Growth and condition of stocked glass and farmed eels in a brackish water system

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Declaration on oath

I hereby declare, on oath, that I have written the present dissertation by my own and have not used other than the acknowledged resources and aids.

Place, date	Björn Kullmann

On the search for the Turboaal ...

“Wenn alle dasselbe denken, werde ich misstrauisch. Es gibt Meinungen, die werden dauernd wiederholt, aber nie wirklich belegt”

(Prof. Dr. Stefan Hell)

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Summary

The recruitment of the European eel *Anguilla anguilla* (Linnaeus 1758) stock has decreased massively since the 1980s and stagnates at historically low levels. In 2007, the European Union (EU) adopted a regulation with measures for the recovery of the European eel (EC No 1100/2007) which aims at the EU-wide protection and the sustainable use of *A. anguilla*. Amongst e.g. the reduction of migration obstacles and the restrictions on fisheries, stocking measures are proposed to increase the silver eel escapement biomass. Based thereon, the German eel management plan has been adopted in 2008 with the objectives to protect the eel stock and to enable a sustainable eel fishery in German waters. Eel stocking measures, nevertheless being entirely reliant on wild eel catches, have been identified as key measure for the sustainable achievement of the silver eel escapement biomass target specified by the EU regulation i.e. a minimum of 40 % compared to the best estimated pristine level.

The aim of the present study was, on the one hand, to provide the basis for a comprehensive evaluation of the benefit from eel stocking measures. And on the other hand, to evaluate the optimization potential of eel management plans by the choice of the stocking form. Therefore, application oriented protocols have been established for the chemical mass-marking of the most commonly used stocking forms i.e. glass and pre-grown farmed eels from aquaculture facilities (Chapter I and II). Special attention has been payed particularly to the carful but efficient handling of the eels and a successfully realized marking success of 100 % using alizarin red S (ARS) at consistently low mortality rates of below 1 % of the total biomass. Furthermore, evidence have been found that the chemical marking of farmed recruits is essential for the accurate ageing and associated calculation of growth rates (Chapter III). This was hypothesised to be a consequence of several size-grading's during the farming process which is necessary to separate the fast from slow growing individuals to reduce loss-

es due to cannibalism. Hence eels are regularly subject to stress which leads to the formation of stress rings on otoliths that are used for the purpose of ageing. It has been demonstrated that these stress rings cannot be distinguished from potentially true annuli in blind readings. This resulted in an overestimation of the age by an average of two and up to seven years and, as a consequence, an underestimation of the growth rate of up to 108 ± 48 %. Moreover, if farmed eels make up a substantial part of a stock, the observed ageing error was found to be relevant for age-based stock assessment models which are likely to underestimate the stock biomass and therefore hamper model based evaluations of management measures.

Furthermore, the farming process is associated with a severe risk of infection with diseases. In commercial eel farms the *eel herpesvirus* (AngHV-1; *anguillid herpesvirus 1*) plays a key role since eels often get deliberately infected to forestall an uncontrolled outbreak (Chapter IV). Controversially, infected eels are still used for the purpose of stocking and disease screenings in advance of the release are rarely carried out. This study provides evidence that uncontrolled stocking measures with farmed eels in the Schlei fjord introduced AngHV-1 in the first place. Furthermore, larger and in particular maturing silver eels have been found to show a clinically relevant virus load which leads to the conclusion that infected eels are very likely unable to contribute to future recruitment.

With respect to a possible improvement of the efficiency of eel stocking measures, a comparative study using glass and farmed eels have been conducted to evaluate potential differences between stocking forms in terms of growth performance, body condition, and benefit-cost-ratio (Chapter V). A total of 117 kg of glass eels and 1040 kg of farmed eels have been stocked in the Schlei fjord at an equivalent purchase cost ratio which has equalled a numerical proportion of 2.3:1 at date of stocking. After two years of growth, recaptured farmed

eels showed a significantly higher mean total length and body weight than stocked glass eels whereby the specific growth rates did not differ significantly indicating that these differences are likely to persist. Derived from the numerical proportion within the recaptured sample the relative mortality was 3.9 times higher in glass eels allowing the conclusion of a more advantageous benefit-cost ratio when using farmed recruits.

The results of this study have shown that efficient mass marking of stocking material comprised of glass and farmed eels is possible whereby ARS is favourable due to its harmless application and detectability using the wide-spread fluorescence microscopy technology. Multinational research collaborations could be initiated on this basis to evaluate a potential net benefit from eel stocking. Contradicting numerous previous studies, evidence has been found concerning the basic suitability of farmed recruits as stocking material inasmuch as they have been marked chemically and the health status has been approved to avoid both the observed ageing error (Chapter III) and the dissemination of diseases (Chapter IV).

Zusammenfassung

Das Nachwuchsaufkommen des Europäischen Aalbestandes *Anguilla anguilla* (Linnaeus 1758) ist seit den 1980er Jahren massiv zurückgegangen und stagniert derzeit auf einem historisch niedrigen Niveau. Aufgrund dessen wurde im Jahr 2007 eine EU Verordnung erlassen (EU VO 1100/2007), die den gesetzlichen Rahmen für umfangreiche Maßnahmen zur europaweiten Wiederauffüllung des Bestandes bildet. Das Ziel dieser Verordnung ist der Schutz der Art *A. anguilla* und eine nachhaltige Nutzung sicherzustellen. Neben Vorschlägen zur Verbesserung der Durchgängigkeit von Gewässern und Einschränkungen der Fischerei werden Aalbesatzmaßnahmen als Mittel zum Zwecke der Erhöhung der Biomasse abwandernder laichreifer Blankaale empfohlen. Darauf basierend wurde 2008 der Aalmanagementplan der deutschen Bundesländer vorgestellt, der neben dem Schutz des Aalbestandes auch dem Erhalt der Aalfischerei dienen soll. Der Besatz von juvenilen Aalen wurde ebenfalls als Schlüsselmaßnahme identifiziert, um eine von der EU vorgegebene Abwanderung laichreifer Aale von wenigstens 40 % der Biomasse sicherzustellen, die ohne menschlichen Einfluss wahrscheinlich stattgefunden hätte.

Das Ziel der vorliegenden Arbeit war auf der einen Seite, die Grundlage für eine Evaluierung der Wirksamkeit von Aalbesatzmaßnahmen zu schaffen, die vollständig von dem natürlichen Aalaufkommen abhängig sind. Auf der anderen Seite sollte überprüft werden, ob und inwiefern Aalmanagementpläne durch die Wahl der Besatzform optimiert werden könnten. Dazu wurden anwendungsorientierte Protokolle zur chemischen Massenmarkierung von den beiden meistgenutzten Besatzformen Glas- bzw. in Aquakulturanlagen vorgestreckte Farmaale etabliert (Kapitel I und II). Im Vordergrund standen insbesondere das schonende und gleichzeitig effiziente Handling des Besatzmaterials und im Ergebnis der erfolgreich realisierte 100%ige Markierungserfolg mit Alizarinrot S (ARS) sowie eine niedrige Mortalität nach der

Markierung von nicht mehr als 1 % der Gesamtbiomasse. Im Weiteren konnte belegt werden, dass die chemische Markierung der Farmaale notwendig ist, um das Alter und die damit korrelierte Kalkulation der Wachstumsraten akkurat durchführen zu können (Kapitel III). Während der Vorstreckungsphase müssen die schnell wachsenden Individuen im Abstand von vier bis sechs Wochen von den langsamer wachsenden Aalen getrennt werden, um die Kannibalismusrate möglichst niedrig zu halten. Dabei sind die Aale regelmäßig Stress ausgesetzt, was zur Bildung von Ringstrukturen auf den Otolithen führen kann, die zur Altersabschätzung genutzt werden. Es konnte anhand von Blindlesungen gezeigt werden, dass diese Stressringe nicht von potentiell echten Jahresringen unterschieden werden können, was zu einer mittleren Überschätzung des Alters von zwei und bis zu sieben Jahren führte und einer daraus resultierenden Unterschätzung der Wachstumsrate von bis zu 108 ± 48 %. Darüber hinaus sind Belege gefunden worden, dass altersbasierte Bestandsmodelle die Gesamtbiomasse durch diesen systematischen Fehler in der Altersabschätzung wahrscheinlich massiv unterschätzen und damit eine modelbasierte Bewertung von Aalmanagementmaßnahmen erheblich behindern könnten.

Im Zusammenhang mit dem Prozess der Vorstreckung steht auch ein erhöhtes Risiko der Infektion mit Krankheiten. In kommerziellen Aalfarmen spielt insbesondere das Aal-Herpesvirus (AngHV-1; *anguillid herpesvirus 1*) eine herausragende Rolle, weil die Aale häufig mit diesem Erreger vorsorglich infiziert werden, um einen unkontrollierten Ausbruch zu verhindern (Kapitel IV). Problematisch ist, dass auch solche Aale weiterhin für Aalbesatzmaßnahmen genutzt werden und der Gesundheitszustand des Besatzmaterials nur selten vor dem Ausbringen der Tiere getestet wird. In der vorliegenden Studie konnte gezeigt werden, dass AngHV-1 durch die Durchführung von unkontrollierten Farmaalbesatzmaßnahmen in den nachweislich ehemals Aal-Herpesvirus freien Aalbestand in der Schlei eingebracht wur-

de. Darüber hinaus wurde insbesondere bei großen und abwandernden Aalen eine klinisch relevante Viruslast festgestellt, so dass vermutet werden kann, dass AngHV-1 infizierte Aale sehr wahrscheinlich keinen Beitrag zur Reproduktion des Bestandes leisten.

Mit dem Ziel einer möglichen Steigerung der Effizienz von Aalbesatzprogrammen wurden vergleichende Untersuchungen zwischen Glas- und Farmaalen im Hinblick auf die Wachstumsleistung, die körperliche Verfassung und die Kosten-Nutzen-Effizienz durchgeführt (Kapitel V). Insgesamt wurden dafür 117 kg Glasaale und 1040 kg Farmaale mit ARS markiert und zwischen 2015 und 2016 in der Schlei ausgesetzt, was einem äquivalenten Anschaffungskosten- und Stückzahlverhältnis von 2,3:1 entsprach. Es hat sich gezeigt, dass die Farmaale nach zwei Jahren des Wachstums eine signifikant höhere Totallänge und ein höheres Körpergewicht aufwiesen, wobei sich die Wachstumsraten zwischen den Besatzformen ab dem zweiten Jahr nicht mehr unterschieden, so dass die beobachteten Unterschiede wahrscheinlich bestehen bleiben werden. Aus dem beobachteten Stückzahlenverhältnis bei den wiedergefangenen Aalen konnte zum einen eine 3,9 Mal höhere Mortalität bei den besetzten Glasaalen abgeleitet und zum anderen eine damit korrelierte höhere Kosten-Nutzen-Effizienz bei Farmaalen festgestellt werden.

Die Ergebnisse der vorliegenden Arbeit haben gezeigt, dass eine effiziente Massenmarkierung von Besatzmaterial bestehend aus Glas- und Farmaalen möglich ist und die Markierung mit ARS zudem den Vorteil einer harmlosen Anwendung und der Detektierbarkeit mithilfe der weit verbreiteten Fluoreszenzmikroskopie bietet. Multinationale Forschungskooperationen könnten auf dieser Grundlage initiiert werden, um den Nettonutzen von Aalbesatzmaßnahmen zu untersuchen und umfassende neue Einblicke in die kontinentale Lebensphase des Europäischen Aals zu ermöglichen. Im Widerspruch zu zahlreichen vorherigen Studien sind Belege für die grundsätzliche Eignung von Farmaalen als Besatzmaterial gefunden wor-

den, sofern diese Rekruten chemisch markiert und ihr Gesundheitszustand vor dem Besatz geprüft wurde, um den Fehler bei der Alterslesung (Kapitel III) und die Ausbreitung von Krankheiten zu vermeiden (Kapitel IV).

General Introduction

The genus *Anguilla* worldwide

The genus *Anguilla* Schrank 1798 consists of 19 species or subspecies including 2 in the Atlantic, 3 oceanic species, 1 in the Western Pacific, and 13 indo-pacific eels (Figure 1a and b; Minegishi *et al.*, 2005; Teng *et al.*, 2009). Genetic analysis revealed that *Anguilla mossambica* (Peters, 1852) and *Anguilla borneensis* Popta 1924 are likely to be the most basal living species within the genus indicating that the Indo-Pacific region is the origin of all other species clades (Aoyama *et al.*, 2001; Minegishi *et al.*, 2005). The spread out into the other world oceans was possible via five different routes (e.g. Tseng, 2016) whereby multi-dispersal events are the most probable scenario supported by genom-based divergence time estimations (Minegishi *et al.*, 2005). All known *Anguilla* species are catadromous and have in common a leaf-like early life stage called leptocephalus larva which is the characteristic feature of all Elopomorpha orders and suborders (Greenwood *et al.*, 1966). *Anguilla marmorata* Quoy & Gaimard 1824 shows the largest distribution range (Teng *et al.*, 2009) while the three species namely the American eel *A. rostrata* Lesueur 1821, the Japanese eel *A. japonica* Temminck & Schlegel 1846, and the European eel *Anguilla anguilla* (Linnaeus 1758) are most important from an economically perspective (Crook, 2010).

The European eel: reproduction and ecology

The European eel *Anguilla anguilla* displays the longest spawning migration amongst eels (Aoyama, 2009) with its starting and end point located in the Sargasso Sea (Figure 2 and Figure 3; Schmidt, 1922). The nursery areas of the eel, however, are the coastal and inland water bodies of Europe, northern Africa, and partly Asia, thus hatched eels migrate mainly

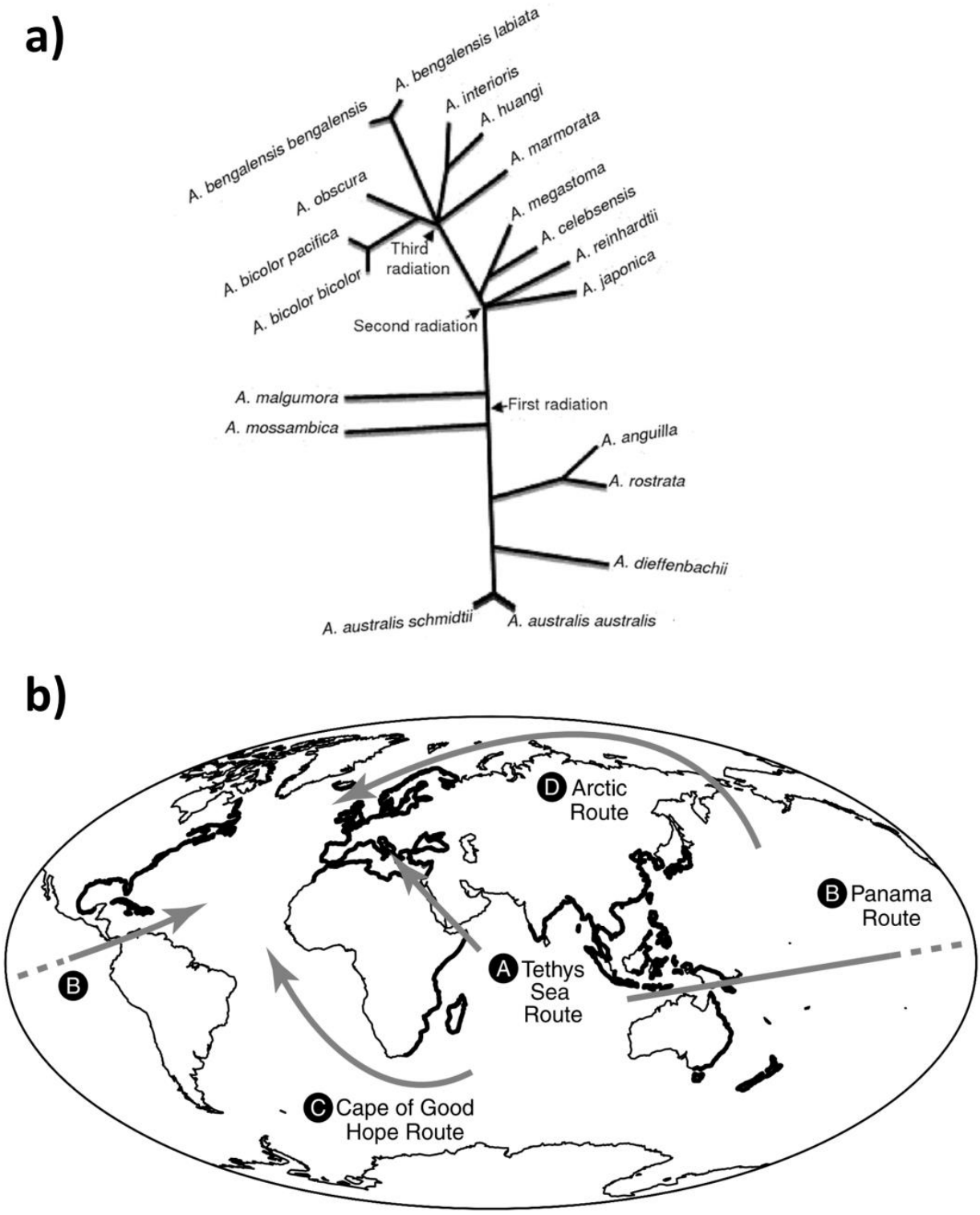

Figure 1 a) Phylogenetic tree of the genus *Anguilla* (figure from Tseng (2016)) and b) worldwide distribution (bold shore lines) and possible dispersal routes (A – D) of *Anguilla* ssp. after Minegishi *et al.* (2005)

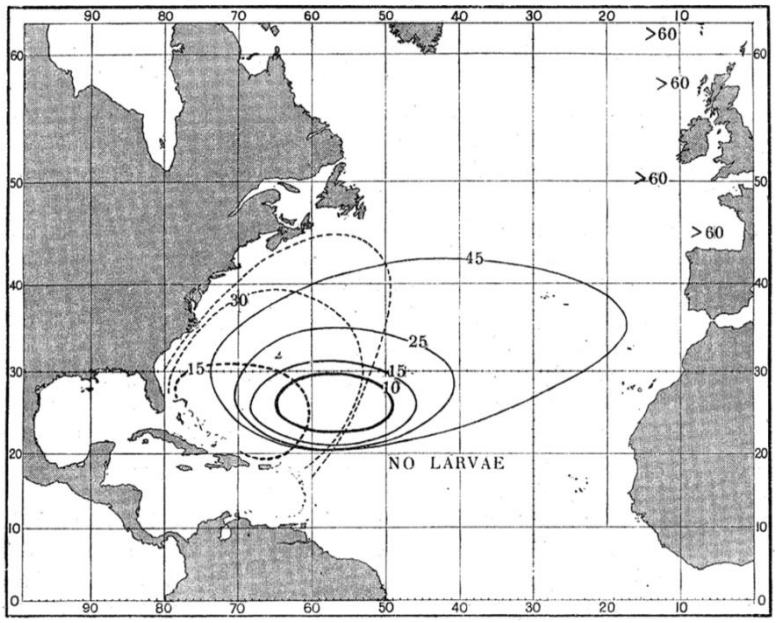

Figure 2 Larval distribution of *Anguilla anguilla* (solid lines) and *A. rostrata* (dashed lines) in the North Atlantic. Numbers indicate the observed maximum total length of larvae. Unchanged from Schmidt (1922).

