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Report of the Workshop of the Working Group on Eel and the Working Group on Biological Effects of Contaminants (WKBECEEL)

25–27 January 2016

Os, Norway

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International Council for the Exploration of the Sea
Conseil International pour l'Exploration de la Mer

H. C. Andersens Boulevard 44–46
DK-1553 Copenhagen V
Denmark
Telephone (+45) 33 38 67 00
Telefax (+45) 33 93 42 15
www.ices.dk
info@ices.dk

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Contents

Executive summary

The Workshop of the working group on eel and working group on biological effects of contaminants (WKBECEEL) met in Os, Norway, 25–27 January 2016, to discuss the subject "Are contaminants in eels contributing to their decline?", chaired by Caroline Durif (Norway) and Bjørn Einar Grøsvik (Norway). There were 19 participants representing 10 countries.

The European eel population has been declining since the 1980s. The Working Group on Eel (WGEEL) has been documenting the decline for at least three decades. The causes for the collapse are multiple: overfishing, habitat reduction, pollution, parasites and diseases, and climate change. The role of contaminants is poorly documented because the final migration and reproductive phase of the eel's life cycle remain unknown. It was therefore recommended to use knowledge from other fish species to evaluate whether it could apply to eels.

The objective of the WKBECEEL was to describe 1) the trends in contaminants in eel, 2) the potential impact of contaminants on lipid metabolism and migration in eel and other species, 3) the impact of contaminants on reproduction in eel and other species, 4) review the impact of contaminants on the genetics of the European eel, and 5) suggest methods to quantify eel quality with regards to contaminants and what could be learned from other species.

Temporal trends of contaminants in eel are still very high, sometimes rendering eels unfit for human consumption. Contaminants clearly remain a health threat for eels now and will remain so for many years because of their long life-cycle and the persistence of legacy contaminants in the environment. Analyses of historical samples (pre-1980s) would help in understanding the dynamics of these contaminants and give us a better grasp on the potential effects of emerging contaminants.

Eels in some areas accumulate high concentrations of lipophilic contaminants, all of which may affect their health and fitness. The concentration is likely to increase at the end of their life cycle, as they fast during their transatlantic migration. Thus, contaminants, as they are released into the blood can cause damage to reproductive organs, affect embryogenesis and larval fitness and survival.

Clear dose-effect relationships for specific contaminants are missing. In other species, contaminants reduce fecundity, lower hatching success and reduce egg quality, induce larval malformation and/or disrupts the endocrine system. Effects on eels are limited to a model which predicts that, depending on eel sensitivity, maternally transferred dioxin-like contaminants (at realistic levels) could cause up to 50% larval mortality.

The limited evidence in other species indicates that there is cause for concern when it comes to the possible effects of contaminants on eel navigation. However, direct research (such as experiments in swim tunnels) is lacking on the effect of contaminants on migration.

Synergistic effects between contaminants and disease agents are very likely. The impact of contaminants at the genomic and transcriptomic level is promising for the development of tools to evaluate biological effects. Ways to incorporate the effects of contami-

nants into quantitative assessments were described. Two eel research proposals are described in the last chapters: a) to standardize fat level measurements in eels, and b) to quantify the effect of contaminants on the reproductive success.

1 Introduction

1.1 Background for the workshop

The European eel is a critically endangered species on the IUCN red list of species (http://www.iucnredlist.org/details/60344/0).

Anguilla anguilla is constituted of a single population with unique spawning grounds in the Atlantic and a widespread growth area ranging from northern Africa and into the Mediterranean, up to northern Norway. Therefore, the status of this species concerning many crucial aspects can only be reported through a strong network of data and scientists covering the North Atlantic, North Sea, Baltic, Mediterranean and North African areas. This has been the aim of the joint EIFAAC/ICES/GFCM Working Group on Eels (WGEEL).

To give advice, the group has traditionally focused on quantitative stock assessment based on glass eel recruitment, more recently on silver eel escapement, and perhaps in the future also on yellow eel abundance (see the eel's life cycle). The decline in the European eel population has now been documented for at least three decades (ICES 1996) and declining trends are evident from some parts of the stock as far back as the 1950s and 1960s (Dekker and Beaulaton 2016). Trends in recruitment clearly show a decline starting in the early 1980s. The collapse may have been caused by overfishing, habitat reduction, introduction of parasites, predation, pollution and probably changes in ocean-atmospheric conditions affecting their early oceanic life-stage (Bevacqua *et al.* 2015; Miller *et al.* 2015; Castonguay and Durif 2015). Although the relative impact of these causes are unknown, contaminants may cause a decrease of migratory and reproductive capacities.

The decrease in recruitment in the eel population during the last 30 years coincided with a strong intensification of agriculture and with the industrial production of various new substances (van Leeuwen and Hermens, 1995; Anonymous 2003; Guhl *et al.* 2014). While the decline of several animal populations attributed to pollution was most evident during the seventies, the population crash in eel actually occurred in the early eighties. This can be explained by the eel's long and variable generation time of up to 30 years (Belpaire *et al.* 2016). The more or less simultaneous decreases in recruitment in the Northern hemisphere eel species (*A. anguilla, A. rostrata* and *A. japonica*), suggest that a common source or multiple causes are involved, reinforcing the argument that specific broadly distributed contaminants over the industrialized world are key elements in the decline (Geeraerts and Belpaire 2010).

Eels are particularly vulnerable to contaminants because of their high lipid content, their trophic position as a top predator, their longevity and their bottom-dwelling habits. Eels stop feeding during their spawning migration to the Sargasso Sea. They will subsist on their fat reserves until reproduction. As lipid reserves decrease during the journey, the levels of lipophilic contaminants in the blood will increase (Robinet and Feunteun 2002; van Ginneken *et al.* 2009).

Organochlorine pesticides and heavy metals were already mentioned in 1996 as a potential cause for the decline of the species and it was noted that the WGEEL would continue to watch for any new contaminant problems (ICES 1996). A probable negative impact of contaminants was mentioned in following reports but no actions were taken. In 2004, it

was requested by those organising the WGEEL that relevant environmental information (water quality and contaminants) be made available in country reports (ICES, 2004). A recommendation on pollution monitoring and the identification of areas producing high quality spawners (low contaminated areas) was included in the 2006 report. In the same report, advances on the effect of contaminants on reproduction were reviewed (ICES 2006). In 2007, the WGEEL established an Eel Quality Database (EQD) compiling eel quality parameters over its distribution area (Belpaire *et al.* 2011; see presentation summary in Annex 7). PCB, flame retardants, pesticides and heavy metals were to be given priority in the database (ICES 2008).

The implication of contaminants in the collapse of eels were summarized by Belpaire (2008) and ICES (2008) and considered again during WKBECEEL:

1) Contamination has been demonstrated as the cause of population collapse of many other biota from the 1970s on (e.g. the collapse of several birds of prey in the 1960s as a consequence of DDT);

2) Many chemicals have been developed and put on the market, simultaneous with the intensification of agricultural and industrial activities during the 1970s. The timing of this increase in the production and release of chemicals may fit with the timing of the decrease in recruitment from 1980 on. On the other hand, the period 1965-1980 was the most dirty in many countries but the collapse coincides with significant clean-up activities in Europe water systems;

3) Eels bioaccumulate many lipophilic, slowly metabolized and excreted, chemicals to a high extent;

4) The more or less comparable decreases in recruitment in the Northern hemisphere Anguilla species, like *A. rostrata* and *A. japonica*, during the last 30 years, might suggest that some new contaminants quickly spreading over the industrialized world, might have contributed to the decline;

5) Many reports have been dealing with direct adverse effects of contamination on individual, population and community level in fish. In eel, many detrimental effects of contaminants on the individual level have been demonstrated, including impact on sub-cellular, cellular, tissue and organ level. Also genetic diversity seems to be lowered by pollution pressure (Maes *et al.* 2013);

6) Considering the high levels of contamination in eels from many areas, endocrine disruption in mature silver eels might be expected, jeopardizing normal reproduction. Dioxin like contaminants have been reported to hamper normal larval development in other species and preliminary observations were made on eel (see Section 5);

7) Lipid levels in yellow eels from Belgium have decreased considerably over the past 15 years. This decrease in lipid levels could be induced by contamination. Low lipid levels may have contributed to a reduction in migration and reproduction success. In areas, such as Scotland, where eels are not or only slightly impacted by contaminants, similar decreases in lipid levels have not been observed (Oliver *et al.* 2015).

In 2010 and 2011, WGEEL attempted to quantify eel quality by constructing indices to evaluate eel quality. The Eel Quality Index (EQI) is based on contaminant concentrations, parasite and virus prevalence. The Reproductive Potential index (RP) is based on lipid

content in eels relative to the distance to the spawning grounds. Obviously, both of these approaches were based on overly simplified assumptions which need some fine-tuning before they can be used as a management tool (WKPGMEQ, ICES 2015).

In the absence of available empirical data on the impact of contaminants specifically on eel and its reproduction success, the WGEEL discussed the possibilities for an exchange of information with WGBEC (Working Group on Biological Effects of Contaminants). It was thus recommended in 2013 to organize a joint workshop between WGEEL and WGBEC under the subject "Are contaminants in eels contributing to their decline?". Prior to this, a workshop on "Development of standardized and harmonized protocols for the estimation of eel quality" met in 2014. Its main outcomes are summarized in Section 1.4. The ultimate objective of both workshops was to develop ways to integrate quality parameters into quantitative stock assessment (Figure 1). Parasites and diseases were not considered in WKBECEEL.

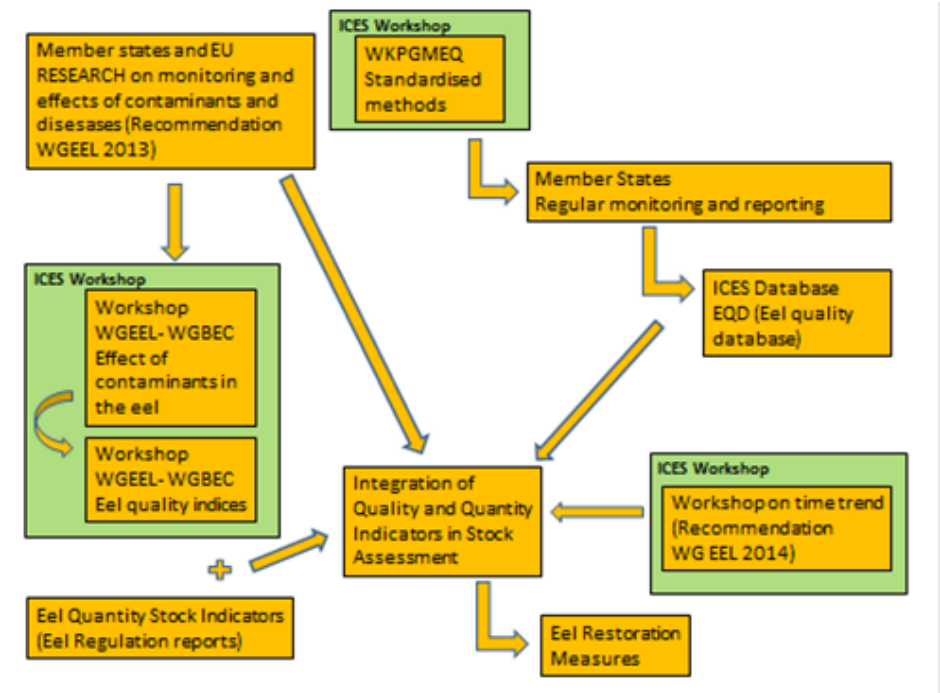

Figure 1. Integrating eel quality indicators in the international advice for stock management and restoration.

1.2 Terms of references and main tasks

The **WKBECEEL** was organized to respond to the following Terms of References (ToRs):

a) Describe the spatial and temporal trends in concentrations of "traditional" and/or "emerging" contaminants in eel (but mainly refer to figures available from WGEEL 2008–2013);

b) Describe the potential impacts of contaminants on reproduction in the European eel, based on science of eel and what can be learned from other species models (including endocrine disruption, effect on sex ratio, maternal transfer of bioaccumulated contaminants toward the eggs and effects on the larvae);

c) Describe the potential impacts of contaminants on lipid metabolism and migration in the European eel based on eel science and what can be learned from other species;

d) Review the impacts of contaminants on the genetics of the European eel;

e) Explore whether there is experience with assessing/qualifying the bioaccumulation + fitness status in other species, which can be helpful for the eel's quality assessment (Eel Quality Index) and to quantify the impact of eel quality.

The report is structured so that the response to each ToRs corresponds to a chapter.

1.3 The eel's life cycle

European eel life history is complex and typical among aquatic species, being a long-lived semelparous and widely dispersed stock. The shared single stock is panmictic (Palm *et al.* 2009) and data indicate the spawning area is in the southwestern part of the Sargasso Sea and therefore outside Community Waters (McCleave *et al.* 1987; Tesch and Wegner 1990). The newly hatched *leptocephalus* larvae use ocean currents to drift to the continental shelf of Europe and North Africa where they metamorphose into glass eels and enter continental waters. The growth stage, known as yellow eel, may take place in marine, brackish (transitional), or freshwaters. This stage may last typically from two to 25 years (and could exceed 50 years) prior to metamorphosis to the silver eel stage and maturation. Age-at maturity varies according to temperature (latitude and longitude), ecosystem characteristics, and density-dependent processes. The European eel life cycle is shorter for populations in the southern part of their range compared to the north. Silver eels then migrate to the Sargasso Sea where they spawn and die after spawning, an act not yet witnessed in the wild. (ICES 2014b).

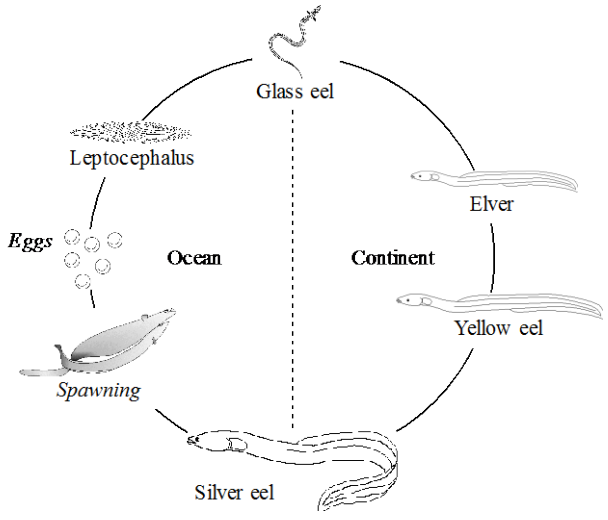

Figure 2. The lifecycle of the European eel. The names of the major life stages are indicated; spawning and eggs have never been observed in the wild and are therefore only tentatively included. (Dekker, 2002).

1.4 Overview of the WKPGMEQ report "Development of standardised and harmonised protocols for the estimation of eels quality"

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) and to inadequate understanding of the effects of quality on reproduction). This has emphasised the urgent need to establish a comprehensive overview with improved spatial coverage of the quality of the eel population across Europe. 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 2012 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 of eel quality and risking inefficient use of resources. Initially, ICES (2009) 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 recommend 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 participants drafted reports describing the framework and methods used in their countries for the assessment of contaminants and diseases in the eel in advance of the workshop. The report provides 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 visualize contaminants in the eel are described. The disease sections 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 have been suggested. The workshop also discussed the future perspectives of using biomarkers of effects to assess eel health. This seems to be the only way possible since 90% of the xenobiotics causing the observable biological effects, such as cytochrome P-450 induction, has unknown structures. 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 need 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 to improve 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.

2 General effects of contaminants in the eel

The eel is a long-living semelparous carnivore with high body fat content, which can accumulate high levels of lipophilic xenobiotics. Eels often reside in contaminated sediments accumulating high levels of lipophilic compounds through gills, skin and contaminated foods (van Leeuwen *et al.* 2002). It 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 and Goemans 2007a), although investigations on otolith microchemistry (at least for yellow eels in lower river stretches) indicate movements between freshwater and saltwater habitats (Marohn *et al.* 2013; Bodles 2016). Yellow eels are 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 habitats they have encountered. Like Cd, PCB have been demonstrated to disturb the fat metabolism in eels (Geeraerts *et al.* 2007; Belpaire *et al.* 2009).

Yellow eels are quite resistant, surviving in poor water conditions (low oxygen), and frequently living in heavily polluted habitats. Eel in the yellow stage are subadults; they do not reproduce in freshwater. Therefore, body burdens are not seasonally affected by a reproductive event and therefore are not associated to changes in lipid metabolism. Unlike iteroparous species, there is no loss of contaminants, specifically associated with annual reproductive processes (fat metabolisation and production and release of gametes). They can stay for a prolonged period in freshwater (on average 9-12 years for males and 16-30 years for females (ICES, 2013), continuing to bioaccumulate xenobiotics, and increasing their levels with age, reaching a maximum prior to silvering and emigration.

Silver eels, which have already started their downstream 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 *et al.* 2016), with residual levels being less than 10% of initial levels. The highly lipophilic compounds, such as the highly chlorinated PCBs, dioxins and flame retardant (PBDE), will not be eliminated during migration. The negative impact of highly contaminated lipid reserves in eel, explaining the stock decrease in Europe, has been suggested by Larsson *et al.* (1990): while the lipid reserves are depleted during migration the lipid based levels of contaminants increase simultaneously. This may cause higher contaminant levels in the blood, what may cause damage to reproductive organs and affect embryogenesis (see Section 5).

Effects of contaminants in eel are complex and are reported at several levels of biological organization, from subcellular, organ, individual up to even population level. Reports documented disturbances of the immune system, the reproduction system, the nervous system and the endocrine system (Belpaire *et al.* 2016).

Several authors have reviewed the effects of pollutants on the eel. Bruslé (1990/ 1991) described and discussed the effects of heavy metals, pesticides and PCBs on eels, listing experimental concentrations of various contaminants over different life stages of the European eel. Further reviews were made by Robinet and Feunteun (2002) and Geeraerts and Belpaire (2010).

Due to the variety of chemicals involved, and the complexity of impacts, a straightforward approach to measure potential impacts on the eel stock is a challenging task. Recently, Belpaire *et al.* (2016) reviewed the role of contaminants in the collapse of the eel stock, and presented a conceptual model of the effects of pollution exposure on the population structure of the European eel. The model showed possible mechanistic relations between the various hierarchical levels of biological response to pollution, from the molecular to the population level. Such a model has the potential to become a framework for the development of an advanced mathematical model with predictive capability.

In the following chapters WKBECEEL further elaborated on the effects of pollution on lipid metabolism (section 4), reproduction (section 5), immune system (section **Error! Reference source not found.**), behaviour and migration (section 6) and genomics (section 8), after a description of the trends in traditional and emerging contaminants susceptible of affecting eels (section 3).

3 Spatial and temporal trends in traditional and emerging contaminants affecting eels

The interest in contaminants having an effect on the reproduction of eel is brought about by the declining recruitment across the period 1980–1990. As eel-recruitment of several eel-stocks has fallen "simultaneously" over the world, while contamination of inland waters with potential toxic contaminants takes place world-wide, the effect of these contaminants should be investigated.

3.1 Temporal and spatial trends of contaminants in eel (reported between 2008 and 2013)

Temporal trends of contaminants in eel are available from long term monitoring studies, as reported by Belpaire *et al.* (2016). Historical contaminants such as PCBs and DDTs are decreasing, but still are ubiquitous in eel even after their ban 40 years ago. This time lag may be explained by the strong persistence of these chemicals and the long generation time in eels. The timing of the increase in the production and release of some of the most toxic chemicals may fit with the timing of the decrease in recruitment from 1980 onwards. As an example the peaking of PCB levels in the environment occurred around 1970s, leading to high bioaccumulation during the growth of elvers and yellow eels at that time. Due to the high levels accumulated during this growth period of peaking PCBs, eels from this cohort would end up with extreme levels of PCBs accumulated in their fat stores, when starting their reproductive journey (on average late 70s). Due to their high body burden these eels would have an impaired migration or reproduction leading to recruitment collapse in the early 1980s. Also Byer (2013) concluded from analyzing time trends of POPs in *A. rostrata* that the decline in the recruitment of American

eels to Lake Ontario corresponds to the time period when eels were highly exposed to dioxin-like compounds.

In the Netherlands, a 30 year data collection of contaminant levels in eel (de Boer *et al.* 2010) showed a slow decrease in PCBs since 1977. In Belgium, the levels of PCB in eels decreased with a modelled rate of 15% per year (Maes *et al.* 2008). Also in the American eel, significant decreases of PCBs and DDTs were reported (Byer *et al.* 2013). Nevertheless, approximately 3 million metric tons of PCB-contaminated waste oils and contaminated equipment still need to be managed, globally contributing to ongoing environmental pollution (Stockholm Convention 2010; Weber 2013). Still, flood events or excavation works play an important role as secondary sources for the contamination of inland water bodies and flood plains (Stachel *et al.* 2004; Lake *et al.* 2015).

Some pesticides and metals decreased following the environmental management of these chemicals (Maes *et al.* 2008). As an example, the concentration of lead in eel muscle consistently decreased between 1994 and 2005 in Belgium, perhaps due to the transition from leaded to unleaded fuels and a reduction of industrial emissions (Belpaire *et al.* 2016). In contrast the concentrations of some metals such as mercury and cadmium showed no time trend (Maes *et al.* 2008).

However, in many areas pollution levels in eel are still a matter of high concern. Levels in eels are often much higher than in other fish species. While most data have been measured in immature yellow eels, contaminant levels generally increase with the time spent in their foraging habitat, reaching a maximum just prior to silvering and spawning migration (Belpaire *et al.* 2016; Freese *et al.* 2015). It should be noted that the large number of available reports all indicate extreme spatial variability in levels for the studied contaminants. This variation is dependent on the variable levels of anthropogenic contaminant sources and pressures. A comprehensive study on pollution load in Belgian eels (>350 sites) reported ranges of Sum 7 PCBs muscle levels between 3 and 12 000 ng/g wet weight (Belpaire *et al.* 2016). Large ranges have also been reported in the levels of metals and pesticides (Maes *et al.* 2008), dioxins (Geeraerts *et al.* 2011), dioxin-like PCBs (Freese *et al.* 2016), volatile organic compounds (Roose *et al.* 2003) and brominated flame retardants (Malarvannan *et al.* 2014; Sühring *et al.* 2015), and this was the case also for studies on European eel in other parts of Europe and in American eels.

