sample</th>
<th>Method</th>
<th>Units</th>
<th>Heavy metals measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sánchez et al. 1998</td>
<td>River Urumea (Basque country EMU)</td>
<td>7</td>
<td>G, L, M (dorsal)</td>
<td>AAS</td>
<td>mg/kg ww</td>
<td>Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn</td>
</tr>
<tr>
<td>Linde el at 1999</td>
<td>River Pigueña (Asturias EMU)</td>
<td>36</td>
<td>L, K</td>
<td>ICP-AES</td>
<td>µg/g ww</td>
<td>Cu, Zn, Pb, Cd</td>
</tr>
<tr>
<td>Linde et al. 2001</td>
<td>River Ferrerias (Asturias EMU)</td>
<td>20</td>
<td>L</td>
<td>ICP-AES</td>
<td>mg/g ww</td>
<td>Cu, Zn, Cd, Pb</td>
</tr>
<tr>
<td>Usero et al. 2003</td>
<td>Andalucía EMU (Atlantic coast)</td>
<td>10</td>
<td>L, M (dorsal)</td>
<td>AAS</td>
<td>mg/kg ww</td>
<td>As, Hg, Fe, Zn, Mn, Cu, Cd, Cr, Ni, Pb</td>
</tr>
<tr>
<td>Bordajandi et al. 2003</td>
<td>River Turia (Valencia EMU)</td>
<td>14</td>
<td>M</td>
<td>AAS</td>
<td>µg/g ww</td>
<td>Pb, Cd, Zn, Cu, As</td>
</tr>
<tr>
<td>Ureña et al. 2007</td>
<td>Albufera lake (Valencia EMU)</td>
<td>12</td>
<td>L, K, G, M (caudal)</td>
<td>ICP-Mass</td>
<td>µg/g ww</td>
<td>Cd, Zn, Hg, Cu, Fe, Pb, Mn, and Pb in blood</td>
</tr>
<tr>
<td>PG- Murcia EMU (2010)</td>
<td>Mar Menor (Murcia EMU)</td>
<td>16</td>
<td>K, L, M</td>
<td>ICP-AES (Pb, Cd, As) and DMA</td>
<td>mg/kg</td>
<td>Hg, Pb, Cd, As</td>
</tr>
<tr>
<td>Esteve et al. 2012*</td>
<td>Albufera lake (Valencia EMU)</td>
<td>49</td>
<td>liver</td>
<td>ICP-Mass</td>
<td>ng/g ww</td>
<td>Hg, Cu, Se, Cd, Zn, Co, Pb, Mn, Cr, Fe</td>
</tr>
</tbody>
</table>

AAS= atomic absorption spectrometry, ICP-AES= inductively coupled plasma atomic emission spectroscopy, ICP-Mass= inductively coupled plasma mass spectrometer, DMA= direct mercury analyzer, * the effect on fish condition was evaluated.

4.2 Interpretation, visualisation and assessment
5. Diseases

5.1 Sampling and analysis of eels for parasitology (*Anguillicoloides* and others)

5.1.1 *Anguillicoloides crassus*

In Spain there is not a routine monitoring network measuring diseases in eels. However, substantial work on eel diseases has been carried out as scientific surveys and experimental work, mostly, but not exclusively, on *Anguillicoloides*. After the first description of this nematode in Spain (Gallastegui et al. 2002) the presence of *A. crassus* has been studied in several Spanish EMUs (Aguilar et al. 2005; Maíllo et al. 2005; Díaz et al. 2006; Márquez Llano-Ponte et al. 2007; Esteve and Alcaide 2009; Costa-Díaz et al. 2010; Martínez-Carrasco et al. 2011a,b; Esteve et al. 2012; Mayo-Hernández et al. 2014, Muñoz et al. 2015). The number of sampled specimens ranged from 48 to 271. In some of those studies, eels were brought back to the laboratory alive and then examined immediately for parasites (Gallastegui et al. 2002; Maíllo et al. 2005; Esteve and Alcaide 2009; Martínez-Carrasco et al. 2011a,b; Esteve et al. 2012; Muñoz et al. 2015), maintained alive in freshwater tanks with aeration and recirculating flow, for up to 4 days before examination (Aguilar et al. 2005) or stored frozen until sampling (Díaz et al. 2007; Costa-Díaz et al. 2010; Mayo-Hernández et al. 2014). Some specimens were sampled after mortality events (Márquez Llano-Ponte et al. 2007).

The majority of those studies only reported the presence of pre-adult and adult parasites in the swim bladder lumen while Costa-Díaz et al. 2010 quantified larval stages (L3 and L4) as well as the presence/absence of L2 larvae in the swim bladder wall and Martínez-Carrasco et al. 2011 quantified all *A. crassus* larval stages (L2, L3 and L4). For this purpose, Martínez-Carrasco et al. 2011a digested swim bladders in freshly prepared 1.5% (w/v) pepsin (1: 10 000 activity) and 1.5% (w/v) chlorhydric acid in distilled water. *A. crassus* morphometric studies (Martínez-Carrasco et al. 2011a; Esteve et al. 2012) and sex determination (Martínez-Carrasco et al. 2011a) have been conducted according to Moravec and Taraschewski (1988) by several authors.

Results are reported using parasitological indices following Bush et al. (1997); prevalence as the percentage of infected individuals; mean intensity, as the mean number of parasites per infected fish; and the mean abundance, as the mean number of parasites per fish.

Regarding the impact assessment, Muñoz et al. (2015) studied the influence of the number of adult *A. crassus* present in the swim bladder of wild eels on the macrophage response (phagocytosis and respiratory burst) as part of the first immune response to pathogens while several studies have evaluated the effect of this nematode in eel condition (Costa-Díaz et al. 2010; Esteve et al. 2012)

In those studies, clinic, morbidity and swim bladder damage have not been recorded. No data are available regarding the prevalence of this parasite in some Spanish ecosystems.

5.1.2 Other, like trematodes?

Several works have studied the parasitofauna of wild eels from Spanish ecosystems (Outerialetal al. 2001; Outerialetal al. 2002; Aguilar et al. 2005; Maíllo et al. 2005; Martínez-Carrasco et al. 2011b; Mayo-Hernández et al. 2014). The number of sampled eels ranged
between 48 and 956 specimens, being yellow or silver eels. Grano-Maldonado et al. 2011 conducted a parasitological study in a subsample of 12 glass eels from a sample with 12600 specimens. Additionally, Sánchez et al. 1992 reported the presence of *Pseudodactylogyrus anguillae* in eels from the Esva river (Asturias) in a sampling conducted with 22 specimens, only gills were observed for parasitological analysis. Table 6 summarizes the different parasitological methods conducted in those studies.

Table 6: Methodology used in parasitological studies conducted with European eels from Spanish environments.

<table>
<thead>
<tr>
<th>PARASITOLOGICAL PROCEDURE</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wet mounts</strong></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>Martinez-Carrasco et al. 2011, Mayo-Hernández et al. 2014</td>
</tr>
<tr>
<td>Viscera</td>
<td>Aguilar et al. 2005</td>
</tr>
<tr>
<td><strong>Eel examination</strong></td>
<td></td>
</tr>
<tr>
<td>Naked eye</td>
<td>Maíllo et al. 2005</td>
</tr>
<tr>
<td><strong>Parasite washing</strong></td>
<td></td>
</tr>
<tr>
<td>9 % NaCl</td>
<td>Outeiral et al. 2001, 2002</td>
</tr>
<tr>
<td><strong>Parasite fixation</strong></td>
<td></td>
</tr>
<tr>
<td>70% ethanol</td>
<td>Martinez-Carrasco et al. 2011, Mayo-Hernández et al. 2014</td>
</tr>
<tr>
<td>4% formalin</td>
<td>Maíllo et al. 2005</td>
</tr>
<tr>
<td>Berland’s fluid + 70% ethanol</td>
<td>Outeiral et al. 2001, 2002, Aguilar et al. 2005</td>
</tr>
<tr>
<td><strong>Parasite staining</strong></td>
<td></td>
</tr>
<tr>
<td>Giemsa, haematoxylin, methylene blue, and preparation in glycerinated gelatine</td>
<td>Maíllo et al. 2005</td>
</tr>
</tbody>
</table>
In those studies several parasites have been reported:

- **Protozoa**: *Trypanosoma granulosum*, *Eimeria anguillae*, *Trichodina pediculus*, *T. jadranica*, *Ichthyophthirius multifiliis*.
- **Myxozoa**: *Myxidium giardi*, *Myxobolus portucalensis*, *Myxobolus sp.*, *Hofferellus gilsoni*.
- **Monogenea**: *Pseudodactylogyrus anguillae*, *P. bini*.
- **Digenea**: *Nicolla gallica*, *Diplostomum sp.*, *Deropristis inflata*, *Bucephalus poly- morphus*, *Helicometra tasciata*, *Lecithochirium musculatus*, *L. ru-toviride*, *Podocotyle angulata*, *Derogenes varicus*, *Lecithaster gibbosus*.
- **Cestoda**: *Bothriocephalus claviceps*, *Proteocephalus macrocephalus*, *Grillotia sp.*, *Tetrephyllidea sp.*
- **Acantocephala**: *Acantocephaloides propinquus*, *A. clavula*.
- **Copepoda**: *Ergasilus gibbus*, *Gnatia maxillaries*.