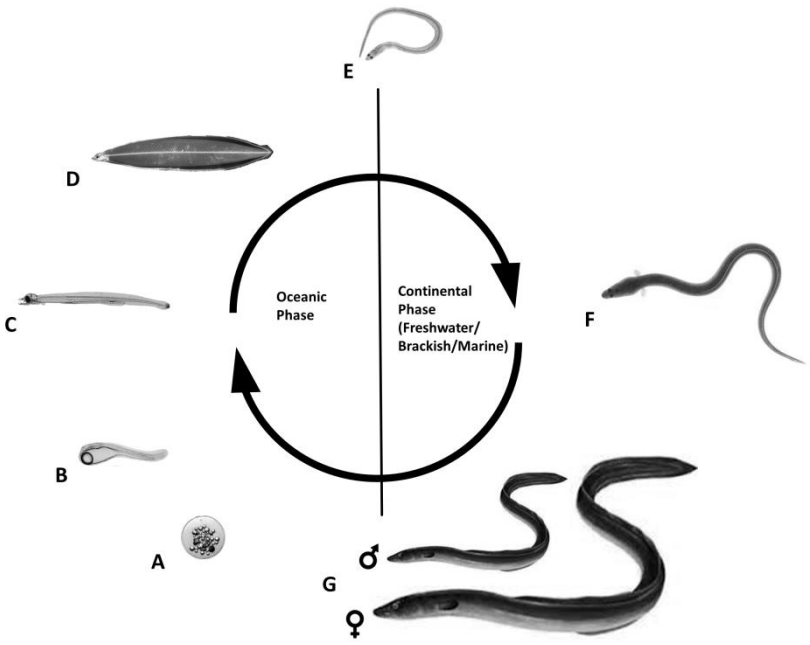

Figure 3 Facultative catadromous life cycle of the European eel. A: Eggs which were never observed in the wild. B: Earliest life stage that was found in the Sargasso Sea. C: Pre-leptocephalus stage. D: Leptocephalus stage. E: Metamorphosis to glass eel completed. F: Yellow eel phase. G: Silver eel phase whereby females are significantly longer at onset of silvering. Escaped silver eels do also belong to the oceanic phase during their trans-Atlantic migration. Modified after Dekker (2004). (A, B, and C from Sørensen *et al.* (2016)).

passively at least 5500 km as leptocephali larvae and unpigmented 'glass eels' before their continental life called 'yellow eel' phase begins (Tesch and Thorpe, 2003). The duration of the early life Atlantic crossing phase is not yet known and cannot be determined empirically e.g. by analysing otoliths due to vagueness of daily rings structure (Antunes and Tesch, 1997; McCleave, 2008; Fukuda *et al.*, 2009). It is assumed that the *A. anguilla* larvae migration takes between 7 months and >4 years (Bonhommeau *et al.*, 2010) but recent indirect approaches like ocean current dynamic simulations and particle flow models revealed a mean duration of 21 months at a mortality rate of approximately >99 % (Bonhommeau *et al.*, 2009).

After roughly 5 – 50 years as yellow eel (Dekker, 2000), a distinct sexual dimorphism becomes apparent when the skin colour changes from yellow to silver. This process called "silvering" starts at a total length of ca. 30 – 45 cm at first in male eels while females are significantly longer and older at the onset of the silvering process (Figure 3G; Tesch and Thorpe, 2003; Durif *et al.*, 2005; Durif *et al.*, 2009; van den Thillart *et al.*, 2009). Besides changes in skin colour from yellowish to silver, the eye diameter and pectoral fin length increases, gonads start to develop and the gastrointestinal tract regresses increasingly (Pankhurst and Sorensen, 1984; Durif *et al.*, 2005). Migrating individuals usually stop foraging and are able to maintain a swimming speed of approximately half a body length per second on their way towards their place of birth (van Ginneken *et al.*, 2005; Wysujack *et al.*, 2015; Righton *et al.*, 2016). A comprehensive satellite tagging study recently presented new insights into the spawning migration and gave evidence that migrants reveal a directed movement towards south west (Righton *et al.*, 2016).

Once eels have left the continental shelf, however, a proof for their directed migration towards their suspected spawning grounds south-west of the Bermuda islands is the offspring

of eel larvae smaller than 2 mm somewhere in the Sargasso Sea (Schmidt, 1922; Pacariz *et al.*, 2014; Westerberg *et al.*, 2017). Larvae surveys showed that spawning occurs most likely between December and May (Kuroki *et al.*, 2017; Westerberg *et al.*, 2017), however, to date no mature eel was ever caught or observed in the Sargasso Sea just as no eggs were ever found.

Exploitation of the stock

All life stages including glass, yellow, and silver eels are commercially exploited. Glass eel fisheries occur mainly at the Bay of Biscay, the North Sea, and the western Mediterranean from Portugal, Spain, France, Italy, and the United Kingdom. The International Council for the Exploration of the Sea (ICES) never reported landings in the official advice because a significant lack of completeness in catch declarations is considered (ICES, 2017a). As a consequence of incomplete data, ICES refrains from calculating total allowable catches and instead follows the guidelines of the precautionary approach that all anthropogenic impacts should be reduced to a minimum. Conflicts arise from strong economic interests and a high world market demand for eel products (Crook, 2010). The majority of yellow and silver eels are caught from both commercial and recreational fishermen near shore or in inland water bodies with eel traps, fyke, stow, and pound nets (Tesch and Thorpe, 2003). Although separate landings statistics do exist for yellow and silver eels, stages are most often determined only by skin colour which is questioned and considered unreliable (Pankhurst, 1982; Pankhurst and Lythgoe, 1982; Durif *et al.*, 2005). In particular, glass eel fisheries are highly profitable and have a long tradition in south Western Europe (Dekker, 2002). In 2015 and 2016 EU countries reported legal glass eel catches between 50 and 60 tonnes with a volatile market price between 300 and 400 € per kilogram depending on availability (ICES, 2016c). Approxi-

mately 40 % of the catch in Europe is used for human consumption while 60 % must be held back alive for recovery measures (European Council, 2007). The amount intended for consumption is not necessarily consumed as glass eel but often exported for the purposes of farming in commercial aquaculture facilities. ICES (2016c) compiled production data for European countries from different sources and estimated that from 2003 to 2015 between 4000 and 9000 tonnes of eels have been produced in eel farms annually.

Status of the stock

The recruitment of the European eel stock decreased massively in recent decades (Figure 4; ICES, 1999; Dekker, 2003, 2016; ICES, 2016c, 2017c). In 2003, scientists raised awareness of an obvious synchronic recruitment collapse of the American, Japanese, and European eel and called for instantaneous countermeasures (Québec Declaration of Concern, 2003). Thereupon, the European Union reacted in 2007 and adopted a regulation (EC No 1100/2007) which requests EU member states to establish eel management plans and provides a timeline for the implementation of recovery measures. Since 2008 the IUCN (International Union for Conservation of Nature and Natural Resources) listed the European eel on its Red List of Threatened Species™ as 'critically endangered' (Jacoby and Gollock, 2014). Moreover, to reduce the massive trade of living glass eels to Asia, the species became listed on Annex II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) making unauthorized trade illegal (https://cites.org/eng/app/appendices.php). Despite the aforementioned international regulations, eel products including live eels are traded illegally up until now due to the high market value and a continued high demand for eels in Asia in combination with an inefficient

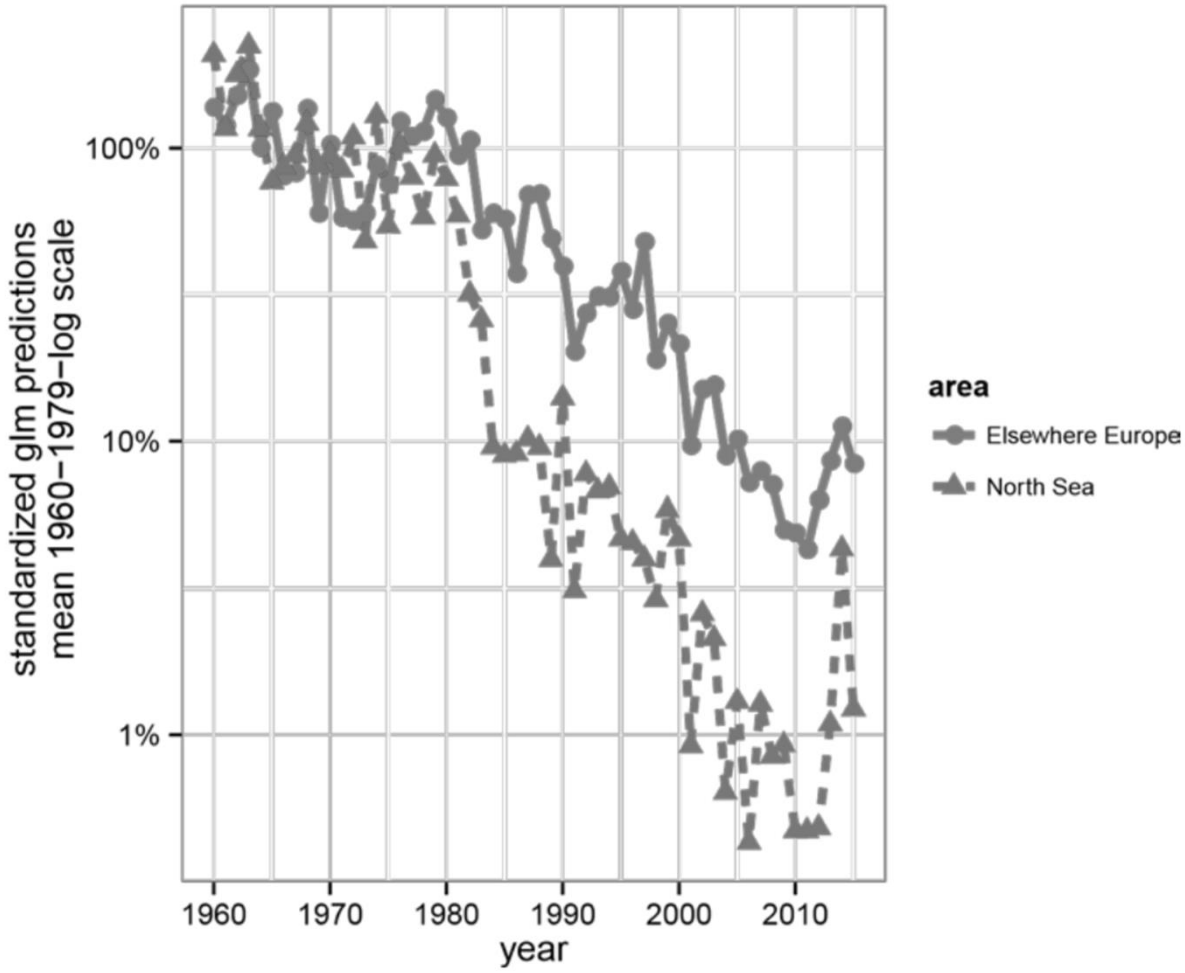

Figure 4 ICES WGEEL recruitment index for the North Sea (dashed line) and elsewhere in Europe (solid line) scaled for the 1960-1979 average. The figure was obtained from ICES WGEEL (2016b).

control system (Crook, 2010; Crook and Nakamura, 2013; Nijman, 2017). Nijman (2017) found evidence that the CITES ban appeared to have only little impact on northern African countries (Morocco, Algeria, Tunisia) live eel exports. By contrast, the annual export volume of refined products (chilled, smoked) increased significantly. Recent molecular evidence for massive live eel export to e.g. Japan was recently provided by Arai *et al.* (2017) who found that the Tone River system in Japan is indeed dominated by the non-native European eel.

Due to the complexity of the eels life cycle with extended periods of migration and others characterized by a pronounced site fidelity (Nzau Matondo *et al.*, 2017; Verhelst *et al.*, 2017) a variety of factors are impacting the stock at different life stages in a diverse manner.

There are natural continental factors like predation mortality due to birds (e.g. cormorant *Phalacrocorax carbo*), natural interspecific competition or parasites like *Anguillicoloides crassus* (Lammens *et al.*, 1985; Cowx, 2003; Kirk, 2003; Bevacqua *et al.*, 2011; Skov *et al.*, 2014). The latter leads to an irreversible cicatrisation of the swim bladder wall which loses its buoyancy function over time (Palstra *et al.*, 2007; Lefebvre *et al.*, 2011). Without a functioning swim bladder, eels have to invest more energy into buoyancy control, which in turn might have a negative impact on their energy budget during the migration to the Sargasso Sea and thus potentially damage reproduction success of the stock. The first record in 1982 of *A. crassus* in Europe (Paggi *et al.* (1982) as reviewed by Peters and Hartmann (1986)), coincides roughly with the beginning of recruitment collapse in the late 1970's. This apparent coherence suggests that *A. crassus* seriously impacts the spawning potential of migrating silver eels. In contrast, Kettle *et al.* (2008) found strong evidence that most recruitment time series across Europe have already been in decline before *A. crassus* have spread all over Europe, albeit an additive negative effect cannot be ruled out.

Anthropogenic non-natural continental factors namely hydropower plants and transverse structures in rivers, reclamation of wetlands and floodplain drainage seriously impaired the eel's migration ability accompanied with tremendous habitat loss across Europe (Feunteun, 2002; Marohn *et al.*, 2014). According to Airoldi and Beck (2007) European countries have lost approximately 50 to 80 % of their coastal wetlands and seagrass meadows since the 1960's which are expected to be important foraging areas and growth habitats for eels (van Liefferinge *et al.*, 2012). Furthermore, halogenated lipophilic persistent organic pollutants and dioxin-like compounds are man-made noxious substances which can have strong negative impacts on all type of animal organ systems (e.g. in fish; King-Heiden *et al.,* 2012). The European Water Framework Directive (WFD) has been adopted to protect European waters

as a non-commercial common resource (European Council, 2000), however, though pollution is decreasing steadily and compliance is high, legacies in the sediment are still the source of contamination in fish (Fliedner *et al.*, 2016). Particularly eels are vulnerable to lipophilic contaminants because of their high lipid content (Robinet and Feunteun, 2002). In addition, more recent studies found high concentrations of teratogenic substances in eels of all stages (e.g. Sühring *et al.*, 2014; Freese *et al.*, 2016). Moreover, evidence has been provided that dioxin-like toxins are maternally transferable suggesting that lipophilic pollution pressure might impact multiple generations of eels (Freese *et al.*, 2017).

Eel regulation and management approaches

The European Union (EU) adopted in 2007 a regulation which aims at the protection and the sustainable use of the European eel stock (EC No 1100/2007). As of this date, EU member states have been requested to establish eel management plans (EMP) in defined eel management units and to inform the European Commission regularly about recent advances in the form of implementation reports. Reference has to be made to the 40 % escapement target which refers to the estimated amount of eels that would have escaped without any anthropogenic mortality.

The achievement of this goal necessitates the reduction of the anthropogenic pressure on the stock and the regulation proposes a number of management options including fishing restrictions such as the introduction of a minimum landing size (MLS) for eels or the further increase of MLS, removal of migration obstacles, combating predators, and stocking measures. Also the temporary switch-off of hydropower plants and so-called “catch and carry” programmes are intended to reduce anthropogenic mortality which results in a higher number of successfully escaping migrants. The latter approach can be described as provi-

sional and temporary solution whenever a permanent removal of migration obstacles is not possible.

The most important and wide spread management measure is stocking of juvenile eels into waters with current low natural recruitment (ICES, 2016e). Especially inland water bodies with numerous migration obstructing features like weirs or dams suffer from a small number of ascending juveniles and an eel population with corresponding escapement can only be maintained with extensive human assistance (Brämick *et al.*, 2016).

Stock enhancement measures

Stocking of fish has been a common measure to enhance fish stocks across all continents (Cowx, 1999; Pearsons and Hopley, 1999) and can be defined as "multiple releases of fish to a stock chronically suffering from poor recruitment with the aim of increasing both fishery recruits and the spawning biomass" (Støttrup and Sparrevohn, 2007).

The practice of eel stocking has a long tradition beginning in the first half of the 19th century in France (Dekker and Beaulaton, 2016b). Glass eels have been caught and transported short distances over land to ponds or lakes in order to harvest the high valuable fish with low catch effort. As the transportation infrastructure in Europe developed in terms of both distance coverage and travel time, eel stocking became possible all over the continent.

Today, the supply of glass eels for stocking purposes is only limited in terms of availability in the European winter and spring. But since shipping of glass eels in cooled transport boxes by air is possible nowadays, recruits can readily be distributed to any recipient water without substantial losses during transport (Kirkegaard, 2010). Another often applied strategy is to raise glass eels to larger elvers prior to stocking (e.g. Angelidis *et al.*, 2012). The pre-grown

farmed eels can be loaded on fish transportation vehicles and shipped over long distances by road without persistent impairments (e.g. Boerrigter *et al.*, 2015).

Pearson and Hopely (1999) defined five tasks in order to plan stocking measures in an environmentally compatible manner: (i) determine non-target taxa and define an acceptable impact level, (ii) determine spatiotemporal overlaps of target and non-target taxa (e.g. inter specific competition), (iii) determine strong ecological interactions, (iv) determine ecological risks, and (v) determine the level of uncertainty. The case of the European eel, however, needs special attention particularly because the status of the stock remains critical and the artificial reproduction is not yet feasible, thus stocked recruits have to be caught in the wild. Walker *et al.* (2009), therefore, developed a guideline for good practice in eel stocking measures. Among others, they highlighted that stocked eels might follow life-history patterns as recently found in American eels (Stacey *et al.*, 2015). In addition, Pavey *et al.* (2015) found evidence that the habitat preference of eels is genetically determined. Furthermore, also the spread of diseases (e.g. viruses and parasites), skewed sex ratios, and a general reduced fitness particularly of eels from aquaculture facilities are pronounced risks that might negate the objective of a stocking programme (Krueger and Oliveira, 1999; Haenen *et al.*, 2012).

Aim of this thesis

By 2017, ten years of Europe-wide eel management passed by, however, the European eel stock remains in a perilous state. Given that eel management plans most often rely on stocking measures to compensate for poor recruitment, there is an imbalance between effort and benefit. Moreover, a general concern about the contribution of stocked recruits has arisen

which necessitates a more holistic approach to evaluate a potential net benefit in eel stocking measures. The entire supply chain of eel stocking material should certainly be monitored in order to enable also deductions about the consequences of the eel removal in the donor habitat until the onset of the spawning migration of stocked individuals in the recipient water. However, the illegal export of glass eels results in tremendous losses to an unknown proportion which interferes with a reliable assessment of catch related mortalities both caused by fishing gear or indirectly by density dependent effects. The present study, therefore, deliberately leaves aside glass eel catch associated factors and is thus focused mainly on the post-stocking phase. In this regard, the ICES recommends chemical marking of stocked recruits prior to stocking to enable conclusions about the further development of stocked recruits in terms of growth, survival, condition, and finally their contribution to silver eel biomass. Eels become stocked in tonnes, however, there is considerable lack of efficient mass-marking techniques to handle these immense quantities.

Within this framework, the objectives of this study were (i) to provide novel application-oriented mass-marking protocols for glass and farmed eels to allow the persistent chemical marking of stocking material in large quantities without substantial losses as basis for large scale monitoring programmes. Moreover, (ii) the chemical marking of particularly farmed recruits has been hypothesised to be crucial since farming related stress rings on otoliths were assumed to interfere with ageing and growth estimations. The present study approached to quantify this error in blind readings and to demonstrate potential effects on local stock assessments (i.e. biomass calculations). (iii) The farmed cohorts in 2015 and 2016 have subsequently been found to be infected with the *anguillid herpesvirus 1*. A follow-up study was conducted in 2016 with regard to the virus prevalence six years after first stocking event in 2010, the impact of the swim bladder nematode on diseased eels, and virus load of

eels of different sizes. Finally, (iv) glass and farmed eels have been released simultaneously in a brackish fjord in a purchase cost equivalent proportion to derive comparative conclusions in terms of growth, mortality, and benefit-cost ratio which overall might be helpful to increase the outcome of eel management plans.