High levels of contaminant (PCDD/PCDF, PCB, PBDE) are still present in the eels from the Rhine making them unsuitable for human consumption (Guhl *et al.* 2014). A study in the Gironde estuary (France), analysed PBDE and PCB contamination in glass and silver eels (Tapie *et al.* 2011). As expected, the levels were two orders of magnitude higher in the silver compared to the glass eels.

Also, spatial differences were reported in eel PAH metabolites measured in bile from European countries, e.g. high individual levels of 1-hydroxyphenanthrene were detected in eel from Germany (Kammann *et al.* 2014) and the United Kingdom up to 7000 ng/ml (Ruddock *et al.* 2003). In contrast about a magnitude lower maximum levels were found in eels caught in Poland (Szlinder-Richert *et al.* 2014) and Morocco (Wariaghli *et al.* 2015).

Moreover, as many of these chemicals often co-occur due to their similar physical properties, origins and distribution patterns, eels are often polluted by a combination of contaminants that might cause impacts in a synergetic or in some cases antagonistic way (i.e., the combined effect being even greater or lesser than the individual components).

The extent of spatial variation in pollution in eel and the potential for impacts on growing, migration and spawning phases, warrants the requirement to take account of this within the eel management for the purposes of stock recovery. The quality of local stocks of eels may be very different between EMUs and even within one EMU. Therefore, international stock assessment requires understanding such heterogeneity in the eel (Belpaire *et al.* 2016).

Time-series of contaminant levels in eels could potentially give insight in which contaminants are involved in the decreased reproduction of eel. Unfortunately, there are no series of levels in eel dating from the seventies. Moreover, many contaminants could not be analysed in those days. Historic samples, if stored properly, could be analysed with the current state-of-the art techniques to detect all (now) known candidate contaminants. There is a time-series of samples stored in the Netherlands of eel from 1978, but specimen showing the environmental pollution from 1960 to 1980 would be better. Also in Belgium, an eel tissue bank from historical samples on a broad spatial scale from the nineties has been preserved. Of course, levels in other environmental compartments (fish-eating birds, other fish species), or in sediments, do not always correlate with the levels in eels. Nonetheless, with current expertise on behaviour of contaminants the levels in historic eels could be "predicted" from the values obtained in other environmental matrices.

3.2 Emerging contaminants

The chemicals which were reported in eels over its distribution area include a variety of well-known toxic substances (see above). However more recently 'emerging' substances have been reported to accumulate in wild eels. Emerging contaminants can be defined as any synthetic or naturally occurring chemical or any microorganism that is not commonly monitored in the environment but has the potential to enter the environment and cause known or suspected adverse ecological effects. In some cases, certain emerging contaminants may have been present for a long time but were not recognised until new detection methods were developed (http://toxics.usgs.gov/regional/emc/). One "recent" example is PFOS, its production started in 1950 but it was declared an emerging contaminant in 2000. As a result PFOS has been banned in the EU for most uses since 2008 (EC 2006). Analysis in eel samples from the specimen bank at IMARES taken between 1978 and 2008 show PFOS levels in Dutch eels from some locations have increased from 30 ng/g in 1978 up to 120 ng/g in the mid-1990s (samples from river Rhine). Since then, a decrease of levels in eels to the levels of the 1970s has been observed (Kwadijk *et al.* 2010).

Belpaire *et al.* (2015) showed that toxic textile dyes were found in the majority of eels from the studied sites. The dye malachite green or its metabolite was found in 46% of the samples. Other examples of emerging contaminants found in eel include musk compounds (Leonards and De Boer 2004), perfluorinated substances (Roland *et al.* 2014), organophosphorus flame retardants and plasticizers (PFRs); (Malarvannan *et al.* 2015) and drugs such as cocaine (Capaldo *et al.* 2012). According to Gay *et al.* (2013) cocaine in eel, at environmental concentrations, behaves like an endocrine disruptor. But in general, the potential effects of these emerging chemicals in the eel are still not well understood and the data series are far too restricted to allow trend analysis.

Pharmaceuticals compounds are designed to have physiological effects, and low levels in the environment may affect fauna. The chemical characteristics of these compounds gen-

erally do not result in high bioaccumulation in organisms like eel (as opposed to PCB bioaccumulation), nor do they bind strongly to sediments. Exposure is therefore mainly through the water phase (uptake by gills, respiration), and can be short-lived (dilution and / or degradation can take place). For example, paracetamol induced significant physiological modifications in the eel, however none yielded clear oxidative stress, maybe indicating effective detoxification mechanism (Nunes *et al.* 2015).

3.3 Mercury impairments

Mercury (Hg) compounds have triggered major environmental and human health concerns particularly the organic moiety, methylmercury. Methylmercury is lipophilic (in contrast to metals in general) and has been shown to have a range of effects in nature and on human health, including immunotoxicity, neurotoxicity and developmental toxicity (Dietz *et al.* 2013). Still, there is a knowledge gap on Hg accumulation in the brain and eyes of fish, its association with biochemical alterations and related impairments of behaviour. Fish behaviour patterns (i.e. locating food, avoiding predators) are mediated by eyes with an appropriate integration of the central nervous system (CNS). Those behaviours can be impaired or lost as a result of Hg exposure (Berntssen *et al.* 2003).

A number of studies are being conducted under the scope of a Portuguese research project (NEUTOXMER – Neurotoxicity of mercury in fish and association with morphofunctional brain alterations and behaviour shifts, FCT financed) in order to disclose the effects of Hg at the neurosensorial level of fish and behaviour. Juveniles of the white seabream (*Diplodus sargus*) are being used in this project since this species represent a good experimental model to investigate the toxicity of Hg. Some innovative results were obtained in this project, namely: (i) eyes and brain were unable to eliminate inorganic Hg (iHg) after 14 days of exposure (Pereira *et al.* 2015); (ii) iHg elicited cellular loss (neurons plus glial cells) in specific brain regions of fish, namely in hypothalamus, optic tectum and molecular layer of the cerebellum after 7 days of exposure. Such brain damage was accompanied by an impairment of motor function and altered mood/stress behaviour in fish (Pereira *et al.* 2016). Recent work showed that the abundance of inorganic mercury is far higher in eel brain than in muscle or liver (Bonnineau *et al.* 2016)

Fish eyes and brain should be considered as target organs in environmental health assessment since they faithfully reflect water and sediment Hg contamination (Pereira *et al.* 2014). It is important to evaluate changes in these organs at structural and functional levels in order to examine to what extent accumulated Hg could compromise neurosensory processes. Moreover, iHg is a relevant neurotoxicant in fish and inductor of behavioural changes at the motor level.

Despite the relevant findings for *D. sargus*, there are no studies on the effects of neurotoxins (e.g. Hg) in the migratory behaviour of the eels. Thus, future studies should search for possible brain alterations, swimming performance changes, feeding behaviour and the possible interference of contaminants in eel's orientation.

4 Relationship between contaminants and lipid metabolism in eels and other species.

4.1 Importance of lipids for eels

In fish species with long distance migrations, storage of somatic energy reserve is essential in fulfilling their life cycle (Jonsson *et al.* 2006). As energy stores are vital for the reproductive migration, lowered fat content will have most consequences in silver eels, affecting migration and reproduction.

Eels accumulate lipids during development at the elver and yellow stage (Boëtius and Boëtius 1985; Tesch 2003). These lipids are mainly stored as triglycerides in their muscle tissue (Pierron *et al.* 2007). Accumulation of energy through lipid storage are necessarily affected by changes in food availability, but other environmental factors involved are pollution, disease agents, 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 reserves are catabolized by the liver to provide sufficient energy to enable migration, gonad maturation and spawning (Pierron *et al.* 2007). Large individuals (females but also shorter males) with high lipid content are considered to have a higher contribution to the spawning stock (ICES 2012). Knowledge of lipid levels may provide 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. Preliminary analyses using field data (parameters such as lipid level, cost of transport, eel size, and distance to Sargasso Sea) demonstrated the heterogeneity of the reproductive potential of local eel stocks (ICES 2012, 2013).

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 28%, lipid levels in the blood start to increase, triggering the production cascade 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); although it is more likely and more specifically increased growth and simultaneous production of growth hormone which trigger silvering (Huang *et al.* 1998; Durif *et al.* 2005). Thus, 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 and 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 suggested that minimal fat levels of 20% are required to allow migration and successful reproduction (van den Thillart *et al.* 2007).

Some silver eels exhibiting low fat levels (<20%) are in any case less likely to reach the Sargasso Sea and spawn successfully (Svedäng *et al.* 1996). It is possible however that

these unfit silver eels may revert back to a yellow stage to fulfill another growth season before starting the reproductive migration (Durif *et al.* 2009).

4.2 Lipid content analysis

Thus, lipid content in eels is a key fitness indicator as they play an important role for migration and maturation. As discussed above fat content in eel muscle has to exceed a certain value for eels to become sexually mature (Larsson *et al.* 1990; Lokman *et al.* 2003; Durif *et al.* 2006) and also, to provide the needed energy for the journey to their spawning grounds, the Sargasso Sea (Boetius and Boetius 1980; Palstra *et al.* 2008; van den Thillart *et al.* 2007). Still, no unified way of measuring fat in eels has been postulated yet. Fat content is measured as lipid concentration in muscle and usually 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 homogenously distributed across the body length (McCance 1944; Tesch 2003; Clevestam *et al.* 2011). It is 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. Therefore, specific and consistent sections of muscle should be collected, as it is vital that comparable areas of the eel are examined to allow for better comparison within samplings. More detailed recommendations are given in WKPGMEQ (ICES 2015).

A number of studies have been carried out in which different methods of lipid measurements in eels were applied. Since lipids are a diverse group of hydrophobic or amphiphilic molecules, different methodologies of measuring lipids may lead to different results, making it difficult to compare these findings, especially if not defined clearly. To approach this problem and allow a better comparability, fat content in eels should be defined as total lipids in relation to dry- and in any case, 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).

Lipid content in fish tissue can also be measured using a number of different gravimetrical or photometrical methods (Bligh and Dyer 1959; Smedes 1999; Inouye *et al.* 2006; Schlechtriem *et al.* 2012). In gravimetrical methods, fat content is usually determined in a homogenized sample of eel tissues by separating lipids from their matrices with (organic) solvents and then by weighing them out after the solvent has evaporated (Smedes 1999; Schlechtriem *et al.* 2012). Doing this, lipid content of eel tissue can be measured on the exact same extract or separate aliquot for contaminant analysis (Voorspoels *et al.* 2004). For better comparability it is important to make sure that the solvents used are capable of extracting total lipids and/or those lipids that are of interest for the respective study. Therefore the method described by Smedes (1999) with modifications by Schlechtriem *et al.* (2012) is recommended.

Differences in the lipid composition of various tissues are often not assessed, although it is recognized that lipid composition likely influences contaminant distribution (Bertelsen *et al.* 1998). It should be noted though, that recent evidences have indicated, that accumulation and partitioning of hydrophobic organic contaminants are strongly affected by lipid polarity class. This is a contrast to a vast majority of studies, which normalized tissue contaminant concentration to total lipids. Total lipids basically comprise of two

groups: Polar and non-polar lipids, which then could be further differentiated into triglycerides, phospholipids, sphingolipids and even more. To get more insight to the kinetics of lipophilic contaminants, it is thus recommendable to (additionally to the methods recommended above) assess the (polar and non-polar) fractions of different lipid-classes in the total-lipid extracts.

Besides analytical laboratory methods, a fat meter hand device has mostly been used as a non-invasive method to determine lipid content in live animals. This method is particularly interesting for fieldwork due to its simple handling and directly accessible results. The measurements with this device are based on an algorithm, which uses the detected water content as the inverse of dry mass (Schoeller 2000) to calculate fat mass. Non-lethal assessment techniques of eels are important where destructive analysis is not applicable or allowable (e.g. if the eel should be kept alive and/or for ethical reasons). Based on work by Klefoth *et al.* (2013), the use of a fat meter was considered a suitable method to non-lethal estimate energetic status in eel and other species. However, WKPGMEQ (2015) pointed out 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.

As previously stated in WKPGMEQ (ICES 2015), the use of fat meters has shown inconsistencies in analysis, specifically across life history stages. Recent studies from Germany and the UK comparing fat content measurement between fat meter and subsequent laboratory analysis brought similar findings to light (presented at this workshop – see Appendix). The results indicated that, while yellow eel fat content is relatively comparable across methods, the same could not be said for silver eels which showed discrepancies in values measured with fat meter compared to lab analytical methods (with fat meter always recording significantly lower fat content).

One suggested hypothesis for this is, that the inconsistencies are due to differences in water content (and the fat meters functionality of its detection) in the different related life stages, as silver eels are known to imbibe water as a function of the physiological processes of "silvering" (Tesch 2003). For individual measurements, analytical laboratory methods are thus considered to produce more accurate and reliable lipid values.

4.3 Lipids and contaminants

4.3.1 General facts

A contaminant that enters the surface water column will redistribute itself between the water and carbon-rich compartments (such as sediment and biota) in the water column. Uptake by biota occurs via:

1) direct uptake from water;

2) ingestion of contaminated food or other suspended particles;

3) drinking contaminated water;

4) direct sorption from sediment.

Contaminant accumulation by aquatic organisms varies depending on the nature/type of contaminant, the organism and environmental conditions. Concentrations of a given

contaminant in biota vary with species, sex, age, body size or weight, surface-to volume ratio, life stage or reproductive state, lipid content, trophic level, vertical distribution, physical condition, tissue or organ analysed, migration pattern, and the season in which samples were collected (Walker *et al.* 2012; EU 2014). Their relative importance depends on the concentration of the contaminant in the water, the place of the species in the food web, the physical and chemical properties of the contaminant, and the possible synergetic activity with other substances or stressors (Nowell *et al.* 1999).

All vertebrates, including eels, can biotransform lipophilic substrates, including organic contaminants, through enzymes generally grouped as phase-1 and phase-2. The main phase-1 enzymes are cytochrome P450s, or mono-oxygenases. The ability of biotransformation enzymes to metabolise halogenated contaminants (such as PCBs, brominated flame retardants, dioxins, etc.) is limited, however, and the greater part of such lipophillic contaminants will only to a small extent be detoxified. PCBs and other lipophilic contaminants will be associated with lipid pools throughout the body (Bruijs *et al.* 2002). While there will be an equilibrium between lipid and blood, the highest internal exposure concentrations for any organism will occur during exposure and under starvation or other conditions causing lipid to be mobilised. Metal contaminants tend to be stored in the liver, though cadmium will predominantly accumulate in the kidney and liver, whilst methylmercury is stored in muscle tissue, but also in the central nervous system.

During the migration and lipid/nutrient redistribution from tissues/liver to gonads, internal exposure to stored contaminants will increase dramatically, even if the plasma is lipid-rich during this process. Such exposure has the potential to cause all the types of toxicity referred to above for the different organic contaminants, particularly immunotoxicity and neurotoxicity. Cadmium will also be redistributed from the liver to ovaries during oogenesis, but will be associated with proteins in blood.

4.3.2 Relationship between contaminants and lipid level

4.3.2.1 In eels

Belpaire *et al.* (2009) have reported a significant decrease of muscle lipid content in yellow eels in Belgium and in pooled samples of yellow eels (30–40 cm length class) from several locations in the Netherlands. The magnitude of the described decrease was considerable, questioning the ability of these eels to start silvering and to achieve their spawning migration. Contaminants may have been involved in the decrease of these lipid levels. However, also other reasons such as a shift in sex ratios in the samples could have led to such a change as suggested by recent unpublished work, (Kotterman *et al.* (in prep). It seems that the decrease in lipid levels, as observed in some pooled samples of eels (n=25, 30–40 cm length class) from certain location was reflective of a changing sex composition. The number of male eels in the pooled samples had decreased and sex-specific analysis of individual eels confirmed that lipid levels were not reduced. Male yellow eels of 30–40 cm have high lipid levels and small, female yellow eels of 30-40 cm contain very low lipid levels. It is noteworthy that the male /female ratio depends on location in The Netherlands and elsewhere, and that these ratios are not constant over time (van de Wolfshaar *et al.* 2014). Notably, there was no correlation between changes in sex composition and contaminant-levels, nor a correlation between lipid-levels and contaminant levels. The analysis of 40 individual silver eels, caught at Hollands Diep at the

onset of their migration confirmed that lipid levels in migrating silver eels had not declined (all eels >20% lipid on wet weight). Another study collecting silver eels, originating both from heavily polluted as well as clean areas. No correlation between condition index and lipid levels was observed (van der Lee *et al.* 2013).

4.3.2.2 In other fish species

Besides eel, many reports propose that a diversity of contaminants may have an impact on lipid levels in fish (see for a review: Adams *et al.* 2012). Some other examples of an overall decrease in fat levels in fish have been reported:

McMaster *et al.* (1991) found reduced lipid stores, smaller gonads and decreased energy commitment to growth in white sucker exposed to paper mill effluents. Rajan 1990 reported a decline in lipids occurring in both muscle and liver of *Cyprinus carpio* when exposed to sublethal concentrations of textile mill effluent and attributed to using energy to mitigate stress. In a study by Munkittrick and Dixon (1988) fish from lakes with elevated levels of copper and zinc had decreased muscle lipids, serum lipids and visceral lipid reserves and reproduction was impaired. Neff *et al.* (2012) examined 35+ years of muscle lipid content data for ten Great Lakes fishes from Canadian waters. The long-term (1970s–2008) temporal trends in lipid contents of these fish revealed that, levels were significantly decreasing in eight of the ten species. Although lipophilic contaminants have declined in the Great Lakes, trends in concentrations of PCBs and dioxins have either stabilized or even increased (Neff *et al.* 2012). Significant decreases in fat levels have also been reported in Baltic herring (*Clupea harengus membras*) since the late 1970s until 2000 (Adjers *et al.* 2000). They were thought to be linked to large-scale oceanographic changes, especially a decrease in availability of the energy-rich marine copepods. Also Flinkman *et al.* (1998) suggested that bottom-up processes mediated via changes in mesozooplankton species composition have induced a longer-term failure in feeding success and a decline in fat content and herring growth. In the German long running monitoring programme, the lipid levels in bream, analysed over a wide variety of locations, does not decrease. In many locations a strong increase is observed, but this is not corrected for any possible influences of age and length changes

(https://www.umweltprobenbank.de/en/documents).

5 Effect of contaminants on reproduction

5.1 Reproduction and larval development in eels

Large parts of the eel's natural reproduction cycle are still considered a mystery and only little is known about the physiology and the developmental processes that eel eggs and larvae naturally undergo. Reasons for that lay mainly in the fact that so far, the complete life cycle of the European eel has not been completed in captivity and only limited knowledge exists about optimal holding conditions and suitable feed for the larvae. Among anguillid species, reproduction and early stage characteristics have been more intensively studied for the Japanese eel, *Anguilla japonica*. Assisted reproduction protocols were established for this species in the 1970s (Yamamoto and Yamauchi 1974; Yamauchi *et al.* 1978; Ohta *et al.* 1996, 1997; Tanaka *et al.* 2001), which led to the first production of glass eels (Tanaka *et al.* 2003; Kagawa *et al.* 2005) as well as second-generation

captive hatchings (Okamura *et al.* 2014; Tanaka 2015). In addition, experimental propagation of American eel (*Anguilla rostrata*) as well as two species of eel from New Zealand (*Anguilla diffenbachii* and *Anguilla australis*) has led to viable eggs and hatched larvae (Lokman and Young 2000; Oliveira and Hable 2010).

Records of the larval development of European eel are scarce, limited to catches of leptocephali in the wild (e.g. Schmidt 1922; Munk *et al.* 2010) and observations made in line with the few, artificially matured eels and their lab-reared offspring (e.g. Sörensen *et al.* 2016). Concerning the European eel, significant scientific effort has been invested but the bottleneck still seems to be first feeding of the larvae.

Artificial sexual maturation of eels was first carried out on male European eels (Fontaine 1936), then later on in females (Fontaine *et al.* 1964). The first eggs were fertilized in the 1970s (Boëtius and Boëtius 1980) followed years later with the production of hatched larvae that survived up to 3.5 days post-hatch (Bezdenezhnykh *et al.* 1983; Prokhorchik 1986; Prokhorchik *et al.* 1987). Fertilized eggs and hatched larvae in limited numbers were produced by Pedersen (2004), adopting the Japanese protocol (Ohta *et al.* 1997).

Recently, advances in assisted reproduction technology and offspring culturing techniques, have enabled repeated production of large batches of viable eggs and larvae that reach the first-feeding stage (Tomkiewicz 2012; Tomkiewicz *et al.* 2013; Mordenti *et al.* 2013; Sørensen *et al.* 2016). This progress has been aligned with the development of techniques for artificial fertilization (Peñaranda *et al.* 2010; Sørensen *et al.* 2013; Gallego *et al.* 2013; Butts *et al.* 2014), embryonic incubation (Sørensen *et al.* 2014, 2015, 2016), and early larval rearing (Politis *et al.* 2014), thereby improving offspring survival. However, no one has yet been able to obtain the larvae to feed.

Given all of the above, very little is known about the effects of contaminants on the development of eel larvae. Clear dose-effect relationships for specific contaminants or pathogens are still missing. One available study by Palstra *et al.* (2006) dealt with effects of Toxicity Equivalents (TEQs) caused by dioxin-like substances on European eel. The authors interpreted the results as a proof of the toxic effects of dioxin like compounds on the development and survival of eel embryos. Unfortunately, for this study only small sample numbers were available and the authors did not measure the actual concentrations of dioxin like compounds, but used chemical activated luciferase gene expression (CALUX) for the detection of effects caused by dioxins and dioxin-like compounds.

For these reasons, findings from studies on other animals and particularly other fish species could be very helpful to find relevant information and maybe even threshold values for the toxicity of different contaminants on the larval development of eels.

5.2 General effects of contaminant on reproduction and larval development

A multitude of reports are available which describe the detrimental effects of contaminants on the reproductive biology of fish.