Results are reported using parasitological indices following Bush et al. (1997). For all parasite species detected infection prevalence was calculated. In some cases mean intensity of infection and mean abundance was additionally calculated. In the case of protozoan parasites no attempt to estimate intensity or abundance was conducted. In some studies the very large numbers of *Pseudodactylogyrus* detected in the gills prevented from identifying all individuals to species level.

Formaldehyde and mebendazole have been used to control monogenean infections in glass eel stored in rearing tanks (Grano-Maldonado et al. 2011).

To understand the costs of co-infection in wild eels, Mayo-Hernández et al. (2015) investigated the relationships among parasites (richness and intensity) and eel’s “health status” (fluctuant asymmetry, splenic somatic index and the scaled mass index) by partial least squares regression.

In those studies, clinic and morbidity have not been recorded. QC-QA status has not been implemented.
5.1.3 Other....

5.2 Sampling and analysis of viral and bacterial diseases

5.2.1 Viral diseases

A recent article reports the presence of viruses in wild eels from Albufera Lake (Valencia EMUs) (Bandín et al. 2014). Herpesvirus, betanodavirus and aquabirnavirus were screened. A total of 179 specimens at different growth stages were analysed. Pools of spleen and kidney were tested for the presence of herpesvirus and aquabirnavirus while brain samples were used for betanodavirus isolation. Monolayers of chinook salmon embryo, bluegill fry, rainbow trout gonad, epithelioma papulosum cyprini, brown bullhead, eel kidney and E-11 cells (a clone of the cell line striped snakehead SSN-1) were used to culture eel virus. PCR was used to herpesvirus detection while nested RT-PCR was performed to detect IPNV and betanodavirus.

In the framework of EMP (Murcia EMU) the presence of betanodavirus, VHSV and IPNV has been monitorized (González et al. 2009, GESAC 2010). GESAC 2010 described a standardized protocol for sampling and diagnosis of betanodaviruses, VHSV and IPNV in wild and farmed fish.

Prevalence was used in those studies to report data while no data regarding clinical pathology was reported.

Additionally, an ongoing study is evaluating the presence of EVEX, IPNV and herpesvirus in 200 wild eels from Andalucía EMU. The two former viruses are detected using RT-PCR and real-time PCR techniques while herpesvirus are detected using real-time PCR.

5.2.2 Bacterial diseases

The assessments of eel health status has been included in several Spanish EMPs for the EMUs, nevertheless no information regarding these assessments was included in the Evaluation Progress Eel Management Plans. Two scientific groups have studied the eel bacterial diseases in Albufera Lake and Mar Menor, coastal lagoons belonging to Valencia and Murcia EMUs respectively (Alcaide et al. 2006, Esteve et al. 2007, 2009; Muñoz et al. 2009; Martínez-Carrasco et al. 2011b). The number of sampled eels ranged between 48 (Martínez-Carrasco et al. 2011b) to 122 specimens (Esteve et al. 2009) and specimens were yellow or silver eels. In 2006, after a mortality event in Sella river (Asturias) 28 specimens were sampled for bacteriology analysis (Márquez Llano-Ponte et al. 2007). In all these studies pathological signs such as haemorrhagic fins, petechiae on the belly, skin damage were recorded in the necropsy room when present. Skin, kidney, spleen, liver and/or body ulcers were the tissues used for bacterial isolation.

Samples were streaked onto tryptone soy agar plates plus 1% (wt/vol) NaCl (TSA-1) and incubated at 28 °C for 24–48 h, in one study sampled were additionally streaked onto agar sangre plates. Colonies of each morphological type were picked and transferred to TSA-1 plates for purification, and then checked for GRAM, oxidase and O/F metabolism. API 20E, API 20 NE and/or API 32E strips (BioMérieux) were used to identify bacterial isolates. In addition, Gram-negative, chemo-organotrophic (O/F) and anaerobic facultative bacterial isolates were also identified using conventional plate and tube tests.
For *V. vulnificus* biotyping, ODC and indole production tests were also used. The final identification of putative *V. vulnificus* isolates was performed by colony hybridization with a *V. vulnificus*-specific alkaline phosphatase labelled DNA probe (DNA Technology, Aarhus, Denmark) directed against the cytolysin gene (hlyA). Moreover, *V. vulnificus* isolates have been also serologically characterized by slide-agglutination with rabbit polyclonal antisera (Alcaide et al. 2006; Esteve et al. 2009). Sanjuán and Amaro (2004) designed and validated a protocol for *Vibrio vulnificus* specific isolation from environmental samples. They used saline eel serum broth for the first step. The selective and differential agar *Vibrio vulnificus* medium was selected for the second step.

Most of the studies have been focus on *Vibrio vulnificus* and *Edwardsiella tarda* but other bacteria have been reported such as *Aeromonas hydrophila*, *A. caviae*, *A. sobria*, *A. salmonicida*, *A. sobria*, *V. mimicus*, *V. fluvialis*, *V. alginolyticus*, *Proteus mirabilis*, *Plesiomonas shigelloides*, *Candida sorbophila*, *Enterobacteria*, *Serratia* spp. and *Staphylococcus* spp.

Results are reported as prevalence according to Bush et al. 1997. The mean lethal dose (LD50) for eels of selected isolates has been assessed by both intraperitoneal injection as well as waterborne infection (water salinity of 0.5% NaCl) (Alcaide et al. 2006, Esteve et al. 2007, 2009). The occurrence of strains that are resistant to antibiotics in freshwater eel farms and experimental facilities has been reported (Alcaide et al. 2005; Andree et al. 2013). Additionally, Roig et al. 2009 determined the antibiotic resistance patterns in a wide collection of *V. vulnificus* strains belonging to the three biotypes that had been isolated worldwide from different sources.

Regarding glass eels, an inspections by customs agents at Barcelona airport discovered 420 kg of contraband glass eels of unknown origin. After confiscation, they were transported to holding facilities. During their captivity period, several peaks in mortality occurred and multiple bacterial strains were isolated from moribund animals. Sequencing of 16S rDNA was used to determine specific identity of the isolates. Physical examinations, histology and antibacterial treatments were reported (Andree et al. 2013).

Additionally, yeast strains isolated from wild eels were identified by restriction fragment polymorphism of the 5.8S rRNA gene and 26S rRNA sequencing (Esteve et al. 2009).

### 6. Perspectives of using biomarkers of effects to assess eel health

Cholinesterase (ChE) activity was measured in plasma, whole blood [using 5,5'-dithiobis(2-nitrobenzoic acid) and 2-PDS as chromophores], brain, and whole eyes of eels exposed to a sublethal concentration of 11.15 mg/L (one-third of the 96-h LC50) of the carbamate herbicide molinate (Sancho et al. 2000a). The results indicate that ChE activity, as well as hematological parameters, may be useful as a diagnostic test for molinate exposure in aquatic organism. The eyes of the European eel were found to be a sensitive biomonitor for acetyl ChE inhibitors like thiobencarb (Sancho et al. 2000b). These authors measured the inhibition of both total and specific acetyl ChE activities in the whole eyes of the yellow eel after exposure to the carbamate thiobencarb.

According to Sancho et al. 2003 gill ATPase activity could be used as an indicator of herbicide exposure in European eels. These authors reported that sublethal exposure of eels to the herbicide thiobencarb produced a significant inhibition of both Mg2+ and Na+, K+-ATPase in gill tissue, however, a lower ATPase inhibition was detected in eel muscle tissues. Animals transferred to clean water during the recovery period recovered ATPase.
activities in both tissues at the end of the studied period. Under their test conditions, muscle water content was also a good indicator of the water quality.

Sancho et al. (2009) stated that the enzymes glutamyl transpeptidase, alanin aminotransferase, alkaline phosphatase and lactate dehydrogenase can be used as good biomarkers of herbicide contamination.

7. International needs (making your results available, and international database)

Depending on the institution and the purpose of each scientific approach, results of some of the studies concerning eel quality, contaminants and diseases are made available through publications in peer reviewed journals, through institutional reports on the respective websites, through reports mentioned in the appendices of the WG EEL country reports or as reports in the EU water framework directive. Unfortunately some reports are also only made accessible in Spanish on regional institute / administrations web pages, which hinder accessibility.

Raw data usually are neither included nor accessible.