Chapter I Mass-marking of glass eels

Chemical marking of European glass eels with alizarin red S and in combination with strontium - *in situ* evaluation of short-term salinity effects on survival, and efficient mass-marking

This chapter is the unedited version of a manuscript that has been published in the Journal of Fish Biology (92(1), 203–213. doi:10.1111/jfb.13508)

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Abstract

This study presents a new chemical double marking technique for European glass eels *Anguilla anguilla* by combing alizarin red S (ARS) and strontium chloride hexahydrate (Sr). Marked eels (double marked with ARS and Sr but also single marked with ARS) were exposed *in situ* to brackish water (15 g L^{-1} artificial sea salt) for 14 days and did not exhibit increased mortalities compared to unmarked eels. Indeed, no mortality occurred in a marked group during the experiments. Moreover, an efficient mass-marking approach with low handling effort for both techniques single ARS and double ARS-Sr is described and was proven to be practicable for large scale stocking programmes.

Introduction

The recruitment of the European eel *Anguilla anguilla* (Linnaeus 1758) has decreased massively since the late 1970's (Dekker, 2016; ICES, 2016d). In 2007, the European Union adopted the so-called 'eel regulation' (EC, 2007) which requests all member states to establish eel management plans (EMP) and to take countermeasures that encompass the recovery of the stock but also the sustainable use. A wide-spread measure implemented in many EMP's across Europe is the relocation of natural recruits referred to as stocking (e.g. ICES, 2016e). Because artificial reproduction is still not feasible, stocking is entirely reliant on wild glass eel catches. Between 2010 and 2015 approximately 122 million glass eels were stocked in the EU (ICES, 2016b), but it is still unknown whether this provides a net benefit defined as "a higher silver eel escapement biomass than would have occurred if the glass eel seed had not been removed from its natural (donor) habitat in the first place" (ICES, 2016e).

ICES (2011a, 2016e), therefore, recommended the chemical marking of stocked recruits to allow future estimation of their contribution to recruitment. Alizarin red S (ARS), strontium chloride hexahydrate (Sr), and barium chloride dihydrate (BC) are considered as ideal markers, but ICES advised against the antibiotic oxytetracycline (OTC). However, BC, in the required concentration, is more poisonous than ARS or Sr (e.g. Wickström and Sjöberg, 2014) and might therefore also be unfavourable. This leaves ARS and Sr, but detection of the latter is laborious as well as technically demanding and time consuming (Wickström and Sjöberg, 2014). In contrast, ARS marks can be detected by standard fluorescence microscopy. A combination of ARS and Sr (ARS-Sr) provides a unique chemical marker which can be applied with two consecutive treatments. On the contrary, the use of the same compound multiple times necessitates a sufficient time interval and food intake to ensure that there is sufficient otolith growth to produce discernible marks (Holmgren, 1996; Caudron and Champigneulle,

2009; Wilson *et al.*, 2009). Only those recaptured eels with ARS marks would have to be checked for Sr marks which would reduce lab time substantially. As a management option, when two stocking cohorts are produced sequentially, they could be marked using alternative combinations (e.g. ARS, ARS-Sr) of rather low-risk compounds. This approach would improve cohort assignment and thus ageing related growth estimations.

The choice of the recipient habitat is also crucial for the obtainment of potential spawners. In brackish water bodies, eels display higher growth rates, a better overall condition, and are less loaded with parasites and dioxin-like PCBs compared to eels in freshwater (Daverat and Tomas, 2006; Melià *et al.*, 2006; Jakob *et al.*, 2009b; Simon *et al.*, 2013b; Freese *et al.*, 2016). Thus, brackish recipient waters with current low natural recruitment might be more promising for stocking measures (ICES, 2009b). It is known that, particularly, glass eels can tolerate rapid transfers from fresh to saline water (Crean *et al.*, 2005). However, this has never been evaluated for chemical marked glass eels, whereby the osmotic shock in combination with marking induces stress might lead to increased mortalities.

The aims of this study were (i) to determine if it is possible to achieve an immediate double mark on otoliths by using ARS and Sr, and to evaluate *in situ* the short-term effects of salinity on survival and (ii) to present an efficient mass-marking technique for ARS and ARS-Sr that reduces handling effort and thus associated stress.

Materials and Methods

This *in situ* experiment was approved by the Office of Food Inspection and Veterinary Affairs of Hamburg (Ref. No 92/14).

Salinity effects on survival of marked eels

For the *in situ* laboratory evaluation a total of 240 glass eels *Anguilla anguilla* originating from the River L´Adour estuary were used with a mean total length (L_T) of 6.41 cm ± 0.15 (standard deviation) and mean body mass (BM) of 0.26 ± 0.07 g. Eels arrived in cooled transport boxes and were slowly adapted to the temperature in the resting tank (200 L; 18 °C; 12 h day/12 h night) where eels recovered from transport stress for two days without food. The single marking (ARS, n = 120) and double marking (ARS-Sr, n = 120) experiments were conducted consecutively. Before each trial 50 % of the eels (n = 60 per trial) were randomly separated and either single marked with ARS (CAS-No. [130-22-3]; 3 h, 0.15 g L^{-1}, 20 °C according to (Simon and Dörner, 2005) or double marked beginning with ARS (according to Simon and Dörner, 2005) and afterwards with Sr (CAS-No. [10025-70-4]; 24 h, 1 g L^{-1}, 19 °C according to Wickström and Sjöberg, 2014) in aerated 20 L tanks. Unmarked eels served as the control group (n = 60 per trial) and stayed in the resting tank during the marking procedures. For the actual experiment, the eels were kept in six aerated 72 L observation tanks (length 800 mm, width 300 mm, height 300 mm) equipped with external biofilters. A two cm layer of sand and perforated bricks were provided as shelter. Three tanks were filled with 48 L freshwater and three tanks contained water (also 48 L) to which 15 g L^{-1} artificial sea salt (RedSea®) was added. Immediately after the marking process, all eels were placed into the corresponding tank without further adaptation. Fish density was 20 eels per tank comprising of ten marked and ten unmarked eels. Groups were separated by a net. Light regime was set at 12 h day and 12 h night. Temperature, pH, and oxygen concentration was measured daily (Hanna HI 9828 multi parameter probe) and nutrient concentrations (nitrite and nitrate) were checked twice weekly with quick tests (JBL™). Eels were fed *ad libitum* with frozen *Cyclops* spp. and dead individuals were removed, if necessary, on a daily basis.

Mass-marking approach

For the mass-marking procedure a heightened rectangular tank (length 300 x width 145 cm), which could be aerated, was used. A constant flow of freshwater was possible but was turned off. At the bottom of the tank a plugged outlet was installed to make the tank drainable over portable knotless net cages on legs. This construction allowed the careful and quick separation of the glass eels from the marking solution. A fine metal lattice covered the spillway to ensure that the glass eels stayed inside the tank. Three hours before the estimated time of arrival of the glass eels, the tank was filled with 1.5 m³ of freshwater (possible maximum of 3.9 m³) and 225 g of pre-dissolved ARS was added (equals 0.15 g L^{-1}). A second tank which was identical to the marking tank was prepared with 3.9 m³ of freshwater and the constant flow was adjusted.

60 kg of glass eels (L_T = 6.72 ± 0.34 cm; BM = 0.24 ± 0.05 g) originating from England were purchased from a commercial glass eel supplier (Aalversandstelle, Deutscher Fischereiverband, Halstenbek). The glass eels arrived in cooled transport boxes (one kg per box), were weighed, and placed in the marking solution without any prior feeding. The stocking density was 40 kg m^{-3} (corresponding 13.8 kg m^{-2}). Temperature, pH, and oxygen concentration was measured half hourly. At the end of the marking procedure (after 3 hours) the marking solution was drained over the net cages as described above, the mortality rate was determined, and the eels were placed in freshwater for a resting phase of 12 h. The next day, the eels were placed in the transport boxes (one kg per box) with approximately 50 to 100 mL of water and transported to the recipient water. Sixty eels were held back and fed *ad libitum* with frozen copepods (*Cyclops* ssp.) for 14 days in one observation tank (see *in situ* experiments) in a 19 °C air-conditioned room to verify the marking success.

The double marking approach (ARS-Sr) was conducted using the same procedure as the single ARS marking procedure. The same tanks were used and the stocking density was identical. Three hours before eels arrived (60 kg; L_T = 6.71 ± 0.33 cm; BM = 0.24 ± 0.08 g), one tank was filled with 1.5 m³ of freshwater and 1500 g of pre-dissolved Sr (equals 1 g L^{-1}) was added. The water parameters were measured hourly for 24 h. Then, the eels were separated from the first marking solution (see above) and were placed immediately in the prepared ARS marking bath (1.5 m³; see above). The Sr solution was collected and disposed of by a disposal company. Sixty eels were held back for 14 days in one observation tank (see above) to monitor marking success.

Mark detection

After 14 days the glass eels were anesthetized with MS-222 (0.012 % aqueous solution) and sacrificed with an overdose of MS-222 (0.1 % aqueous solution) before freezing (-20 °C). L_T and BM were measured and the sagittal otoliths were removed and stored dry in 2 ml plastic tubes. One otolith per fish was embedded in thermoplastic Crystalbond 509 (Buehler®) on glass slides, ground with abrasives (grade P1200, Buehler®) to the primordium (Figure 5a), polished with alumina-powder (0.3 µm Micropolish II, Buehler®) and tap water on a synthetic cloth (MicroCloth, Buehler®) and cleaned with deionized water on another synthetic cloth. All prepared otoliths were checked for fluorescent marks using a light microscope (Leica DM 2500) equipped with a UV lamp (CoolLED *p*E-300-W) and UV filters for specific wavelengths (530-580 nm). The control-group fish did not show any mark (Figure 5b), but an ARS mark with a maximum emission wavelength at 580 nm appears as yellow glowing

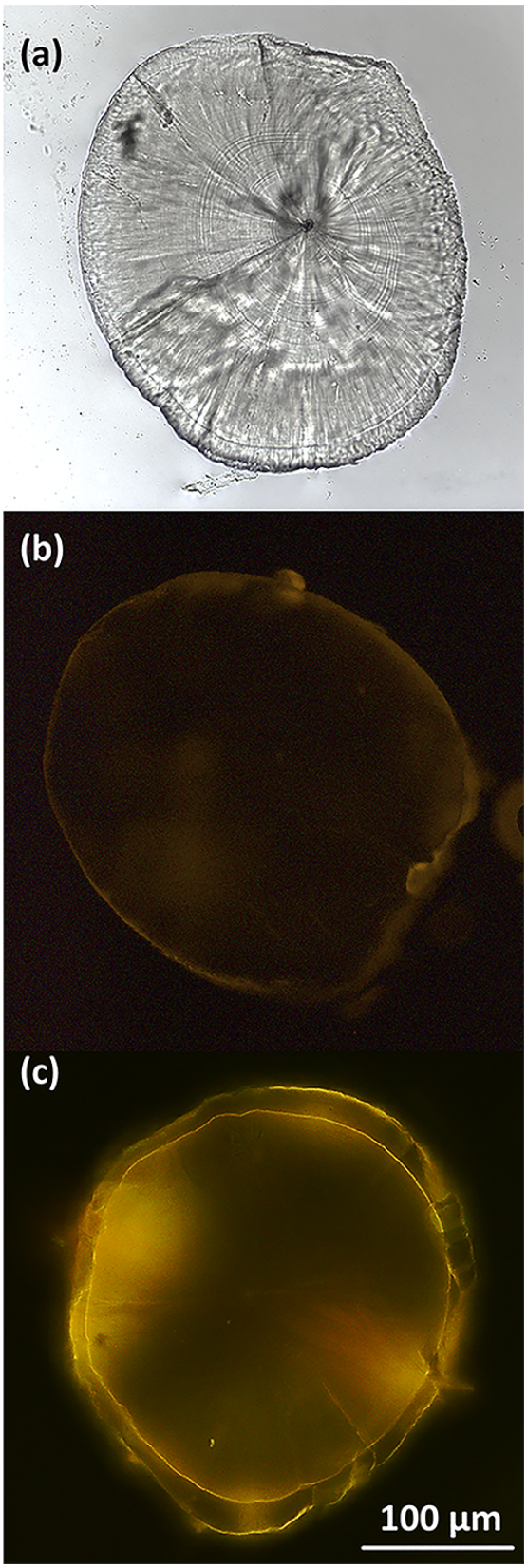

Figure 5 Thin section preparations of glass eel otoliths that were removed 14 days after the marking process (a) under bright field conditions of a fluorescent microscope and under dark field conditions with filtered light (530-580 nm) in (b) without an ARS ring (control group) and (c) where the ARS ring appears as yellow glowing band close to the edge where distinct refraction artefacts can be present. The scale bar is suitable for all photographs.

band in marked fish (Figure 5c). The double marked otoliths were first prepared as described and checked for an ARS mark. Secondly, the same preparations were carbon coated and Sr marks were detected using an electron-microscope (Leo 1525 Field Emission Scanning Electron Microscope) via X-ray fluorescence microanalysis detector (Ametek OCTANE PLUS) on a transect running from the core to the edge (Figure 6).

Statistical analysis

The software R (R Core Team, 2017) was used to statistically compare water temperature, oxygen concentrations and pH between replicate tanks and between freshwater and brackish water treatments . Results were described as significant if the calculated probability of error was below 5 per cent ($P < 0.05$). Because data distribution was always non-normal and homoscedasticity not satisfied, the non-parametric Kruskal-Wallis test (KWT for > 2 groups) and Wilcoxon test (WT for n = 2 groups) was used for all statistical tests.

Results

Salinity effects on survival

There was no marking induced mortality, neither during the single ARS nor the double ARS-Sr marking procedure. In general, as soon as the eels were placed in the observations tanks, the marked as well as unmarked eels immediately searched for shelter in the bricks and sand layer. Food was always provided from the beginning but it took five days until all eels started feeding regardless of marking or water treatment. Within all tanks for the ARS and ARS-Sr trial nitrite levels remained stable, close to 0 mg L^{-1} and nitrate accumulated over time but did not exceed a concentration of 1 mg L^{-1}.

During the single ARS marking trial neither water temperatures (KWT, H = 11.53, d.f. = 2, $P > 0.05$) nor oxygen concentrations (KWT, H = 5.69, d.f. = 2, $P > 0.05$), nor the pH values (KWT, H = 0.01, d.f. = 2, $P > 0.05$) differed significantly between freshwater replicate tanks. Thus mean values (± standard error) were presented (c.f. Table 1). The same was true of the brackish water replicates (temperature: KWT, H = 12.37, d.f. = 2, $P > 0.05$; oxygen: KWT, H = 3.25, d.f. = 2, $P > 0.05$; pH: KWT, H = 0.54, d.f. = 2, $P > 0.05$). Between water treatments, temperatures (WT, w = 895, $P > 0.05$) and oxygen concentrations (WT, w = 829.5, $P > 0.05$) did not differ significantly, but the pH value of the brackish water was significantly higher compared to the freshwater (WT, w = 1461, $P < 0.05$). L_T of the glass eels was not significantly different between tank compartments (KWT, H = 12.12, d.f. 11, $P > 0.05$). No mortality occurred during the 14 days of observation (Table 1).

During the double marking ARS-Sr experiment the parameter pattern was the same as for the ARS experiment. Neither the freshwater parameters (temperature: KWT, H = 14.19, d.f. = 2, $P > 0.05$; oxygen: KWT, H = 8.25, d.f. = 2, $P > 0.05$; pH: KWT, H = 0.15, d.f. = 2, $P > 0.05$) nor the brackish water parameters (temperature: KWT, H = 10.62, d.f. = 2, $P > 0.05$; oxygen: KWT, H = 11.21, d.f. = 2, $P > 0.05$; pH: KWT, H = 1.03, d.f. = 2, $P > 0.05$) differed significantly between corresponding replicates. Temperatures (WT, w = 741.5, $P > 0.05$) and oxygen concentrations (WT, w = 741.5, $P > 0.05$) did not differ significantly between salinity treatments, but the pH value of the brackish water was significantly higher compared to the freshwater due to bicarbonate in the salt (WT, w = 1648.5, $P < 0.05$). L_T of the glass eels did not differ significantly between tank compartments (KWT, H = 5.44, d.f. 11, $P > 0.05$). One dead individual was observed in a freshwater control group after 10 days of observation (Table 1).

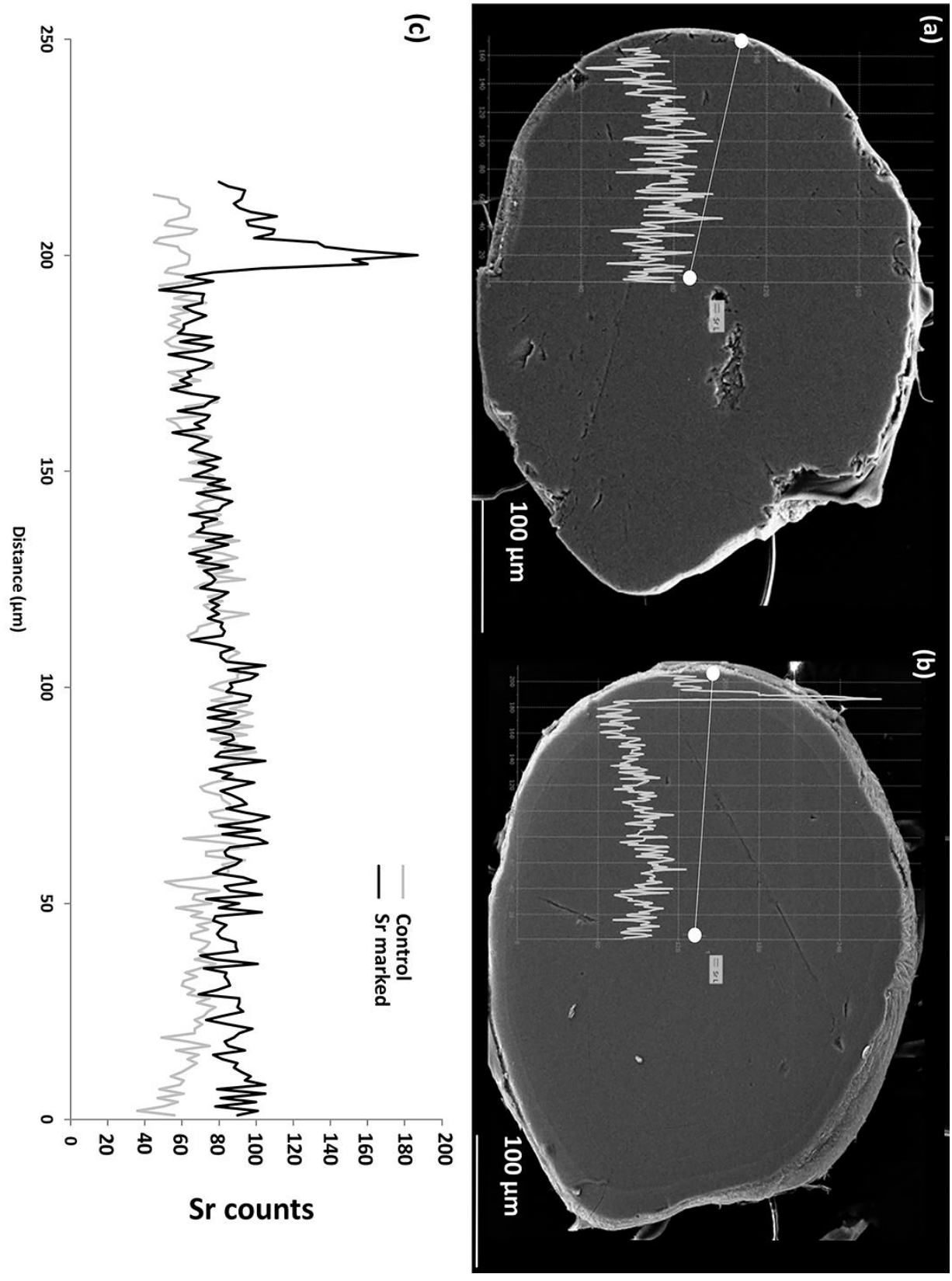

Figure 6 Carbon coated thin section preparations of glass eel otoliths under an electron microscope (a and b). Strontium profiles (grey line) of an unmarked eel (a) and a SR marked eel (b), shown on different scales. White dots indicate start (in the centre) and endpoints of X-ray fluorescence microanalysis transect. SR-peak appeared close to the outermost edge of the otolith (b). (c) SR profiles of an SR marked eel (black line) and an unmarked eel (grey line), shown on the same scale.