Bengtsson (1980) reported that adult minnows (*Phoxinus phoxinus*) exposed to PCB (Clophen A50) suffered from delayed spawning, and offspring hatched earlier. PCB at high environmental levels, reduced fecundity and hatching success in the common barbel *Barbus barbus* (Hugla and Thomé 1999). Other reproductive anomalies observed upon PCB exposure include inhibition of spermatogenesis and various testicular abnormalities

(Sangalang *et al.* 1981; Freeman *et al.* 1982) as well as disruption of reproductive endocrine function (Khan and Thomas 1996).

Yellow perch (*Perca flavescens*) from metal-impacted Canadian lakes exhibited dose-dependent decreases in plasma sex hormone concentrations and gonadosomatic index (GSI) along a metal contamination gradient (Levesque *et al.* 2003). According to Boyle *et al.* (2008), natural metal contaminated diet can have profound effects on reproduction in fish. Laboratory studies have shown that vitellogenin (VTG) synthesis, an egg-yolk protein, is reduced in rainbow trout injected with high doses of cadmium (Cd) (Olsson *et al.* 1995).

The sensitivity of fish early life stages for the effects of POPs has been demonstrated by various researchers (see for example: Henry *et al.* 1997; King-Heiden *et al.* 2012; Walker and Peterson 1994 for dioxins; Mhadhbi *et al.* 2012; Usenko *et al.* 2011 for PBDEs; and Sisman *et al.* 2007; Soffientino *et al.* 2010; Murk *et al.* 1996; Wilson and Tillitt 1996; Zabel *et al.* 1995a, 1995b for –dioxin like- PCBs). Contaminants may interact with the embryonic development and growth of fish larvae. Relatively low levels of chlorinated hydrocarbons in ovaries also has negative effects on embryonic development of North Sea whiting (von Westernhagen *et al.* 2006). PCB exposure of eggs induces embryonic malformations in several species (Helder 1980; Walker and Peterson 1991; Walker *et al.* 1994; Stouthart *et al.* 1998).

Sühring *et al.* (2015) found evidence of maternal transfer of several persistent organic pollutants and displayed a transfer of these substances (PBDEs, BFRs and other halogenated flame retardants) in line with the redistribution of lipids from muscle tissue to gonads and eggs. BFRs can cause developmental effects, endocrine disruption, immunotoxicity, reproductive, and long term effects, including second-generation effects in chub (*Leuciscus cephalus*), bream (*Abramis brama*), and perch (*Perca fluviatilis)* (Hajslova *et al.* 2007). Norman *et al.* (2007) reported a dose related increase in the number of atretic oocytes in female zebrafish exposed to a BFR mixture, which might indicate disturbed ovulation. Exposure to BFR at high dose (100 nM/g) resulted in lowered spawning success. A reduced hatching success was seen in offspring from fish exposed to the BFR high dose. Uptake in adult fish and maternal transfer was shown for the BFR mixture in a parallel study (Rattfelt *et al.* 2009).

Lahnsteiner *et al.* (2005) reported that in male brown trouts (*Salmo trutta*) exposed to estimated BPA concentrations of 1.75 and 2.40 µg/L semen quality was lower than in controls in the beginning of spawning (reduced sperm density, motility rate, and swimming velocity) and in the middle of spawning (reduced swimming velocity, at 2.40 µg/L BPA also reduced sperm motility rate). Therefore, production of high quality semen was restricted to the end of the spawning season and delayed for approximately 4 weeks in comparison to the control. At BPA exposure levels of 5.00 µg/L only one of eight males gave semen of low quality (reduced semen mass, motility rate, and swimming velocity). The percentage of ovulated females was similar for the control group and the groups exposed to estimated BPA concentrations of 1.75 and 2.40µ g/L, whereas at 5.00 µg/L BPA females did not ovulate during the investigation. No effect was observed on the quality of eggs (egg mass, percentile mass increase during hardening, egg fertility). Comprehensive reviews about the effects of certain contaminants (PBDEs, heavy metals) on fish reproduction were made by Yu *et al.* (2015), Sfakianakis *et al.* 2015 and King-Heiden *et al.* 2012. These include thresholds of contaminant effects for various fish species.

5.3 Effects of contaminants on reproduction of eels

5.3.1 Maternal transfer of bioaccumulated contaminants towards egg and effect on hatching

The specific predisposition of eels towards xenobiotics, due to their biology as semelparous, sediment related predators with high body fat contents, make them specifically vulnerable to lipophilic contaminants (see Section 0). This naturally led to concerns among the scientific community that the reproductive capacity of eels are particularly threatened by xenobiotics. Due to a homogeneous distribution of the POPs within the lipids in the female tissue, the lipid-normalized concentration in the eggs that the eel produces will be comparable to the maternal tissue (Russell *et al.*, 1999). These maternally transferred POPs could cause negative effects on the developing offspring after fertilization (Tietge *et al.* 1998; Olsson *et al.* 1999; Nakayama *et al.* 2005; Ishibashi *et al.* 2006; Belpaire *et al.* 2016, Figure 3). Hence the POP concentration in the tissue of the mother fish represents the minimum toxic pressure for the developing offspring. Compared to fully developed fish, larvae are relatively sensitive to toxicants (McKim 1977; Hutchinson *et al.* 1998) as a consequence of the critical development of organs and tissues during this life phase of the fish (Foekema *et al.* 2012).

While progress in artificial reproduction of eels is being made, few data are available on the actual transfer of toxic compounds from mother to offspring in these species. During maturation of female European silver eels, about 60 g fat per kg eel is incorporated in the oocytes (Palstra *et al.* 2007). Given the vital importance of lipids in the egg-maturation process (Nassour and Léger 1989), a deficiency of lipid reserves available for gonad maturation may lead to a decrease of egg production with consequences on reproductive success (Henderson and Tocher 1987). In eel, 1.72 g eggs can be produced with one gram of fat (van Ginneken and van den Thillart 2000).

In the absence of hard biochemical proof, recent publications based on modelled scenarios have attempted to describe the kinetics of contaminants against body burdens (Brinkmann, Freese and Pohlmann *et al.* 2015; Foekema *et al.* 2015). Foekema *et al.* (2015) studied the effect of dioxin-like compounds on eel reproduction. The sensitivity of eel larvae for dioxin-like compounds was estimated based on the sensitivity distribution of larvae of other teleost fish species (Stevens *et al.* 2005). If European eels are among the 1% most sensitive fish species, 50% larval mortality due to maternally transferred dioxin-like contaminants can be expected for tissue concentrations in migrating eel of 43 pg TEQ/g lipid weight. At 14 % of the sampled sites in The Netherlands and 11% of the Belgium sites this level is exceeded. If eel larvae are among the 5% most sensitive fish species, the critial concentration for 50% larval mortality (241 pg TEQ/g lw) is exceeded in one location in The Netherlands and two locations in Belgium.

In a study by Freese *et al.* (2016 in prep.), the authors measured the actual transfer of dioxin-like PCBs, PCDDs and furans in gonad tissue and eggs of wild caught, artificially matured eels. Van Ginneken *et al.* (2009) found that PCB exposure led to swelling of the yolk sac and especially the pericardium (elevated wet weights of embryos/larvae), thus showing disturbed hydromineral balance (oedema). This indicates a disturbed water balance, which in adult fishes induces a stress response, partly controlled by the Hypothalamus Pituitary Interrenal (HPI) axis via cortisol. Remobilization of pollutants to gon-

ads might also occur with some heavy metals, as demonstrated for cadmium. Pierron *et al.* (2008) also found a negative effect of cadmium on sexual maturation of female silver eels and on spawning migration by altering the lipid accumulation process. After 30 days of Cd exposure, a significant metal accumulation was observed in the kidney, the liver, the gills and the digestive tract of Cd exposed eels. Thereafter, during the maturation phase, which unfolded in Cd-free seawater, a significant increase in Cd content in gonads and kidney of Cd pre-contaminated eels was observed. This was associated in these animals with a significant decrease in Cd content of gills and digestive tract.

In some of these studies, evidence is provided, that lipophilic contaminants are redistributed to gonadal tissue and eggs during gonadal maturation of eels. The distribution process are primarily driven by the lipid dependent logarithms of the octanol-water partition coefficient (log KOW) of the respective compounds. It still has to be considered though, that the log KOW is not always a good estimate for associations between chemicals and lipid, as bioavailability of those chemicals is also influenced by their physicochemical properties such as molecular weight, shape and degree of hydrophobicity.

Although, octanol is used as a surrogate for biological lipid, it cannot simulate barriers to uptake, such as steric hindrance by membranes, and functions instead as a simple measure of linear partitioning (Elskus *et al.* 2005). Lipids are a group of inhomogeneous class of biomolecules, with different chemical characteristics leading to evidence that POPs partition differently among lipid classes.

In summary, Figure 3 presents the two possible pathways along which contaminants may impact eel reproductive success (Belpaire *et al.* 2016).

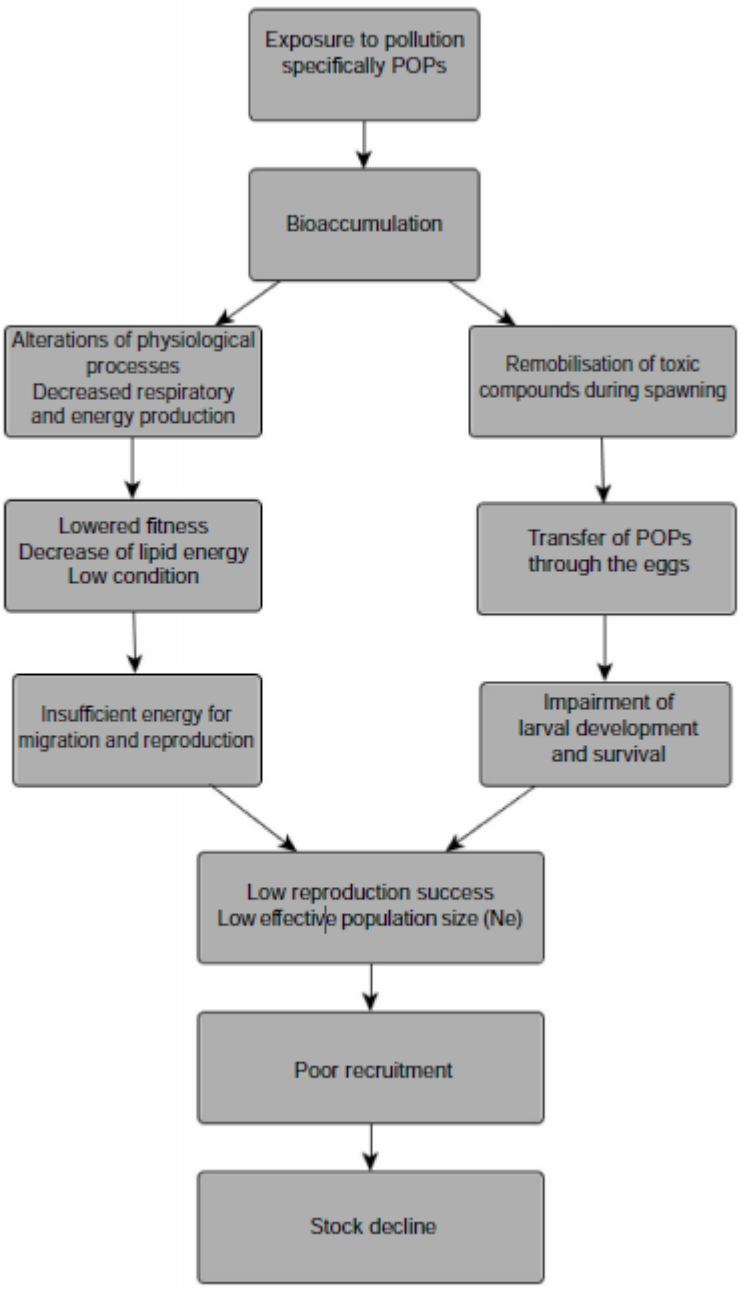

Figure 3. Taken from Belpaire *et al.* 2016: Mechanistic model of the impact of reprotoxic and persistent organic pollutants (POPs) on the stock of the Europan eel. Environmental pressure by lipophilic contaminants leads to internal exposure through uptake from water, food and sediment. Eels are particularly sensitive to bioaccumulation of POPs and the concentrations of these compounds in eels attain much higher levels than in other species. The effects of the high body burden of contaminants may act through two possible pathways. One way is through the disturbance of the lipid metabolism, resulting in lowered lipid reserves and a decrease in condition (fitness). As a result, energy stores are insufficient to fuel their reproductive migration or eels arrive in inadequate condition which does not allow normal reproduction. Alternatively, lipophilic contaminants are released from the fat during migration as the fat stores are gradually depleted. Their reprotoxic properties impede the quality and the survival of the developing eggs/larvae, resulting in diminished recruitment.

5.4 Endocrine disruption

Endocrine disruptors are exogenous chemicals or chemical mixtures that can interfere with any aspect of hormone action. They can act directly on any number of proteins that control the delivery of a hormone to its normal target cell or tissues (WHO 2012).Endocrine disrupting compounds (EDC) include natural hormones and phytoestrogens, synthetic hormones (e.g. 17-alpha ethynylestradiol (EE2), and industrial/commercial compounds (such as alkylphenols, POPs (like PCBs, chlorinated pesticides, brominated compounds and PFOS), pharmaceuticals, and phthalates) (http://toxics.usgs.gov/regional/emc/endocrine_disruption.html; WHO 2012). Exposure to endocrine-active contaminants can cause endocrine disruption, which can have severe impacts on fish populations.

In a whole lake study in Canada, Kidd *et al.* (2007) demonstrated that exposure to 5 ng/L ethinylestradiol (EE2) dramatically increased VTG concentrations in male fathead minnow, pearl dace and lake trout (by 1 900-24 000-fold), while the effects were much less marked in male white sucker (by up to 118-fold; Palace *et al.* 2009). The results demonstrated strong evidence that chemical exposure is associated with a suite of male reproductive abnormalities (intersex and abnormal spermatogenesis). This compromised their reproductive capabilities and ultimately lead to the collapse of a "wild" population (Kidd *et al.* 2007). Still it is a challenge to detect such impacts in field locations with mixed pollution situation.

Intersex, the presence of both male and female characteristics within the same fish, is one manifestation of endocrine disruption in fish. It has been observed in many fish populations in streams across the United States and Western Europe. Endocrine disruption can result in adverse effects on the development of the brain and nervous system, the growth and function of the reproductive system, and the response to stressors in the environment. The following are some recent examples of USGS studies on endocrine disruption in fish.

A population of fish downstream of a sewage treatment plant in Colorado, USA was dominated by females, and 18–22% of fish exhibited intersex (Vajda *et al.* 2008). The occurrence of intersex can be particularly high during the spawning season. A higher incidence of intersex occurs in streams draining areas with intensive agricultural production and high population when compared to non-agricultural and undeveloped areas (Blazer *et al.* 2007).

The breeding behaviour of males exposed to nonylphenol (degradation product of surfactants found in industrial and household detergents) varied significantly with exposure level (Schoenfuss and others, 2008). Low doses "primed" the males for breeding competition, whereas higher exposures inhibited their breeding behaviour.

PCB, PCDD/PCDF, PBDE have been suspected to impair aquatic organisms due to their endocrine disrupting mode of action (Blanchet-Letrouvé 2014).

In vitro tests have shown various agonistic and antagonistic activities of PBDE on steroid receptors (Hamers *et al.* 2006; Legler 2008; Ren and Guo 2013).

The effects of contamination on the reproductive endocrine system of fish are well documented (Kime 1995). Field studies have found reproductive impairment associated with high concentrations of chemical contaminants (Slooff and de Zwart 1983; Stott *et al.* 1983;

Johnson *et al.* 1992). Life cycle tests with chemical stressors have shown that intersexual interaction and development can be impaired at concentrations that do not affect embryonic development, hatching, or growth (Folmar 1993). Reproductive hormones and vitellogenin may be suppressed in fish exposed to xenobiotic chemicals in the field or laboratory (Folmar 1993). Endocrine disruption in freshwater fish presenting intersex individuals with ovotestes, has now been reported from many places and in many freshwater and marine fish species (Gimeno *et al.* 1998). Indirectly, endocrine disruption might also affect fat storage due to specific chemicals, some of them mimicking the steroid hormone estrogen (Turner and Sharpe 1997), which may be particularly harmful for long distance migrating species, such as the eel. PCBs are known as endocrine disruptors and effects have been shown in many fish. There is also a large body of evidence on the endocrine (hormone) disrupting properties of alkylphenols. Jobling and Sumpter (1993) used rainbow trout (*Oncorhynchus mykiss*) hepatocytes in an in-vitro study focusing on estrogenic (capable of mimicking the action of the female hormone estrogen) chemicals (including alkylphenols) in sewage effluents discharged into UK rivers and estuaries. Disruption in gonadal development of wild roach (*Rutilus rutilus L.)* is manifest in a variety of ways, ranging from malformation of the germ cells and/or reproductive ducts to altered gamete production. Intersex fish were also found to have an altered endocrine status and an elevated concentration of plasma VTG (Jobling *et al.* 2002a; Bjerregaard *et al.* 2006). Under natural conditions, VTG is only produced by mature female fish as a yolk precursor and has therefore been widely used to detect exposure to compounds with estrogenic properties (Versonnen *et al.* 2004; Gillemot 2003). Intersexuality also influences reproductive success. Gamete production is reduced in intersex roach. Moreover, sperm motility (percentage of motile sperm and curvilinear velocity) and the ability of sperm to successfully fertilize eggs and produce viable offspring is reduced in intersex fish compared with normal male fish. This documents a relationship between the morphological effects (e.g. intersex) of endocrine disruption and the reproductive capabilities of any wild vertebrate (Jobling *et al.* 2002b). From a monitoring program in British rivers it has been proven that steroidal estrogens play a major role in the appearance of intersex. Their appearance shows correlation with the location and severity of pollution by estrogen-like compounds (Jobling *et al.* 2006).

In eel, Versonnen *et al.* (2004) investigated plasma VTG content, measured in 142 eels sampled at 20 different locations in Belgium, in relation to the internal pollution levels (PCBs, organochlorine pesticides, metals). No correlations were found between VTG content and weight, length, condition, fat content, contaminants or date of sampling. 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 (Livingstone *et al.* 2000; Peters *et al.* 2001) of eel sampled at different locations in the UK during different seasons, 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 (Versonnen *et al.*, 2004). The fact that yellow eel might be relatively insensitive (regarding VTG levels) to waterborne endocrine disrupters is also confirmed by Burzawa-Gerard and Dumas-Vidal (1991) and Luizi *et al.* (1997) who found that high doses of (injected) E2 (17b-estradiol; at least 5 x 0.5 μg g-1 w.w. during 12 days) were needed to induce VTG production in immature eels (Versonnen *et al.*, 2004). The onset of maturation in the European eel only takes place during a period of prolonged swimming which might be a necessary physiological stimulus (van Ginneken *et al.* 2007). It is there-

fore possible that endocrine disrupting effects of pollutants become apparent during the starvation period during migration or during the spawning itself (Versonnen *et al.* 2004; Figure 5.1). Therefore, research under experimental conditions (swim tunnels) with silver eels is recommended.

5.5 Sex determination in eels

Catadromous eels enter continental habitats as sexually undifferentiated glass eels and develop into males and females before migrating back to sea as silver eels. Females develop ovaries directly from the ambiguous primordial gonad (Geffroy *et al.* 2013) whereas males pass through a transitional intersexual stage before developing testes.

Eels have sex-specific life-history strategies. Males may grow faster than females initially, but females attain a greater age- and size-at-metamorphosis than males. Male fitness is maximized by maturing at the smallest size that allows a successful spawning migration (a time-minimizing strategy) whereas females adopt a more flexible size-maximizing strategy that balances pre-reproductive mortality against fecundity.

Although heteromorphic sex chromosomes have been identified in some species, the sex of developing gonads is labile and gender is determined principally by environmental factors. Individuals experiencing rapid growth prior to gonad differentiation tend to develop as males, whereas eels that grow slowly initially are more likely to develop as females (Davey and Jellyman 2005). Paradoxically, males tend to predominate under conditions of high density, which may be because a "quick growth-early maturation strategy" increases an individual's chances of survival during periods of intraspecific competition.

High temperatures and saline conditions have also been proposed to favour development as males but experimental studies have failed to demonstrate a clear effect of either on sex determination. High proportions of female silver eels migrating from some upstream areas, lakes and large rivers may be due to low population density or poor conditions for growth in these habitats (Davey and Jellyman 2005), or that only females reach these headwaters because the males have emigrated from freshwater before upstream migration rates would have caused them to reach these waters. Further work (Geffroy *et al.* 2012) showed that density alone could not explain the determination of sex into a male at high density and female at low density.

The condition factor of individual fish at early stages can explain partly the sex determination with an initial elevated condition factor leading to the determination into a female. All the more because elevated condition factor eel are more likely to migrate upstream, eels will tend to determine into female where the competition between individuals is lower. The determination into male is more likely in a habitat where the inter-individual competition is high, where compensatory growth will allow low condition factor eel to maintain. The evaluation of the habitat carrying capacity by the fish is also an important factor explaining the determination of eels.

Geffroy, *et al.* showed that eel somehow evaluate the environmental conditions in such that when the environmental conditions are favourable for growth or improving, they will determine into a female. Intersexual eels have rarely been observed (Geffroy *et al.* 2012) and could not be related to contamination.

Sex steroids (oestrogens and phytoestrogens) have a strong feminizing effect on undifferentiated individuals and are most effective when targeted at younger eels and administered at high doses for prolonged periods (Davey and Jellyman 2005). As a conclusion, it seems that the impact of contaminants such as oestrogens or hormone like organic compounds on eel sex determination may be low, and has never been reported for conditions observed in the wild environment.

6 Effect of contaminants on behaviour and migration

Behaviour links physiological function with ecological processes, therefore behavioural indicators of toxicity are well adapted to assess the effects of aquatic pollutants on fish populations (Scott and Sloman 2004). Contamination in eel can bring about a mobilisation of (specific) contaminants during migration, and therefore cause a reduction of the fitness of potential spawners. This has been considered one of the key factors that can contribute to decrease the probability of successful migration and reproduction, and it is with reference to this possibility that in recent years the WGEEL has considered the risks of reduced biological quality of escaping silver eels.