8. Other issues, remarks,

9. References


Report on the methods and assessment of eel quality (contaminants and diseases) in:

Sweden

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Introduction
This country report has been drafted in preparation of the ICES Workshop of a Planning Group on the Monitoring of Eel Quality: “Development of standardized and harmonized protocols for the estimation of eel quality.” (WKPGMEQ), held at INBO Brussels from 20 to 22 January 2015.

This report aims to give an overview of methodologies and assessments concerning eel quality, contaminants and pathogens done on a broader scale in Sweden. It does not intend to claim for completeness of all available assessed methods in all Swedish research facilities and/or institutes.

Currently, Swedish eel research focus on genetic studies, population dynamical studies and evaluation of actions plans taken to save the eel population. Sweden took part in the eeliad research project, a four year EU-funded project (2008 to 2012) with the objective to improve the understanding of the marine ecology and biology of European eels. A project on implications of body size and swimming distance on migration and maturation of the European eel was published in 2011, and data is continuously collected according to the EU Data Collection Framework (DCF).

2. Framework national cases and national guidelines (on contaminants and health)

2.1 Contaminants
Up to date, no official routine monitoring network measuring contaminants in eels is set up in Sweden, and thus no national guidelines are available. Sampling of fish in context with monitoring environmental quality or food laws are made spontaneously by the respective organs such as Swedish University of Agricultural Science or National Food Agency and therefore do not follow routine regularities. Results of some of these investigations are made accessible by national reports or by scientific publications. A report on levels of Dioxins and PCB in fish, including eel, was published in 2012 (Rapport 21-2012
Dioxin- och PCB-halter i fisk och andra livsmedel 2000-2011) by the Swedish National Food Agency.

2.2 Diseases
No official routine monitoring network measuring diseases or disease agents in eel is set up in Sweden at present. Eels have been monitored and sampled in connection with quarantine at entry, or when moved from west coast to east coast. However, no national guideline documents exist.

An ongoing project by The Swedish Agency for Marine and Water Management, SwAM, in cooperation with National Veterinary Institute, seeks to investigate disease among wild cod and flounder in the Baltic Sea. A few eels have been included and sampled due to illness and acute mortality.

3. General issues on eel sampling for contaminants and/or diseases in your country.
Treatment of the sampled eels on site and in the laboratory.

3.1 Ethics and legislation
Experimental work complied with the standards and procedures stipulated by the Swedish Ministry of Agriculture (made under licence N130/12 and C16/14).

Sampling sites are described and characterized through GIS data, season, air temp, water temp, water quality (pH, salt content etc.). Generally, i.e. in Sweden’s DCF program, we collect the date of sampling and two different kinds of geo reference data for our sites. More historical datasets may include more detailed data on temperature (air & water) as well as for water quality parameters.

The DCF program collects WGS84 as well as RT90 coordinates for the sampling sites.

3.2 Sampling procedures
Sweden’s DCF program (the freshwater part) currently collects 750 adult eels from 6 sites. This is done in collaboration with locally active fishermen commissioned to collect 125 adult eels per site and year.

The fishermen are provided with general instructions on how to sample and handle the collected fish. Samples are to be representative and without any kind of sorting with respect to size and collected during what they, (the fishermen) consider to be the seasonal fishing peak, typically during late summer early autumn. The fish are to be deep frozen directly after catch pending transport to our institute. (Full instructions can be provided if needed.)

The eels are collected using traditional stationary big pound nets (älbottengarn). In the general instruction, the fishermen have been asked to select fishing gear in sites that they consider as long term recurring fishing sites (to facilitate our comparisons).

The choice to use fishermen to do our collection is done for practical and economic reasons. It would be too time consuming and expensive to do independent collection and sampling on site with our own staff. The obvious disadvantage is the risk of sampling bias of some sort (and also variations in sampling techniques (bias) between fishermen). We are also constrained by the short period in which they do their regular fishing i.e. we
only get samples from a short period of the season. The possible effects of this can be elaborated on if needed.

In addition to this, young recruiting eels are sampled along the coast using the “Drop Trap technique” (Westerberg et al., 1993; ICES, 2009) and in rivers using standard electro-fishing techniques. This sampling is also part of Sweden’s DCF-program. Sampled eels are typically analyzed in the same way as the adult eels. *Anguillilcoloides crassus* are searched for in all eels, irrespective of size and sex for eels above 250 mm (if possible). Eye size and fin length are only measured in larger eels. When to sample? Impact on stages. (Please see above)

### 3.3 Stage, gender, morphometrics

Only *Anguilla anguilla* occurs in Sweden, so there is no need for distinguishing eel species. To distinguish eel stage, we compute Pankhurst’s eye index (Pankhurst, 1982) and Durifs silver index (Durif, 2005). For DCF purposes we rely on Durifs silver index. In historical datasets only subjective stage classifications may be available. Gonads are inspected macroscopically to distinguish gender when necessary.

Morphometric measurements are taken from frozen (i.e. thawed) eels in the majority of cases. Millimeters and grams are used. The accuracy ranges between 0 and 2 decimals (mm-1/100 mm) depending on the measurement in question. Measurements of body length and weight are adjusted to compensate for shrinkage due to freezing (Wickström 1982).

### 3.4 Treatment

Live storage is kept to a minimum (contracted fishermen are instructed to deep freeze all samples directly after catch). However, fishermen may on occasion use coolers to store fish for shorter periods of time (this is done in accordance to their own permits). In cases of our own field work, we sometimes anaesthetize eels in the field using large plastic barrels for fish storage (i.e. anaesthetizing and recovery).

To euthanize/anaesthetize eels we (the Institute of Freshwater Research at Swedish University of Agricultural Sciences, SLU) use benzocaine.

Fishermen are instructed to label the eel samples in connection to the collection (labeling should include lake, site, date of catch, number of eels, etc. but is inconsistent). After examination (analysis) at the institute, all saved samples (e.g. otoliths) are labelled using a unique number corresponding to individual eels. The Institute of Freshwater Research as well as the Swedish University of Agricultural Sciences currently is in the process of certifying all data sampling and data storing procedures which will mean that e.g. labelling and storage (includes both physical samples as well as data) in the future will follow a very strict protocol.

How and where to store samples? Prior to, and after analysis. Samples are stored according to need and protocol. For those occasions we save tissue samples they are stored in deep freezer and e.g. otoliths are stored in a dedicated archive. Also see above.

There is no tissue database or tissue bank. We do not save tissue samples routinely.
4. General issues on eel condition (fitness, lipid levels)
Size of eels, expressed as body length and body weight, is necessary in assessment and used e.g. for estimations of life time mortalities etc. Body length and weight are of course an indirect measure of condition. However, condition as such is not yet incorporated in the assessment. When evaluating the possible value of silver eels released through trap & transport activities, the size, condition and maturity stage are rather subjectively taken into account as a “quality value” (cf. Cleestam et al. 2011).

4.1 Condition
Condition is calculated according to Fulton (1904) and Ricker (1975), i.e. W/L^3.

4.2 HSI
Hepatosomatic index (HIS), perspectives and limitations: See GSI below.

4.3 GSI
Gonadosomatic index (GSI): Is currently not used. We do have historical datasets containing GSI, HIS and digestive tract index (DTI) as described by Durif et al. (2005). In our view these are all valuable parameters for describing an eel population (especially GSI and DTI), although they are time consuming to acquire, and in our view these index need to be “calibrated” against a measurement of hormone activity (see Durif et al., 2005) signaling onset of the maturation to be really useful and to “fine tune” for geographical differences in maturation pattern we feel might exist.

4.4 Lipid levels
Sweden’s current DCF program does not include any lipid analysis. However, historical datasets exist that include analysis of lipids.
Eel stages are always distinguished between. See below.
Historically, lipid levels data are expressed as per cent total fat mass per wet mass muscle according to EC-method B (Anon., 1998). Muscle tissue samples taken 5 cm behind the anal cavity were used for fat analysis (Clevestam et al., 2011).
When sampling live eels in the field, we occasionally use the Distell Fish Fatmeter to measure fat. The resulting data seem reasonable but have not been corroborated by chemical fat analyses. However, comparisons on frozen eels were not positive, as there were no (significant) relation between Fatmeter and gravimetric fat analyses.

4.5 Other condition related issues
- NA
5. Contaminants

5.1 Sampling and analysis

We have no activities in this area at the moment. Although between year 2000 and 2010, the Institute of Freshwater Research have provided Sweden’s National Food Agency as well as The Swedish Museum of Natural History with eels from freshwaters for analysis of contaminants. Analysis included PCBs, dioxins and mercury compounds. If needed, the current DCF program as well as other activities at the Institute of Freshwater Research provides excellent opportunities to collect tissue samples for future contaminant analysis.