Table 1 The i*n situ* evaluation of short-term salinity effects on survival of single (ARS) and double (ARS-SR) marked glass eels during the 14 days of observation.

	Single				Double			
	Freshwater		Brackish water		Freshwater		Brackish water	
	Marked	Control	Marked	Control	Marked	Control	Marked	Control
N	30	30	30	30	30	30	30	30
Mortality (N)*	0	0	0	0	0	1	0	0
Temperature (mean ± S.E.;°C)	18.7 ± 2.8		18.6 ± 4.8		18.6 ± 2.8		18.5 ± 2.0	
Oxygen (mean ± S.E.; mg L^{-1})	9.5 ± 1.4		9.4 ± 2.4		9.4 ± 1.4		9.3 ± 1.0	
pH (mean ± S.E.)	7.6 ± 1.1		7.9 ± 1.2		7.4 ± 1.1		7.7 ± 0.8	

Single, marked with alizarin red S (ARS); Double, marked with alizarin red S and strontium (ARS-SR); N, number; * total number of dead glass eels at the end of the observation time (14 days)

Mass-marking approach

During the single ARS marking procedure the temperature and pH remained stable and sufficient aeration was maintained (Table 2). The eels appeared agile and no obvious signs of health impairment were observed. At the end of the procedure a mortality of 0.56 % was determined. The marked eels were placed in the freshwater tank to enable them to recover from marking stress. Mortality was determined again when the eels were placed into the transport boxes the next day but no further increase was observed. Sixty randomly selected eels were held back for 14 days. No mortality occurred during this phase and all eels showed a distinct ARS mark on the otoliths.

Table 2 Water parameters, mortality, and marking success of the single ARS and double ARS-SR mass-marking approaches.

	Single	Double	
	ARS	SR	ARS
Date	13 May 2016	02 June 2015	03 June 2015
Temperature mean ± S.E.; °C)	11.7 ± 2.1	13.9 ± 5.7	14.3 ± 3.8
Oxygen (mean ± S.E.; mg L^{-1})	6.5 ± 2.7	5.7 ± 2.3	5.4 ± 1.4
pH (mean ± S.E.)	7.6 ± 2.4	7.7 ± 3.2	7.9 ± 2.1
Mortality (%)	0.56	0.58	
Marking success (% (N))	100 (60)	100 (60)*	

Single, single marked with alizarin red S (ARS); Double, marked with ARS and strontium (ARS-SR); S.E. standard error; *All 60 otoliths were clearly double marked

The ARS-Sr double mass-marking procedure started with the Sr bath at a conductivity of 1140 µS cm^{-1}. The relevant water parameters for oxygen concentration and pH were stable for Sr and ARS bathes within an optimal range (Table 2). After 24 h of Sr and an additional 3 h of ARS bath the mortality was determined at 0.58 %. After 12 h the mortality was determined again but no further increase was observed. Sixty glass eels were held back for evaluation of the marking success which were all clearly double marked (Table 2; c.f. Figure 6).

Discussion

Artificial breeding of European eels is not yet feasible. It is therefore widely accepted that stocked eels should be marked or tagged in some way to allow the efficiency of stocking programmes to be evaluated. The choice of marker must be in accordance with the defined objective of a study (Nielsen, 1988). In particular if life-time traceability is the intended result

and there are large quantities of fishes to be marked, only chemical mass-marking of bony structure seems feasible and will result in a permanent mark.

In this study, a new successful marking technique is presented by combining ARS and Sr, two already established marking compounds (e.g. Simon and Dörner, 2005; Wickström and Sjöberg, 2014; Caraguel *et al.*, 2015). Previous reports of combined markers for eels used chemical markers (like alizarin complexone, Sr, and BC) with passive integrated transponder (PIT) tags (Holmgren, 1996; Wickström and Sjöberg, 2014). However, PIT tags are unsuitable for mass-marking of small glass eels. The other option is to use the same compounds multiple times to get the desired number of marks on the otoliths (Holmgren, 1996; Iglesias and Rodríguez-Ojea, 1997; Caudron and Champigneulle, 2009; Wilson *et al.*, 2009; Wickström and Sjöberg, 2014). A particular disadvantage of this method is, however, that an adequate time interval between markings is necessary in order to allow otolith growth via food intake. In this regard, the initial feeding of glass eels is a critical phase and can be associated with high mortalities (Heinsbroek, 1989; Kirkegaard, 2010; Angelidis *et al.*, 2012; Hirt-Chabbert *et al.*, 2012). In addition it can take up to two weeks until all eels start feeding (Egginton and Johnston, 1984). Moreover, eels and fish in general should be fastening for 24 to 168 h before handling to reduce their metabolism and them less vulnerable (Ashley, 2007; EFSA, 2008; Boerrigter *et al.*, 2015) which might lead to food deprivation induced stress (Midwood *et al.*, 2016). The double marking ARS-Sr method presented in this study, in contrast, provides a unique new chemical marking technique that can be achieved in two consecutive applications without the need for a time interval between marks and the associated risks.

Strontium can only be detected electron-microscopically via X-ray fluoresce or by laser ablation which is both laborious and expensive (e.g. Wickström and Sjöberg, 2014). Assuming that sampling of chemically mass-marked individuals is random, sample size is very limited if

only Sr was used. ARS in comparison can be detected by standard fluorescence microscopy. Since otolith preparation for ARS and Sr detection is almost identical in the first steps, a large sample could be checked first for ARS marks and only such marked otoliths would necessarily be scanned for an additional strontium mark. This leads to a substantial reduction of lab time, and thus improvements of the monitoring in terms of both time and cost efficiency.

The *in situ* evaluation of short-term salinity effects for ARS and ARS-Sr marked glass eels revealed that increased mortality would not be expected if marked eels are stocked in brackish near-shore waters (c.f. Table II). Simon and Dörner (2005) previously found that ARS had no effect on the mortality of glass eels compared to unmarked eels within the first three weeks under freshwater conditions. This was later successfully validated for a longer period of 192 days (Simon *et al.*, 2009). Josset *et al.* (2016) observed ARS mass-marked and unmarked glass eels *in situ* over 15 days in freshwater tanks and found also no differences. It was also previously shown that unmarked glass eels can tolerate rapid transfers between different salinities (e.g. during stocking activities) without increased mortalities (observed for 21 days; Crean *et al.*, 2005). The present study provides the very first evidence that the marking procedures for both ARS and ARS-Sr do not negatively affect glass eels that were stocked in brackish water bodies at least on a short-term. This is particularly relevant as the eel is facultative catadromous (Tsukamoto *et al.*, 1998) and recruits arrive at the coast before they enter riverine systems. Hence, stocking of coastal habitats with current low recruitment is closest to natural conditions and, additionally, eels are not hindered to migrate into inland waters.

The chemicals for the *in situ* experiment and the mass-marking approach were applied in a reverse order. This amendment of the mass-marking protocol became necessary for a practical-logistical reason associated with the Sr solution disposal. Despite this inconsistency, the

impact of the order on dye assimilation, survival or effectiveness of otolith labelling was considered to be negligible. Evidence may come from the low mortality and no obvious differences in the distinctness of the ARS as well as the Sr marks.

The evaluation of life-time durability of ARS and Sr marks would be desirable but difficult given the European eel's multi-decadal life-span (e.g. Tesch and Thorpe, 2003). For Sr, longevity could be derived from otolith microchemistry studies that investigate life history traits assuming that incorporated elements will not vanish over time (Tzeng *et al.*, 1997; Marohn *et al.*, 2013). In contrast, fluorochromes like ARS or OTC might lose their fluorescent nature. In this regard, Verreault *et al.* (2010) found American silver eels *Anguilla rostrata* (Lesueur 1817) with L_T between 57.0 and 66.8 cm originating from a stocking programme in the St. Lawrence estuary that displayed OTC marks. It might be concluded that fluorochromes are life-time detectable but further evidence, especially, for ARS is needed.

It is known that the handling of glass eels influences survival after release (Josset *et al.*, 2016). Within the context of monitoring programmes, the marking process might be particularly critical and stress should be reduced to a minimum. If the number of fish increases (e.g. in large scale stocking programmes) the facility where the marking procedure takes place should be suitable for that purpose. Caraguel *et al.* (2015) described an approach of marking a mass of 90 kg glass eels with ARS in 26 oxygenated 70 L tanks. This has the advantage of spreading the risk in case of an accident. On the other hand, handling effort may be increased although neither handling procedure nor acute marking induced mortality was described or reported. The acute mortality presented in this study at 0.56 and 0.58 % for ARS and ARS-Sr, respectively, was not substantially higher than the normal transport mortality of up to 0.5 % (Kirkegaard, 2010). Caraguel *et al.* (2015) as well as Josset *et al.* (2016) evaluated the post-stocking mortality at the stocking site using fish held in net enclosures for the first

15 days and using fish recaptured by electrofishing over seven months after release. A similar experimental set up could be used in the future to evaluate potential long-term sublethal effects of the mass-marking procedure presented in this study.

In summary, the present study describes a new chemical marking technique for glass eels (ARS-Sr), provides evidence for a negligible salinity effect on survival of marked eels in brackish water, and describes a gentle and low handling effort mass-marking procedure for both single ARS and double ARS-Sr techniques. Details for the practicable implementation are provided and prospective study designs are discussed. The ARS-Sr marking method could help to evaluate the efficiency of stocking programmes which could lead to significant progress towards the recovery of the European eel stock.

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Chapter II Mass-marking of farmed eels

Mass-marking of farmed European eels (*Anguilla anguilla* (Linnaeus, 1758)) with alizarin red S

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Summary

The Working Group on Eel of the International Council for the Exploration of the Sea (ICES) regularly reports that a significant amount of stocked eels in Europe was pre-grown in aquaculture farms prior to stocking – so called 'farmed eels'. The ICES advices chemical marking of stocked recruits to ensure their traceability throughout all life stages. To date, however, there was a lack of knowledge concerning the most suitable chemical substance and its application on farmed eels. The aim of this study was to fill this gap by presenting successful attempts of marking those eels with alizarin red S (ARS). An ARS concentration of 150 mg L^{-1} buffered with 150 mg L^{-1} Tris(hydroxymethyl)methylamine applied as an immersion bath over 9 h was sufficient to mark a total of 3572 kg of farmed eels (6.5 – 8.0 g mean body weight). The marking success was 100 % on otoliths and highest stocking density of up to

67.1 kg m^{-3} (corresponding 54.0 kg m^{-2}) turned out to have no effect on mortality which was consistently below 1 %.

Introduction

The recruitment of the European eel stock (*Anguilla anguilla* (L.)) decreased in recent decades to historical low levels (e.g. Dekker, 2016; Dekker and Beaulaton, 2016a; ICES, 2016c). In 2007, the European Union adopted a regulation called 'Establishing measures for the recovery of the stock of European eel' (European Council, 2007) which requests its member states to establish eel management plans (EMP) to ensure the sustainable use and recovery of the stock. Many EMPs have implemented stocking measures to enhance the size of the stock which are, however, entirely reliant on wild caught glass eels (total length (TL) 5.4-9.2 cm; Dekker *et al.*, 1998) or farmed young yellow eels (TL ca. 15-20 cm, fattened from glass eels). Thus stocking of the critically endangered European eel (Jacoby and Gollock, 2014) is the reallocation of natural recruits in order to create a "net benefit" defined as "a higher silver eel escapement biomass than would have occurred if the glass eel seed had not been removed from its natural (donor) habitat in the first place" (ICES, 2016e). Because of the complex life cycle of the European eel and the wide range of habitats it can inhabit (Tesch and Thorpe, 2003) it will be difficult to estimate the effect of stocking measures on the spawning stock biomass (ICES, 2016e). An essential prerequisite is to mark all eels that were redistributed for conservation purposes to be able to distinguish between natural and stocked recruits after re-capture. Therefore, comprehensive chemical marking of stocked recruits is advised to ensure traceability through all life stages (ICES, 2016b, 2016c, 2016e). If looking at the total numbers – from 2000 to 2015 between approximately 7 and 51 million

eels (glass and young yellow eels) were annually stocked in the EU (ICES, 2016b) – only chemical mass-marking is feasible to handle these large quantities. However, chemical marking techniques are almost exclusively available for glass eels or very small elvers (Alcobendas *et al.*, 1991; Simon and Dörner, 2005; Wickström and Sjöberg, 2014; Caraguel *et al.*, 2015), although since 2010 between approximately 18 and 85 % of all stocked eels were reported as "young yellow" (ICES, 2016b).

The aim of this study was to present the results of successful attempts of mass-marking farmed young yellow eels with alizarin red S (ARS) to make this technique available for scientists and eel stock managers across Europe.

Materials and Methods

For this study a total of 3572 kg of farmed eels were purchased from a commercial eel trade company between 2008 and 2016 (Aalversandstelle, Halstenbek, Germany). From 2008 to 2015, 50 % deionized water was used for the preparation of the marking solution to reduce the conductivity. The chemical compounds were added 4 to 12 h before eels arrived to ensure that ARS and buffer (Tris(hydroxymethyl)methylamine) were dissolved properly (Table 3). ARS concentration was constantly at 150 mg L^{-1} but buffer concentration was increased from 140 mg L^{-1} in 2008 to 150 mg L^{-1} from 2011 onwards. At arrival ten times 100 eels were randomly taken out of the transport tanks and weighed to get the mean individual body weight (6.5 – 8.0 g) and to check their health status. 500 of these eels served as unmarked control group during the marking process and were held in a 250 L tank with constant water flow. The other eels were placed at once into the staining bath tanks without adaptation (2008 – 2015). But in 2016, dilution with demineralized water was not possible

Table 3 Date, experimental set up and used eels for the attempts between 2008 and 2016 to mark farmed eels with 150 mg ARS L^{-1}

Date	Duration (h)	Total eel weight (kg)	Stocking density (kg m^{-3})	Stocking density (kg m^{-2})	BW (g)	V (m^3)	TRIS (mg L^{-1})	Tank
07.05.2008	12	200	20.0	3.5	8.0	10.0	140	1 x A
24.05.2011	12	600	20.0	10.6	7.0	30.0	150	4 x B
27.05.2014	9	700	23.3	12.4	7.1	30.0	150	4 x B
22.09.2015	9	1132	37.7	20.0	6.5	30.0	150	4 x B
20.07.2016	9	940	67.1	54.0	6.6	14.0	150	4 x C

ARS, alizarin red S; TRIS, buffer (Tris(hydroxymethyl)methylamine); BW, body weight; V, summed volume of all tanks; Tank A, length 590 cm x width 590 cm, filled with 10.0 m^3 freshwater diluted with 50 % demineralized water, oxygenated (technical O_2); Tank B, diameter 300 cm, each filled with 7.5 m^3 freshwater diluted with 50 % demineralized water, oxygenated (technical O_2); Tank C, length 145 cm x width 300 cm, each filled with 3.5 m^3 freshwater, aerated

and eels were placed into marking tanks (still filled only with freshwater) first. After 10 h of recovery from transport stress, pre-dissolved ARS and buffer was added subsequently. During the marking process water parameters (pH, oxygen concentration, temperature) were measured hourly and conductivity initially using the multi parameter probes Hach HQ40 d (between 2008 – 2014) or Hanna HI 9829 (2015 and 2016). Stocking density was increased stepwise from 20.0 kg m^{-3} (corresponding 3.5 kg m^{-2}) at the fist marking attempt in 2008 up to 67.1 kg m^{-3} (corresponding 54.0 kg m^{-2}) in 2016. The tanks were oxygenated (2008 – 2015) or aerated (2016). The duration of the staining bath was 12 h in 2008 and 2011 but then reduced to 9 h from 2014 onwards. At the end of the marking process the marking solution was slowly replaced with freshwater (2008 – 2015). Or the solution was drained over a net to separate the eels which were placed in a prepared freshwater tank immediately after-

wards (2016) and the morality was determined. Not less than 10 h after the marking process the marked eels were transported to the recipient water. For evaluation of the marking success a random selected subsample out of all used tanks (up to 50 individuals) was hold back and fed *ad libitum* with chironomidae larvae from 2 to 11 months. The eels then were sacrificed with an overdose of MS-222 (0.1 % buffered with 0.2 $NaHCO_3$). For mark detection the sagitta otoliths were used. They were removed by longitudinal dissection of the head, cleaned and stored dry in 2 mL tubes. One otolith per eel was cut on a transversal plane in two pieces and embedded with the cut surface down in thermoplastic wax (Crystalbond® Buehler) on a glass slide. Both pieces were ground to the primordium as described by Simon *et al.* (2013). These thin section preparations were checked for ARS marks using a light microscope (Leica DM 2500) equipped with a light source (CoolLED *p*E-300-W) and a light filter for wavelengths between 530 and 580 nm. Using this set up, an ARS mark appears as yellowish glowing band. For the assessment of the mark quality, the marked preparations were categorized in distinctive, faint, and absent (c.f. Figure 7).

A marking success of 100 % and a mortality rate below 1 % were defined as target criteria.

Results

All of the five attempts to mark young yellow eels with ARS led to a marking success of 100 % on otoliths and mortalities of less than 1 % between 2008 and 2016 (Table 4). No health impairment was ever observed during the marking processes. 150 mg ARS L^{-1} buffered with 150 mg TRIS L^{-1} was sufficient to mark eels up to 8.0 g body weight adequately. The pH value kept stable between 7.6 ± 0.2 and 7.9 ± 0.1 within all attempts. Oxygenation of the staining

Table 4 Date, mean water parameters ± SD, observed mortality, marking success, and mark quality for the attempts between 2008 and 2016 to mark farmed eels with ARS

Date	pH	O_2 (mg L^{-1})	Temperature (°C)	Conductivity (µS cm^{-1})	Mortality (%)	Marking success (% (N))	Mark quality (% (N))†	
							Distinctive	Faint
07.05.2008	7.7 ± 0.2	8.6 ± 1.2	17.1 ± 1.9	321	< 1.0	100 (43)	95 (41)	5 (2)
24.05.2011	7.9 ± 0.1	13.8 ± 4.5	16.1 ± 0.8	260	< 0.1	100 (20)	85 (17)	15 (3)
27.05.2014	7.6 ± 0.2	10.0 ± 1.5	16.6 ± 0.2	249	< 0.1	100 (10)	100 (10)	0 (0)
22.09.2015	7.8 ± 0.4	9.7 ± 2.4	13.8 ± 0.2	274	< 1.0	100 (50)	90 (45)	10 (5)
20.07.2016	7.6 ± 0.1	5.9 ± 1.4	16.6 ± 0.3	397	< 0.01	100 (50)	86 (43)	14 (7)

SD, standard deviation; N, number; ARS, alizarin red S; † see also Figure 7

solution (2008 – 2015) provided oxygen concentrations between 8.6 ± 1.2 and 13.8 ± 4.5 mg L^{-1} but also aeration turned out to be adequate for a sufficient oxygen supply in the marking solution (5.9 ± 1.4 mg L^{-1} in 2016). It was possible to mark up to 1132 kg of farmed eels simultaneously (2015) and also a stocking density of up to 67.1 kg m^{-3} (corresponding 54 kg m^{-2}) did not lead to elevated mortalities (consistent < 1.0 %; cf. Table 3 Table 4). Omitted dilution with demineralized water in 2016 and the subsequently added pre-dissolved chemicals did not impair the marking success (100 %) or eel's health (mortality < 0.01 %). No mortality was ever observed in the unmarked control group during the marking procedure.