For instance, pollutants could affect fine sensory processing of water currents and odours necessary for synchronization of the internal clock, which in turn influences migration onset. Indeed, pollutants may damage olfactory neurons (Halpern 1982) and may also affect a variety of behaviours through the upsetting of sensory, hormonal, neurological, and metabolic systems (Scott and Sloman 2004).

6.1 General effects of contaminants on swimming in fish

Swimming behaviour of fish is impaired by exposure to a diversity of contaminants. Alterations in swimming behaviour have been detected during exposures to various contaminants at concentrations as low as 0.7 to 5% of their LC50 values and at concentrations that subsequently inhibited growth after longer periods of exposure (Little and Finger 1990). The physical capacity to swim against water flow tends to be affected at relatively high toxicant concentrations and often presages mortality. Orientation to water flow, however, is altered at sublethal concentrations (Little and Finger 1990). A meta-analysis, based on 39 papers and synthesizing the effects of pesticides, identified deleterious effects on swim speed and general activity of amphibians and fish by a decrease modelled to be equal to 35% and 72% respectively (Shuman Goodier and Propper 2016). The effects varied across chemical classes, which likely reflect underlying physiological processes. Pyrethroids, carbamates, and organophosphates all produced a large decrease in swim speed. In this meta-analysis, even sub-lethal concentrations of pesticides had a strong effect. Endosulfan (an organochlorine pesticide) impaired the swimming kinematics and exploratory behaviour of zebrafish (Pereira *et al.* 2012).

Decreased locomotor performance was observed in minnow after exposure to heavy metals (Kolok *et al.* 1998). Other endocrine disruptors, such as DDT and atrazine on the contrary increased activity in goldfish (Weis and Weis 1974; Saglio and Trijasse 1998). Adult zebrafish (*Danio rerio*) also showed hyperactivity after exposure to a concentration as low as 1% of the LC50-24h within five hours (Huang *et al.* 2014). Marentette *et al.* (2012) highlight that behavioural changes in a laboratory can be quite different from measurements in the field. Their findings showed that round goby (*Neogobius melanostomus*) from

relatively cleaner areas were more active than individuals from highly contaminated habitats when tested in a laboratory. However, field sampling did not reveal differences in travelled distances or residency times.

6.2 Effects on swimming in eels

Lipids and their constituent fatty acids are, along with proteins, the major organic constituents of fish, and they play major roles as sources of metabolic energy for growth, reproduction, and movement particularly migration. This might be especially important for the catadromous eel, whose biological cycle includes a transoceanic migration of over 5000 km.

In 2005, van Ginneken *et al.* demonstrated, by experimental work in swim tunnels, that fasting European eels are able to swim the distance corresponding to the spawning area in the Sargasso Sea, with a remarkably high swimming efficiency and at low energy costs. This implies that sufficient energy reserves and an efficient metabolism could be more critical for this species to successfully spawn compared to other fish species. That is why the eel could be very vulnerable to persistent toxic contaminants released from fat stores during migration, which could interfere with energy metabolism in addition to reproduction (Section 5).

Given the difficulty to study physiological changes and toxicological effects in eel during migration, little is known about effects of PCB-exposure on spawning migration of eels. During this period the starving eel relies completely on its energy stores in adipose tissue. Further experiments by van Ginneken *et al.* 2009, tested the hypothesis that release of PCBs from fat may interfere with eel physiology and energy metabolism. Their results showed that PCB-concentrations on a lipid basis are 2.8–14 times higher in swimming compared to resting animals. However, in order to increase the PCB levels 3-14 times on lipid weight, the eels had to spend their lipid reserves for a very large part. Lipid analysis did not show this, what makes this large increase very unlikely. A more realistic scenario is the increase of levels of lipophilic contaminants on lipid base by a maximum of 33% as shown by Foekema *et al.* 2015.

During the 6000 km migration to the Sargasso Sea, much fat will be used for energy consumption causing the PCB concentrations to increase. This could enhance the chance of interference with the energy metabolism of the eel and possibly with the thyroid status reducing the chance of the animals to reach their spawning grounds (van Ginneken at al. 2009). When eels reach their spawning grounds, elevated PCB-levels may also interfere with steroid hormone function and therefore with reproduction and hatching success of the larvae (see Section 5).

6.3 Effect on orientation/navigation mechanisms

The main sensory systems thought to contribute to short and long distance migrations in fishes are vision (e.g. detection of the polarized light field), olfaction (e.g. odour signatures of rivers), inertial detectors and mechanoreception (e.g. tidal flow) and magnetoreception (detection of the earth's geomagnetic field); (Secor 2015). Thus, any contaminant-related deficit in the normal function of the central nervous system and sense organs could potentially disrupt fish migrations (Baatrup 1991). Further, any contaminant-related effect on the swimming ability or resilience of fishes could affect the success of

their migrations. Additionally, any contaminant-related deficits in the sensory systems that underlie mate-finding or reproductive behaviour on the spawning grounds could also have negative repercussions. There is limited information about these possible effects in fishes, and even less for eel, particularly when it comes to reproductive behaviour.

Carvalho *et al.* (2008) reported that exposure of larval stages of *Salminus brasiliensis* to realistic water concentrations of phenanthrene impaired vision and foraging skills. Torreiro-Melo *et al.* (2015) reported that exposure of the guppy *Poecilia vivipara* to phenanthrene affected swimming speeds, trajectories and prey capture. Claveau *et al.* (2015) reported that methylmercury (MeHg) exposure increased activity in non-migrant glass eels but not migratory behaviour. Contamination affected mitochondrial structure and metabolism, suggesting a higher oxidative stress and activation of antioxidative defence systems in non-migrant glass eels, implying increase in energy expenditure and a higher vulnerability to predation in non-migrant glass eels in the wild. Goulding *et al.* (2013) exposed juvenile rainbow trout (*Oncorhynchus mykiss*) to deltamethrin at environmentally realistic concentrations. They reported transient decreases in swimming performance that appeared to be recoverable. Also, Zakon (2015) discusses the possibilities for adaptation of sensory systems to pollutants and other environmental drivers.

The limited evidence indicates that there is cause for concern for the possible effects of contaminants on eel migration. However, direct research on the effect of contaminants on migration and orientation is lacking.

6.4 Effects on olfaction

Cadmium has been recognized for some time as a potent environmental pollutant with the capability of disrupting olfactory-mediated behaviours (Bertin et Averbeck 2006; Sullivan *et al.* 1978). Failing to respond to chemical cues in the environment could adversely affect foraging, reproduction and predator avoidance.

Alteration of anti-predator behaviour was observed in zebrafish raised with relatively low concentrations of cadmium (Kusch *et al.* 2008). Exposure to low levels of cadmium throughout development may alter neurogenesis, subsequently resulting in long-term impairment of chemical cue perception.

Because the vertebrate olfactory system is characterized by continuous neurogenesis throughout life (Bettini *et al.* 2006; Lazzari *et al.* 2013), damage to the olfactory receptor neurons would only be short-lived and concern the early-life stages (i.e. glass eels).

7 Relationship between parasites and contaminants

The effect of parasites and diseases were voluntarily disregarded during the workshop, however we emphasize the relevance of the interaction between contaminants and disease agents with respect to their effect on the eel, as described previously in the review of Geeraerts and Belpaire (2010). These interactions may be complex. Contaminants may induce disease outbreaks, and can influence the immune system of the eel. Alternatively, some parasites may bioconcentrate specific contaminants and hence may suppress the detrimental effect of these chemicals on the eel. In 1977, Rødsæther *et al.* (1977) exposed eels to copper contaminated freshwater and reported mortality due to vibriosis (*Vibrio*

anguillarum). Eels kept in non-contaminated freshwater (6 pg Cu/L) remained healthy. Rødsæther *et al.* (1977) suggested that *V. anguillarum* is a common inhabitant of eels, and copper can change a commensal association between fish and bacterium to one of pathogenicity. This may have illustrated a decrease in immune response which was induced by copper.

Toxicants like PCBs have been related to resistance to diseases, viruses and parasites. Sures (2006) demonstrated that parasites may: (1) influence the metabolism of pollutants such as PCBs, in infected eels, (2) interact with pollution in synergistic or antagonistic ways, and (3) induce physiological reactions in eels which were thought to be pollutant-induced.

Experimental studies have shown that alterations in pollutant uptake and accumulation in different intermediate and final hosts due to parasites are very important in the field of ecotoxicology. Sures (2006) points that in addition to such alterations, there is a close interaction between the effects of pollutants and parasites which seems to be mediated at least partly by the endocrine system, which itself is closely related to the immune system in fish.

Fat oxidation (e.g. as a result of contamination) could lead to immuno-suppression, thus to reduced resistance to diseases, viruses and parasites. In combination the effects of contaminants in European eel with swimming, the EVEX virus causes haemorrhages and anaemia resulting in the death of the animals after 1000–1500 km (van Ginneken *et al.* 2005). Sures and Knopf (2004) showed that in eels 3,3,4,40,5-pentachlorobiphenyl (PCB 126) suppresses the humoral response and that it is associated with increased incidence of infection by the swimbladder nematode *Anguillicoloides crassus*. Experimentally infected eels with *A. crassus* and exposed to high concentrations of Cd and PCB 126 showed a significant increase of *A. crassus* specific antibodies in the peripheral blood 61 days p.i., indicating that it was not the invasive larvae but the adult worms which elicit the antibody response. The exposure to PCB 126 resulted in a complete suppression of the antibody response. Sures and Knopf (2004) also indicated that the Cd concentrations (21.7 ± 12.8 µg/L; mean ± S.D.) applied in their study were not high enough to suppress the immune response of European eels. Furthermore, as eels are able to withstand environmental pollution and tend to accumulate heavy metals to a very high degree (e.g. Mason and Barak 1990; Sures *et al.* 1994), it seems likely that this species is not sensitive enough to show alterations in its immune response at low levels of Cd pollution. The toxicity of PCB 126 seems to be related to a structural similarity to 2,3,7,8-tetrachlo- rodibenzo-p-dioxin (TCDD), the most toxic of all halogenated aromatic hydrocarbons (HAH) (Regala *et al.* 2001). The degree of immunotoxicity of PCBs correlates with the degree of binding affinity to the cytosolic aryl-hydrocarbon receptor (Kafafi *et al.* 1993), which is a well-described transcription factor for a variety of gene products, including cytochrome P4501A (Hahn and Stegeman 1994).

The lack of parasites or parasitic lesions in the skin of DHAA-treated animals indicated that the above reported abrasive action of DHAA also may be adverse to parasite fixation, preventing this kind of infestation (Pacheco and Santos 2002).

Some parasites have been demonstrated to bio-concentrate specific contaminants such as metals, lowering the concentration of these chemicals in host tissues and hence suppressing the detrimental effects on the host. The intensity of the accumulation is parasite-

specific and may be influenced by external factors. The effect of salinity and the mode of application (oral versus aqueous) on the lead accumulation in different eel tissues and its parasites *A. crassus* (Nematoda) and *Paratenuisentis ambiguous* (Acanthocephala) was investigated by Zimmerman *et al.* (1999). Waterborne as well as dietary lead exposure caused an increase in the metal levels of different eel tissues and its parasites. The mode of lead application had a significant influence on the distribution of lead in the fish tissues, and the resulting metal concentrations were approximately 20–2000 times higher in *P. ambiguous* than in *A. crassus*. These differences may be due to the different microhabitats and nutrient uptake mechanisms of both parasite species (Zimmerman *et al.* 1999). Eira *et al.* (2009) give evidence for the possible role of cestode infection in metal metabolization/storage processes in host tissues. They studied the effect of *Proteocephalus macrocephalus* on the accumulation of trace elements (Cr, Cu, Ni, Pb, Zn) in contaminated eel. Results showed decreased levels of Ni and Cr in kidney and liver tissue of eels while *P. macrocephalus* individuals accumulated these contaminants. Bioaccumulation of Cu, Cr and Pb in *A. crassus* varied according to eel co-infection with *P. macrocephalus*. But *A. crassus* is also able to excrete mercury in detoxification processes as is shown by Palikova and Barus (2003) who found less mercury in *A. crassus* tissue compared to the tissue of its final host eel. Low efficacy of mercury accumulation (and also of other heavy metals) that they found in *A. crassus* enables to exclude in this parasite-host system, the influence of the parasite on decreasing the heavy metal concentrations in body tissues of infected eel specimens.

8 Impact of contaminants at the genomic and transcriptomic level in European eel

8.1 Environmental genomics

The field of environmental genomics involves the application of toxicogenomics to environmental contamination and wildlife. Environmental genomics seeks to improve our understanding of how organisms respond, at the genetic level, to changes in their external environment, in order to develop a stronger basis for environmental risk assessment. The genome responses are diverse and may vary between species, depending on toxicokinetics and toxicodynamics.

Toxicology is the study on how contaminants are taken up, distributed and excreted in organisms, while toxicogenomics studies the mechanistic effects of contaminants in wildlife. Combined, these fields of research aim at understanding how contaminants affect organisms depending on their biology and ultimately to identify species-specific molecular biomarkers.

Aiming at understanding how environmental contaminants affect organisms, numerous toxicogenomics studies have been conducted in fish, including European eel. "Omics" technologies include transcriptomics, proteomics and metabolomics. Microarray technology, today being increasingly replaced by direct sequencing, has been applied in hundreds of fish studies to identify genome-wide responses to contaminants and to predict novel molecular biomarkers at the transcriptional level. Pathway analysis based on altered transcription has identified contaminant-specific responses to environmental contaminants, even at exposure levels that do not lead to significant tissue accumulation.

Proteomics technology has also been commonly used in search for effects of contaminants in fish. For this, a well characterized and fully annotated reference genome is preferentially required. Metabolomics technology, predicting the cellular outcome of contaminants in terms of metabolites, is also more and more being used in fish studies. Today, these methods are increasingly applied in combination to decipher how environmental contaminants influence wildlife at the gene, protein and metabolite level following exposure. Epigenetics, the study of changes in organisms caused by modification of gene expression rather than alteration of the genetic code itself, can also be studied with "omics" technology. Both organic pollutants and heavy metals known to accumulate in fish have been shown to be able to affect DNA methylation patterns in fish. In this sense, studies have shown that certain chemicals can affect fish across generations, in that exposed F0 animals seriously influence the well-being of non-exposed F1, F2 or later generations through altered DNA methylation or histone modification.

8.2 Transcriptomics and Pollution

Transcriptomic approaches allow the measurement of the activity of thousands of genes at once, providing a snapshot of which genes are being actively expressed in a particular moment in time. Methods used include the traditional microarrays and more recently, RNA-seq, which takes advantage of the recent advances in NGS technology.

Transcriptomic approaches have the potential for identifying and studying the genetic basis of traits affecting fitness and identify genes whose expression changes in response to environmental perturbations (e.g. pollution, parasite infestation) and thus become candidate genes for being involved in the response. In particular, the effect of pollutants on eels has been the focus of a few recent studies.

Pujolar *et al.* (2012) developed a microarray specific for eels consisting of 15 000 annotated genes that was applied to detect differentially expressed genes between polluted sites in Italy (highly polluted river Tiber vs. lowly polluted Bolsena lake). As expected, genes related to detoxification were upregulated in the polluted sites relative to the clean sites, including CYP3A, which takes part in phase I of the xenobiotic metabolism and glutathione-5-transferase that takes part in phase II, or glutathione peroxidase, which is involved in oxidative stress. Surprisingly, several metabolic related genes were downregulated[1], in particular genes from the mitochondrial chain and oxidative phosphorylation. In order to doublecheck this pattern, the study was also conducted in parallel in clean and polluted sites in Belgium (Pujolar *et al.* 2013a). Again, detoxification genes were upregulated, while energy-related genes were downregulated in animals from polluted sites. Although metabolism was not measured, results point to a poor energetic status of eels experiencing a high pollution burden. A reduced metabolism is alarming in the case of eels due to the high energetic demands needed to conduct the spawning migration of adults to the Sargasso Sea. Although speculative, it might support the notion that poor quality of spawners could be responsible for the eel decline.

[1] Downregulation is the process by which a cell decreases the quantity of a cellular component, such as RNA or protein, in response to an external variable. An increase of a cellular component is called upregulation (Wikipedia).

In relation to the quality of spawners, there has been much talk in recent years about how pollutants mobilize to the gonads during the spawning migration and how it might jeopardize egg and larval development. In this sense, Baillon *et al.* (2015a) studied the effect of pollutants on gonad development in female silver eels. Transcriptomic analysis was conducted by means of an array of 1000 candidate genes comparing artificially matured females from clean vs. polluted sites. An impairment of gonad development was suggested for females from the polluted site, as over-expressed genes[2] were involved in mechanisms of protection against oxidative stress or DNA repair. Baillon *et al.* (2015b) also compared the global hepatic transcriptome of European and American eels under a pollution gradient using RNA-seq. Specific signatures were revealed for the different pollutants measured, the most important of which were related to cadmium, arsenic, lindane and the hepatosomatic index (HSI).

Recently, there is increasing evidence of the role of epigenetics in cases of high pollution burden. Pierron *et al.* (2014) investigated the epigenetic effect of low-dose cadmium exposure in European eel. Results showed a positive association between cadmium exposure and DNA methylation. Genes showing hyper-methylation were involved in intracellular trafficking, lipid biosynthesis and phosphatidic acid signalling pathway. Ultimately, the study shows how DNA methylation can occur as a response to pollution.

8.3 Integration of -Omics into Eel Management

The role of omics technologies using global and without a priori (candidate biomarkers) approach is important in the discovery of new biomarkers of exposure and effects (ICES 2015, WKPGMEQ). WKPGMEQ reviewed and listed a number of recent and promising biomarker studies in the eel, and further discussed future perspectives and research needs. Currently, information on biomarkers cannot yet be extrapolated to reliably evaluate the potential of eels to successfully migrate and reproduce in their marine spawning habitat.

Integrating genomic studies into management has become a reality in the past five years, mainly due to the advances in next generation sequencing (NGS) techniques. In the past, studies based on genetic markers have been widely used to advance the knowledge on eel biology that eel managers demanded, including the estimation of the genetic diversity of the species, inference of the population genetic structure, estimation of the effective population size, studies on hybridization between European and American eel, studies on selection/local adaptation, etc. However, such studies were traditionally conducted using a very limited and small number of genetic markers. For instance, in the 1990s and 2000s, studies based on around 20 microsatellite markers were the standard on many genetic surveys. This was due to the shortage of genomic sequence resources available for eels. Only five years ago, no genome sequencing had been conducted for any eel species and the number of sequences available in Genbank for the European eel consisted of only 121 expressed sequence tags (ESTs), 404 nucleotide sequences and 232 proteins.

As mentioned above, the advances in the speed and cost of NGS techniques have changed all this and nowadays the number of markers available for eels is extraordinary

[2] Excessive expression of a gene by producing too much of its effect or product (such as in cancers). Merriam-Webster dictionary.

to say the least. NGS methods are now conducted in parallel, which allows for fast sequencing. Costs are also now relatively low and decreasing so that even non-model species can be sequenced, unlike in the very beginning, where sequencing efforts were mostly directed to model organisms such as drosophila.

In eels, the revolution in NGS methods allowed in the first place the sequencing and annotation of the eel transcriptome (Coppe *et al.* 2010), and subsequently the complete genome sequencing of European eel (Henkel *et al.* 2012a) and Japanese eel (Henkel *et al.* 2012b). While both genomes are qualified as "draft genomes" (not fully completed and assembled due to technical limitations), they provide a rich source of data for any future genetic or genomic study.

One kind of NGS method that has been very successfully used in eels is RAD (Restriction Associated DNA) sequencing or RAD-seq. This is a genotyping-by-sequencing method in which only a reduced representation of the genome is sequenced (generally around 1%). RAD-seq has revolutionized the field of population genetics since it can provide data on hundreds of thousands of SNPs distributed all over the genome. For instance, in the case of eels, RAD-seq has been used to generate over 350 000 SNP (single nucleotide polymorphism) markers (Pujolar *et al.* 2013b). These SNPs have then been used to study many key aspects on the biology of European eel that is vital for its management from population structure to hybridization.

In this sense, the information that "omics" has provided so far is fundamental. First regarding panmixia (the existence of a single randomly mating population in European eel). The panmixia hypothesis was challenged by the study of Wirth and Bernatchez (2001) that suggested a pattern of Isolation-by-Distance across Europe. However, later studies proved that the pattern of Isolation-by-Distance was not temporally stable and that temporal differences always exceeded geographic differences (Dannewitz *et al.* 2005; Pujolar *et al.* 2006, 2011). Later, the study of Als *et al.* (2011) provided conclusive evidence for panmixia after conducting the most extensive study to date in terms of samples and markers. Genetic differentiation was low and not significant (F_{ST}= 0.00024), even when comparing larvae collected in situ in the Sargasso Sea (F_{ST}= 0.00076), which provides very strong support for panmixia in European eel. The recent study of Baltazar-Soares *et al.* (2014) seemed to apparently find differences within Europe but re-examination of the data showed that differences were due to extremely low samples sizes (N=10 and lower) and biased sampling.

Recently, the use of an "omics" approach, RAD-sequencing, has provided conclusive evidence for panmixia at the genomic level (Pujolar *et al.* 2014a). Samples from 8 distinct European locations (from Morocco to Iceland) were RAD-sequenced and the patterns of genome-wide genetic diversity across locations were examined using over 450 000 SNPs. No differences were found in values of genetic diversity and overall genetic differentiation was virtually zero (F_{ST}= 0.0007) and not significant, suggesting that most of the genome is homogenized by gene flow.