5.1.1 PCBs

In the investigation reported by National Food Agency in 2012, sampling was, as far as possible, made in accordance with EG 1883/2006. Whole body with muscle and subcutaneous fat tissue, no head or organs. Dl-PCB and i-PCB were measured. Reported on fresh body weight basis.

5.1.2 Pesticides – NA (see above)

5.1.3 brominated Flame Retardants – NA (see above)

5.1.4 Dioxins – NA (see above, 5.1.1)

5.1.5 Metals – NA (see above)

5.1.6 PAHs – NA (see above)

5.1.7 Others – NA (see above)

5.2 Interpretation, visualisation and assessment

Sweden follows details and threshold limits on Dioxin and PCB-levels in eel as per the EU regulations, based on WHO’s toxic equivalent (TEQ) updates from 2005. All Swedish samples of eel have shown results below maximum allowable levels. Examples of visualisation below, from Rapport 21-2012 Dioxin- och PCB-halter i fisk och andra livsmedel 2000-2011 by the Swedish National Food Agency:

Dioxin and PCB-levels in eel caught 2000-2010 in the fresh water lakes of Vänern, Hjälmaren, Mälaren and the Baltic Sea. Number (N) of pooled samples analysed, the median (min-max) of average length (cm) and weight (kg) and the concentration of dioxin and PCB (based on fresh body weight).
Body fat varied between 14-26%. The levels are highest in the samples collected in northern Lake Vänern. The contribution from dl-PCBs in this sample of PCDD/F-PCB is over 60%, but PCDD/F-PCB concentration of 8.2 pg TEQ2005/g fresh weight is still below the threshold for eels, set to 10 pg PCDD/F-PCB TEQ2005/g fresh weight.

Dioxin and dioxin-like PCB levels in eels from the Baltic region. The limit of PCDD/F in eel is 3.5 (dash line) and for PCDD/F-PCB it is 10 pg TEQ2005/g fresh weight (solid line).

The limit on I-PCBs for eel is 300 ng/g fresh weight, higher than for other fish, and all pooled samples of eel shows levels below the limit.
6. Diseases

6.1 Sampling and analysis of eels for parasitology (*Anguillicoloides* and others)

6.1.1. *Anguillicoloides crassus*

- The swimbladder (and some connecting tissue) of the eel is macroscopically examined for presence of *Anguillicoloides crassus*. This routine screening of all analysed eels with respect to prevalence and intensity of *A. crassus* has been performed since the end of the 1980th.
- The number of parasites is recorded. Some additional (inconsistently recorded) notes on damages may also exist (e.g. swimbladder damages).
- Measurements of *A. crassus* prevalence and intensity are routinely computed.
- Reports are visualised graphically as well as in table format.
- Literature references have been used in combination with models to discuss and predict impact in reports and scientific publications (Clevestam et al., 2011).

6.1.2 Other, like trematodes?

NA

6.1.3 Other.....

Inconsistent notes on various eel damages may exist. This includes a variety of causes from e.g. fungus, parasites like *Ichthyophthirius*, cormorants, and damages from fishing gear, superficial haemorrhages etc.

6.2 Sampling and analysis of viral and bacterial diseases

6.2.1 Viral diseases

- In case of suspected disease/ acute mortality, or temporary investigations, eels are sent to National Veterinary Institute where they are necropsied. If findings indicate suspected viral disease, samples are taken from spleen, kidney, gills, heart and/or CNS for cell cultures. (General cells sensitive for IPN and VHS). Growth of virus is further cultured and typed. Examples of found virus diseases are IPN, herpes and eel rhabdovirus.
- QC-QA follows the procedures of the National Veterinary Institute.

6.2.2 Bacterial diseases

- At necropsy, standard routine fish necropsy protocol is followed, with examination of skin, gills, fins, eyes, muscle tissue, organs etc. Bacteriology samples are always taken from kidney as well as organs with pathological changes. Samples are cultured on blood agar, and if myxobacteria are suspected, on KDMC. If symptoms of myxobacteria are present, smear from gills and/or organs are taken. Bacteria are typed according to ordinary identification protocol or Maldi tof. Results are recorded in the database of the institute.
7 Perspectives of using biomarkers of effects to assess eel health
- NA

8 International needs (making your results available, and international database)
- **Through publications in scientific journals.** When *Anguillicoloides crassus* first was discovered in Sweden some papers were published on the occurrence of the parasite in Swedish waters (Höglund et al. 1993, Wickström et al. 1998). Clevestam et al., 2011 focus entirely on eel quality and use e.g. lipid analysis and *A. crassus* levels to model and predict reproductive success.
- **Through institutional reports.** Data on condition, maturity stage, parasite load and when available fat content is used and presented in internal, national reports when relevant.
- **Through the national contact point of the ICES Working Group on Eels (WGEEL) and the yearly eel country reports.** Certain quality measurement/parameters (e.g. *A. crassus*) are collected and reported annually in the annual Country Reports presented at WGEEL.
- **Through reporting to the ICES WGEEL Eel Quality Database.** Available and relevant “quality data” are normally reported to the ICES WGEEL Eel Quality Database.
- **Through reporting in the framework of the CFP.** Within our DCF-programme data on condition, maturity stage and *Anguillicoloides crassus* are collected but not formally reported on in the Annual Report to EU.

Raw data are accessible but usually not presented in any kind of report or scientific paper, nor as supplementary information.

9. Other issues, remarks

In addition to our DCF dataset, the Institute of Freshwater Research at Swedish University of Agricultural Sciences (SLU) has large historical datasets containing both morphometric data, typically measurements of body length, body weight, eyes, pectoral fin, parasites, sex and stage. We also have a comprehensive archive of otoliths.

We would also like to stress the importance of using a common and strict protocol for stage determination, possibly with national calibrations using hormone levels as a tool. If comparisons are to be made between eel populations from different countries / geographic areas and make sense, it is necessary to compare silver eels with silver eels (for some studies applying all of Durif’s stages when grouping eels).

The Institute of Coastal Research (at SLU) also collect eels from the Baltic Coast as a part of Sweden’s total DCF program. Results from their samplings are reported on in their annual report to EU DCF and their country report to WGEEL.
10 References


Available at:
https://www.fiskeriverket.se/download/18.7920eb4612b67b1eed280005259/slutrapport_radda_alen _o_alfisket.pdf/


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Report on the methods and assessment of eel quality (contaminants and diseases) in:

United Kingdom

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Introduction

This country report has been drafted in preparation of the ICES Workshop of a Planning Group on the Monitoring of Eel Quality: “Development of standardized and harmonized protocols for the estimation of eel quality.” (WKPGMEQ), held at INBO Brussels from 20 to 22 January 2015.

Due to the United Kingdom being made up of a number of regions, there are a collection of competent authorities for monitoring and regulating freshwater fisheries. This document mainly refers to England and Wales and thereby delivery of regulation, monitoring and managing of freshwater fisheries that is completed by the Environment Agency. Inclusions in this document have also been provided by Northern Ireland (Agri-Food & Biosciences Institute) and Scotland (Marine Scotland).

There are 11 Eel Management Plans (EMPs) for England and Wales, including 1 shared with Scotland, 1 for the remainder of Scotland, and 3 in Northern Ireland including 1 shared with the Republic of Ireland. Most of the UK EMPs have been set at the River Basin District (RBD) level, as defined under the Water Framework Directive. The RBDs in Northern Ireland deviate slightly from those defined for the WFD, owing to their trans-boundary nature. The North Western International EMP is a trans-boundary plan with the Republic of Ireland.

England & Wales: There is a national approach to the monitoring of fish health across England and Wales. This includes: monitoring of health prior to movements; disease investigations; monitoring through WFD eel specific surveys and visual inspections in the wild. There are efforts underway to produce an eel specific dissection protocol by the Environment Agency, National Fisheries Services (NFS) Brampton (in progress. Lewin et al. 2014).

In England and Wales all eel health work is completed on wild eel and recreational fisheries. There is currently no aquaculture production for eel. Ad hoc eel sampling has taken
place in England and Wales for over 40 years, by a number of different organisations, universities and institutions.

_N. Ireland:_ Overall policy responsibility for the supervision and protection of eel fisheries in Northern Ireland, and for the establishment and development of those fisheries rests with the Department of Culture, Arts and Leisure (DCAL). The Agri-Food and Biosciences Institute for N. Ireland (AFBI) are employed by DCAL to provide the scientific basis for eel management in Northern Ireland.

2. Framework national cases and national guidelines (on contaminants and health)

2.1 Contaminants

_England & Wales:_ There is no monitoring network and there are no immediate plans for the Environment Agency to routinely monitor contaminants in eels as part of an on-going programme.

A number of studies have included assessments of contaminants in eel (references of general study). Recent work by Centre of Ecology and Hydrology (Jurgens et al. 2013) looked at contaminants in a small number of eels from English rivers but is not currently funded as an on-going monitoring programme.