Immediately after the marking process, no fluorescent mark was visible on the prepared otoliths (Figure 7a). An on-growing phase of two months after the marking process was found to be sufficient for clear identification of the ARS mark on the otolith preparations. The mark was distinctive on 85 – 100 % of the all prepared otoliths across all treatments (Table 4; c.f. Figure 7 b and c).

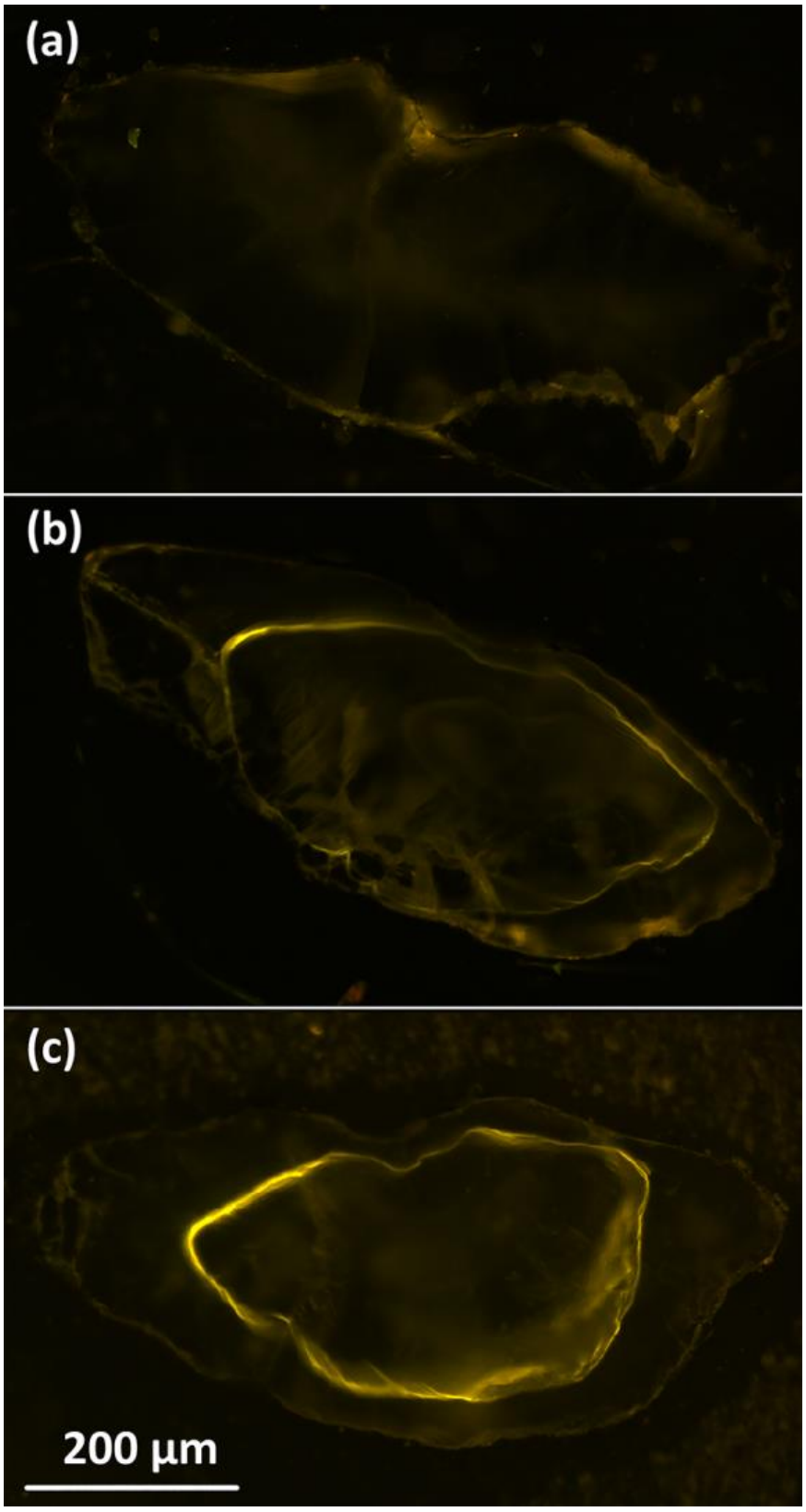

Figure 7 Photographs of thin section preparations of otoliths from ARS marked eels (a) immediately after the marking process without a detectable fluorescent mark. (b) With an on-growing phase of eleven months showing a faint ARS ring and (c) after eleven months with a distinctive mark. The scale bar is suitable for all photographs.

Discussion

The presented mass-marking technique revealed highest possible marking success at acceptable low mortality levels over several years and attempts, thus the mass-marking feasibility was proven. The vast majority of the observed mortality could be attributable to handling or transport since no mortality occurred in the unmarked control groups and dead individuals showed commonly external damages. A clear distinction, however, between the mortality caused by transport, marking stress or other reasons was not possible because the transport related mortality was not separately determined. But the use of different marking tanks at various stocking densities (Table 3) and the consistent low proportion of dead individuals (Table 4) provides evidence that the presented method is rather harmless to eels. Especially the approach in 2016 of adding all chemicals pre-dissolved after a post-transport resting phase of 10 h turned out to be the most convenient procedure regarding both animal welfare and handling effort.

Lievremont *et al.* (1982) found pH values between 4 and 8 to be ideal for the binding of ARS and calcium. When ARS was added, the pH value often dropped from ca. 8 to < 7. Though these conditions were still suitable for ARS and calcium binding but were considered as too stress full for the eels to be marked. TRIS buffer (150 mg L^{-1}) was added that kept pH value stable between 7 and 8, thus served as stress reducing compound. From 2008 to 2015, the freshwater (spring water) was diluted with 50 % demineralized water to reduce probability of ARS binding by dissolved Ca^{2+} ions. Because bivalent calcium ions can form an ARS-Ca complex by chelation process (Virtanen and Isotupa, 1980; Lievremont *et al.*, 1982), less ARS would be available for the intended incorporation into the skeletal parts of *A. anguilla*. Dilution was not possible in 2016 but no effect on marking success (100 %) or mortality (0.1 %) was observed, which leads to the conclusion that conductivity might be neglected to a cer-

tain level. However, Marohn *et al.* (2011) attempted to mark farmed eels with ARS under marine conditions (salinity = 38) over 23 h but no fluorescent mark was detectable. Thus, a very high conductivity or salinity is indeed negatively impacting the marking success.

The aeration of the marking solution in 2016 was a further improvement of the marking technique because oxygenation may lead to gill damages during the marking process (Brauner *et al.*, 2000). Even at the highest stocking density of 67.1 kg m^{-3} the oxygen concentration kept stable only by aeration and no health impairment was observable suggesting that even higher densities might be possible.

The marking success control group had to be held back for at least two months to ensure sufficient otolith growth. Without growth increment, the ARS mark was located at the outermost edge of the otolith and could easily be mistaken for a refraction artefact, and vice versa (c.f. Figure 7a). Low mark quality seemed to be associated more with the quality of the thin section preparations than with the duration of the immersion bath. No specific pattern between different treatments was observed (Table 4). The control group was kept for a maximum of eleven months which was sufficient for the assessment for the marking success and short-term retention rate but not for its durability. The ARS mark might vanish over time but the longevity must be a subject of a long-term monitoring programme as eels can become 10 years and older (e.g. Tesch and Thorpe, 2003). In this regard, Verreault *et al.* (2010) presented oxytetracycline marked American silver eels originating from a stocking programme in the St. Lawrence estuary. It could be concluded that some fluorochromes can maintain its fluorescent nature in otoliths over several years until eels start their spawning migration. This is a key issue for spawning stock assessments in combination with estimations about the contribution of stocked eels to silver eel escapement biomass and future recruitment. In this context, the evaluation of potential differences between marked and unmarked individuals

in terms of growth and survival could be evaluated under laboratory conditions as presented by Simon *et al.* (2009) for glass eels.

The present study enables comprehensive evaluations of stocking measures with farmed eels within the framework of EMP's across Europe which might lead to substantial improvements and thus to a recovery of the stock.

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Chapter III Biased age-based stock assessment

Age-based stock assessment of the European eel (*Anguilla anguilla*) is heavily biased by stocking of unmarked farmed eels

This chapter has been submitted to the Journal Fisheries Research and is currently under review

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Abstract

Stocking of farmed eels is a commonly used management measure across Europe, and partly Asia, to refill local stocks that chronically suffer from poor recruitment. During the farming process, increased growth and stress related annulus-like rings are being formed, which have been hypothesised to bias ageing and growth estimations. Alizarin red S (ARS) marked European eels (*Anguilla anguilla*) from eel farms were used to demonstrate that these stress rings cannot be distinguished from potential true annuli. Two readers overestimated the age on average by approximately 2 and up to 7 years in blind readings. In addition, a significant positive correlation between the estimated age and the number of counted stress rings was

observed. The individual number of rings inside the ARS mark (i.e. prior to stocking) was used to correct the estimated age which led to an increase in the calculated growth rate between 18 ± 16 % and 108 ± 48 %. Furthermore, an age-based cohort model indicated that the stocking-related ageing error strongly affects total biomass estimates with potential effects on silver eel escapement, depending on the proportion of stocked recruits. Chemical marking of all farmed recruits in the future is proposed to enable statistically necessary individual age corrections.

Introduction

Ageing is a key tool in fisheries science and fish stock management. For this purpose, calcified structures from fish are used to gather information reaching from a single individual up to the population level (Campana and Thorrold, 2001). Frequently used structures are otoliths and analyses most often refer to age estimation but can also be used for investigations on otolith chemistry, and microstructure analyses (Begg *et al.*, 2005; Campana, 2005; Sandin *et al.*, 2005).

In particular, the age structure of a fish population and associated cohort analyses are crucial for stock assessments, total allowable catch estimations and questions regarding general population dynamics (Hilborn and Walters, 1992; McAllister and Ianelli, 1997; Berkeley *et al.*, 2004). In this regard, the accuracy of the age estimation is of major importance and careful validation is essential (Beamish and McFarlane, 1983). Because most population parameters such as (fishing and natural) mortality and spawning stock biomass estimates are age-based, ageing errors have a strong influence on forecast models, thus affecting fisheries management (Bradford, 1991; Richards *et al.*, 1992; Reeves, 2003; Bertignac and Pontual, 2007).

Management of the semelparous European eel *Anguilla anguilla* (Linnaeus 1758) is complicated because there is only one single panmictic stock dispersed across European and North African continental waters (Froese and Pauly, 2017). Due to the large distribution area and the complex catadromous life cycle, there is a significant lack of data which is why the International Council for the Exploration of the Sea (ICES) is still unable to conduct a reliable stock assessment. As a result, no TAC was ever published for this stock (ICES, 2017a). Because of the severe recruitment decline since the 1970's (e.g. ICES, 2016a) and the great uncertainties in the data, ICES adopted the precautionary approach following the guideline to keep "all anthropogenic impacts as close to zero as possible"(ICES, 2017a).

With the objective to create a comprehensive data resource for a reliable stock assessment, the European Commission requested the EU member states to include the European eel into the large scale Data Collection Framework (DCF; EU COM, 2009) which by then had its focus mostly on marine, commonly exploited fish stocks. ICES was requested to advise on the data collection and, amongst others, highlighted length distributions, age profiles, growth rates, and the sex ratio (ICES, 2012). More recently, ICES (2017d) reviewed data requirements for a reliable stock assessment and age was identified as key parameter especially for the estimation of both, natural and anthropogenic mortality. The fundamental difficulties in age estimation of European eels, however, are the pronounced phenotypic plasticity and their high longevity of up to more than 15 years (e.g. Marohn *et al.*, 2013; Simon, 2015). In older eels the probability of stress related rings increases (e.g. Berg, 1985), and more importantly, the annuli formation pattern differs remarkably between habitats due to its dependency on environmental factors such as temperature, salinity or food availability (e.g. Campana and Thorrold, 2001). This would imply an almost impracticable need for habitat dependent age

validation (mark and recapture) in *Anguilla anguilla* as well as consideration of possible habitat shifting (e.g. Marohn *et al.*, 2013).

Because ageing techniques for the European eel differ remarkably between countries, ICES made efforts to standardize the ageing technique and recommended the "crack and burn" protocol. In addition, ICES provided advice for the interpretation of the winter ring structures (annuli) (ICES, 2009c, 2011b). Stocking measures with farmed eels – an important management option in many eel management plans across Europe and partly Asia (e.g. Shiraishi and Crook, 2015; ICES, 2016c; Kaifu *et al.*, 2018) – is a potential source of biased age

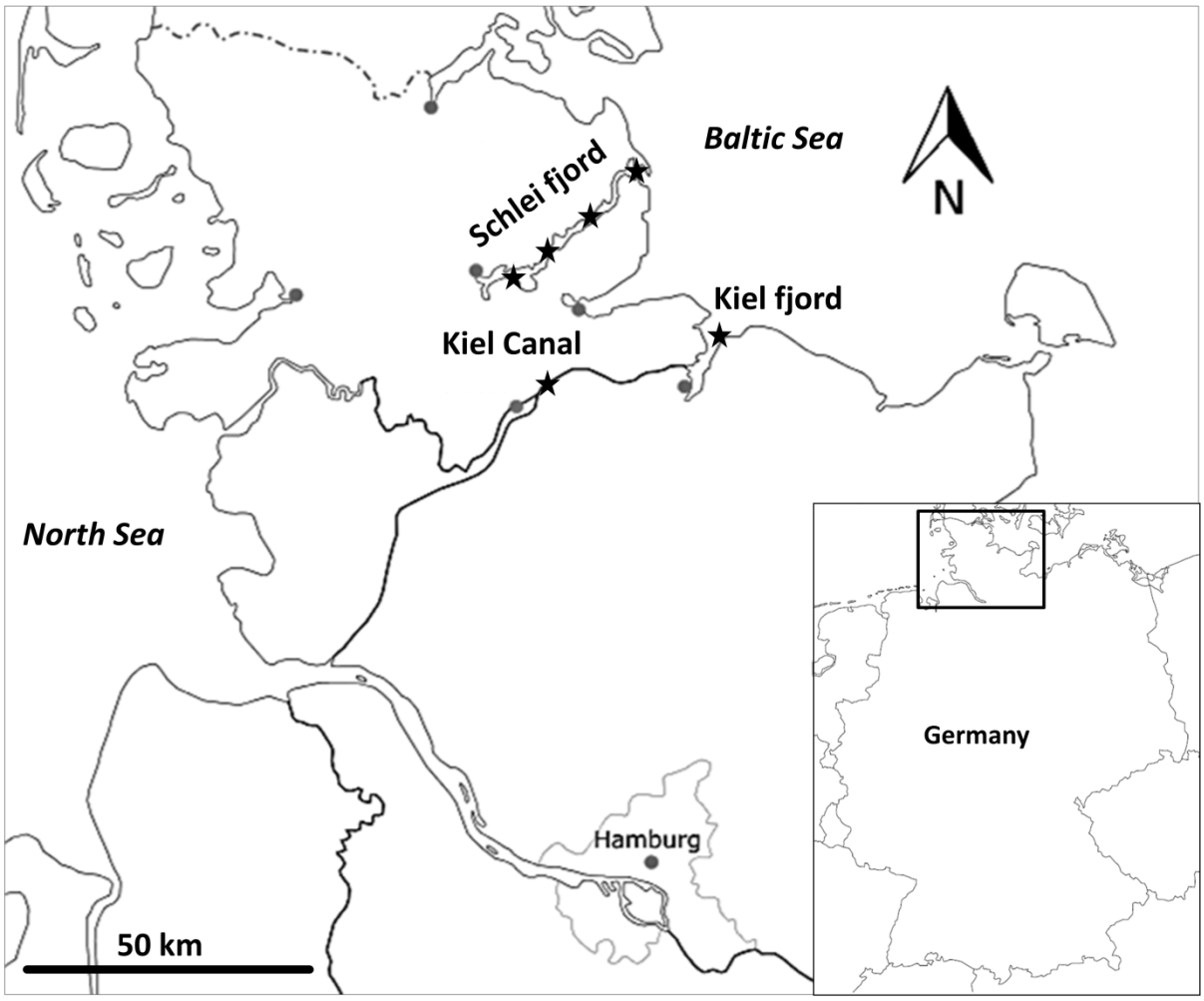

Figure 8 Overview map of the sampling locations (stars) at the western German Baltic Sea coast and the Kiel Canal.

readings in *A. anguilla* and probably *A. japonica* because the stress-related formation of wintering-like zones on otoliths was hypothesised to interfere with ageing and subsequent growth estimations (Simon *et al.*, 2017). Since 2010 up to 51 million eels have been stocked in European waters on an annual basis of which up to 85 % were pre-grown in aquaculture facilities prior to stocking (ICES, 2016a). During the farming process, eels are subject to stress caused by multiple size-grading's (Knights, 1987; Kamstra, 1993) and additionally can be – deliberately or accidentally – infected with the *eel herpesvirus* (Kullmann *et al.*, 2017a) which may also lead to stress related ring structures on otoliths (Oliveira, 1996). In addition, the transport, marking, and stocking process itself causes stress (Boerrigter *et al.*, 2015; Josset *et al.*, 2016) which overall may result in systematic misidentification of the zero band (ICES, 2009c) leading to an overestimation of age in farmed individuals (Simon *et al.*, 2017).

The aim of this study was to test whether knowledge of the individual recruitment history (IRH) has significant impact on age estimation with associated effects on growth and population estimates in the European eel. To quantify the possible error caused by false discrimination of stress rings from true annuli, we analysed blind readings from otoliths of recaptured alizarin red S (ARS) marked recruits previously reared in aquaculture facilities. Furthermore, we exemplified the effects of this error on population estimates in a simple age-based cohort model.

Material and Methods

Study sites and stocking measures

The used eels were obtained from three different brackish water bodies in northern Germany (Figure 8), the Kiel Canal (KC), the Schlei fjord and the German Baltic coast. The KC connects the North and Baltic Sea via the River Elbe (53.887664°N, 9.136134°E) and the city of

Kiel (54.366047°N, 10.150314°E). It is 98 km long, 11m deep on average, and characterized by a salinity gradient decreasing from ca. 10 (east) to 3 (west). The Schlei fjord (54.595976°N, 9.852501°E) covers an area of 5400 hectare. It is ca. 2 – 3m deep on average and shows a salinity gradient decreasing from 19 (east) down to ca. 4 (west). Dam building measures narrowed its opening down to approximately 100 m but the fjord is still connected with the Baltic Sea which itself shows a salinity range of roughly 10 to 20 and a mean depth of 15 – 20 m at the Kiel fjord.

From 2006, approximately 12 t of farmed eels with mean body mass between 6.5 and 8.4 g have been stocked in the KC. From 2009 on-wards 45.5 % of farmed eels have been marked with ARS prior to stocking (Kullmann *et al.*, 2017b). Taken together the Schlei fjord as well as the western German Baltic Sea coast has been the recipient water of 3 t of ARS marked farmed eels between 2015 and 2016.