That European eel is constituted by a single population has important implications. First, this means the species shows random larval dispersal (larvae do not return to the feeding grounds within Europe where the parents migrated from). When for instance a silver eel leaves the continent from the Mediterranean and goes to reproduce to the Sargasso Sea, the progeny will not necessarily return to the Mediterranean Sea and might end up in

Iceland or the North Sea. If larval dispersal were not random, we would expect genetic differences across Europe, which we do not see in the data. It also means that eels cannot locally adapt; eels experience natural selection and the best individuals are selected in each environment. However, all selective advantage is lost in the next generation since the progeny from a silver eel well-adapted to the warm Mediterranean might end up in Scandinavia, or the progeny of a silver eel adapted to high pollution might end up in a clean area. In conclusion, due to panmixia and lack of homing, heritable transgeneration local adaptation is not possible in eels. It would be interesting to further discuss the implications that this has on the evolution of the species and how European eel can cope with environmental and anthropogenic changes. On a more pragmatic implication, the existence of a single panmictic populations means that, from a genetic point of view, transfer of individuals can be done all across Europe without risking genetic erosion or mixing of individuals with different genetic background, as European eel is constituted by a randomly mating single population. Hence restocking should be straightforward in the case of European eel, since there are no differences within Europe and, moreover, there is no possibility for heritable local adaptation. However, it should be taken into account that even if there are no issues from a genetic point of view, there could be issues from an ecological point of view as there is high selective pressure during early life stages after glass eels arrive in a given environment (Pavey *et al.* 2015) and adults might show imprinting problems and might not be able to find their way to the Sargasso Sea from their relocation site (Durif *et al.* 2013).

Another interesting topic addressed with genomics data recently is effective population size (Ne). Earlier studies based on few microsatellite markers suggested an Ne for European eels of 5000–10 000 individuals. However, recent estimations based on whole genomic data (Pujolar *et al.* 2013b) suggest a much higher effective population size. RAD-sequencing and generation of over 350 000 markers suggested an extremely high nucleotide diversity for eels, Pi= 0.005, which number of SNPs per RAD locus ranging from 2 to 22. From the obtained value of nucleotide diversity and using a conservative mutation rate, the Ne can be estimated in around 100 000 individuals. The value obtained from RAD data is one order of magnitude higher than the one obtained with microsatellite markers, which puts doubts in the adequacy of the latter for Ne estimation. It should be taken into account that this Ne of 100 000 individuals is the "long-term" effective population size and should not be confused with the current effective population size.

Genomics has also allowed to advance immensely the knowledge on hybridization between European and American eels (Pujolar *et al.* 2014b). Again using a RAD-approach, the authors identified SNPs showing a FST value of 1 fixed between species and constructed an array of 96 diagnostics SNPs that allowed not only to identify F1 hybrid classes but also backcrosses and late-generation (post F1) hybrids for the first time. Genotyping of a large number of individuals in Europe showed that hybridization mostly occurs in Iceland, where 10.7% of individuals are hybrids. Most of those are F1 hybrids from European eel female and American eel male crosses but first and second generation backcrosses were also detected. By contrast no F1 hybrids were found in mainland Europe, and only few individuals were identified as old backcrosses (introgression occurred 5 or 6 generations ago). Similarly, also very few hybrids were found in the analysis of larvae from the Sargasso Sea.

So for management implications, managers can be sure that hybridization is not an issue in mainland Europe and that the number of hybrids is negligible, and only would be of importance in case one included Icelandic eels.

Overall, the information detailed above on genetic differentiation, genetic diversity, no possibility for local adaptation, effective population size or hybridization is the kind of information on the biology of eels that genomic studies can provide for people working on eel management and can be integrated with data from other fields such as physiology, fisheries data.

WKPGMEQ (2015) recognized 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).

9 Integrating quality into quantitative stock assessment

9.1 Summary of quality indices for eels described in the WKGMEQ report

9.1.1 Eel quality index (EQI)

The EQI was developed by ICES (2010–2012). It is derived from so-called Eel Quality Classes which were defined based on quantitative distribution of an extensive set of data (means per location) for PCB, OCP and metals in Belgium (Belpaire and Goemans 2007). 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 can be found in WKPGMEQ (ICES 2015).

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 or at the catchment or EMU level: 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).

Using these quality classes, the EQI is calculated according the average value of the quality classes for the measured contaminants:

$$EQI_{CONT} = \left[\sum_{i=1}^{n} conta\min ant.classes / n \right]$$

With n= the number of contaminants

The disadvantage of this method is that it considers all the contaminants as equally important in the assessment of eel quality.

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

Maes *et al.* (2005, 2013) have used an integrated index for metals. They calculated a relative bioaccumulation index by dividing (standardising) the individual concentration of heavy metal i (Ci) by the maximum observed concentration (Ci 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:

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

with n the total number of metals, Ci the individual concentration of heavy metal i, Ci 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 Ci max . If in one site Ci 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 Ci 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 Ci max representative for all European habitats, it is important to recalculate/define Ci 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 (WKPGMEC ICES 2015).

9.1.3 The reproductive potential index

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 body 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 (also see section 0).

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).

Net fat content at start of migration = Body mass * % 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 and 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

Mean COT (g fat) =Body mass (kg) * 14.5 (mg fat/kg/km)* 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:

ER_{ind} = Net fat content at start of migration – COT

or

ER_{ind} = (Body mass * % Lipids /100) - (Body mass (g) * 0.0000145 * DSS (km)

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

$RP_{ind} = ER_{ind}$ (g fat) * 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 (RP_{EMU}). Individuals with a negative or zero ER_{ind} 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 ER_{ind}, the RP_{EMU} can be calculated using the following equation:

$RP_{EMU} = \Sigma RP_{ind}\ ER > 0 / N_{ind}\ ER > 0) * N_{EMU}\ ER > 0$

$N_{EMU}\ ER > 0$ = number of female silver eels with $ER_{ind} > 0$ leaving the catchment

$N_{ind}\ ER > 0$ = number of female silver eels with data on lipids and body mass and with a calculated

$ER_{ind} > 0$

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.

The reproductive potential index would need to be standardized by other confounding factors such as: temperature, growth conditions (environment productivity) during the yellow stage.

9.2 Determination of contaminant thresholds for which there are biological effects

9.2.1 PCB and DDT

In absence of clear relationships between body burden in muscular and ovarian eel tissue, field concentrations measured in eel muscles were compared to the ovarian threshold concentration in whiting (WGEEL 2009). Values above which impairment of reproductive success was likely to occur were: > 200 $\mu.kg^{-1}$ wet weight for ΣPCB: > 20 $\mu.kg^{-1}$ wet weight for ΣDDT, and > 10 $\mu.kg^{-1}$.wet weight for dieldrin (von Westernhagen *et al.* 2006). In fish, studies of gonad to muscle ratios of PCBs indicate at least five times greater concentrations in eggs compared to muscle (WGEEL 2008).

These threshold values for PCBs, DDTs and dieldrin were compared with eel contaminant data from the INBO Flemish Eel Pollutant Network (Belgium) (2463 eels collected in the period 1994-2005):

- 50% of yellow eels had ΣPCB (CB 118, 153, 138, 180) concentration in their muscle above the threshold (>200 ng/g).

- 87% of yellow eels had ΣDDT concentration in their muscle above the threshold (> 20 ng/g).

- 37% of yellow eels had dieldrin concentration in their muscle above the threshold (> 10 ng/g).

Taking data from another study (Hoogenboom *et al.* 2006) in The Netherlands 82% had ΣPCB (CB 118, 153, 138, 180) concentration in their muscle above the threshold (> 200 ng/g).

Contrarily, eels from the Thames (Jürgens *et al.* 2009) had PCB and DDT mean concentrations lower than the threshold values for most of the eels that were analysed (35 eels from two sites).

Threshold values were also compared with the data of contaminants measured in eel from several European rivers/lakes (ICES 2008). Overall, the body burden of PCB, DDT and dieldrin in eels over Europe is so high that in many eels we may expect negative effect on normal reproduction , although large variations between catchments or countries are noticeable.

Embryonic malformations are typical for PCB-exposed eggs and indicate negative interference from dioxin-like contaminants (Helder 1980, Walker and Peterson 1991, Walker and Peterson 1994, Stouthart *et al.* 1998) in other species. Monitoring studies (van Leeuwen *et al.* 2002) show that most silver eels have too high TEQ values. So, matured eels with values > 1 ng TEQ/kg gonad would in fact not participate in successful production of viable offspring (Foekema *et al.* 2016). A difficulty remains on how to extrapolate this threshold value to reference values for eels that have not matured yet and per kg muscle. However, fats inclusive of accumulated PCBs that were originally in the muscle have

been incorporated in the oocytes of the mature female. Under this assumption (worst case) we can extrapolate the found values to ng TEQ/kg muscle in wild silver eels. As already mentioned, Palstra *et al.* (2006) observed a negative correlation between embryo survival time and TEQ (toxic equivalent) levels in the gonads implying TEQ-induced teratogenic effects. The disrupting effects occurred at levels below 4 pg TEQ/g wet weight gonad, which are below the EU maximum consumption limit for dioxin in human food (Table 2 in Palstra *et al.* 2006).

9.2.2 Mercury

A threshold value for negative effects of Hg on fish is between 0.3 and 0.7 mg Hg/kg in the whole body homogenates which are at least about a quarter lower than the concentrations in the fillet (Sandheinrich and Wiener 2011). Therefore, Guhl *et al.* (2014) concluded that a deleterious effect of the Hg concentrations measured in eels from the Rhine was unlikely. It is however critical to differentiate between inorganic and organic Hg. For mammals (humans), threshold levels for Hg effects have been decreasing continuously the last couple of decades (Dietz *et al.* 2013). Behavioural effects have been shown in adults following early life exposure in grayling (Fjeld *et al.* 1998). Exposure through maternal transfer could therefore have consequences for adult eel.

9.3 Describing biological effects in other species and what can be used for eels

9.3.1 The WGBEC approach for other species

ICES WGBEC has through the last two decades identified biological effect methods for marine fish (and invertebrates that are diagnostic of contaminant exposure and effects). For the most contaminant-specific, ecologically relevant and widely used methods, assessment criteria have been developed. Data for a given species and methods from unpolluted locations were used to develop a "background assessment criteria (BAC)" (90 percentile of such data) for the above methods. Species included were Atlantic cod, dab, flounder, red mullet and haddock, but there was not sufficient data for all methods and species to develop criteria. For some biological effect methods, an "ecotoxicological assessment criterion (EAC)" could be developed. This required data indicating deleterious environmental effects at a given level of the biological effect response in question (ICES 2009). The criteria are species specific and would need to be developed specifically for eel.

The assessment of environmental quality and the design of monitoring programmes to evaluate environmental quality are best undertaken on the basis of combinations of appropriate sets of chemical and biological measurements (Vethaak *et al.* 2016). WGBEC used selected biological effects methods combined with chemical measurements to develop a framework for assessing the environmental quality at any given location (with regard to contaminants) (Davies and Vethaak 2012; Vethaak *et al.* 2016). The framework comprises three compartments: water, sediments and biota (fish, mussel, gastropod). The FISH component of the integrated assessment is summarized in Figure 4. This framework also requires chemical analyses of both organisms and abiotic matrices, as well as hydrographical information.

According to ICES WGBEC, 2010, the following criteria should be prioritized for any effect-based method prior to implementation on a national or international level (Hylland *et al.* 2016): (i) the method should be able to separate contaminant-related effects from natural processes or the influence of other stressors, including knowledge of confounding factors, (ii) there should be some knowledge of dose-dependency, (iii) the mechanism of toxicity should at least partly be understood, (iv) quality assurance should be established, and finally (v) assessment criteria must be established for responses in relevant species.

Chosen biological effects should be highly responsive to contaminant stress while being moderately affected by other endogenous or exogenous factors. Criteria such as increased disease prevalence (Vethaak *et al.* 2011; Lang *et al.* 2015) or reduced individual condition/growth (Hansen *et al.* 2004) are strongly affected by factors other than contaminants, so even though they are ecologically relevant, it is not possible to link effects directly to contaminant exposure. As will be evident from the framework shown in Figure 4, assessment of individual fish will comprise both contaminant-specific methods (subcellular) as well as health-directed methods (tissue responses and whole organism).

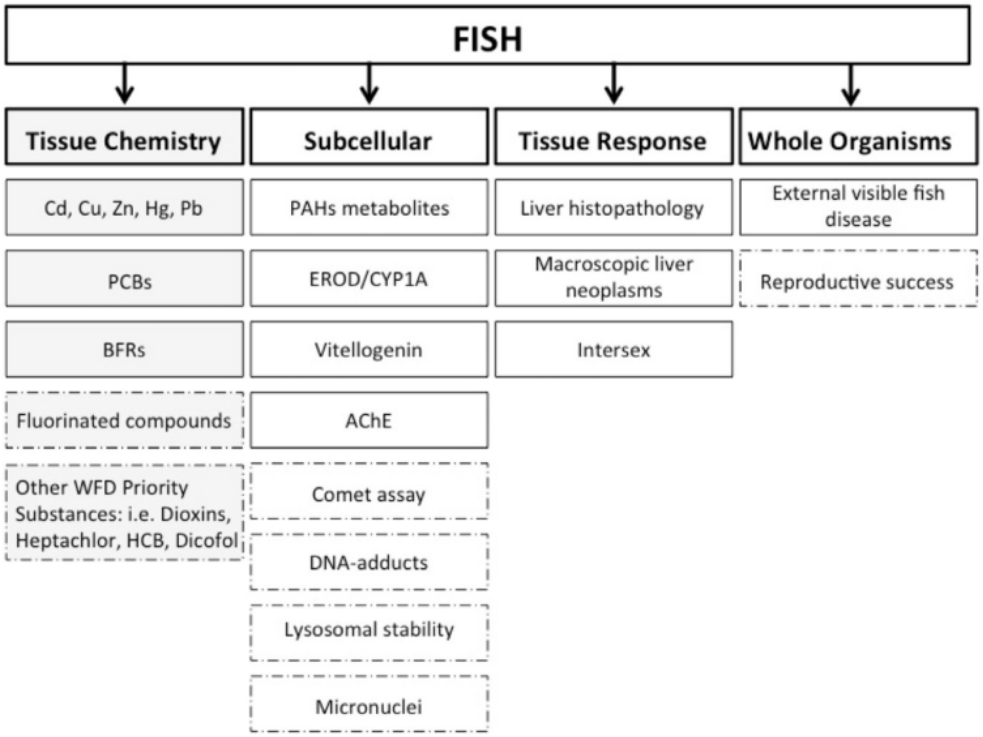

Figure 4. From Vethaak *et al.* 2016. Determinands and measurements included in the fish component of the ICES/OSPAR integrated monitoring framework. Solid lines, core methods; broken lines, additional methods. PCBs, polychlorinated biphenyls; BFRs, brominated flame retardants; AChE, acetylcholinesterase. WFD, Water Framework Directive. WFD priority substances are required in biota under Directive 39/2013/EU. Supportive factors for biota are not shown (details can be found in OSPAR (2013b).

Contaminants in the tissues of an organism are not necessarily biologically active. It is therefore not surprising if tissue residues do not correlate well with biological responses. This also means it is not possible to convert directly from concentrations to effects or

vice-versa. For lipophilic contaminants, there will be an equilibrium between concentrations in tissues and concentrations in plasma, potentially causing responses. The highest internal exposure will however be experienced at the moment of exposure and during fast/starvation or other physiological processes that cause mobilization of lipids. Such mobilization is clearly very relevant to eel, which will mobilize lipid resources both during migration and spawning.

The nature of the association of contaminants with cells may make it possible to generalise over effects, as observed with high concentrations of lipophilic contaminants causing narcotisation (Hylland *et al.* 2016).

In Germany, an approach was developed to link contaminant effect monitoring to fisheries, focussing on assessment of fish embryonal aberrations and fish disease (Dethlefsen *et al.* 1984; Lang 2002; von Westernhagen *et al.* 1987, 1989; Wosniok *et al.* 2000). Large spatial variation and very high frequency of aberrations in the embryos from some species were observed, e.g. dab (*Limanda limanda*) and whiting (*Merlangius merlangus*); (von Westernhagen *et al.* 1989). The frequency of aberrations decreased in the 1990s, coinciding with decreased inputs of persistent pollutants from the Rhine and Elbe/Weser. A similar decrease was seen for prevalence of liver tumours in fish from the same area (reviewed by Hylland *et al.* 2006a).

Table 1. From Hylland *et al.* 2015. Biological effects methods recommended by ICES WGBEC.

Method	Species	Countries	Times	Comment
Bulky DNA adduct formation	Fish	NO, SE, UK	25	
AChE inhibition	Fish, molluscs, crustaceans	FR, ES, IE, UK	22	
Metallothionein induction	Fish, mussels	ES, IE, IT, NO, SE	26	
EROD or P4501A induction	Fish	BE, ES, FR, IE, NO, SE, UK	14, 23	
ALA-D inhibition	Fish	NO		
PAH bile metabolite concentration	Fish	BE, IE, GE, NL, NO, UK	39	
Alkylphenol bile metabolite concentration	Fish	NO		
Lysosomal stability (including NRR)	Mussels, oyster	IE, IS, IT, NL, NO, UK	36	
Lysosomal stability using histochemical quantification	Fish, mussels	ES, GE	34	
Early toxicopathic lesions, preneoplastic and neoplastic liver lesions by histopathology	Fish	GE, UK	38	
External visible lesions and parasites	Dab, flounder, cod	GE, UK	19	
Vitellogenin induction	Male, juvenile fish	GE, IE, NO, UK	31	Species-specific antibody
Intersex	Male dab, flounder, eelpout	GE, IE, UK		
Reproductive success	Eelpout	DK, GE, SE		One species only
Scope for growth	Mussel	ES, IE, IS, UK	40	
Imposex	Neogastropods	DK, ES, FR, IE, NO, UK	24	
Intersex	Periwinkles	NL	37	
Histopathology	Mussels	DK, ES, FR, IE, IT, NO, UK		
Embryo aberrations	Amphipods	SE	41	Field only

9.3.2 The case of the eel

WKPGMEQ (ICES 2015) listed the biomarkers that have been used to describe the health status of eels (table). The workshop concluded that most results were obtained during laboratory studies and that field data is still needed. The applicability of these biomarkers, identified by WKGMEQ and developed under laboratory conditions, should be evaluated in the wild population as adequate responses to multiple stresses and at concentrations measured in the field.

Table 2. From WKPGMEQ (ICES 2015). Recent biomarker studies on the European eel at different biological levels.

Biological level	Biomarkers	References
Behaviour	Exciting phase, ataxia and death	Brusle 1991
	Swimming performance	Palstra *et al.* 2007a; Palstra *et al.* 2007b
Fecundity	Eggs production/Gonad development	Palstra *et al.* 2006; Palstra *et al.* 2007a; MacNamara and McCarthy 2012; MacNamara *et al.* 2015
	Embryo development	Palstra *et al.* 2007a
	Vitellogenin	Livingstone *et al.* 2000; Versonnen *et al.* 2004
Tissue	Gill structure	Gony 1990; Santos *et al.* 1990; Pacheco and Santos 2002; Lorin-Nebel *et al.* 2013
	Lipid reserve/ Fatness	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
	Skin disruption/renal tubule alterations	Santos *et al.* 1990; Pacheco and Santos 2002
Cellular	DNA integrity/apoptosis	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
	Blood component / cell structure	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

Biochemical	Glycemia/ acid lactic/ proteins levels	Sancho *et al.* 1997; Fernández-Vega *et al.* 2002a; Teles *et al.* 2004; Teles *et al.* 2005
	Hormonal concentrations (E2, cortisol etc.)	Teles *et al.* 2004; Teles *et al.* 2005; Teles *et al.* 2007; Oliveira *et al.* 2008
	Reactive oxygen species (ROS)/ Glu- tathione (GSH) /lipid peroxidation (LPO)	Pena *et al.* 2000; Regoli *et al.* 2003; Ahmad *et al.* 2006; Gravato *et al.* 2006; Guimarães *et al.* 2009; Gravato *et al.* 2010; Nunes *et al.* 2014
Enzyme activity	glutathione S-transferase GST	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

For the European eel, it is important to consider biological effects methods that have the ability to separate contaminant-related effects from influences caused by other factors (Vethaak *et al.* 2016). In this sense, condition and growth may not solely reflect the biological effect of contaminants. For this particular aim, variation in lipid levels may also reflect other processes than exposure to contaminants and therefore be inadequate.

Among the descriptors that have been used by WGBEC in the integrated assessment approach on fish, PAH metabolites and EROD activity could be used to quantify biological effects of contaminants.The PAH metabolites method is applicable to eels as these have measured in the bile fluid of European eel and the method has been intercalibrated on a European scale (Kammann *et al.* 2013). The activity of EROD, a metabolism enzyme, covaries with concentrations of PAH metabolites. More studies are required to test whether other descriptors would be appropriate for eels.

9.4 Approaches to integrating quality parameters into quantitative stock assessment

9.4.1 The management framework of eel

Within Europe, the eel stock, fisheries and other anthropogenic impacts are managed in accordance with the European Eel Regulation No 1100/2007, "Establishing measures for the recovery of the stock of European eel" (European Council, 2007). This regulation sets a framework for the protection and sustainable use of the stock of European eel of the species *Anguilla anguilla* in Community Waters, in coastal lagoons, in estuaries, and in rivers and communicating inland waters of Member States (MS) that flow into the seas in ICES Areas III, IV, VI, VII, VIII, IX or into the Mediterranean Sea. The Regulation sets the national management objectives for Eel Management Plans (EMPs); (Article 2.4) to reduce anthropogenic mortalities (in the long term) so as to permit with high probability

the escapement to the sea of at least 40% of the silver eel biomass relative to the best estimate of escapement that would have existed if no anthropogenic influences had impacted the stock. Under the EC Regulation, MS should monitor their eel stock, evaluate current silver eel escapement biomass and post-evaluate implemented management actions aimed at reducing eel mortality and increasing silver eel escapement.

Outside Europe, the Eel Regulation is not binding but the whole-stock (international) assessment requires data and information from both EU and non-EU countries. Some non-EU countries provide such data to the ICES assessment and have developed EMPs outside the Regulation.

9.4.2 The 'international' stock assessment approaches

There are several approaches to the international or whole-stock assessment of the European eel. These are summarised below to provide context for this discussion, but are discussed in detail in reports of the WGEEEL and associated workshops (most recently ICES, 2016).