No national framework specifically for sampling eel contaminants. However, there is extensive guidance on appropriate sampling of a variety of contaminants in OSPAR guidance (for marine) and recent biota standards guidance. These relate specifically to chemicals rather than eels.

_N. Ireland:_ Contaminant analysis is not performed on a regular basis on eel populations across N. Ireland or in the fisheries at Lough Neagh or Lough Erne as it is not seen as a major issue given that historic testing proved it to be of no significance.

_Scotland:_ No current programme to monitor contaminants.

2.2 Diseases

_England & Wales:_ Eel health and diseases in England and Wales are monitored by the Environment Agency through mortality investigations, targeted surveillance and screening of eels prior to re-stocking.

Since 2013, two eel-specific mortalities have been reported from still water fisheries in England. Field investigations and detailed post-mortem examinations confirmed the primary cause for these losses to be Anguillid herpesvirus 1 (AngHV-1). These events, combined with previous outbreaks reported in 2009 and 2010 (Armitage et al. 2013), bring the total number of mortalities associated with this virus in England to four.

All four outbreaks of AngHV-1 have involved large eels, measuring between 70 and 120 cm in length. These fish had estimated ages of between 17 and 29 years and many eel examined showed morphological characteristics of silvering. Affected eels were lethargic and unresponsive with signs of external haemorrhaging, skin lesions and severe gill necrosis. Histopathological examinations revealed marked necrosis, haemorrhage and inflammatory changes within the gills, kidney, skin, liver and spleen.
Post mortality sampling suggested that up to 70% of the eel populations were lost from these waters. It is proposed that the onset of silvering, with associated physiological changes and migration pressure, were triggers for these disease events, which so far have all occurred in still waters with barriers to escapement. Further sampling is underway to assess the prevalence, persistence and impact of the virus within these waters.

Since 2011, efforts have been made to establish the distribution of AngHV-1 in wild eels in England and Wales. This collaborative study between the Environment Agency and Cefas, has involved taking blood samples from live eels captured and returned during routine monitoring activities. To date, 685 eels, from 36 rivers in 11 RBDS have been tested for antibodies to AngHV-1. An additional 429 glass eels have been tested, from 14 sites in 5 RBDS. This work has confirmed that AngHV-1 has a relatively widespread distribution, but exists at a low prevalence (~5%) in most of the rivers sampled. This work will help inform existing disease risk assessments for this virus. Efforts are also underway to assess the presence and distribution of other eel viruses in England.

Anguillicola crassus is widely distributed throughout England and Wales. Since 2009, adult eels from 32 rivers have been examined for this parasite. Of these, 26 rivers were found to be infected, with up to 93% of eels harbouring nematodes. A small number of catchments and isolated rivers in North Wales and Northern England remain either sparsely infected or tentatively free of the parasite. Studies are underway in collaboration with Salford University to confirm and progress these findings.

Efforts have also been made to establish A. crassus infections in other life stages of eels. During 2011, a sample of 200 silver eels obtained from the River Avon, Hampshire for research purposes was examined for parasites. A. crassus was found in 85% of these fish, with infections ranging from 1 to 58 parasites (mean = 8.2). Since 2011, eight samples of glass eels were also examined for parasites during routine health checks prior to stocking. Two samples from the Great Ouse and River Severn revealed A. crassus, with 37%–53% infected respectively. These included a number of heavy infections resulting in total occlusion of the swim bladder. Other notable infections included Dermocystidium anguillae in 20% of these fish, with cysts engulfing large areas of the gills.

Collaborative studies

A number of collaborative projects are underway to progress understanding of European eel health interactions. This includes development of a standardised protocol to harmonise assessments of eel spawner quality and maximise retrieval of data from UK monitoring activities (Lewin et al. 2014).

A study in collaboration with Southampton and Cardiff Universities was also conducted to assess the influence of parasites on the behaviour and passage of silver eels in freshwater. This involved observations of 150 silver eels in response to a range of flow regimes within flume facilities. It has been shown that infections of A. crassus alters the behaviour of silver eels, causing avoidance of high flow velocities, potentially delaying downstream migration (Newbold et al. in press). This could have important implications for eel passage, escapement and eel spawner quality.

The Environment Agency and Cefas, Weymouth are collaborating on development of diagnostic tests for pathogenic eel viruses. It is hoped that this will facilitate the detection
of important viruses of eel during both mortality investigations and potentially fish movement activities.

**N. Ireland:** All work is performed on live, fresh material. Eels are sampled regularly as part of a long-term research programme which investigates all life stages throughout the year. Yellow eel catches are sampled weekly over 20 weeks (from May to September). A sample of 20 yellow eels is chosen to reflect all size ranges caught, and analysed for age and length. In addition, the entire, ungraded landing of two fishing crew on one day each month is sampled, usually comprising 400–600 eels captured by long line and a similar number by draft net, to enable comparison between methods. Every eel is measured for length and the total catch recorded.

In the Lough Neagh/Bann RBD, samples of 10 eels chosen to reflect all size ranges in the catch are removed every week over a 12 week period and analysed for age and length. At weekly intervals the previous nights haul is measured for length. The number analysed can vary widely but on average covers at least 400 fish within a night’s catch of >1 t. The weekly silver eel samples are also analysed for weight, sex, age, stomach contents, the prevalence and intensity of *A. crassus*, and gastrointestinal endohelminths. Sex ratio of the silver eel population is also estimated by counting the numbers of individuals contained in the graded 15 kg boxes which the fishery then sell. Eels are graded as small (males) and large (females), based on a length-sex key derived from previous sampling.

In Lough Neagh, the glass eel/elvers are monitored for the presence of *A. crassus*, and the weekly samples of yellow eels are also examined for weight, sex, age, stomach contents, the prevalence and intensity of *A. crassus*, and gastrointestinal endohelminths. The undersized yellow eels (<40 cm long) captured via long line are returned to the Lough at the point of capture. Every month 100 undersized eels are sampled at the fishery, their hook location recorded and in conjunction with analysis of the catch composition; attempts are made to quantify possible losses to the fishery through hook mortality.

Preliminary analysis indicates that a larger proportion of small eels (<40 cm) are captured by draft nets whereas more of the larger eels (>60 cm) are taken on long lines. Furthermore, there was significant variation in the numbers of small eels captured by long lining dependent upon bait type (earthworms caught more) and hook size (larger hook caught fewer small eels).

For the first time in 50 years permission to carry out a fyke net survey on Lough Neagh was granted by the LNFCS in the summer of 2013. At the time of writing, part one of this survey has been undertaken with the repeat to take place late September. In 2014 a new Queens University PhD funded directly by the LNFCS began examining all aspects of the biology of the Male eel based on L. Neagh.

**Scotland:** There is no national level monitoring for eel diseases. At one time SEPA sampled eels to assess pollutants in muscle tissue (one paper was published and a second maybe in review). At this time Marine Scotland Science requested that they examine the swim bladders for signs of *A. crassus* (which was found at 2 of the c. 30 locations sampled).
3. General issues on eel sampling for contaminants and/or diseases in your country. Treatment of the sampled eels on site and in the laboratory.

3.1 Ethics and legislation

England & Wales: In England the Environment Agency is responsible for implementation of EU Eel Regulations. Capture fisheries are regulated by Section 27 of the Salmon and Freshwater fisheries Act. As such, authorisation is required and allowed effort is limited and controlled. The introduction of eels is regulated under the Keeping and Introduction of Fish Regulations 2015. Introductions must meet conditions of source and health status of fish. Access and passage of eels are regulated to allow accessibility of waters. Cyfoeth Naturiol Cymru (Natural Resources Wales) is responsible for delivery of these roles in Wales.

A significant ethical consideration is the sacrificial sampling of eels to assess health and spawner quality. Whilst this is an essential means of monitoring, efforts have been made to develop and conduct non-destructive sampling of eels for specific disease issues e.g. eel viruses.

N. Ireland. The competent authority for live fish movements is the Dept of Agriculture and Rural Development (DARD). As eels are not listed as a susceptible or vector species by DARD they are not covered by their Aquatic Animal Health Regulations.

3.2 Sampling procedures

Sampling sites vary depending on different monitoring activities (disease/mortality investigation; health check sample for fish movement; project work).

Disease/mortality investigations: These involve both riverine and still water environments, including heavily managed and intensively fished waters. For all disease investigations, a standardized questionnaire (Environment Agency Fish Mortality Questionnaire) is used to ascertain important information about all sites. Site details (date sampled; site name and address; National Grid Reference; Live Fish Movement code; contact name and phone numbers); Fishery details (water type; type of fishery; fish density; angling pressure; size and depth; inlets/outlets; adjacent land use); Water quality (DO; temp; pH; ammonia; water clarity; habitat; algal bloom); Mortality details (date losses started; rate of losses; other waters on site; recent fish movements; obvious signs of pathology; details of previous losses); Fisheries management details (biosecurity; angling close season; fishery management details; unusual events prior to losses).