ARS mark identification, ageing, and growth estimations

For this study a total of 100 eels from the Schlei fjord, the Kiel-Canal, and Kiel fjord were used (Table 5). Total length (TL in mm) and weight (BW in g) was measured. Otoliths were removed by longitudinal dissection of the head and stored dry in plastic tubes. For each individual, one otolith was cut on a transversal plane and embedded in thermoplastic wax (Crystalbond 509, Buehler®) on a glass slide. The cut surface was investigated for the ARS mark without grinding using a light microscope (Leica DM 2500) equipped with a UV lamp (CoolLED *p*E-300-W) and UV filters for specific wavelengths (530-580 nm). If an ARS mark was visible, the longest diameter was digitally measured (Figure 9 1a - 3a), using the pro-

gramme “Leica Application Suit X”. For the purpose of ageing, the otolith piece with the sized ARS mark was extracted out of the wax and prepared according to the “crack & burn” protocol (ICES, 2009).

Blind reading trial

A photograph was taken at each stage of the double prepared otolith piece to standardize the reading procedure between readers. Each otolith was independently read by two experienced eel otolith readers familiar with “crack & burn otoliths” from European eels. First, the readers had to decide on the basis of the photographs whether they do allow the age estimation of the individual and, secondly, to identify and clearly mark all annuli (using the software ‘ImageJ’ (https://imagej.net) or ‘Adobe Photoshop’ (https://adobe.com)). The readers were deliberately not informed about the ARS marks but had access to accompanying information concerning the individual sampling location and the specific sampling date. This approach enabled the subsequent discrimination between potential annuli outside the ARS ring and definite stress rings inside the fluorescent mark (Figure 9 1b to 3b). Hereafter, the terms “estimated age” for the total individual number of counted rings and “corrected age” (i.e. minus the individual number of counted rings inside the ARS mark) were used. Estimates of age were considered an “agreement between readers” if the number of individual rings counted was identical, independent of the individually marked rings.

Estimation of growth parameters

For further analysis, otoliths were not included if both readers did not agree on the readability of the photograph. The growth rate was estimated as TL at catch minus TL at stocking

Table 5 Numbers (n), total length (TL), body weight (BW) and sampling periods of recaptured farmed eel with an ARS mark from the different sampling locations.

Location	n	TL ± SD (mm)	TL range (mm)	BW ± SD (g)	BW range (g)	Sampling periods
Kiel fjord	15	298 ± 55	219 – 349	44 ± 23	16 – 66	30/06/2016 – 08/10/2016
						21/06/2017 – 22/06/2017
Kiel Canal	45	511 ± 42	450 – 610	220 ± 88	124 - 397	26/04/2017 – 14/08/2017
Schlei fjord	40	325 ± 57	256 – 435	59 ± 29	23 – 131	18/06/2016 – 31/08/2016
						18/05/2017 – 19/08/2017
Total	100	409 ± 106	219 – 610	131 ± 95	16 – 397	18/06/2016 – 08/10/2016
						26/04/2017 – 19/08/2017

N, number; TL, total length; BW, body weight

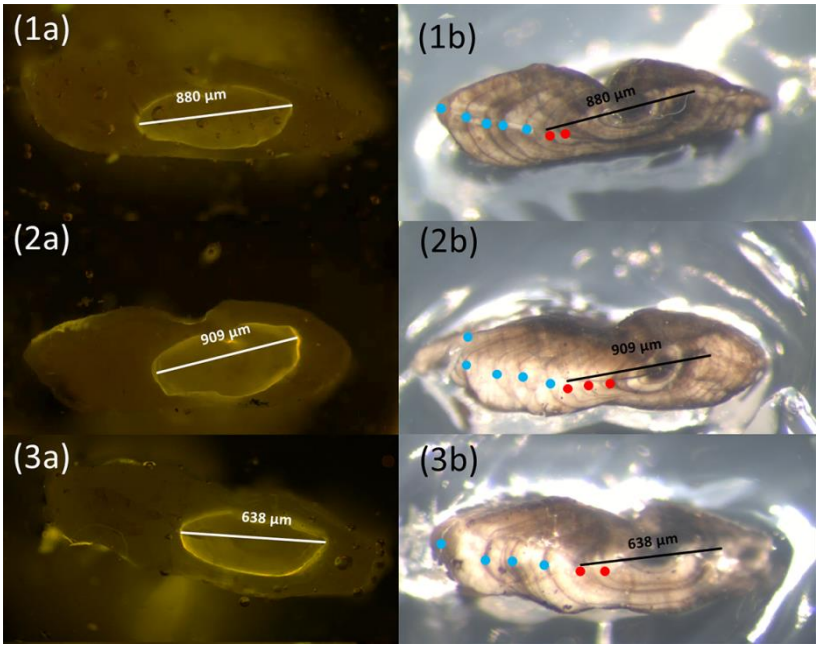

Figure 9 Eel otoliths prepared for the ARS mark identification under fluorescent light (1a – 3a) and the same one burned for the purpose of age estimation (1b – 3 b). Red dots indicate what could be mistaken as annuli but are actually inside the mark ring shown by the black bar. Blue dots show the position of what was considered as potential true annulus outside the mark ring. All eels were caught in April 2017 thus the outer edge was counted as annulus (ICES, 2009).

(mean of 171 mm across all years) divided by the mean estimated and corrected age, respectively. The growth rate for BW was estimated accordingly with a mean BW at stocking of 6.3 g across all years. The individual estimated ages were therefore averaged between readers, assigned to the respective lower age classes (Table 6) and then corrected by averaged individual number of rings inside the ARS mark (c.f. Figure 10).

Additionally, length (L_∞) and weight (W_∞) growth was calculated with the estimated and corrected age via iteration according to the von Bertalanffy growth function (VBGF) as reviewed in Beverton and Holt (1957) and Ricker (1975):

$$L_t = L_\infty\left[1 - e^{-k(t-t_0)}\right]$$

and

$$W_t = W_\infty\left[1 - e^{-k(t-t_0)}\right]^3$$

Where L_t and W_t are the calculated asymptotic maximum TL and BW at age t (n annuli), k is the growth coefficient and t_0 is the abscissa intercept. Because all investigated eels are known to be stocked pre-grown recruits the ordinate intercept was fixed at TL_0 equals 171 mm and W_0 equals 6.3 g for length and weight (see above), respectively. The individual ages (estimated and corrected) were averaged between readers and used as input data for the estimation of VBGF parameters.

Statistics and Modelling

For statistical analysis, the program R was used (R Core Team 2017). The significance level for all tests was $\alpha = 0.05$. For comparison of the estimated and corrected age between readers, the Wilcoxon test was used to test for significant differences from paired differences of 0 (Campana *et al.*, 1995). To calculate the effect of the IRH on estimates of the stock biomass, we followed the approach from Pohlmann *et al.* (2016), using the corrected and un-

corrected growth function. Briefly, the procedure is based on the virtual population analysis (VPA) by Gulland (1965), where the number of individuals in age-group n+1 is defined by the number of individuals in age-group n and the associated mortality rates. Natural mortality per age-group was calculated separately for either growth function as described by Bevaqua *et al.* (2011), for an intermediate stock density and a hypothetic annual mean temperature of 10°C. The biomass per age-group was subsequently calculated via the respective VBGF and the length-weight relationship as $W = 0.003*TL^{3.040}$ (n = 100 eels; c.f. Table 5). For the sake of simplicity, we assumed that all individuals are recruited in age group 0 ($n = 1x10^6$) and cormorant predation, as well as anthropogenic mortalities, was 0. Since the growth function is based on specimen with a maximum corrected age of 9 years, number of recruits and biomass per age group was calculated up to this age. Accordingly, it was not possible to calculate meaningful estimates of escapement and we therefore did not account for emigration, though eels of higher age groups were well within a size range where silvering can occur (silvering may first occur in female eels at length > 450mm though the majority of silvering occurs at length > 600mm; e.g. Oeberst and Fladung, 2012). Nonetheless, the calculated changes in biomass will ultimately translate to escapement and are therefore considered the best approximation based on the available dataset.

Results

Blind reading trial

The readers considered 88 and 66 % of the otolith photographs (n = 100) as reliable for age estimation, respectively, and agreed on the readability of 64 % (n = 64). Estimated ages ranged between 1 and 14 annuli (Table 6). Readers agreed on the age in 45.3 % of the analysed otoliths with an overall mean deviation of 1.1 ± 1.4 annuli. The uncorrected readings

from both readers differed significantly (Wilcoxon test, W = 0, $P < 0.001$). Between zero and seven annuli were counted inside the ARS mark ring (pooled mean = 2.17, pooled median = 2; Figure 10; Figure 11a), and between zero and nine annuli outside the mark (Figure 11b). The agreement on the number of rings inside and outside the ARS mark was 48.4 % and 65.6 % with at an overall mean deviation of 0.4 ± 0.9 and 0.8 ± 1.1 annuli, respectively. When solely considering the counted number of rings inside the ARS mark, the readings differed significantly between readers (Wilcoxon test, W = 23, $P < 0.001$). However, focusing on

Table 6 Annual growth rates for the total length using the mean estimated age and after age correction with indication of the mean underestimation per age group.

Estimated mean age (n annuli)[†]	Corrected mean age (n annuli)[‡]	n	TL ± SD (mm)	Estimated mean growth rate ± SD (mm a^{-1})	Corrected mean growth rate ± SD (mm a^{-1})	Mean underestimation of growth rates ± SD (%)[§]
1	0	1	298 ± NA	127 ± NA	NA	NA
2	1	5	302 ± 57	66 ± 29	118 ± 26	75 ± 55
3	1	8	320 ± 50	50 ± 17	102 ± 50	106 ± 78
4	2	13	355 ± 61	46 ± 15	83 ± 27	95 ± 69
5	2	4	383 ± 64	42 ± 13	85 ± 12	108 ± 48
6	4	7	480 ± 29	52 ± 5	76 ± 13	49 ± 27
7	5	6	522 ± 41	50 ± 6	62 ± 15	18 ± 16
8	5	6	502 ± 41	41 ± 5	58 ± 9	41 ± 30
9	5	7	522 ± 45	39 ± 5	61 ± 8	59 ± 34
10	7	5	509 ± 40	34 ± 4	48 ± 8	42 ± 25
11	8	1	530 ± NA	33 ± NA	45 ± NA	38 ± NA
12	7	1	595 ± NA	35 ± NA	61 ± NA	71 ± NA

TL, total length; SD, standard deviation; NA, not available [†] averaged number of counted rings outside the ARS mark ring (cf. Figure 9 1b to 3b, red dots) rounded down [‡] estimated age minus the average number of counted rings inside the ARS mark (cf. Figure 9 1b to 3b, blue dots) rounded down [§] percent increase compared to the estimated growth rate

the number of rings outside the fluorescence mark, age readings differed slightly but not significantly (Wilcoxon test, W = 30, *P* = 0.059). In general, the pooled estimated age was significantly higher than the pooled corrected age (Wilcoxon test, W = 0, *P* < 0.001).

The R^2 value for the counted rings inside the ARS mark was 0.3879 indicting a high deviance between readers. In addition, one reader (reader Y) consistently counted more rings than the other (Figure 11a; Figure 12a). Disregarding the stress rings inside the ARS mark, the R^2 value increased by a factor of 2.24 (Figure 11b). In general, the deviance between readers tended to be higher when the estimated age increased (c.f. Figure 12 a and b) and there was a significant positive correlation between the estimated age and the number of counted stress rings (Spearman rank correlation, ρ = 0.516, *P* < 0.0001; Figure 11c).

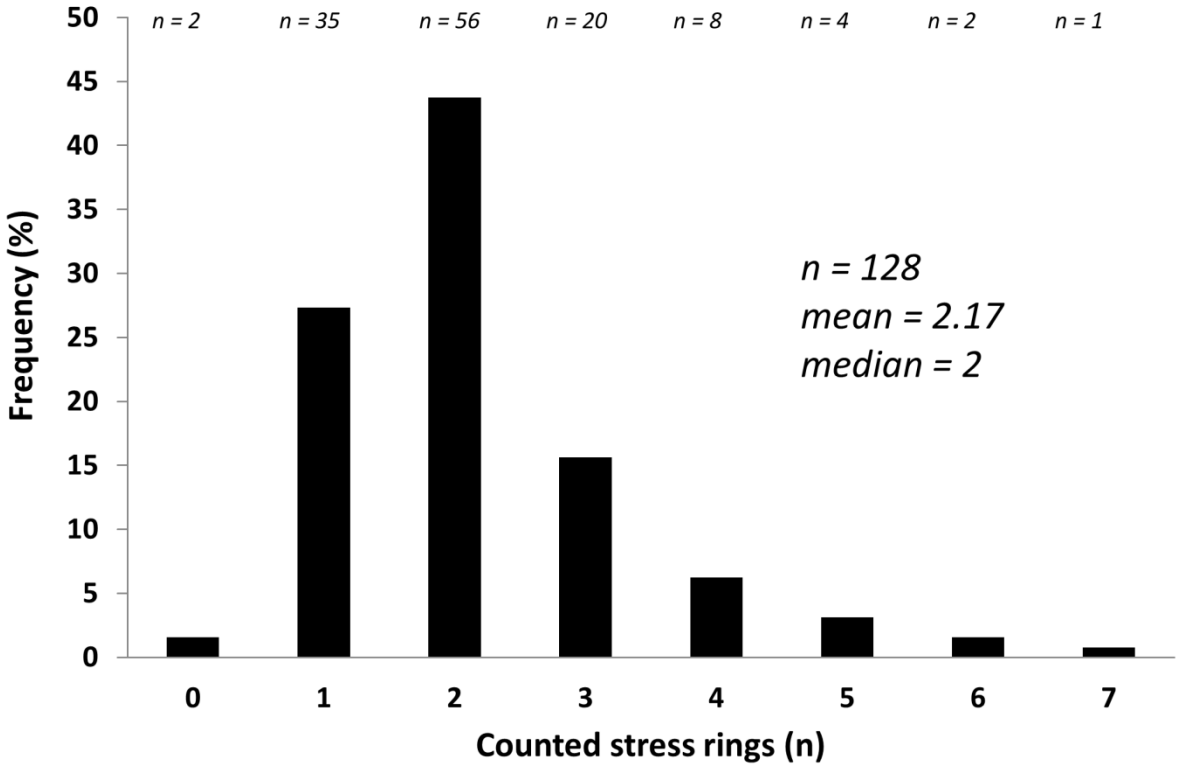

Figure 10 Pooled frequency and number of counted stress rings inside the alizarin red S mark during the blind reading trials. The readers agreed on the readability of 64 otoliths (i.e. n = 128).

Cohort shift and impact on growth estimation

The number of counted rings inside the ARS ring was averaged and rounded down to correct the estimated mean age by the readers in the blind reading trials (Table 6). The estimated mean growth rate using the estimated age was 47.6 ± 17.6 mm a^{-1}. After correction, the mean growth rate increased significantly to 76.4 ± 30.0 mm a^{-1} (Welch T-test, t = -6.5356, df = 97.901, $P < 0.0001$). This corresponds to a significant underestimation of the growth rates for total length between 18 ± 16 % and 108 ± 48 % (Table 6) with higher changes in younger age classes.

Impact on population parameters

Using the average estimated age from the blind reading trials, the growth function approached to an asymptotic length (L_∞) of 707 mm by a growth coefficient (k) of 0.12 (Figure 13a). After age correction, L_∞ decreased by 18 % to 578 mm, while the growth coefficient increased to 0.30 (i.e. 158 % higher). The uncorrected growth function for weight revealed an asymptotic weight (W_∞) of 405 g (by a k of 0.16). After age correction, W_∞ decreased by 24 % to 308 g and the growth coefficient increased by 113 % to 0.34 (c.f. Figure 13b).

Impact on modelled biomass and number of recruits per age group

The estimated biomass of age class 1 increased by 83 % compared to the initial model (0 % farmed eels in the stock) without substantial changes in numbers (Figure 14). The most sizable underestimation of biomass was observed in the age-3 cohorts with a maximum under

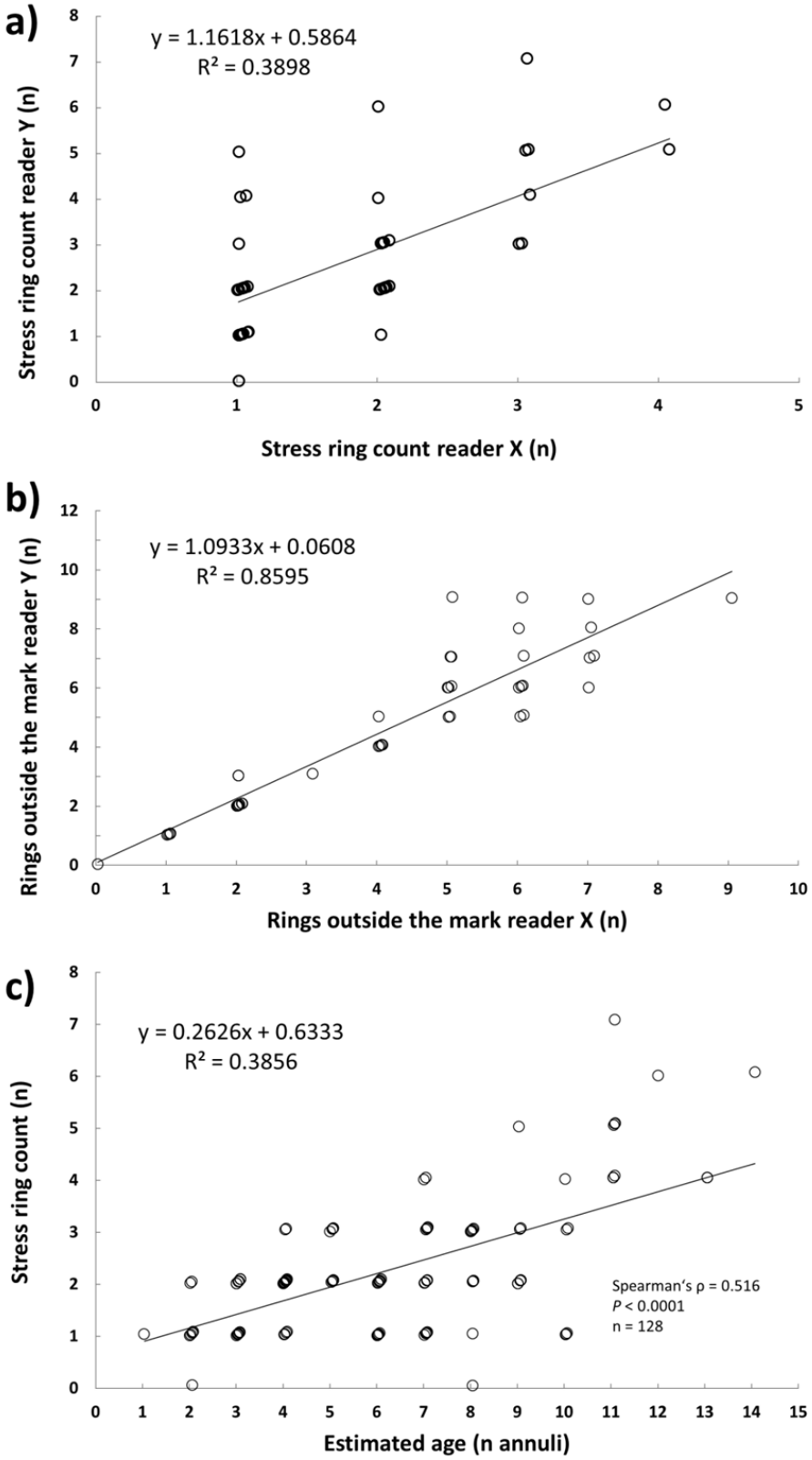

Figure 11 (a) Comparative presentation of the number of counted stress ring (i.e. rings inside the alizarin red S mark) of the two blind readers and (b) number of identified rings outside the mark. c) Significantly positive correlation between the estimated age and the number of counted stress rings inside the ARS mark.