9.4.2.1 Recruitment indices

Recruitment time-series have been collated by the WGEEL since the early 1980s and these, along with the much less complete fisheries landings, have formed the basis for the provision of advice on the status of the eel stock since that time. The trend in recruitment for the European eel is derived from long-term time-series collected in estuaries scattered over all of Europe.

The WGEEL has collated information on recruitment from 51 time-series.

Figure 5. Location of the recruitment monitoring sites in Europe, white circle = glass eel, blue circle = glass eel and young yellow eels, yellow square=yellow eel series.

Some of the time-series date back to the beginning of 20th century (yellow eel, Gőta Älv, Sweden) or 1920 (glass eel, Loire, France), but no time-series are complete from then until now.

The 'WGEEL recruitment index' (ICES 2008) is a statistical prediction using a Generalised Linear Model (GLM). The GLM has a reference period set of 1960–1979, and presently includes twelve yellow eel and 39 glass eel series.

In 2015, the WGEEL glass eel recruitment indices were 1.2% of the 1960–1979 reference level in the 'North Sea' series, and to 8.4% in the 'Elsewhere' series. The 'recruiting yellow eel' index has also fallen to 11% of the level during the reference period.

The recruitment indices approach is quite simple and relies on the most reliable series available for eel. However, this kind of approach also has the disadvantages of simplicity in that (i) it cannot be used to make future predictions, (ii) it ignores the complex spatial structure of the stock, and (iii) it is very difficult to explain changes using the method alone, e.g. a positive increase may be the result of appropriate management measures but may also result from favourable environmental conditions.

9.4.2.2 Escapement biomass and anthropogenic mortalities based on European eel Regulation

The Eel Regulation (European Council, 2007) specifies a limit reference point for the biomass of the escaping silver eel of 40% of what would have existed if no anthropogenic influences had impacted the stock. A mortality limit of lifetime mortality $\Sigma A = 0.92$ can be shown to correspond to the 40% biomass limit (Dekker 2010; ICES 2011a; 2011b). In principal, this approach of the international assessment consists of the post hoc summing up of stock indicators, based on estimates for:

- $B_{current}$, the amount of silver eel biomass that currently escapes to the sea to spawn, corresponding to the assessment year;
- B_0, spawner escapement biomass in absence of any anthropogenic impacts;
- B_{best}, spawner escapement biomass corresponding to recent natural recruitment that would have survived if there was only natural mortality and no stocking, corresponding to the assessment year;
- ΣA, the sum of anthropogenic mortality rates, i.e. $\Sigma A = \Sigma F$ (the fishing mortality rate, summed over the age groups in the stock.) + ΣH (the anthropogenic mortality rate outside the fishery, summed over the age groups in the stock) or %SPR, the ratio of actual escapement $B_{current}$ to best achievable spawner escapement B_{best}.

This international stock assessment is based on national data or 'stock indicators', as reported by Member States annually to ICES and every 3 years to the European Commission (see national reports and ICES 2013a).

The approaches used by Member States to estimate their stock indicators are variable, both between countries and in some cases between eel management units within countries. The 2014 report of the Joint EIFAAC/ICES/GFCM WGEEL (ICES 2014) describes the range of methods in some detail, but they can be broadly grouped into those that apply methods based on:

- direct catch or counting of silver eels;

- yellow eel abundance;
- fishery catches;
- comparison with similar habitats elsewhere.

In particular, those methods reliant on yellow eel data convert these to silver eel estimates using principles of eel life history such as growth rates, natural and anthropogenic mortality rates, rules about sex differentiation and about the size and/or age when yellow eel transform to silver eel, and length to weight conversions. These life history relationships are either derived from habitat-specific data or often from generalised relationships reported in the scientific literature. As eel data are rarely available for all inland and saline waters within a management unit, it is common to extrapolate estimates from 'data rich' to 'data poor' environments.

9.4.3 Include the impacts of contaminant in quantitative stock assessment

9.4.3.1 Categories of impact

The effects of contaminants can be diverse and complex (see earlier chapters) but for considering their inclusion in quantitative stock assessments, we group them into those that are:

i) Lethal within the same life stage: Those that are lethal to eels soon after exposure, or at least during the same life stage, may be the simplest to incorporate in stock assessments, because they can be treated as any other mortality rate like fishing or passage through hydropower turbines. Direct lethal effects of contaminants have been reported after fish kills due to some specific spills or accidents. However, reports of such events are relatively rare (ICES 2013b). Nevertheless, these spills may have significant local impact as have been reported e.g. from the Rhine (Sandoz accident in 1986), from Lake Balaton (in 1991 and 1995) and from the Meuse (in 2007) (Geeraerts and Belpaire 2010). Of course, this principle applies only to the life stages that are monitored, and therefore not to any impact that occurs during oceanic migrations or spawning itself.

ii) Lethal with latent effects: Taking account of those that are lethal after some significant period of time depends on whether or not that mortality occurs within a life stage that is included in the assessment. An impact on glass eel that causes mortality in yellow eel can be included as a mortality in the same way as (i) above. An impact that does not occur until the silver eel has 'escaped' from the eel management (assessment) unit, cannot be incorporated directly in that assessment.

iii) Sub lethal: These are the impacts that somehow hinder the reproductive probability of the eel but they do not kill it.

   a. Where these have a direct effect on reproductive potential, that effect could be incorporated by applying a reducing factor to the silver eel escapement estimate for those eels whose 'quality' was insufficient to be expected to reproduce successfully. The challenge is to have enough information on cause and effect, and on the levels of contaminants in the local eel 'stock', to be able to derive this proportional impact. The Re-

productive Potential (RP) indicator proposed in ICES (2013b) is one approach to derive this but it is described as a probability of success or failure, whereas the probabilities can be considered anything between 0 and 1.

b. Alternatively, the effect could be indirect on reproductive potential by affecting an earlier life history process. For example, an effect might lengthen the time required for an eel to achieve the size and energy stores necessary to become a silver eel, and that increased time would increase the probability that the eel is killed before it can become a silver eel.

9.4.3.2 Incorporating contaminant effects in assessments based on recruitment indices

The assessment of stock status based on recruitment indices compares present-day values with those in the period 1960–1979. If the environmental levels of certain contaminants differed between the reference period and recent times, then this might help explain the recruitment decline. Of course, this ability relies on being able to confidently describe an effect of contaminants. Furthermore, the relative effect over the time period would have to be negative to help explain the decline, but it could be argued that some contaminants are less abundant now than they were in the 1970s and therefore that the 'contaminant effect' might be less now than the reference period. Of course, this is a very simplistic view of a very complex situation which does not account for the lag due to the very long generation time in eel, compared for example to birds of prey. Nevertheless, even if contaminant effects could explain part of the decline, this would not get around the fact that recruitment today is only a few percent of what it was in the 1970s.

9.4.3.3 Incorporating contaminant effects in assessments based on estimates of biomass and anthropogenic mortality rates

As noted above, there are a variety of approaches taken to estimate escapement biomass and mortality rate stock indicators for eel management units. In the table below, we use a simplified version of an approach based on yellow eel proxies to illustrate the variety of ways that contaminants could be incorporated in estimates of silver eel biomass and anthropogenic mortality rates.

Ref	Possible steps to estimate silver eel biomass	Possible method to incorporate contaminant effects
	ESTIMATE SILVER EEL PRODUCTION	
1	Summer surveys count yellow eel numbers, by length class	
2	Where gender is not measured, assign gender using life history model, e.g. sex ratio /density	Vary the sex ratio /density model
3	Apply growth and natural mortality to estimate numbers of silver eel in autumn	Vary growth rate; vary natural mortality rate
4	Number of silver eels derived from probabilities of silvering at length, for males and females	Vary length at silvering
5	Numbers converted to biomass using length/weight relationship	Vary weight at length
6	Extrapolate sites to basin rivers, lakes, estuary	Exclude contaminated habitats

7	Extrapolate basin to Eel Management Unit (kg/ha)	Exclude contaminated basins
	ESTIMATE SILVER EEL ESCAPEMENT subtract anthropogenic impacts	
8	Fisheries	Vary susceptibility to fisheries
9	Habitat loss (including barriers to migration)	Vary susceptibility to density dependent effects
10	Turbines and pumps	Vary susceptibility to turbines/pumps
11	Contaminants	Direct mortalities
12	Diseases and parasites	Vary susceptibility to diseases and parasites
13	Predators	Vary susceptibility to predation

The next step in developing this combined understanding would be to incorporate what we know or believe about the effects of contaminants into a stock assessment model and explore how these affect the predicted outcomes.

One challenge to modelling this next step, and to the approach in general, is whether or not the geographic scales at which eel and contaminant data are available and applied in assessment process are consistent or not. A lack of coincidence in geographic scale does not necessarily preclude the use of contaminant effects, but it may require some generalisations. For example, the life history process modelling steps 3-5 in the table above are fixed for the entire river basin and therefore differential effects could not be modelled between tributaries that are more or less contaminated. There will be solutions to this, however, and the challenge is only raised here so that it can be addressed.

The process outlined above will produce estimates of silver eel escapement biomass, for the appropriate time period (typically recent or historic reference). The 'recent' is compared with the 'historic reference' and the target is to exceed 40%. There remains the challenge of accounting for contaminant effects after 'escapement'. Any impacts of contaminants on oceanic migration, spawning and return of recruits to continental waters, still have to be addressed within the assessment process. This is where a classical stock-recruitment relationship would be very useful but deriving this for European eel is proving very difficult.

9.4.4 Further considerations

The objective of the Regulation is to reduce anthropogenic mortalities (Article 2.4). Therefore, where contaminants cause mortality in eel, it is implicit in the Regulation that MS should address this. However, this is not explicitly handled in the assessments of most or perhaps any EMPs. This absence may be because evidence of direct mortality is rare, and/or there is no obvious physical location for the pollution event (especially for diffuse pollution) unlike for fisheries, hydropower or habitat loss. This lack of physical identity may also complicate the taking account of contaminants in estimating the 'pristine' escapement, especially where the target is based on data from pre-1980 as instructed in the Regulation, because there were probably effects of contaminants in this period.

The assumption is that a proportion of today's escapement is not going to reproduce because of contaminants, and so we have to reduce our escapement estimates to account for this proportion. That principle raises the potential to write off some eel based on qual-

ity parameters, as has been suggested in some countries; but do we know enough to be sure that they will not contribute to reproduction in some way? The knowledge could also affect other management measures, in particular stocking, where there might be closed areas where no eel should be taken for stocking, or where no eel should be stocked. Incorporating sub-lethal effects adds another level of complexity.

Finally, all of the above must be considered in terms of how the eel environment has changed since before the recruitment decline became most obvious in the early 1980s. As our knowledge of contaminants and their effects becomes ever greater, it is easy to assume that the perceived situation is worse now than it was during the eighties. However, the opposite may be true for some contaminants because increased controls on their production use has reduced their presence in the eel environment.

10 Research proposal: Development of calibration curves for using a Fat Meter on wild eels

The development of accurate and non-destructive methods to measure lipids in eel, is crucial in the further development of lipid-based indices for application in eel stock assessments.

There are studies emerging that suggest low correlations between fat measures of the Fat Meter versus laboratory analyses, especially for certain life stages and/or sizes or sexes of European eels.

One possible explanation for this observation is that the standard calibration curves supplied with the meter are not appropriate for all stages of wild eel, because they are derived from cultured eels. Therefore, we propose to test this hypothesis by developing calibration curves for wild eel and testing these across eel of different length, condition, life stage and region across their natural distribution.

The approach for developing the calibration curves will be based on the guide from Distell, the Fat Meter (supplied 26 January 2016).

The Laboratory measures will be made using standard methodology (insert) performed by a laboratory accredited or one that follows the Good Laboratory Practice (GLP).

This project requires the collection of new data. We propose to sample eels across the length and fat range from Lough Neagh (Northern Ireland), Germany, Mediterranean – actually we should compare places that are high, medium and low fat content. Also other biotic factors such as sex and life stage should be taken into account.

The proposal therefore is for a joint project to (1) collect these existing and new data, (2) develop new 'wild' calibration curves and (3) test these curves in additional eel stocks at more sites across the distribution of the European eel, representing the variety of life history.

The outputs of the project will be directly relevant to scientists in a number of countries. Therefore the project should be funded through an international initiative.

11 Research proposal: "Towards understanding and quantifying the effects of contaminants on the reproductive success of the European eel and integration in stock wide assessments"

This research proposal was drafted by WGEEL in 2012 and further developed during WKBECEEL.

Our knowledge on the impact of pollutants on the reproductive phase of the European eel is currently mostly speculative because of the lack of experimental data. Observations of individuals spawning in the wild is still yet not feasible, however silver eels caught in rivers can be artificially matured to the reproductive stage thus offering new possibilities for research on the effect of contaminants on reproduction and maternal transfer.

WKBECEEL noted that essential issues to assess the importance of eel quality for reproductive success, such as evaluations of the effects of specific contaminants on the ability for eel to migrate and reproduce, are currently not included in ongoing research projects. WGEEL 2012 recommended specific research on these issues, and addressed to EU and funding agencies the request to support research resulting in a better understanding of the eel's sensitivity towards contaminants with respect to survival, migration and reproduction success.

Given the urgent need for this experimental work, WKBECEEL recommends initiating an internationally coordinated research project with the aim of improving the understanding and quantification of the effects of contaminants on the reproductive success of the European eel for integration in stock wide assessments. Such a coordinated project could be initiated within the EU Framework Programme for Research and Innovation (EU Horizon 2020, http://ec.europa.eu/research/horizon2020/index_en.cfm?pg=h2020) funding scheme. Therefore, WKBECEEL discussed and listed the most urgent requirements and outlined the objectives of such an international project, taking into consideration the presence of expertise within the eel scientific community and new technological developments.

11.1 Overall objective of the research proposal

International stock assessment requires the development and integration of approaches to quantify the effects of eel quality on reproductive potential and integrating these into stock assessments. Contamination by (especially lipophilic) compounds bioaccumulating in the yellow eel during its continental growth period has been shown to affect several fitness related parameters in the silver eel at the onset of the reproductive migration. Contamination may result in lowered lipid levels and hence insufficient energy for migration and reproduction. Reprotoxic effects of these compounds may also affect the gonad quality (endocrine disruption, altered gametogenesis, decreased fecundity, altered sperm quality), and subsequently the reproduction success, in terms of number of larvae survival. Significant gaps in scientific knowledge have been recognized, such as to what extent and at what level these contaminants affect the eel reproductive success. A flow chart identifying the different levels of potential impact by contaminants is given in Figure 6 and presents the various effects of contaminants that need to be experimentally assessed. The results will provide input data needed to integrate eel quality estimates into stock wide assessment. The overall objective is to carry out experimental and modelling work aiming to better understand and quantify the effects of contaminants on the

reproductive success of the European eel and to integrate eel stock quality parameters in stock wide assessments.

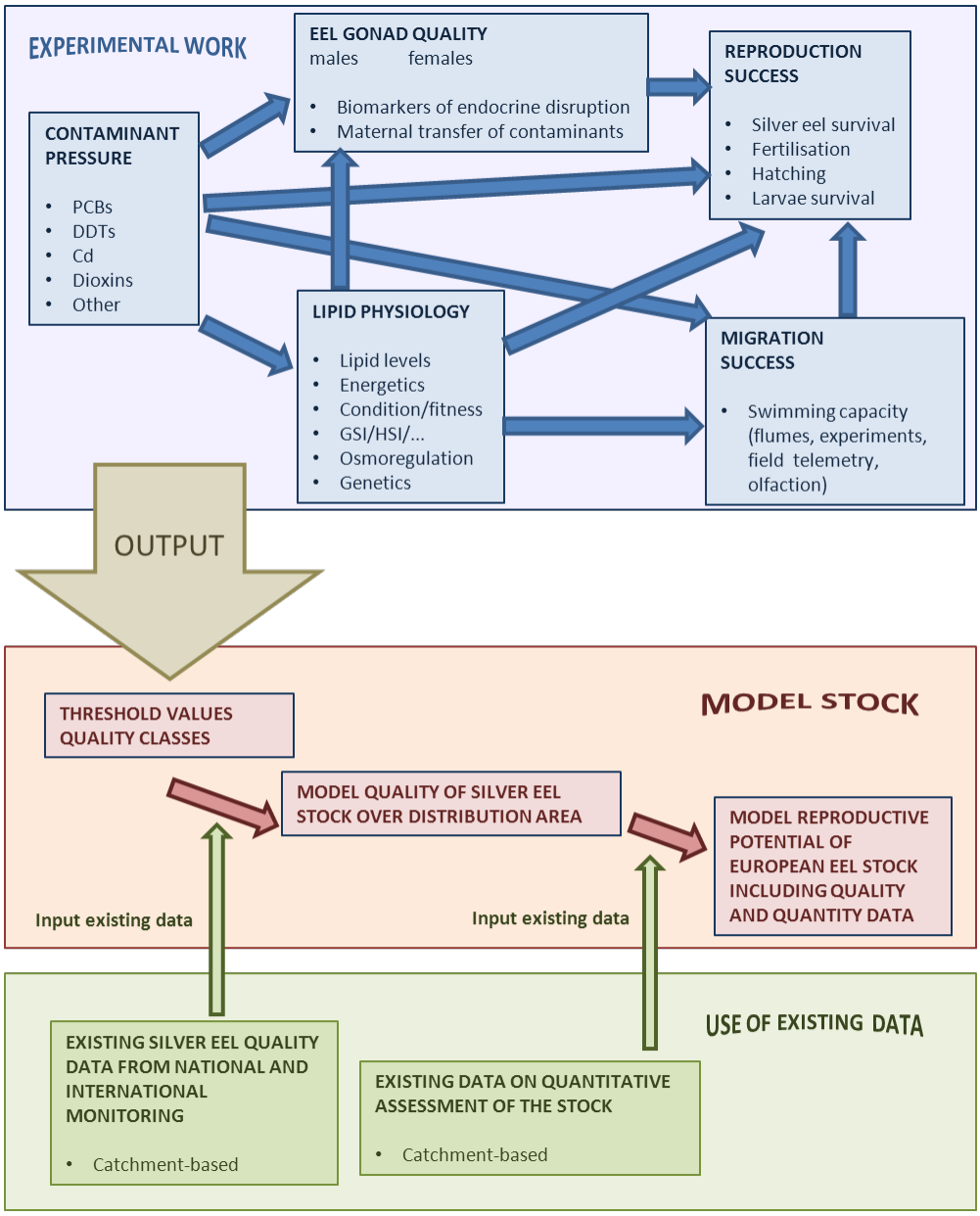

Figure 6. Different levels of potential impacts by contaminants and various effects of contaminants that need to be experimentally assessed.

11.2 Experimental work

Identifying the contaminants that are most likely to affect eels

Thousands of compounds may affect the European eel negatively, and many are known to bioaccumulate. PCB, DDT, Cd and dioxins are recognized as priority substances for future impact studies. Contaminants that are highly lipophylic (like PCBs) are likely to have an effect even after migration. Others like triclosan, pharmaceuticals or modern type pesticides may have large effects during freshwater life stage, but are lost during migration.

In combination with the measurements of contaminant loads we propose to use biomarkers (bile, EROD[3], see summary by Ulrike Kamman in Appendix, see also WKPGMEQ). Data are already available for analysis and modelling the impact of many contaminants.

Impact of contaminants on lipid physiology

Lipid reserves and energetic contents of silver eels leaving their catchment vary considerably. In other species, contaminants have been reported to cause the impairment of lipid metabolism, resulting in lowered muscle lipid levels.

This work package also depends on the standardization of non-destructive lipid measurements and the understanding of the discrepancies in lipid levels observed between yellow and silver eels (see project proposal: section 10).

Exposure experiments will be done in laboratory/aquaculture facilities on young elver and yellow eel stages to quantify the effect of pollutants on growth, condition, lipid and energetic metabolism, but also other biomarkers (proteomics, genomics, etc.).

Impact of contaminants on migration

Some values regarding the swimming efficiency and the cost of transport of silver eels migrating to the spawning grounds are available but include a variety of uncertainties (See ICES 2012). Flume experiments are required for a better estimate of the energetic requirements of reproductive migration. Further, flume studies will also be carried out on contaminant exposed eels to obtain quantitative estimates on swimming speeds and costs of transport. Although some data exist on the direct and indirect effects of contamination on the swimming capacity and energetic requirements, this needs to be further addressed.

Impact of lipid reserve and lowered fitness on migration

Migration success of male and female silver eels leaving their catchment depends on parameters such as body size, energetic content and DSS. Catchment based threshold values of fitness related parameters need to be defined to enable stock wide assessments.

Thresholds could also be determined in laboratory experiments using swim tunnels.

[3] EROD activity is induced by planar PAHs, planar PCBs and dioxin/furans.

Impact of contaminants and impairment of lipid metabolism on eel gonad quality

Contamination has been reported to induce impairment of gonads in a large number of species, but this is poorly studied in eels. Levels of maternal transfer of contaminants from muscle fat into the ovaries needs quantification. The impact of specific compounds in both ovarian and testicular tissue of eel needs to be addressed. Recent advances in eel reproduction techniques make it now possible to assess biomarkers for endocrine disruption (including histology) after exposure of maturing eels to contaminants (fecundity, sperm quality, GSI, Vtg, etc.). Impairment of lipid metabolism and lowered energy reserves will have an impact on gonads and fecundity, which needs quantification.

Impact of contaminants on reproduction success

Reproduction experiments using eels exposed to variable internal levels of contaminants will enable to quantify the impact of contaminants on reproduction success, both in males and in females. Biomarkers may include fertilization success, hatching of eggs, and survival of the larvae.

11.3 Modelling work: integrating biological thresholds into a quantitative stock assessment model using field data

By integrating threshold values from the experimental work, biological characteristics of individual eels as well as populations, the effect of different contaminants on the production and quality (size, fat content, gonad quality, toxic load, migration success, etc.) of silver eels can be revealed, quantified and integrated in a form that will be representative of catchments and modelled over the distribution area. Existing (current and past) silver eel quality data from national and international monitoring will specifically be used together with output data from the experimental work to develop models of the quality of silver eel stock over the distribution area. Further, existing data on the quantitative assessment of the stock will be integrated in a combined model of the reproductive potential of European eel stock including both quality and quantity data.