Health check supporting fish movements of glass eel, elver or yellow eels: these may be still water or riverine sites and information obtained will include site address; National Grid Reference; EA area; RBD, Fish Movement reference code. For these sites fewer site characteristics would be recorded (for instance water quality parameters would not be collected). (Environment Agency Fish Health Check Submission Form).

Project work: this would be dependent on site and specifics of the work. Type of water, NGR, location, would be taken as standard. Water quality parameters may be included.

Environment Agency eel specific monitoring surveys for WFD: site name, river, survey purpose, catchment, date, National Grid Reference, water chemistry (conductivity, ammonia, temp, DO, pH), fishing method, survey strategy, use of stop nets, pool depth (me-
tretes), riffle depth (metres), site length/width, survey area, conditions (weather, flow, turbidity, shade), flow type, substrate, dominant plant species, sources of cover, non-native species, land use and potential impacts. (Environment Agency Fish Population Survey Field Sheet).

Sampling procedures also depend on type of analysis to be completed. Mortality/disease investigation: a representative sample (eels showing symptoms of disease) of 5-6 eels would be required. These may be hand netted from margins, netted, or electrofished. For a mortality investigation, freshly dead eels may be suitable for tissue collection for virology screening only.

Health checks: 30 fish total need to be examined for a health check sample, however only 10 of a species are required. Glass eels may be collected by netting or captured whilst going over barriers.

Project work: dependant on specifics of work, but most likely to be electro-fished. This is an efficient way of sampling wild eels (particularly on rivers). Example: HVA distribution project: 30 yellow eels per site (eels over 300mm ideal due to ease of getting blood sample efficiently); aim for 1 site per RBD; 3 years of study (2011-2013). For each fish examined; length noted to nearest mm; general condition and evidence of clinical disease. Only tissues collected were blood samples and all eels returned alive. For any destructive sampling, there will be a site by site sensitivity on what number of eels may or may not be collected for examination. This is based on expert knowledge of sites and environmental/anthropogenic sensitivities.

N. Ireland: Standard Operating Procedures (SOPs) are in place to ensure that operations are carried out in a consistent manner. SOPs are in place to standardise measurement of morphometrics; swim bladder examinations; gastrointestinal tract removal; stomach content analysis and endohelminth identification. SOP 25 (code: FWFISHNO25v.1) allows the collection of data on lengths and weights of eels measured in the field; assessment of the infection status of swim bladders with A. crassus; removal of the intestinal tract; collection of data on feeding habits; and infections parameters of endohelminths. These analyses are performed on both yellow and silver eel life stages from a range of sites across N. Ireland with a more intensive focus on the commercial fishery on Lough Neagh.

3.3 Stage, gender, morphometrics

England & Wales: All routine health examinations and mortality investigations are performed in the laboratory following submission of live eels. All measurements and observations are based on the examination of fresh material following euthanasia of live eels. Targeted monitoring for specific diseases may be conducted in the field, generally non-destructive sampling on anaesthetized fish.

Eel stage is distinguished by techniques as described by Durif et al. (2005). Gender is distinguished either by microscopic examination or histopathology.

How do you measure and express morphometrics (weight, length, accuracy issues, units)? Living animals? Or after fixation, freezing?

England & Wales: Laboratory examinations following mortality/disease investigations and health checks: weight to nearest 0.1gram and length to nearest mm. If destructive sam-
pling, swim bladder lengths measured using electronic calipers to nearest 0.1mm. Measurements will be taken on euthanized eels and therefore accurate.

Environment Agency eel specific monitoring surveys: every eel captured has length measured to nearest mm and weight to nearest gram. Measurements are taken on live eels.

*N. Ireland:* All work is performed on live, fresh material. Yellow eel catches are sampled weekly over from May to September. A sample of 20 yellow eels is chosen to reflect all size ranges caught, and analysed for age and length. In addition, the entire, ungraded landing of two fishing crew on one day each month is sampled, usually comprising 400–600 eels captured by long line and a similar number by draft net, to enable comparison between methods. Every eel is measured for length and the total catch recorded.

In the Lough Neagh/Bann RBD, samples of 10 eels chosen to reflect all size ranges in the catch are removed every week over a 12 week period and analysed for age and length. At weekly intervals the previous nights haul is measured for length, weight and sex. Sex ratio of the silver eel population is also examined by counting the numbers of individuals contained in the graded (depending upon size) 15 kg boxes. The fishery records the number of boxes of small (male) and large (female) eels sold, and from this the sex ratio and number of silver eels can be estimated.

In Lough Neagh, the weekly samples of yellow eels and silver eels are examined for weight, sex and age. Sex ratio of the silver eel population is also estimated by counting the numbers of individuals contained in the graded 15 kg boxes which the fishery then sell. Eels are graded as small (males) and large (females), based on a length-sex key derived from previous sampling.

### 3.4 Treatment

*Live storage. How, how long, which water system?*

*England & Wales:* Fish maintained in holding facilities (Environment Agency, National Fisheries Laboratory) prior to fish health investigations. Secure holding tanks fed by borehole water (constant 10.6°C and pH 7.6) as a flow through system. Waste water is ozonated prior to discharge. Tubes added to tanks during maintenance of eels to act as habitat, to reduce activity of eels. For health checks, eels generally held for no more than 3 days. For specific project work, eels may stay in tanks for up to a week prior to laboratory examination.

Glass eels: generally arrive in polystyrene boxes or oxygenated poly bags. They will stay within these until examination, which generally takes place within two days. Water changes and aeration may be added if required.

Mortality/disease investigation: live eels will arrive in site source water, and will remain in this until euthanized. All eels from mortality/disease events are examined immediately upon submission to the laboratory.

*N. Ireland:* Work is completed on situ on the lake shore/processing factory at Toome (for Lough Neagh samples).

*How do you euthanize/anaesthetize eels (= animal experiment)? Ethics considerations.*
-Mortality/disease investigations and health checks: live eels will be euthanized in benzocaine solution prior to examination.

-HVA distribution project: eels anesthetized in benzocaine (approximately 100mg/l) as non-destructive sampling. Eels strictly monitored prior to blood sampling and during recovery.

*N. Ireland*: Clove oil and acetone anaesthetic solution (3ml clove oil in 500ml acetone). Stored and transported in glass jar that solution prepared in. To prepare anaesthetic: 10ml of clove oil solution dissolved in 10 litres of freshwater.

*Hygiene measures, what measures do you use not to cross contaminate samples from various sites, protective clothing (gloves, etc.), human health considerations.*

*England & Wales*: Strict biosecurity measures in place involving good laboratory practice, protective clothing, and disinfection procedures. All equipment thoroughly disinfected with Virkon Aquatic and waste water treated through on site ozone plant prior to discharge.

*N. Ireland*: The SOPs used for this work detail measures designed to avoid cross contamination and given that the vast bulk of the work is carried out in situ at the various sites studied we carry separate sets of analytical kit for each RBD to prevent the spread of introduced species such as zebra mussels and *or A. crassus.*

*Labelling issues/techniques.*

*England & Wales*: Every eel examined is given its own unique reference number. This is consistently used throughout all sampling for that individual (i.e. on paperwork, parasite collection, bacteriology swabs, histopathology, virology etc.).

*How and where to store samples? Prior to, and after analysis. Do you have a tissue database and a tissue bank.*

Parasites; fixed in appropriate chemicals/fixatives and stored in on site archives or if for external ID/use, in fume cupboard prior to dispatch. Samples for histopathology routinely fixed in Neutral Buffered Formalin for 48 hours prior to processing. All histopathology material archived. Blood samples; chilled at +4°C prior to centrifuged. Whole blood, pellet and serum frozen at -80°C prior to analysis. If muscle/liver tissues collected, frozen and archived at -20°C.

4. General issues on eel condition (fitness, lipid levels)

*England & Wales*: Condition and lipid levels are not routinely monitored during eel examinations in England and Wales.

4.1 Condition

*England & Wales*: Length and weight data recorded as standard, allowing assessments of condition e.g. using Fulton’s Condition Factor. Though aware of apparent limitations to such measures and not completed routinely.
4.2 HSI

England & Wales: Not routinely collected. Not routinely taken, but has been included in specific studies, measured as per Durif et al. (2009)

N. Ireland: forms part of the PhD on male eel.

4.3 GSI

England & Wales: Not routinely collected. However, would use the formula: GSI = mass of gonad/body mass x 100 (Durif et al. 2009)

N. Ireland: forms part of the PhD on male eel.

4.4 Lipid levels

England & Wales: Analysis of lipid levels is not routinely included in health assessments of eel. Lipid samples for specific research taken on skinned dorsal muscle.