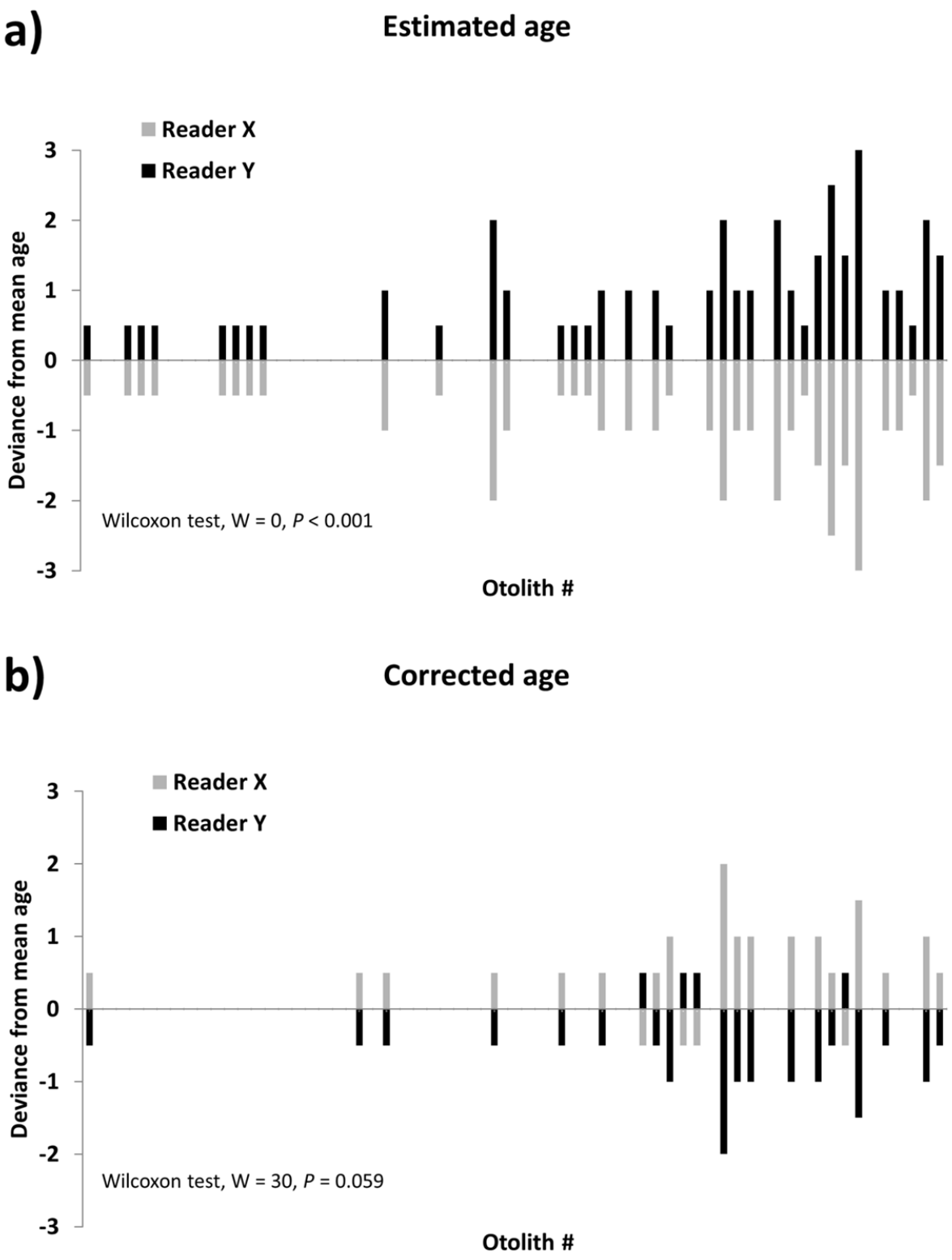

Figure 12 Deviance from the mean age for the estimated (a) and corrected (b) age. Otoliths were order according to the mean estimated (a) and corrected (b) age from low (left) to high (right).

estimation of 121 % assuming 100 % farmed eels in the stock. Thereafter, with increasing age, the estimated change in biomass decreases to a 39 % underestimation in age class 9, while the estimated number of recruits per age group increases constantly towards a maximum underestimation of 14 % in age group 9. The onset of silvering (i.e. escapement) may occur at a total length of 450 mm corresponding to an age of 4 and 6 years for the corrected and uncorrected growth function, respectively. Under the assumption of 100% stocked recruits in the population, total biomass and number of individuals were 111 % and 6 % higher at the onset of silvering (i.e. age 4) as compared to the uncorrected model estimates (i.e. assuming 0% stocked recruits)

Discussion

The objectives of the present study were (i) to investigate whether farming related annulus-like rings on “cracked & burnt” otoliths can be identified by readers and (ii) to quantify this error and exemplify the effect of the observed ageing error on total biomass estimates in an age-based model. The findings clearly demonstrate that stress rings cannot be discriminated from potential true annuli on otoliths of recaptured eels in blind readings, which was associated with a significant age overestimation, potentially resulting in false assumptions of stock biomass and stock structure.

The present study is not a typical mark and recapture experiment because different cohorts with identical ARS marks have been stocked simultaneously, thus the true age of the eels is not known.

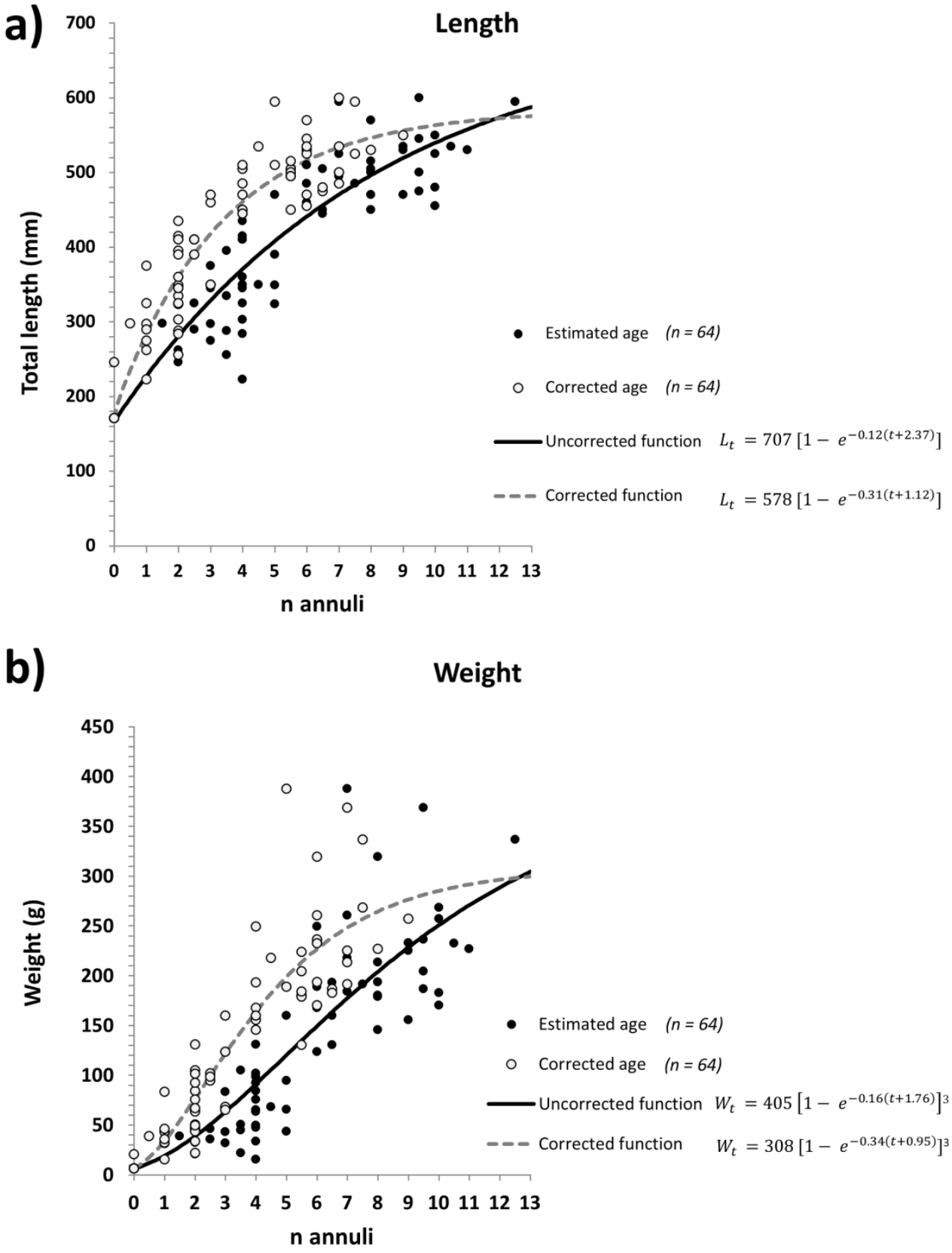

Figure 13 (a) Fitted von Bertalanffy growth function (VBGF) for the total length using the estimated and corrected mean age and (b) the VBGF for weight respectively.

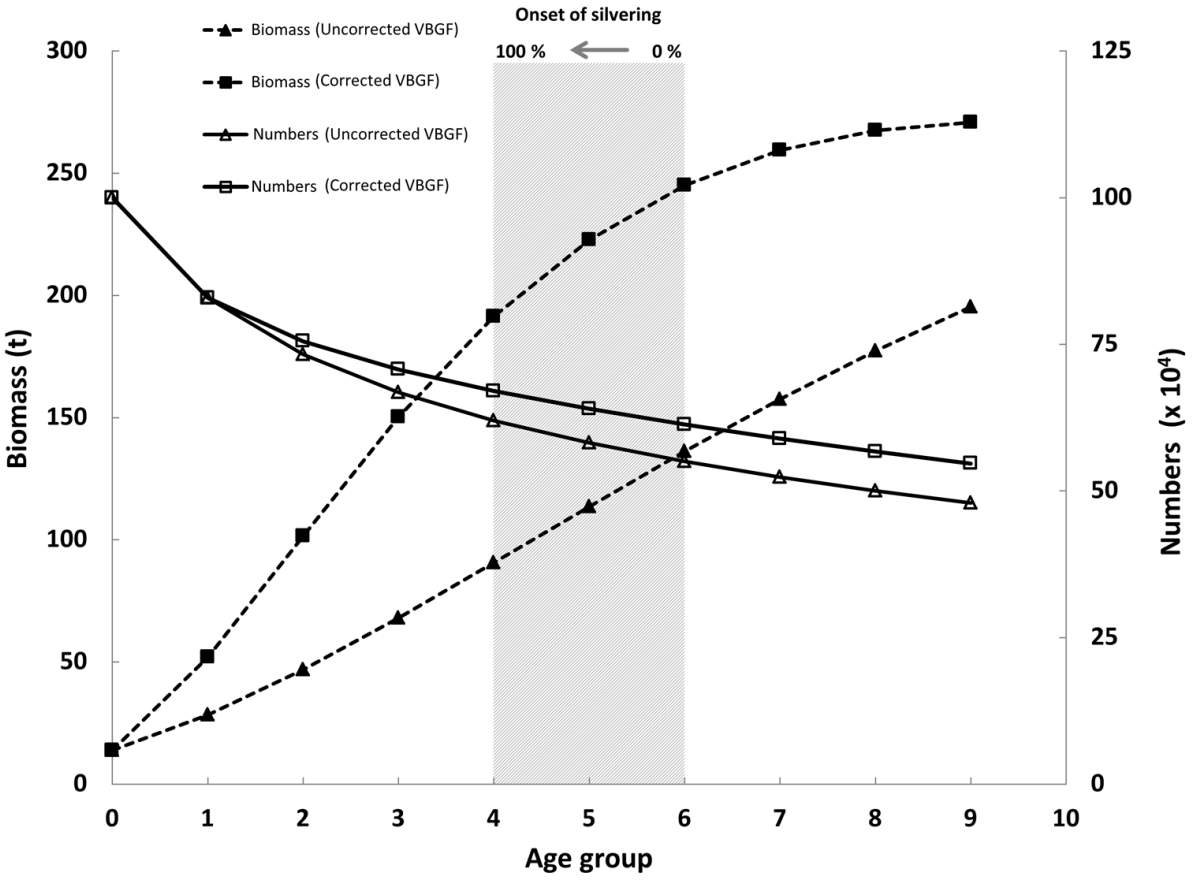

Figure 14 Modelled estimates of total biomass and number of recruits per age group per $1x10^6$ age-0 recruits using the corrected and uncorrected von Bertalanffy growth function (VBGF). The grey bar indicates the earliest onset of silvering (i.e. age at a total length of 450 mm) for the assumption of 0% (uncorrected VBGF) and 100% (corrected VBGF) farmed recruits in the stock.

Accordingly, it was solely possible to distinguish between definitely false annuli (rings inside ARS mark) and potential true annuli (outside the ARS mark). As a consequence of this approach, only the absolute overestimation could be quantified precisely (Figure 10) and then used to exemplify the underestimation of the growth rates which were up to 108 ± 48 % higher after age correction (Table 6).

The estimated age differed significantly between readers and agreement on the number of rings was low (45.3 %). After age correction, however, the agreement increased considerably (65.6 %) and age readings did not differ significantly any more. This indicates that especially

the stress rings inside the ARS mark with an agreement of only 48.4 % caused the observed variations between readers before age correction (c.f. Figure 11a and b; Figure 12a and b). Hence, knowledge of the IRH (i.e. stocked as farmed eel) allows statistically necessary corrections of age. Moreover, despite having extensive experience in eel ageing, both readers in this study were unable to identify stress rings in 98.5 % (n = 65) and 98.9 % (n = 87) of the readable otoliths from farmed eels in the blind reading trial (Figure 10).

The use of photographs as basis for the blind reading trial and quantification of the age overestimation might be considered a source of error since focus adjustment was not possible. Yet, the methodology was also used by ICES (ICES, 2009c, 2011b) for comparative inter-reader calibration analysis and is therefore considered admissible. In addition, both readers have extensive experience in ageing of eels, and rated the readability of the photographs (readable or not readable). Age readings were only used for further analysis in the case of agreement on the readability between readers, further ensuring the quality of readings. In any case, potential uncertainties (e.g. by rings that are not sufficiently visible on the photographs) applied to both readers. Therefore they are unlikely to introduce bias and are considered of no concern in the present study.

Demonstrative examples of the importance of age validation and errors in age-based stock assessments are the eastern Baltic Sea cod *Gadus morhua* Linnaeus 1758 (EBC) and northern European hake *Merluccius merluccius* (Linnaeus 1758) (NEH) stocks. The present status of the EBC stock is considered as unclear because of poor age reading precision due to low visual contrast between growth zones (Hüssy *et al.*, 2016). Consequently, ICES is unable to conduct a reliable age-based stock assessment and thus advices the precautionary approach due to insecurities concerning recruitment and stock size, mainly driven by uncertainties in age readings (ICES, 2017b). A tagging study on European hake has revealed a twofold underesti-

mation of the growth rate than previously assumed for NEH stock assessment (Pontual *et al.*, 2006). Using a corrected age-length-key (ALK), the stock biomass estimate decreased as a result of a skewed catch-at-age matrix towards younger individuals with consequences on medium-term stock predictions, the appraisal of fishing mortality, and thus management advice (Bertignac and Pontual, 2007).

In contrast to the above described effects of ageing errors, the findings of the present study will affect stock assessments only if farmed recruits make up a substantial part of the specific stock. In this case, however, using a respective age-based model (Pohlmann *et al.*, 2016); Figure 14), it was demonstrated that stock assessment approaches which rely on the conversion from age to length are particularly sensitive to changes in the VBGF parameters and thus considerably affected by the observed error. This further adds to the previously described problems in the application of age-based models due to the phenotypic plasticity in eel body growth (De Leo and Gatto, 1995; Melià *et al.* 2006). Based on our results, the underestimation of growth caused a substantial underestimation of total biomass because of two mutually reinforcing effects (i) as a function of body length, individual weight per age group is heavily underestimated, and (ii) the number of recruits per age group is underestimated because natural mortality is negatively correlated with size (Figure 14). Consequently, stocking of unmarked farmed eels introduces bias in ALK, and it can be concluded that, without knowledge of the IRH, the observed error is likely to affect e.g. estimates of silver eel escapement in age-based stock assessment models. It should be noted, however, that the degree of uncertainty will largely depend on the structure of the respective model, and thus corrections have to be considered on a case-by-case basis. A generalized reduction of the age might be an option for the adjustment of age matrices but the significantly positive relationship between the estimated age and the number of counted stress rings must be consid-

ered (Figure 11c). This necessitates detailed information about the amount of stocked recruits per age class in order to correct age readings and thereby the growth functions properly.

In conclusion, stress rings cannot be discriminated from true annuli, which results in a considerable overestimation of the age in farmed eels. It was shown that this systematically ageing error significantly impacts age, growth and related population estimates, which results in biased age-based stock assessment models. Since farmed eels play a key role in many management plans in Europe, particularly across the Baltic distribution range (ICES, 2016e), the IRH must be available to otolith readers in order to make the corrections if necessary. Moreover, the observed ageing error is also likely to be relevant for stock assessments of the Japanese eel *Anguilla japonica* because, firstly, stocking of farmed eels is also commonly used to refill local stocks (Shiraishi and Crook, 2015; Kaifu *et al.*, 2017). And, secondly, ageing of *A. japonica* is likewise based on cracked and burnt otoliths (Okamura *et al*., 2007) whereby age structured models are the fundament of Japanese stock assessments (Tanaka, 2014). The identification of the recruitment history should therefore be possible on otoliths but in a time and monetary efficient manner. This determination is certainly possible via microchemistry using Sr:Ca ratio or stable isotope incorporation patterns (Tzeng *et al.*, 1997; Kaifu *et al.*, 2018). However, this approach is comparably costly, rendering the comprehensive chemical marking of otoliths of farmed recruits prior to stocking is the only practicable solution in large scale monitoring programmes like the EU DCF (Alcobendas *et al.*, 1991; Simon and Dörner, 2005; Wickström and Sjöberg, 2014; Caraguel *et al.*, 2015; Kullmann *et al.*, 2017b; Kullmann *et al.*, 2018). This leaves the use of standard fluorescent microscopy as a common and wide spread technology in order to allow multi-national research collaborations including e.g. North African countries. Without the chemical marking of farmed recruits, stocking

of eels from aquaculture facilities is a considerable source of error for model-based evaluation of management measures and, therefore, severely impedes a reliable stock assessment.

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Chapter IV Spreading of the *eel herpesvirus*

Anthropogenic spreading of *anguillid herpesvirus 1* (AngHV-1) by stocking of infected farmed European eels, *Anguilla anguilla* (L.), in the Schlei fjord in northern Germany

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Abstract

The Schlei fjord in northern Germany is the recipient water of a comprehensive eel, *Anguilla anguilla* (L.), stocking programme. Since 2015 stocked eels become alizarin red S marked, but to date no control mechanism is implemented in this stock enhancement measure to prevent anthropogenic spreading of diseases. Consequentially, it was possible that 2015's and 2016's farmed stocking cohorts (in total ca. 1040 kg) were subsequently tested positive for *anguillid herpesvirus 1* (AngHV-1). For this study 100 eels (total length 24.3 – 72.9 cm, age ca. 1 – 6 years) were caught in 2016 and investigated with regard to AngHV-1 infection, parasite load (*Anguillicoloides crassus*) and body conditions. 68 % of the eels were found to be virus positive while larger specimens were more often infected. In addition, a fitted generalized linear model (area under the curve = 0.741), demonstrated that an increase of individual total length, is accompanied with an icreased risk of clinically relevant virus loads.