12 Conclusions

In 2015, the European eel population is still at an all-time low, following a rapid decline which began in the 1980s. The usual anthropogenic suspects have been implicated in this decline: habitat loss caused by dams, habitat fragmentation, mortality from hydropower turbines, fisheries, chemical contamination, parasites, and climate warming affecting the oceanic part of their life cycle (Castonguay and Durif 2015). Management of the eel populations implies assessing the separate contributions of these threats to the decline in recruitment and spawning stock biomass.

At present, the impacts of contaminants on eels is not taken into account in the quantitative stock assessments that form the basis of national and/or international management plans. The driver for such an adjustment of stock assessments is that in ignoring or failing to include the impacts of contaminants (or parasites or diseases) we might be underestimating anthropogenic mortality rates and overestimating the silver eel escapement biomass proxy for spawning stock biomass.

Direct evidence of the contribution of chemical contamination to the decline of eels however is still unavailable. Despite a century of research on eels, key aspects of their oceanic and continental life history remain a mystery. The major limitation in understanding the impact of contaminants is that naturally spawning adults have never been found and that possibilities of rearing eel larvae in a laboratory are still very limited.

Therefore, the aim of WKBECEEL was to join efforts between WGEEL and WGBEC to use knowledge from other fish species to characterize the biological effects of contaminants on eels.

The information reviewed here and in the first workshop on eel quality (WKPGMEQ, ICES 2015) highlights again that contaminants have probably contributed to the collapse of eel stocks. Impacts have been reported at subcellular, organ, individual and even population levels. Many gaps in our knowledge remain, especially concerning the impacts (dose-effect relationships) of contaminants and diseases on migration and reproduction success of the European eel. These are difficult to fill even using knowledge from other species because of the uniqueness of the eel's life cycle (see 1.3).

Although, contaminant levels (such as PCBs and DDTs) have been banned 40 years ago, contaminated wastes and equipment are still a cause for concern in the environment. Other pesticides, metals and other emerging contaminants are at very high levels in eels with certain substocks being unfit for human consumption (Chapter 3).

The more or less simultaneous decreases in recruitment in the Northern-hemisphere eel species (*A. anguilla*, *A. rostrata* and *A. japonica*), suggest that a common source or multiple causes are involved, reinforcing the argument that specific broadly distributed contaminants over the industrialized world are key elements in the decline (Geeraerts and Belpaire 2010). One way to obtain more evidence for this would be to analyze historical samples collected before the decline (pre- 1980s).

Contaminant levels are characterized by a very high spatial variability which is reflected in the eels. Bioaccumulation in eel is especially high since reproduction only occurs once in its lifetime (Belpaire and Goemans 2007). Pollutants primarily accumulate in fat reserves. Whether they play a role in the level of lipids is not straightforward. Neither in eels nor in other species could lipid content decreases or increases be linked to trends in contaminants. However, it is evident that, during migration, as eels fast and lipid reserves are depleted by 30–40%, lipophilic contaminants will reach high concentrations in the blood and will attain vital organs and gonads (Belpaire *et al.* 2016). Based on the many documented cases of impaired reproductive capacities related to toxicants in fish, there are reasons to suspect negative biological effects of contaminants during eel reproduction. Among the different disturbances are fertility, endocrine disruption and larval deformities after maternal transfer causes for concern in eel (Chapter 5). Effects of pollutants on swimming behaviour in fish have generally been described during short laboratory experiments and a parallel with the lifecycle of eels was not possible. Other possible effects but on which we can only speculate relate to impairments of some of the sensory systems (vision and olfaction). In any case negative effects would probably be short-lived and have little consequences at the population level. Although WKBECEEL did not consider diseases and parasites, it was mentioned that interactions between pollutants and pathogens may worsen the negative biological effects. For example, pollutants will make eels more vulnerable by depressing the immune system (Chapter 7). An interesting ex-

ample of thiamine deficiency (Annex 5) was brought up during the workshop further illustrating the consequences of anthropogenic discharges. Recent results in genetics point to a poor energetic status of eels experiencing a high pollution burden. This will undoubtedly have consequences on the high energetic demands during the spawning migration.

Attempts to characterize the quality of eels have used variables such as the percentage of fat, the distance to the spawning grounds, body mass, contaminant load (number and mean concentration) .Defining thresholds over which biological effects is not possible because there have been too few eco-toxicological studies on dose and effect of contaminants on eels. Using thresholds from other species is not adequate. The Integrated Assessment approach used by WGBEC is interesting and deserves a further investigation. However, this method also require data indicating deleterious environmental effects at a given level of the biological effect response in question. This is a major research topic which should be addressed in the form of a research project (Chapter 11) on quantifying the biological effects of contaminants. Although the full life cycle has not been completed in captivity, it is possible to rear European eel larvae up to the first feeding stage (i.e. up to 20 days, Butts *et al.* 2014). This leaves some opportunities for future research to determine toxicant thresholds inducing deleterious biological effects.

Future research should also focus on improving the standardisation of measurements of fat levels in eels. At the silver migrating stage, this parameter could constitute a proxy for migratory and reproductive capacities (Chapter 10). The set-up of an internationally coordinated research project with the aim of improving the understanding and quantification of the effects of contaminants on the reproductive success of the European eel will be crucial to allow integration of eel quality issue in stock wide assessments.

Annex 1: List of participants

Name	Address	Phone/Fax	Email
Lennart Balk	Department of Environmental Science and Analytical Chemistry (ACES), Stockholm University SE-10691 Stockholm, Sweden	phone: +46 (0)8 674 77 21 Mob: +46 (0) 73 692 34 68	lennart.balk@aces.su.se
Claude Belpaire	Research Institute for Nature and Forest (INBO) Kliniekstraat 25 1070 Brussels Belgium	mobile: +32 475 678992	Claude.Belpaire@inbo.be
Howard Browman	Institute of Marine Research (IMR) Austevoll Research Station 5392 Storebø Norway		Howard.browman@imr.no
Eleonora Ciccotti	Universitá di Roma Tor Vergata Dipartimento di Biologia Via della Ricerca Scientifica 00133 Rome Italy	phone: +390672595969 Mob: +39 3346079886:	ciccotti@uniroma2.it
Francoise Daverat	50 avenue de Verdun F CESTAS cedex 33612 France	+33 5 57 89 08 01	francoise.daverat@irstea.fr
Caroline Durif (chair)	Institute of Marine Research (IMR) Austevoll Research Station 5392 Storebø Norway	+47 976 27269	caroline.durif@imr.no
Derek Evans	Agri-food and Biosciences Institute AFBI HQ Newforge Lane Belfast BT9 5QX UK	+44 28 9025 5551	derek.evans@afbini.gov.uk
Marko Freese	Institute of Fisheries Ecology Palmaille 9 22767 Hamburg Germany	+49 4102 70860 21	Marko.Freese@thuenen.de
Bjørn Einar Grøsvik (chair)	Havforskningsinstituttet Postboks 1870 Nordnes 5817 Bergen, Norway	+47 934 12 859	Bjoern.einar.groesvik@imr.no
Ketil Hylland	Department of Biosciences University of Oslo PO Box 1066, Blindern N-0316 Oslo Norway	+47 41451694	ketilhy@ibv.uio.no

Ulrike Kamman	Institute of Fisheries Ecology Palmaille 9 22767 Hamburg Germany	+49 40 38905198	Ulrike.Kammann@thuenen.de
Michiel Kotterman	Institute for Marine Resources and Ecosystem Studies (IMARES) Haringkade 1 1976 CP Ijmuiden The Netherlands	+31 317487132	Michiel.kotterman@wur.nl
Tinka Murk	Marine Animal Ecology Group Wageningen University NL 6708 WD Wgeningen The Netherlands	+31317483233	Tinka.Murk@WUR.nl
Pål Olsvik	National Institute of Nutrition and Seafood Research (NIFES) PO Box 2029 Nordnes N-5817 Bergen Norway	+47 41459367	pal.olsvik@nifes.no
Marti Pujolar	Aarhus University Ny Munkegade 114 Aarhus 8000 Denmark		martipujolar@gmail.com
Joana Raimundo	IPMA Rua Alfredo Magalhães Ramalho, 6 1495-006 Lisboa Portugal	+351 213027073	jraimundo@ipma.pt
Lisa Sigg	Department of Environmental Science and Analytical Chemistry (ACES) Stockholm University 106 91 Stockholm Sweden	+4687647269	lisa.sigg@aces.su.se
Anne Berit Skiftesvik	Institute of Marine Research (IMR) Austevoll Research Station 5392 Storebø Norway	+47 91866526	anne.berit.skiftesvik@imr.no
Alan Walker	Cefas Lowestoft Laboratory Pakefield Road Lowestoft Suffolk NR33 0HT United Kingdom	phone +44 (0) 1502 562244 mobile +44 (0) 7766 475301	alan.walker@cefas.co.uk

Annex 2: Agenda

Monday 25 January 2016

12:00 Lunch/Check-in

13:00 Welcome – Quick introduction of participants

13:15 Assessing eel quality: background for the workshop and overview of WGEEL work - Caroline Durif

13:35 The Eel Quality Database – Claude Belpaire

Overview of the WKPGMEQ report (ICES 2015)- Claude Belpaire

Experiences from Belgium- – Claude Belpaire

14:05 Update on the eel regulation.

The international stock assessment and integrating quality in the quantitative assessment– Alan Walker

14:25 Overview of activities of WGBEC – Ketil Hylland

14:55 Eel and conger eel data from Portugal - Joana Raimundo

14:10 Measuring lipids as a fitness index – Derek Evans

15:25 Effects of contaminants on reproductive functions of A. anguilla - Francoise Daverat

15:40 Coffee break

16: 00 PAH metabolites and EROD measured in eel - Ulrike Kammann

16:15 Developmental anomalies as integrated indicators of environmental stress in larval, juvenile and adult fish - Eleonora Ciccotti and Clara Boglione

16:30 Experience with transcriptomics based effects in fish after exposure to different contaminants - Pål Olsvik

16:45 Impact of contaminants on genomics - Marti Pujolar

17:00 Lipid levels in Dutch yellow eels 1980-2015, changes and causes - Michiel Kotterman

17:15 Biochemical observations suggests thiamine deficiency in Northern Hemisphere wildlife - Lennart Balk

17:30 Thiamine status in European and American eel - Lisa Sigg

17:45 Conclusions, description of tasks/report

18:00 End

19:30 Dinner

Tuesday 26 January

8:30 Description/Assignment of tasks/work in sub-groups

12:30 lunch

13:00 work in sub-groups

15:30 Coffee break

16:00 Plenary – Summaries from sub-groups

18:30 End of day 2

Wednesday 27 January

8:30 – 12:30

Coffee served at 10:00

Reading draft report

Next steps: beyond WKBECEEL

Project proposal

Annex 3: Recommendations

RECOMMENDATION	ADRESSED TO
1. WKBECEEL reiterates the recommendation of WGEEL to initiate an internationally coordinated research project: to quantify the effects of contaminants on the reproductive success of the European eel, for integration in stock wide assessments.	SCICOM
2. WKBECEEL 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'-DDD, 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.	SCICOM, WGCATCH
3. Develop the work programme of WKBECEEL to evaluate the list of biological effects that have been used over the years by WGBEC for their suitability for characterizing eel quality. Among these identify adequate biomarkers for assessing the health of the local eels as adequate responses to multiple stresses and at concentrations measured in the field.	WGRECORDS, SCICOM, SSGEPI, SSGEPD

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Annex 5: Thiamine deficiency

Populations of many animal species in the Northern hemisphere are currently declining more rapidly than can be explained by known causes, such as habitat loss, climate change, overfishing, and classical environmental pollutants. In a worldwide perspective, these ongoing population declines and the loss of species have been described as the sixth mass extinction (Barnosky *et al.* 2011), and naturalists have proposed this development as the most serious environmental threat to life on our planet (Rockström *et al.* 2009). For several species this negative development has been paralleled by an observed altered behaviour, paralysis, and excess mortality, especially during the last two decades.

Recent discoveries have resulted in a scientific hypothesis (Balk *et al.* 2009), which implies that a wide range of wild animal species, belonging to different animal classes, are suffering from an anthropogenic thiamine (vitamin B_1) deficiency, which causes a number of disorders and, as an ultimate consequence, substantial population declines. Affected animal classes include fish (Fitzsimons, 1995, Åkerman and Balk, 1998; Balk *et al.* 2016), birds (Balk *et al.*, 2009, Paton *et al.*, 1986), reptiles (Ross *et al.*, 2000, Sepulveda et. al., 2004), and bivalves (Balk *et al.* 2016). In the aquatic environment, beside several Salmonides, the well-known negative global trend (migration, reproduction, survival) in anguillid eel (*Anguilla anguilla*, *A. rostrata*, and *A. japonica*) (Dekker 2003) populations gives reason to summon thiamine deficiency among the numerous possible causes of the eel population decline (Fitzsimons *et al.*, 2013; Balk *et al.* 2016).

Thiamine is a water-soluble vitamin essential for all vertebrates and invertebrates (Gothilf and Waites 1968(. Here, 'essential' means that it must be obtained from the food. Thiamine is present in four major forms in the tissues of the body, either non-phosphorylated or phosphorylated with 1–3 phosphate groups. It is mainly the non-phosphorylated form of thiamine (T) that is absorbed from the intestine. Inside the cells, T is phosphorylated in an enzymatic reaction, so that thiamine diphosphate (TDP) is formed. TDP acts as an essential cofactor for several life-sustaining enzymes in basic metabolism. For example, the neurons in the brain have been considered to be particularly sensitive to thiamine deficiency-induced acidosis. Even minor changes of the thiamine status in animals may cause severe systemic effects and damage. Apart from the direct role of thiamine as a cofactor in thiamine dependent enzymes, it has another important function in the body. A fraction of the TDP is further phosphorylated to thiamine triphosphate (TTP), which is necessary for the functioning of nerves (Nakagawasai 2005). Two central and direct consequences for individuals affected by thiamine deficiency are impairment of the immune system (Kumar and Axelrod 1978; Pletsity *et al.* 1979, Prasad *et al.* 1980; Pletsityi and Pletsity 1987; Shoji *et al.* 1994; Shoji *et al.* 1998; Fattal-Valevski *et al.* 2005) and damage to the blood-brain barrier (Harata and Iwasaki 1995; Calingasan and Gibson 2000). These effects of thiamine deficiency result in increased susceptibility to pathogens and toxic substances, respectively. We have, in fact, already encountered numerous examples of infections in birds that most probably are secondary to thiamine deficiency (Balk *et al.* 2009; Balk *et al.* 2010; Balk *et al.* 2016), yet mistakenly thought to be primary causes for the mortality.

As a consequence, the clinical and biochemical symptoms of thiamine deficiency are often expected to be more related to the concentration of toxic metabolites and their detoxification, than to the absolute thiamine concentration in the tissues.

Thiamine deficiency as an environmental problem has also been recognized by the International Union for Conservation of Nature and Natural Resources (IUCN), an organization with the purpose to inform the general public, the scientific community, and responsible authorities about current threats against biodiversity (Vié *et al.* 2009). They regularly publish a "red list" with endangered species, globally and nationally. The Swedish branch of IUCN (ArtDatabanken) has suggested two priority areas where further scientific knowledge is urgently needed (Gärdenfors *et al.* 2010). One of the priority area suggested by IUCN is the problem with thiamine deficiency in fish and birds, and possibly also other classes of animals. All thiamine circulating in the ecosystems (terrestrial and aquatic) is produced by the green plants (including algae), and the possibility that this natural process may be endangered makes this scientific work urgent to carry out.

In summary, lethal thiamine deficiency has by now been demonstrated in several species from different geographical areas and ecological niches, including terrestrial, freshwater, brackish, and marine environments. The working hypothesis is that the described ongoing thiamine deficiency is unnatural and occur as a consequence of antropogenic discharges. Briefly, the mechanism behind this deficiency and the causative agent is unknown, however thiamine deficiency may originate from an insufficient amount of thiamine within their diet, low uptake from the diet, and/or increased metabolism and excretion of thiamine.

Annex 6: Glossary of contaminants and technical terms

BFR: brominated flame retardants

BPA: Bisphenol A

POP: persistent organic pollutants; generally has high lipid solubility (log Kow > 5) and low degradability; expected to be subject to long-range transport, e.g. PCBs, dioxins, chlorinated pesticides, brominated flame retardants

PCB: polychlorinated biphenyls; 209 theoretical congeners with very different mechanisms of toxicity; widely used chemicals initially introduced in the 1930s, global production discontinued in the 1970s, but still widely found in e.g. building materials.

PCDD/PCDF: polychlorinated dibenzo p-dioxins and dibenzofurans. Extremely toxic to some organisms and life stages, e.g. fish larvae, but large species variations. Strong inducers of CYP1A through binding to Ah-receptor.

HCB: hexachlorobenzene; industrial by-product, but also produced through incineration; a range of biological effects, including immunosuppression and reproductive toxicity.

PBDE: Polybrominated diphenyl ethers. These are also potential endocrine disruptors. They are also suspected to affect neurobehavioural development (Muirhead *et al.* 2006). Tomy *et al.* (2004) found evidence for lower plasma levels of thyroine in Lake Trout with BDE concentrations similar to those found in eel from the Rhine.

PFAS: perfluoroalkyl substances; also referred to as PFC – perfluorinated compounds; includes PFOS, PFOA. Extremely persistent substances. As of now, PFOS comprises at least 90% of the PFAS in fish. Amphiphilic – both hydrophobic and hydrophilic parts. Interact with membranes, but also affect other biological processes.

PFOS: perfluorooctane sulfonate. Numerous effects are reported (refs in Gulh *et al.* 2014): hepatic damage, disturbance of DNA metabolism, and adverse effects on protein expression (Hoff *et al.* 2003; Roland *et al.* 2014).

PFOA: perfluorooctane acid. Highly persistent (as other PFCs), same general mechanisms of toxicity as PFOS, but less potent (according to current knowledge).

Non-essential metals: metals with no know physiological function, e.g. mercury, cadmium and lead.

Essential metal: metals with a physiological function, such as copper, zinc, iron,

Heavy metal: there is no clear definition of this term, but it is most commonly used to describe toxic metals (mercury, lead, cadmium), sometimes including metalloids, particularly arsenic; whichever density is used, most transition metals will be in-cluded; it is therefore preferable to use the term "trace metal".

Mercury: non-essential metal mainly found as methylmercury in biota (at least fish); neurotoxicity is possibly the most important mechanism of toxicity for methylmercury, but also immunotoxic and reprotoxic; has been shown to cause neurodegenerative effecs at very low concentrations.

Cadmium: non-essential metal; accumulates in kidney and liver; many mechanisms of toxicity: kidney failure, cardiovascular effects (mammals), bone formation, general cellular toxicity (possibly due to interaction with zink).

Lead: non-essential metal; accumulates in bone and viscera (two compartments); inhibits synthesis of heme at low concentrations and have been shown to have behav-ioural impacts following exposure to extremely low concentrations during develop-ment (mammals).

PAH: polycyclic aromatic hydrocarbons; generated from combustion of organic ma-terial and present in oil; main inputs from fires, oil and use of fossile fuels; very dif-ferent biological effects from 2-, 3-, 4- and 5-ring PAH mixtures; oil-derived dominated by 2- and 3-ring, combustion-derived mainly heavier. Carcinogenic, immunotoxic (predominantly heavier).

EROD: ethoxyresorufin O-deethylase activity; model substrate specifici to cytochrome P4501A(1), the main CYP isoform to be regulated by the Ah (arylhydrocarbon) receptor (AhR) and induced by planar organics such as dioxins, coplanar PCBs and benzo(a)pyrene.

Cytochrome P450 (CYP): large family of enzymes; the main phase-1 enzymes (first step in biotransformation); catalyses oxidation and reduction reactions (including e.g. dehalogenation); different substrate specificities for different isoforms.

Legacy contaminants: Contaminants that are have been known to be problematic for decades and are regulated (many of them are prohibited) but with which we are still struggling; e.g. PCBs, brominated flame retardants, dioxins, mercury, cadmium.

TEQ: Toxic Equivalents report the toxicity-weighted masses of mixtures of PCDD, PCDF, and PCB. The value is more meaningful to toxicologists than the total number of grams. It is defined by the sum of concentrations of individual compounds (Ci) multiplied by their relative toxicity (TEF).

$$TEQ = \sum [C_i] \times TEF_i$$

TEF: Toxic equivalency factor expresses the toxicity of dioxins, furans, and PCB in terms of the most toxic form of dioxin, 2,3,7,8-TCDD. There have been several systems over the years in operation, such as the International Toxic Equivalents for dioxins and furans only, represented as I-TEQ_{DF}, as well as several country-specific TEF. The present World Health Organisation scheme, represented as WHO-TEQ_{DFP}, which includes PCBs is now universally accepted.

Teratology is the study of abnormalities of embryonal development. It is often thought of as the study of human congenital abnormalities, but it is broader than that, taking into account other non-birth developmental stages, including puberty; and other non-human life forms, including plants.

Annex 7: Summary of presentations made by the participants

The eel quality database – Claude Belpaire

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. The international context, future perspectives in eel health assessments and the need of an international database have been discussed in Chapter 8 of the WKPGMEQ report (ICES, 2015).

As such, the EQD (Eel Quality Database) has been initiated by ICES WGEEL (Belpaire *et al.*, 2011) and further developed by Belgium (Research Institute for Nature and Forests, 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 of the WKPGMEQ report (ICES, 2015).

The EQD allows currently to include data on morphometrics, weight, length, stage, lipid levels, condition, age, results on contaminants and diseases, as well as a number of descriptors concerning site location (e.g. geo-reference, watershed, water body and typology, anthropogenic pressures, …) and survey descriptors (team, owner, contact, sampling method, date and time of survey, framework, reference to sampling protocol, etc).

However, the long-term management of the EQD needs a structural basis and is currently hampered by insufficient resources. ICES (2009) 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. This recommendation was repeated by the WKPGMEQ (ICES, 2015).