N. Ireland: Measure regular fat content levels in both yellow and silver eel using a fat meter.

4.5 Other condition related issues

N/A

5. Contaminants

5.1 Sampling and analysis

England & Wales: The Environment Agency does have a national monitoring programme for the analysis of contaminants (EQS Directive). However, examinations on eel are not included within this. Opportunities have been taken in the past to collect and store tissues (muscle and liver) from eel specific research projects to support future analysis of contaminants.

EQS Directive and the requirements for biota sampling for various priority substances had to be based on previous guidance issued by the European Commission. Guidance Document 25 stated “Because of their protected status, eels should only be used for existing trend monitoring (to continue existing monitoring programmes) and for this species the principle of conservation has to be respected.” Consequently, it was not possible for England to consider using eels for biota monitoring and as such roach and trout are sampled instead.

The EQS Directive specifies that monitoring of PAHs must be done in invertebrates/crustaceans/molluscs (we used signal crayfish). This is because fish metabolise PAHs and so are not expected to have high levels of these contaminants. Preliminary results even from crayfish suggest that PAHs are not a significant issue in England. Due to last years analysis of contaminant levels from biota monitoring, we are able to give an indication about likely contamination levels in eels.

N. Ireland: Contaminant analysis is not performed on a regular basis on eel populations across N. Ireland or in the fisheries at Lough Neagh or Lough Erne as it is not seen as a major issue given that historic testing proved it to be of no significance.
Scotland: looked at replacing eel in their contaminant monitoring due to their conservation status. (Macgregor 2010)

5.1.1 PCBs – N/A

5.1.2 Pesticides – N/A

5.1.3 Brominated Flame Retardants – N/A

5.1.4 Dioxins – N/A

5.1.5 Metals – N/A

5.1.6 PAHs – N/A

5.1.7 Others?

5.2 Interpretation, visualisation and assessment

Which threshold values to use? Maximum allowable levels? EU WFD levels in biota. Ecotoxicological thresholds. Human health limits. Give a list of contaminant threshold values (legal or indicative ones) used in your country.

Do you use contaminant quality classes for interpretation, visualisation and assessment?

How do you visualise your results? Give examples?

England & Wales: Although no national routine programme to analyse contaminants, there has been various work completed: Doyotte et al. (2001); Edwards et al. (1999); Juergens et al. (2009); Knights (1997); Macgregor et al. (2010); Mason (1993); McHugh et al. (2010); Peters et al. (2001); Ruddock et al. (2003); Weatherley (1997).

The UK follows details and threshold limits as per the EU Eel regulations. These tissue concentrations of contaminants are recognized by ICES as having a potential impact on effective spawner stock biomass.

6. Diseases

6.2 Sampling and analysis of eels for parasitology (Anguillicola and others)

England & Wales: Eels are screened for parasites and clinical signs of disease prior to all eel and elver stocking activities. This is a requirement of Section 30 of the Salmon and Freshwater Fisheries Act 1975 (to be replaced with The Keeping and Introduction of Live Fish (England and River Esk Catchment) Regulations 2015). Routine fish health screening involves the examination of 30 eels, which may be obtained through national monitoring activities (electro-fishing) or in the case of elvers, from fish passes or licensed fishermen.

Parasitology is also an important component of mortality investigations following suspected disease outbreaks in the wild. Virology, bacteriology and histopathology are also conducted as standard (detail below). Where possible, samples comprising six to ten moribund or clinically diseased eels are submitted for examination, although investigations will be conducted on whatever is available at the time of these events. Such samples may be obtained through hand netting the water margins, electro-fishing or fyke netting.
In all such cases, parasitological examinations are conducted on live eel samples, prior to lethal anaesthesia. Dead and frozen eels are not suitable for full parasitological examination. Detailed dissections are performed with examinations of all major organs using both low and high power microscopy. For any heavy, unidentified or notable parasite infections, accompanying tissues are fixed for histopathology. Digital images are taken of gross changes and infection characteristics. Tissues for histopathology are routinely fixed in Neutral Buffered Formalin (NBF), although different fixatives may be used for hard tissues (e.g. Bouins, Davidsons).

Parasitological examinations are also conducted as part of targeted monitoring activities or specific research projects, which may be performed by government laboratories, universities or other fish health professionals. The sampling and approaches used in these cases may differ depending on the questions posed, samples needed and resources available.

A national protocol is being developed to standardise necropsy procedures for eel health assessments (Lewin et al. 2014; in prep). For routine parasite screening and national disease investigations the following tissues are routinely examined for parasites:

- External examination of skin, fins, eyes, vent and mouth using low power microscope
- Skin scrape and mucus from dorsal, lateral and ventral surfaces (examined under high power)
- Heart, gills (all arches from one side), eye (from uppermost side), body cavity, stomach and gastro-intestinal tract, gall bladder, gonads, muscle (long shallow fillet), and nares. For each of these, the organ is examined under low power microscopy and dissected for parasites.
- Kidney, liver, spleen and gill squashes are prepared and examined under high power magnification. Blood examinations are included at this stage.
- Swim bladder removed whole to a petri dish of saline, opened and examined for parasites using low power microscopy and base illumination.

Parasites are identified through either wet preparations or morphological examinations of fixed, mounted, cleared and stained specimens. Parasites that are difficult to confirm through morphological examinations alone, may also be fixed for molecular identification.

Table 1. Routine approaches to identification and handling of parasites.

<table>
<thead>
<tr>
<th>Parasite Taxa</th>
<th>Handling &amp; 1st Fixation</th>
<th>Mount / ID / Storage</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monogenea</td>
<td>80% ethanol</td>
<td>Fresh mount / 80% ethanol</td>
<td>Pugachev et al., 2009</td>
</tr>
<tr>
<td>Digenea</td>
<td>Hot saline</td>
<td>Paracarmine mount / 80% eth</td>
<td>Gibson et al., 2002</td>
</tr>
<tr>
<td>Cestoda</td>
<td>Hot saline</td>
<td>Paracarmine mount / 80% eth</td>
<td>Chubb et al., 1987</td>
</tr>
<tr>
<td>------------------</td>
<td>--------------</td>
<td>-----------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>Protists</td>
<td>Dry smear</td>
<td>Dry mount</td>
<td>Lom &amp; Dyková, 1992</td>
</tr>
<tr>
<td>Nematodes</td>
<td>Berlands</td>
<td>80% ethanol, cleared with glycerol or creosote</td>
<td>Moravec, 1994</td>
</tr>
<tr>
<td>Acanthocephala</td>
<td>Tap water</td>
<td>80% ethanol</td>
<td>Brown et al., 1986</td>
</tr>
<tr>
<td>Crustacea</td>
<td>80% ethanol</td>
<td>Fresh mount / 80% ethanol</td>
<td>Fryer, 1982</td>
</tr>
<tr>
<td>All – molecular ID</td>
<td>100% ethanol</td>
<td>100% ethanol / RNA later</td>
<td></td>
</tr>
</tbody>
</table>

For each eel examined, the following information is routinely recorded.

- Total length (mm) and weight (g)
- General external condition, with evidence of any gross abnormalities
- Sex and life stage
- Age of eels (from otoliths)
- Identity of all parasites to species level (with some exceptions e.g. Trichodinids to genus)
- Number and location of parasites (if not exact parasite count, then at least an indication of infection level - low, moderate and heavy based on experience)
- Presence of any exotic or non-native parasites
- Prevalence, intensity range and mean intensity within the population (Bush et al., 1997).

For any new, novel or non-native parasite detected during eel health examinations, fish movement controls may be imposed pending further assessments of distribution and pathogenicity. These procedures are structured around a national risk assessment framework for the management of non-native fish parasites (Williams et al. 2011).

*Anguillicola crassus* (revision back to original name based on recent molecular review)

*A. crassus* was first recorded in England in 1987 (Kirk, 2000) and has since spread rapidly throughout England and Wales. The parasite has been the subject of numerous research studies and parasitological surveys, which have confirmed the parasite’s widespread distribution and wide intermediate host range. This parasite has received considerable attention and been the subject of numerous papers and reviews (Kennedy and Fitch, 1990; Pilcher and Moore, 1993; Ashworth and Kennedy, 1999).

During eel dissections, the number of adult female nematodes as well as male, young adult and larval stages within the swim bladder wall are recorded separately (Moravec,
2006). Evidence of gross pathological changes is recorded prior to removal of the swim bladder. Once removed from the body cavity, the swim bladder is dissected with a single cut along its length and parasites removed from the lumen and counted. In certain studies, total mass of parasites has also been measured from each eel (Newbold et al. in press). The swim bladder wall is laid flat and also examined under low power microscopy for nematodes (Environment Agency). In recent samples, the length of the swim bladder has been recorded with digital callipers prior to removal. Assessments of impact are made through histopathology but combined with observations of gross changes including wall thickening, opacity, haemorrhage and discolouration. To avoid distortion during fixation, the swim bladder is fixed flat and transverse sections processed for histopathology.