Experiences from eel contaminant research in Flanders (Belgium) – Claude Belpaire

As the eel is a long-lived, carnivorous, benthic and lipid-rich species, it is particularly prone to the accumulation of noxious chemical compounds, especially lipophilic contaminants like polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and brominated flame retardants (BFRs). At the Research Institute for Nature and Forest (INBO), a monitoring network was set up to measure contaminants in the eel over Flanders during a 15-year research programme, starting from 1994. More than 3000 eels from 376 locations were analysed for PCBs, OCPs, heavy metals and some other compounds.

The data showed clearly that eels in their yellow stage are excellent chemical bioindicators; contaminant profiles in the studied eels are fingerprints of the contamination pressure on the site where they grow up.

Results generated status reports and distribution maps of eel pollution for some 30 substances. Most substances are present all over Flanders, but there is considerable variation between river basins, dependent on land use. Contaminant analysis in eel is able to pinpoint specific pollution sources, like some volatile organic compounds in very specific locations, very high BFR levels in eels from areas with intensive textile industry, or high lindane levels in some rivers under agricultural pressure. We could demonstrate that

banned chemicals like DDT are still in use in some places. Within the study period, trend analysis indicated significant reductions in PCBs and many OCPs. Also for some heavy metals (lead, arsenic, nickel and chromium), concentrations decreased in the eel, but this was not the case for cadmium and mercury.

Self-caught eels are much esteemed by fishermen, but considering the eel's high contaminant body burden, consumption constitutes a potential risk for human health. On many sites levels of specific compounds exceed legal maximum levels.

Several contaminants were assessed for their potential impact on the eel population. Contaminants may impact on various levels of biological organisation from molecular, individual to population and community, hence the nature of the effects varies to a very wide extent. Despite a very high internal load of endocrine disrupters, we did not find any effects on vitellogenin levels in immature yellow eel. However, 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 strongly polluted eels a reduced genetic variability was observed. New advances in gene expression profiling, using either microarrays or RNA-seq, offers the opportunity to investigate the effects of pollutants at the genome-wide level and also showed significant impacts.

It was further demonstrated that fat stores and condition decreased significantly during the last 15 years in eels in Flanders and The Netherlands, jeopardizing a normal migration and successful reproduction of this long-distance migrator. Belpaire *et al.* (2009) hypothesized that pollution is a major driver for this decrease in fat reserves.

Part of the work presented here has been summarized in two reviews. Geeraerts and Belpaire (2010) reviewed the potential effects of contamination on the eel and Belpaire *et al.* (2016) presented a simplified concept describing how reprotoxic chemicals may influence the status of the stock of the European eel.

The international stock assessment and integrating quality in the quantitative assessment – Alan Walker

The driver for incorporating contaminant effects in quantitative stock assessments is that without this, managers might be underestimating anthropogenic mortality rates and overestimating the silver eel escapement biomass proxy for spawning stock biomass.

The effects of contaminants that are Lethal can be included directly as mortalities. More challenging however is to take account of the effects that are Sub lethal, i.e. impacts that somehow hinder the reproductive probability of the eel but they do not kill it.

The mainstay of the international or whole-stock assessment of the European eel is indices of recruitment based on long-term time-series collected in estuaries scattered over Europe. If contaminants had had a greater or lesser influence on recruitment during the reference period than recent times then it might be possible to introduce a raising or reducing factor applied to either the reference period or the most recent period, to make it a more balanced comparison between the two time periods. However, this would not get around the observation that recruitment in recent years has been at a level that is a very small fraction of that which occurred during the reference period.

More recently, the international stock assessment is being developed using national data or 'stock indicators' of silver eel escapement biomass and anthropogenic mortality rates

that are reported by Member States annually to ICES and every 3 years to the European Commission. The approaches used by Member States are variable, both between countries and in some cases between eel management units within countries. The effects of contaminants could be applied in circumstances where they affect modelled eel life history processes such as growth rates, natural and anthropogenic mortality rates, rules about sex differentiation and about the size and/or age when yellow eel transform to silver eel, and length to weight conversions, or where they affect the eels susceptibility to other anthropogenic impacts.

It must be borne in mind, however, that the biomass and mortality rate stock indicators reported by Member States culminate in the silver eels escaping from national waters. As such, any impacts of contaminants on oceanic migration, spawning and return of recruits to continental waters, still have to be addressed within the assessment process. Update on the eel regulation.

Overview of activities of WGBEC – Ketil Hylland

Eel and conger eel data from Portugal – Joana Raimundo

Estuaries are ecosystems where freshwater, including dissolved substances and suspended particles from weathering processes and anthropogenic activities, meet seawater. The pathway and fate of solutes is driven by the water circulation, biogeochemical processes and exchanges between compartments of the estuary.

Concentrations of essential and non-essential elements in aquatic organisms reflect primarily metabolic mechanisms to meet requirements of individuals, as well as response to their availability in food and environment. Eel have been used as a bioindicator of contaminants (De Boer and Hagel 1994; Belpaire and Goemans 2007; Belpaire *et al.* 2008) and many authors have reported (high) levels of a variety of xenobiotics in the eel.

In 1992 eels (*Anguilla anguilla*) were collected in the Sado estuary (Portugal) to determine levels of metals and organic contaminants in different tissues (muscle, skin, liver and viscera). The Sado Estuary is an ecosystem where human pressures and natural values compete. The estuary is the second largest in Portugal, located on the west coast of Portugal. Most of the estuary is classified as a natural reserve but it also plays an important role in the local and national economy. There are several industries, mainly on the northern margin of the estuary. The elemental partition was evaluated and different associations were found: Cd concentrations were higher in the liver, Cu and Pb in the skin and Zn in the viscera. Associations are probably related to the role of these tissues in the metabolism, and detoxification and elimination mechanisms. High levels of the PCB congeners 153, 138 and 180 were found in the muscle of eels. The comparison of metal concentrations determined in 1992 with levels presented by Neto *et al.* (2011) in eels from the Tagus estuary (the largest in Portugal), showed higher levels in eels from 1992. This difference suggest that mitigations measures made in the last one to two decades in both estuaries are probably leading to a decrease of contaminants in the environment. Levels of tPCB and tDDT were related with lipid content (%) in eels and other species (*e.g. Carcinus maenas*, *Dicentrarchus labrax*, *Liza aurata*). In species presenting higher lipid content, such as in eel, the concentrations of the organic compounds were also elevated. Taking into account that eels are good indicators and sensitive to environmental pollution, laboratory experiments were performed exposing eels to pesticides and evaluating the

genotoxic effects and possible recover (Guilherme *et al.*, 2014; Marques *et al.*, 2014 a, b). The results confirmed the induction of DNA damage by the pesticide formulations Garlon®, Roundup® and Decis® in *A. anguilla* at environmental concentrations. And that, even after the exposure cessation, eels still presented genetic damage, highlighting the genetic hazard associated to the addressed agrochemicals, reinforcing the hypothesis of long-lasting damage.

Concentrations obtained in eels were further compared with other Anguilliformes, *Conger conger*. Concentrations in conger eels were lower presumably related with species biology, habitats and feeding habits.

Further studies, determining concentrations in eels and evaluating associations from tissue to sub-cellular level should be done, in this way contaminant effects can be search which can be of extremely importance to fish and to population welfare.

Measuring lipids as a fitness index: The Lough Neagh experience – Derek Evans, Warren Campbell and Cathy Chauhan

Lough Neagh Eels are renowned for their high lipid content, attributed primarily to their Chironomid diet in the lake and this high lipid content is a key feature of their Protected Geographical Indicator (PGI) status. As a food processor, LNFCS commenced nutritional analysis in Sept 2014, at the launch of their new ready-to-cook packs. The objective was to accumulate nutritional information over a number of years to get a meaningful average for back of pack (BOP) consumer information. This testing was based on strict industry standards involving ISO accredited laboratory analysis of eel and amongst a suite of metrics gathered noted total fat content using Nuclear Magnetic Resonance (NMR).

Lipid content analysis in Lough Neagh yellow and silver eels has also been monitored over the previous 2 years by AFBI using a fat metre, providing data used to populate the ICES European Eel Quality Database (EEQD), inform the Neagh Bann Eel Management Plan, and evaluate spawner reproductive potentials during broodstock trials for the EELHATCH project in Denmark.

Comparisons between the 2 lipid measurement approaches indicated discrepancies within the overall fat results. Fat content in yellow eel was found to be replicable between and fat metre and lab NMR method. However, wide ranging discrepancies were recorded when the analyses shifted to silver eels, with NMR method recording consistently significant higher levels of total fat. The implications of these erroneous findings were discussed in relation to the needs of accurate fat content recordings for PGI, Food Labelling and the calculation of energetic costs for silver eel Spawning Migration.

PAH metabolites and EROD measured in eel – Ulrike Kammann

Polycyclic aromatic hydrocarbons (PAH) are important environmental contaminants resulting from incomplete combustion of e.g. coal and petroleum or from natural sources such as degradation of biological materials, which has led to the presence of these compounds in sediments and to the formation of fossil fuels. For the aquatic environment wastewater, atmospheric deposition and petroleum spillage are further prominent sources. PAHs and their intermediate metabolites may induce toxic or mutagenic effects in fish (Brinkmann *et al.* 2010).

PAH metabolites in the bile fluid are widely accepted as measures for PAH exposure in fish because of the rapid metabolization of PAH in vertebrates (Meador *et al.* 1995). As a consequence, PAH metabolites in fish are recommended as core monitoring parameters in European Seas (HELCOM 2012; OSPAR 2015). High performance liquid chromatography (HPLC) is used for the determination of PAH metabolites in European eel (Kammann 2007). The analytical method has been intercalibrated on a European scale (Kammann *et al.*, 2013).

Eeel from different countries vary in PAH metabolite concentration. For example 1-hydroxypyrene as main metabolite in eel from the United Kingdome (Ruddock *et al.*, 2003), Germany (Kammann *et al.*, 2014), Poland (Szlinder-Richert *et al.*, 2014) and Morocco (Wariaghli *et al.*, 2015) indicated different overall contamination levels.

In Germany significant differences of PAH metabolite concentrations in eel were found between the different river systems. For the river Elbe the concentrations of PAH metabolites covaried with the activity of the metabolism enzyme EROD (etoxyresorufin-O-deethylase) measured in the same fish. This indicates that PAH or contaminants with similar sources and distribution patterns might be responsible for elevated EROD levels in eel (Kammann *et al.*, 2014).

Bile pigments measured as indicator of the feeding status of the fish turned out to be a measure for starvation. Bile pigments can be used to identify starving silver eels which might be in a premigratory stage. This could help to identify the proportion of migrating eel in a river which is of special importance for eel management plans (Kammann *et al.*, 2014).

Morphological anomalies and ecological insights as indicators of environmental stress in fish: which issues could contribute to eel quality assessment ? Ciccotti, L. Tancioni and C. Boglione

The aim of the presentation was to illustrate some work on fish ecology carried out in the Fish Ecology Lab besides eel research, to find possible overlapping issues than can apply to eel quality assessment. Some research topics in fact concern the use of fish as an integrated tool for ecosystem risk assessment, that allow an integrated picture of responses at environmental stress at different levels of biological organization. Levels of survey that were illustrated were: subcellular (micronucleus test in fishes), cellular (sense organs –olfactory cells and lateral line cells in normal conditions and with anomalies), tissue and organ level (skeleton anomalies as integrated descriptors of environmental quality). Some of these could give useful insights also for effects of contamination on eel.

Experience with transcriptomics based effects in fish after exposure to different contaminants – Pål A. Olsvik

Trancriptomics is increasingly being used to assess environmental impacts of contaminants. With lowering analytical costs, sequence data available for more species and better annotation, transcriptomics offer a relatively straightforward and easy way to evaluate the negative impacts of contaminants in wild fish. Using microarray and direct sequencing (RNA-seq), we have in several wild fish studies applied global transcriptomic screening in search for molecular markers of exposure. These studies, involving fish species such as the Atlantic salmon (*Salmo salar*), Atlantic cod (*Gadus morhua*), tusk (*Brosme brosme*) and burbot (*Lota lota*), have contributed to the identification of markers affected by environmental contaminants such as crude oil, heavy metals and POPs. By combining

transcriptomics and metabolomics, we have seen that these two omics' technologies provide complementary results, further supporting the implementation of using transcriptomics in environmental monitoring. Downstream functional analysis, using tools like Gene Ontology (GO), KEGG and Ingenuity Pathway Analysis (IPA), can help identify pathway patterns, mechanisms and detailed data on upstream regulators recognition and transcription factors. In general, our studies show that the transcriptome of wild fish seems to reflect site-specific contamination. Transcriptomics is particularly useful to identify pathway patterns and general mechanisms. Caution need to be taken due to the fact that wild fish are outbred, and often show variation in transcription due to size differences, sex and annual behaviour (migration, food source). To find good controls can for these reasons sometimes be a challenge.

Impact of contaminants on genomics – Marti Pujolar

Recent advances in Next Generation Sequencing (NGS) methods have allowed the complete genome sequencing of European eel. Genotyping-by-sequencing methods (i.e. RAD-sequencing) are revolutionising the field of population genetics and can provide data on thousands of markers distributed across the genome. In the case of the European eel, the RAD approach has been used to generate data for 350 000 markers that suggest: (1) conclusive evidence for genomic panmixia (one single population) in European eel, (2) a large effective population size (>100 000 individuals) and (3) presence of hybrids limited to Iceland. Besides genomics, transcriptomic approaches have been used to study the effect of pollution on European eel. Analysis of microarray data comparing eels from clean vs. polluted eels in a replicate study conducted in Italy and Belgium showed a lower expression of genes related to energy and metabolism in eels from polluted sites. This could suggest a possible low energetic status of eels with high pollutant burden that might affect or even impair the spawning migration to the Sargasso Sea.

Lipid levels in Dutch yellow eels 1980–2015, changes and causes – Michiel Kotterman

Eel populations are declining, and among the suggested causes are pollution-related reduced lipid levels. This study analyses monitoring data from 1979 till 2015 with special attention to the sex of the analysed animals.

Until 2011 in Dutch PCB-monitoring studies most eel samples (30–40 cm) were pooled without distinction between males and females. However, as male eels mature at smaller sizes than females, the focus on monitoring smaller eel (30–40 cm) creates a bias for males with associated higher lipid reserves. Our analysis reveals that the male /female ratio is location dependent in The Netherlands, and that these ratios are not constant over time at some locations.

We were able to show that indeed lower lipid levels in the pooled samples coincide with a relative low number of males in the samples. The lower male ratios may be caused by falling stock densities, a phenomenon observed in whole of Europe. Contamination levels of the eels, notably PCBs and dioxins, were not related to changes in sex-ratios.

Sex-specific analysis of individual eels confirmed that lipid levels are not reduced. Male yellow eels of 30–40 cm have high lipid levels and small, low-lipid female yellow eels of 30–40 cm still develop into large, high-lipid eels. Contamination levels of PCBs and diox-

ins in individual eels, on wet weight, were positively related to lipid levels. This was observed in any type of location, heavily polluted or relatively clean.

Analysis of individual silver eels at the onset of their migration confirms that lipid levels have not declined and are not limiting a successful migration and spawning.

Biochemical observations suggests thiamine deficiency in Northern Hemisphere wildlife – Lennart Balk

- Recent discoveries have resulted in a scientific hypothesis, which implies that a wide range of wild animal species, belonging to different animal classes, are suffering from an anthropogenic thiamine (vitamin B1) deficiency, which causes a number of disorders and, as an ultimate consequence, substantial population declines. Affected animal classes include fish, birds, reptiles, and bivalves.
- Thiamine is a water-soluble vitamin essential for all vertebrates and invertebrates. Thiamine is present in four major forms in the tissues of the body, either non-phosphorylated or phosphorylated with 1–3 phosphate groups. Inside the cells, T is phosphorylated in an enzymatic reaction, in which a kinase adds two phosphate groups so that thiamine diphosphate (TDP) is formed. TDP acts as an essential cofactor for several life-sustaining enzymes in basic cellular metabolism, such as transketolase, pyruvate dehydrogenase, and α-ketoglutarate dehydrogenase. A fourth thiamine dependent enzyme is branched-chain α-keto acid dehydrogenase (BCKDH). This enzyme is present in the mitochondria and metabolizes derivatives of the branched amino acids valine, leucine, and isoleucine. The fifth known thiamine dependent enzyme is 2-hydroxyacyl-CoA-lyase, which is present in the peroxisomes and catalyzes the α-oxidation of lipids. Apart from the direct role of thiamine as a cofactor in thiamine dependent enzymes, it has another important function in the body. A fraction of the TDP is further phosphorylated to thiamine triphosphate (TTP), which is necessary for the functioning of nerves. In the light of these facts, it is easily realized that even minor changes of the thiamine status in animals may cause severe systemic effects and damage. In summary, sublethal effects of this deficiency cause disturbed cellular metabolism of lipids, proteins and sugar, and among the most severe and important secondary effect of these disturbances is the impairment of the immune system.
- Thiamine status was investigated in both European and American eel of sev-eral different life stages (elvers, yellow- and silver eels). Thiamine deficiency could be observed both with biochemical measurements of thiamine depend-ent enzymes and by chemical analysis of thiamine concentrations.
- Thiamine status was found to be low in European elvers, and could be improved simply by bathing the elvers in a thiamine solution.
- Swimming endurance of American yellow eels was notably reduced for individuals with lower amount of thiamine in the white muscle tissue.
- White muscle thiamine concentration of migrating silver eels as high as 16 nmol/g have occasionally been observed, however the mean value of European and American eels lies around 2–4 nmol/g, levels that most probably are too low

for the eels to manage to migrate all the way to the spawning area in the Sargasso sea, and produce healthy offspring.

- A chemical substance causing thiamine deficiency may exert its effect either by decreasing the uptake of thiamine or by increasing its consumption (metabolic degradation and/or excretion), or both. Thiamine deficiency may also arise from insufficient amounts of thiamine in the food. Thiamine is produced mainly by green plants, phytoplankton and to some extent by bacteria and fungi. Consequently, this is the sources for this essential vitamin for the entire ecosystem.

Thiamine status in European and American eel – Lisa Sigg

Thiamine (vitamin B_1) status together with sub-lethal effects of thiamine deficiency was investigated in European and American eel of several different life stages (elvers, yellow- and silver eels). Brain, liver, and muscle tissues was investigated using both biochemical measurements of thiamine dependent enzymes (transketolase and α-ketoglutarate dehydrogenase) and chemical analysis of thiamine concentrations. Thiamine status in European elvers was found to be low, and could be improved simply by bathing the elvers in a thiamine solution, a method that have been very successfully used to cure Swim up syndrome, Early mortality syndrome (EMS) and M74 in for example Lake Trout and Atlantic Salmon fry. Swimming endurance of American yellow eels was notably reduced for individuals with lower amount of thiamine in their white muscle tissue. White muscle thiamine concentration of migrating silver eels as high as 16 nmol/g have occasionally been observed, however the mean value of European and American eels is several times lower (2–4 nmol/g), at levels that most probably are too low for the eels to manage to migrate all the way to the spawning area in the Sargasso Sea, and at the same time produce healthy offspring with a sufficient amount of thiamine in the eggs. Poor thiamine status was found in all three life stages of eels and is hypothesized to be derived from an insufficient amount of thiamine within their diet.

Annex 8: WKBECEEL terms of reference

2014/2/SSGEPD07 A **Workshop** of the **Working Group on Eel** and the **Working Group on Biological Effects of Contaminants** (WKBECEEL) will be established under the subject "Are contaminants in eels contributing to their decline?". WKBECEEL will be chaired by Caroline Durif, Norway, and Bjørn Einar Grøsvik, Norway, and will meet in Os, Norway, 25–27 January 2016 to:

a) Describe the spatial and temporal trends in concentrations of "traditional" and/or "emerging" contaminants in eel (but mainly refer to figures available from WGEEL 2008–2013).

b) Describe the potential impacts of contaminants on reproduction in the European eel, based on science of eel and what can be learned from other species models (including endocrine disruption, effect on sex ratio, maternal transfer of bioaccumulated contaminants toward the eggs and effects on the larvae).

c) Describe the potential impacts of contaminants on lipid metabolism and migration in the European eel based on eel science and what can be learned from other species

d) Review the impacts of contaminants on the genetics of the European eel.

e) Explore whether there is experience with assessing/qualifying the bioaccumulation + fitness status in other species, which can be helpful for the eel's quality assessment (Eel Quality Index) and to quantify the impact of eel quality.

WKBECEEL will report by 10 March 2016 (via SSGEPD) for the attention of WGEEL, WGRECORDS and SCICOM.

Supporting information

Priority	During previous meetings WGEEL (2008–2013) made considerable progress in understanding and describing the potential impact of contaminants on the European eel stock. During the last sessions WGEEL 2012 and WGEEL 2013 indicated that the WG would clearly benefit from a joint cooperation with experts from other ICES WGs, and specifically WGBEC. The experience and knowledge concerning the effect of contaminants in other species, as present within WGBEC, is anticipated to be very beneficial to make further progress in understanding the role of contaminants in the eel stock decline.
Scientific justification	The stock of the European eel *Anguilla anguilla* is in decline and there is an increasing awareness that poor health status due to contaminants might be a key element in this decline and might be a hindrance to recovery. Several studies have recently been initiated to study the degree and the effects of pollution on the eel, resulting in an increasing quantity of information that demonstrates the negative impact of pollution on eel. These advances in the science of the effects of contaminants on the eel have beer reviewed recently (e.g. Geeraerts *et al.*, 2010; by Elie and Gerard, 2009, and WGEEL 2008-2012). However, essential issues to assess the importance of eel quality for reproductive success, such as to evaluate the effect of specific contaminants on the ability for eel to migrate and to reproduce have still to be developed. The joint workshop will review all sources of

	information (including work on other species) to better understand how contaminants in eels contribute to their decline.
Resource requirements	
Participants	WGEEL and WGBEC Working Group Participants, and other experts. The Workshop is anticipated to be attended by some 15-20 members and guests.
Secretariat facilities	Sharepoint
Financial	
Linkages to advisory committees	WGEEL,WGBEC and ACOM
Linkages to other committees or groups	WGRECORDS, SSGEPD, SCICOM
Linkages to other organizations	FAO EIFAAC, GFCM, EU DG MARE, EU DG ENV