Other parasites
Parasite surveys spanning more than 40 years have revealed that wild eels harbour a diverse range of parasites (Copland, 1981; Coneelly and McCarthy, 1986; Kirk, 2000; Kennedy, 2001). Information on the parasite fauna of eels has previously been collated in national checklists of British freshwater fish (Kirk, 2000). This information is currently being updated (Brewster et al. in prep). To date, 49 different parasites have been recorded from eels in freshwater. In addition, recent studies have focussed on the pathological importance of parasite infections in elvers, yellow eels and silver eels. Reading et al. (2009) conducted a survey of eel parasites combining morphological and histopathological characteristics of these infections. This work was based on parasitological examinations of over 200 eels from 6 catchments sampled between 2008 and 2013. This work highlighted that, in addition to A. crassus, other parasite infections can cause marked pathological changes with potential to compromise eel health and fitness (e.g. Pseudodactylogyrus spp., Dermocystidium anguillae in elvers).

N. Ireland: Anguillicola crassus is now considered to be ubiquitous throughout Northern Ireland.

North East RBD
It was first recorded from the North East RBD in 2010 where it was found in eels sampled from the Quoile system (N = 52, prevalence 30% mean intensity <1 worm per infected eel). In 2011, A. crassus was found in other lakes connected to this initial location, but was not detected in three other areas.

North Western International RBD
The first records of A. crassus in Ireland were from this RBD in July 1998. No new data are available since the last report.

Neagh/Bann RBD
A. crassus was first found in Lough Neagh yellow and silver eels in 2003, and its spread has been monitored via the analysis of over 3000 yellow and over 1000 silver eels from 2003 to 2013. Prevalence has always been higher in silver than yellow eels, but has reduced in both stages since 2005 (Table 2).
Table 2. Prevalence (% eels sampled) of *A. crassus* in Lough Neagh yellow and silver eels.

<table>
<thead>
<tr>
<th>YEAR</th>
<th>% YELLOW EELS</th>
<th>% SILVER EELS</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>93</td>
<td>100</td>
</tr>
<tr>
<td>2008</td>
<td>67.3</td>
<td>86</td>
</tr>
<tr>
<td>2009</td>
<td>53.6</td>
<td>73</td>
</tr>
<tr>
<td>2010</td>
<td>48.8</td>
<td>80.7</td>
</tr>
<tr>
<td>2011</td>
<td>56.7</td>
<td>74</td>
</tr>
<tr>
<td>2012</td>
<td>40.5</td>
<td>55</td>
</tr>
<tr>
<td>2013</td>
<td>50.9</td>
<td>70</td>
</tr>
</tbody>
</table>

6.2 Sampling and analysis of viral and bacterial diseases

*England and Wales:* Screening for viral and bacterial diseases is conducted as a routine component of national mortality investigations in freshwater fisheries. These involve the examination of moribund or clinically diseased eels that are submitted to the laboratory live for post mortem examination. Where sampling of live eel is not possible, or where dead/frozen material is available, only virology samples are taken. Depending on condition, gross pathological changes may be noted.

During such laboratory investigations, the following information is routinely recorded for all sample submissions:

- Total length (mm) and weight (g) of each eel
- General external condition, with evidence of any gross abnormalities or clinical signs of disease.
- Digital images of any such abnormalities as reference for further examinations/tests
- Sex and life stage of eel
- Age (from otoliths)

Similarly, detailed information about the characteristics of the mortality (timings, duration, scale of losses, species affected), environmental parameters (water type, temperature, pH, ammonia, algal blooms, turbidity, adjacent land use) and management activities (recent stocking, stock densities, anthropogenic influences) are collected to support laboratory examinations (see earlier section on mortality questionnaire).

*N. Ireland:* there is no routine monitoring for viral or bacterial diseases of eel but this facility is available on request from DARD Fish Health Unit and AFBI Veterinary Science Division (VSD).
6.2.1 Viral diseases

*England & Wales:* Between 2009 and 2013, four outbreaks of Anguillid herpesvirus 1 (AngHV-1) have been confirmed in still water fisheries in England (Armitage *et al.* 2013; Williams in prep).

Armitage *et al.* (2013) provided detail of approaches for confirmation of AngHV-1 during mortality investigations. Samples of kidney, spleen and brain are routinely taken from each eel during necropsy. These tissues are pooled (totalling 1 g) for each eel sample and stored in standard viral transport medium (Glasgow modification of minimal essential medium (GMEM) supplemented with 9% NBCS (New born calf serum), 1% glutamax, 1% penicillin streptomycin, 0.2% TRIS (TRIZMA Base 120 g L⁻¹), 0.5% sodium bicarbonate and 1% antibiotic antitoxicycotic stabilised solution) at 4 °C. Samples of gill are also taken and handled in the same way. Detection of AngHV-1 was confirmed through nested PCR following cell culture on Epithelioma papulosum cyprini (EPC, Fijan *et al.* 1983), bluegill fibroblast (BF-2, Wolf *et al.* 1966), fathead minnow (FHM, Gravell & Malsberger 1965), Chinook salmon embryo (CHSE-214, Lannan *et al.* 1984) and eel kidney (EK-1, Chen *et al.* 1982).

For all mortality investigations subject to viral and bacterial screening, additional samples of gill, liver, kidney, spleen, heart, skin and gastro-intestinal tract are fixed in NBF for histopathology (Armitage *et al.* 2013). Similarly, during disease outbreaks, additional gill samples have been taken for electron microscopy. These are routinely fixed in 2% glutaraldehyde 0.1-M phosphate buffer (pH 7.2) and used to visualise virion replication.

Following the aforementioned outbreaks of AngHV-1 in England a national monitoring survey was conducted to assess the distribution of the virus in wild eels. The ability to culture AngHV-1 in eel kidney (EK-1) cells allowed the development of an ELISA to detect antibody to the virus in eel serum (Way *et al.* 2014). The ELISA has been used to detect high levels of antibody to AngHV-1 in surviving eels at three of the previous mortality sites. The ELISA has also been used to screen for antibody in blood samples from live eels collected during routine monitoring activities throughout England and Wales. This has ensured non-destructive sampling of large numbers of eels in RBDS throughout E&W. Opportunities for screening eels for viruses prior to stocking are being explored, but are not part of current monitoring activities. Work is also underway to develop appropriate diagnostic tools for the detection of other important eel viruses.

*N. Ireland:* No EVEX, and no other pathogens of major concerns.

6.2.2 Bacterial diseases

*England & Wales:* Routine sampling for bacterial pathogens involves taking swabs from the kidney, plated onto Tryptone soya agar (TSA), Columbia sheep blood agar and Thiosulphate citrate bilesalts sucrose (TCBS) cholera agar and incubated at 22 °C for 14 days (Armitage *et al.* 2013). Swabs will also be taken from other tissues (e.g. skin) in the presence of clinical disease.

Bacteria are identified using standard microbiological and biochemical tests (Whitman 2004; Austin & Austin, 2014). To date, the bacterial pathogens recovered during eel disease examinations have all been species of *Hydrophila, Vibrio* and *Pseudomonas.* Pseudomo-
nas anguilliseptica was previously isolated from farmed eels in Scotland (Stewart, 1983) but has not been recovered from wild eels in England.

7. Perspectives of using biomarkers of effects to assess eel health

*England & Wales:* Some work has been completed on development of biomarkers in River Thames eel population (Linvingstone *et al.* 2000)

8. International needs (making your results available, and international database)

*Are the results of eel quality assessments in your country made available to the international community?*

*England & Wales:* Results gathered from assessments and eel health examinations will be published through Environment Agency reports, specific to the type of work completed (disease investigation/health check screen etc.). Specific work will also be made available through publication in scientific journals. Other methods of reporting results include through ICES Working Group for Eel, ICES yearly country reports and by reporting to the EU in the framework of the WFD.

If resources allowed, the reinstatement of the Eel Quality Database would provide a beneficial and informative platform to upload data on eel contaminants and disease, and to compare data on a European level.

*N Ireland:* all data is made available yearly upon request from WGEEL for inclusion in the EEQD.

9. Other issues, remarks

Available resources

Cost of analysis

National programmes vs. Small scale projects

Collaborative work – maximisation of tissues

Destructive sampling techniques to endangered species – ethics

Missed opportunities to collaborate between institutes and organisations

Improvement of communication

As stated in the Report of the Joint EIFAAC/ICES/GFCM Working Group on Eel 2014, monitoring eel quality is an expensive undertaking, and at the moment no guidance is available to prioritize what assessments should be conducted that will give meaningful information. While this is potentially important work, it should be evaluated against other data deficiencies and research needs to ensure that it is the highest priority area for improving the assessment and management of eel. A standardized assessment approach would provide a means to address gaps in data reporting, and to allow comparative analysis across Europe.

10. References