A. crassus turned out to be an important stressor for eels, because parasite and virus load revealed a significant positive correlation. The results of this study evidently show the urgent need of a diseases containment strategy for eel stocking programmes.

Introduction

As a consequence of the massive recruiment decline of the European eel, *Anguilla anguilla* (L.), since the 1970's, the European Union adopted the so called 'eel regulation' in 2007 (European Council, 2007) which requests its member states to establish eel management plans (ICES, 2015; Dekker, 2016; Dekker and Beaulaton, 2016a; ICES, 2016b, 2016c). These plans include measures for the recovery of the stock of the European eel and defined specific goals. One goal of high priority is the 40 % target level of escapement of silver eel biomass compared to best estimated pristine levels. Proposed measures are based primarily on reduction of mortality (anthropogenic and natural) and reallocation of natural recruits ('stocking'). The latter is highly controversial because, firstly, these measures are entirely reliant on glass eel (total length (TL) 6-8 cm) catches and, secondly, no clear evidence for a "net benefit" defined as "higher silver eel escapement biomass than would have occurred if the glass eel seed had not been removed from its natural (donor) habitat in the first place" (ICES, 2016e) could be provided yet (ICES, 2016b, 2016c). A German study was able to show, however, that stocking, especially in inland fresh water bodies, is important to meet the local silver eel escapement target of the EU (Brämick *et al.*, 2016). But the contribution of eels from inland freshwater bodies to future recruimtent is questioned (Tsukamoto *et al.*, 1998; Marohn *et al.*, 2013).

In general stocking programmes are associated with serious threats and care should be taken regarding the source of the fish, their health status, the habitat quality of the recipient

water, and biosecurity (van Ginneken *et al.*, 2004; Bartley *et al.*, 2006; EFSA, 2008; Freese *et al.*, 2016; ICES, 2016d). In particular a disease control programme should be a compulsory part of stock enhancement programmes in order to inhibit anthropogenic spreading of diseases or parasites (Haenen *et al.*, 2002; van Ginneken *et al.*, 2004; Bartley *et al.*, 2006; Walker *et al.*, 2009; Peeler and Feist, 2011; Haenen *et al.*, 2012; van Beurden *et al.*, 2012; Armitage *et al.*, 2014; European Commission, 2014). This is particularly the case for the critically endangered European eel (Jacoby and Gollock, 2014) because artificial reproduction is still not feasible and stocking is merely the reallocation of already rare natural recruits. In addition, eels are often stocked as farmed eels (TL 15-20 cm), which means that they were fattened in high densities (> 100 kg m^{-3}) in commercial fish farms over several months prior to stocking (EFSA, 2008; Nielsen and Prouzet, 2008; van Beurden *et al.*, 2012). These conditions are associated with an elevated risk of disease outbreaks that can cause high mortalities (Sano *et al.*, 1990; Haenen *et al.*, 2002; Haenen *et al.*, 2009; Haenen *et al.*, 2012; Armitage *et al.*, 2014).

In eel aquaculture the *anguillid herpesvirus 1* (AngHV-1) plays a key role. The virus was characterized by Sano *et al.* (1990) and is present in most eel farms today (EFSA, 2008). Infected eels may show pathological changes affecting the skin, gills, and liver associated with haemorrhages, necrosis, and lesions (Davidse *et al.*, 1999; Haenen *et al.*, 2002; Hangalapura *et al.*, 2007; Lepa and Siwicki, 2012). AngHV-1 can form a persistent stage in the host (van Nieuwstadt *et al.*, 2001), and Hangalapura *et al.* (2007) could prove its high virulence by showing that it is transferable to naïve eels by bath immersion. Furthermore, AngHV-1 was also isolated from eels with no clinical signs, which means that some individuals reveal a natural resistance but can act as carrier hosts (van Nieuwstadt *et al.*, 2001). Though those eels appear healthy, stress (e.g. high water temperatures, low oxygen concentrations) might trigger a

disease outbreak with high mortalities (Haenen *et al.*, 2002; Haenen *et al.*, 2010; van Beurden *et al.*, 2012). In aquaculture, usually no more than 10 % of infected eels die from virus infection (Davidse *et al.*, 1999; Haenen *et al.*, 2002), and afterwards the survivors are more resistant and less vulnerable for a repeated disease outbreak (EFSA, 2008; ICES, 2009a). In order to prevent uncontrolled outbreaks in more vulnerable fast on-growing stages, farmers deliberately infect juveniles by bath immersion with water contaminated with AngHV-1 (EFSA, 2008; ICES, 2009a; Jakob, 2009). Hence, purposely AngHV-1 infection might prevent an eel farm from even greater economic losses but from a conservation point of view, treated eels are disqualified for stocking purposes (e.g. van Ginneken *et al.*, 2004; ICES, 2009a; Haenen *et al.*, 2012; ICES, 2016d).

In the wild parasite load could be a constant stressor to a fish. In eels especially the infestation with *Anguillicoloides crassus* (Kuwahara, Niimi & Itagaki 1974) is known to lead to mechanical damage in the swim bladder wall (Würtz and Taraschewski, 2000; Gollock *et al.*, 2005; Palstra *et al.*, 2007). This causes elevated cortisol levels in the blood (Sures *et al.*, 2001) and might induce diseases notably when eels are afflicted by a virus infection (Haenen *et al.*, 2010). Finally, if infected eels were never exposed to high stress levels during their yellow eel phase, they metamorphose to sexual maturing silver eels and their 5000 to 7500 km spawning migration to the Sargasso Sea can undoubtedly be considered as energy depleting and stressful (van Ginneken *et al.*, 2005; Haenen *et al.*, 2009; van den Thillart *et al.*, 2009). Even if infected eels are able to spawn, the egg quality might be low and, additionally, it is unknown whether their descendants will also be infected.

In 2006, a study found very low prevalence of AngHV-1 in eels from northern Germany (Jakob *et al.*, 2009b). Especially eels in marine and brackish habitats including the Schlei fjord were found to be AngHV-1 free. Jakob *et al.* (2009b) mentioned this with special reference

to find appropriate locations for stocking measures. Since 2010 the verifiably former AngHV-1 free brackish Schlei fjord (Federal State of Schleswig-Holstein) in northern Germany (Figure 15) is the recipient water of a comprehensive but unmonitored stocking programme (Table 7).

No virological analysis of stocked eels was performed between 2010-2014. However, all eels stocked in 2015 (500 kg) and 2016 (540 kg) were subsequently analysed and the results showed that all of the farmed eels were infected with AngHV-1 (Table 7). It is believed that all farmed eels stocked in the Schlei fjord between 2010-2014 were also infected with AngHV-1.

In 2015 and 2016 all eels (glass and farmed eels) were alizarin red S (ARS) marked prior to release into the Schlei fjord. During the marking process in 2015 (2 x 250 kg farmed eels in two 145 cm x 300 cm tanks each filled with 3.5 m³ freshwater; 0.15 g ARS L^{-1} buffered with 0.15 g TRIS[2] L^{-1}; 9 h) the eels became apathetic and showed clinical signs of a disease. The mortality was low (0.88 %) but increased dramatically during the release the next day. In 2016s marking process (3 x 180 kg farmed eels in three 145 cm x 300 cm tanks each filled with 3.5 m³ freshwater; 0.15 g ARS L^{-1} buffered with 0.15 g TRIS[1] L^{-1}; 9 h) the farmed eels were not apathetic but showed the same clinical signs as in 2015. Mortality indeed was remarkably low (<0.001 %) and did not increase during the day of release, but eels appeared stressed. Due to a lack of legal obligation, all eels were stocked despite obvious clinical signs of a disease.

[1] *Tris*(hydroxymethyl)-aminomethane

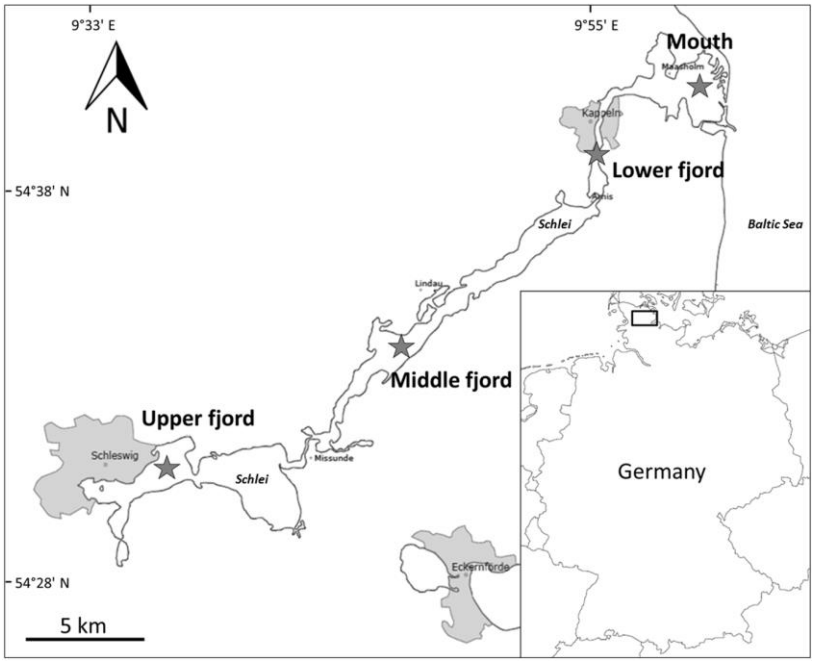

Figure 15 Location of the Schlei fjord in northern Germany. Stars indicate the approximate position of the sampling sites.

Table 7 Known stocking measures of European eels, *Anguilla anguilla*, in the Schlei fjord since 2010. Note that there is no reference material available before 2015

Year	Stocking form	Mass (kg)	TL ± SD (cm)	BW ± SD (g)	AngHV-1 test?	AngHV-1 present?
2010	E_G	0	–	–	–	–
	E_F	320	–	–	–	–
2011	E_G	50	–	–	–	–
	E_F	570	–	–	–	–
2012	E_G	0	–	–	–	–
	E_F	650	–	–	–	–
2013	E_G	11	–	–	–	–
	E_F	580	–	–	–	–
2014	E_G	60	–	–	–	–
	E_F	400	–	–	–	–
2015	E_G	57	6.53 ± 0.32	0.22 ± 0.05	No	–
	E_F	500	18.25 ± 1.83	7.57 ± 2.74	Yes	Yes
2016	E_G	60	6.72 ± 0.34	0.24 ± 0.05	No	–
	E_F	540	17.97 ± 1.56	7.41 ± 3.02	Yes	Yes

E_G, wild caught unpigmented glass eel; E_F, farmed eel; TL, total length; BW, body weight; SD, standard deviation; –, unknown/not available

The aim of the present study was to investigate the potentially negative effects of an unmonitored stocking programme on a local eel stock when no health requirements on the stocking material are imposed. A high *eel herpesvirus* prevalance was hypothesised and high infection rates were especially expected in larger eels, because risk of contagion should be increased over time.

Materials and methods

Study area

The study area was the Schlei fjord in northern Germany (54.595976 °N, 9.852501 °E; Figure 15). It is a narrow (width between 0.14 and 4.10 km) and shallow (mean depth from 2 to 3 m) brackish inlet of the southwestern Baltic Sea. The surface covers an area of 54 km² and there is a salinity gradient decreasing from ca. 19 g L^{-1} (east) to ca. 4 g L^{-1} (west) (LANU, 2001). The opening of the Schlei ford to the Baltic Sea is approximately 100 m wide.

Morphometrical measurements and indices

For this study a total of 100 eels were caugth with fyke nets between July and September in 2016 at four different stations in the Schlei fjord (Figure 15, Table 8). Eels were sacrificed and deep frozen at circa -20 °C. After thawing total length (TL) in cm, body weight (BW) in g, mean pectoral fin (PFL) length in mm, mean eye diameter (ED) in mm, swimbladder length (SBL) in cm, liver weight (LW) in g, and number of swimbladder nematodes, *Anguillicoloides crassus*, were measured or counted to determine the followig indices:

- Silvering stage as function of TL, PFL, and ED according to highest computed S_i score (Durif *et al.*, 2005; Durif *et al.*, 2009)
- swimbladder index (SBI in %) = SBL x TL^{-1} x 100 (Palstra *et al.*, 2007)

- parasitation intensity (PInt) = n *A. crassus* host^{-1}
- hepatosomatic index (HSI in %) = LW x BW^{-1} x 100

Sagitta otoliths were removed by longitudinal dissection of the head, cleaned and stored dry in 2 ml plastik tubes. One otolith was used for age estimation after the cracking and burning protocoll and ageing recommendations according to ICES (2009c). Ages are presented as number of annuli (winter rings). The second otolith was used for ARS mark detection. Therefore, otoliths were cracked on transversal plane and embedded in thermoplastic wax (Crystalbond® Buehler) on glass slides. Both pieces were ground to the primordium as described by Simon *et al.* (2013) and Simon and Dörner (2014). These preparations were checked for ARS marks using a light microscope (Leica DM 2500) equipped with a light source (CoolLED *p*E-300-W) and a light filter for wavelengths between 530 and 580 nm, where an ARS mark appears as yellow glowing band. A definite discrimination between stocking forms (glass or farmed eel) was also possible because the ARS mark position in glass eel otoliths is conspicuous closer to the core than in farmed eels. For ageing of marked eels, the ARS mark was defined as age 0.

Table 8 Numbers, total length (TL), body weight (BW), and sampling period/date of examined eels from the different sampling locations in the Schlei fjord

Sampling site	n	TL ± SD (cm)	TL range (cm)	BW ± SD (g)	BW range (g)	Sampling period/ date
Upper fjord	25	42.3 ± 10.9	29.8 – 69.1	181.2 ± 184.4	47.0 – 910.1	31. Aug 2016
Middle fjord	25	43.1 ± 13.5	27.0 – 64.9	181.8 ± 156.2	36.1 – 492.6	01. July – 28. Sept 2016
Lower fjord	25	42.4 ± 12.4	29.0 – 72.9	190.3 ± 204.0	40.9 – 902.3	01. July – 30. Aug 2016
Mouth	25	39.5 ± 13.8	24.3 – 64.4	159.9 ± 164.6	21.4 – 603.0	18. Aug – 22. Aug 2016
Total	100	41.8 ± 12.6	24.3 – 72.9	178.3 ± 175.9	21.4 – 910.1	01. July – 31. Aug 2016

n, number of eels; TL, total length; BW, body weight; SD, standard deviation

Quantitative PCR (Polymerase Chain Reaction)

For virus detection in individual eel's ca. 0.5 g of pooled organ samples (liver, brain, gills, spleen) were taken from each eel and fixed in 99.5 % isoproanol. The DNA was extracted from ca. 15 mg of tissue pools after mechanic lyses in a QIAgen Tissuelyser II (Qiagen, Germany) with the QIAamp DNA Mini Kit (Qiagen, Germany) according to the manufacturer's manual. After isolation the quantity of DNA was evaluated in a Nanodrop ND-1000 spectrophotometer (Thermo Scientific, USA) and stored at -80 °C. Real-time quantitative PCR was used for amplification of a fragment of the AngHV-1 ORF109 (GenBank1 accession no. NC_013668) by the use of AHV1_O109_qF1 and AHV1_O109_qR1 primers and the AHV1_O109_qP1 (Table 9). The quantitative PCR reaction mix contained 1 x master mix (Maxima Probe qPCR Mastermix, Fermentas, Germany), 300 nM of forward primer, 900 nM of reverse primer and 200 nM of the fluorescent probe, and 5 µl of DNA template. The qPCR was performed in duplicates on a Stratagene Mx 3005P thermocycler (Agilent, USA). The qPCR profile included an initial denaturation step at 95 °C for 10 min, followed by 40 cycles of 95 °C for 30 s, 60 °C for 30 s. AngHV-1 virus genome copies quantification was performed using a recombinant DNA plasmid. Briefly: the end-point PCR product amplified with the primers AHV1_ORF109_F1 and AHV1_ORF109_R1 (Table 9) cusing the Advantage 2 PCR kit (Clontech, USA), was ligated into the pGEM–T Easy vector (Promega, USA) and propagated in

Table 9 Oligonucleotides used in PCR reactions. Primers indicated with "P" were used end-point PCR to create plasmid. Primers/probe indicated with "Q" were used in quantitative PCR

Primer/probe	5`-3` Sequence	Use
AHV1_ORF109_F1	CTTTGGGGACGCCGAGGA	P
AHV1_ORF109_R1	GAGCAGGTTCACGGACAA	P
AHV1_O109_qF1	GCGATTGACGGTGATGTTG	Q
AHV1_O109_qR1	ACCTTGCCTCTGGTTTGGAG	Q
AHV1_O109_qP1	[FAM]-TGTGAGCTACGTGCGA-[BHQ1]	Q

JM109 competent *Escherichia coli* bacteria (Promega, USA). The plasmids were isolated with the GeneJET™ Plasmid Miniprep Kit (Fermentas, Germany). A standard curve from 10^1 to 10^7 of gene copies was prepared and used for quantification of the copy numbers from each sample. The results are presented as negative or positive for AngHV-1 or as the amount of virus positive eels where more than $1x10^4$ AngHV-1 genome copies (AGC) 250 ng DNA^{-1} were found – a virus load that could be considered as clinically relevant with present clinical signs of a disease (e.g. for *cyprinid herpesvirus 3* infection in *Cyprinus carpio* L.; Adamek *et al.*, 2014).

Statistical analysis

For statistical analysis, the software R (version 3.3.2) was used (R Core Team 2017). Results were described as statistically significant, when the estimated probability of error was below 5 per cent ($P < 0.05$). Data distribution was checked for normality by using the Shapiro-Wilk (SWT) test. Depending on the result, assumption of homoscedasticity was tested by performing the Bartlett-Test (SWT, $P > 0.05$) or the Fligner-Killeen-Test (SWT, $P < 0.05$). When variances were considered as equal the T-Test (2 groups) or ANOVA (followed by Tukey post-hoc test for more than 2 groups) was used to detect statistical differences. In case of heterogeneity of variances, the Wilcoxon test (2 groups) or the Kruskal-Wallis test (> 2 groups) followed by Nemenyi's post-hoc test according to Pohlert (2016) was performed.

For the generalized linear model (GLM) calibration Mc Fadden's R^2 coefficient of determination was calculated according to Mangiafico (2016). The discriminative ability of the model was assessed using the Area Under the Curve (AUC) index (e.g. Hein *et al.*, 2007). AUC indices range from 0.5 (no discrimination) to 1 (perfect discrimination). An AUC index of 0.5 indicates a random classification, values above 0.6 indicate a bad, values above 0.7 an accepta-

ble, values above 0.8 an excellent and values above 0.9 an outstanding discrimination (Hosmer and Lemeshow, 2000). Additionally, the Hosmer-Lemeshow Test for 'Goodness of Fit' after Lele *et al.* (2016) was conducted, whereby p-values above 0.05 indicated that there is no significant lack of fit.

Results

Silvering and life history stages, age composition and occurrence of ARS marks

The caught eels for this study (n = 100) belonged to the silvering stages I (52 %; undifferentiated males and females), FII (36 %; female growth phase), FIII (10 %; female pre-migration stage), and FIV (2 %; female growth stopped and migration begins). Examined eels could also be grouped into the stages of young yellow eels (stage I, 52 %), yellow adults (stages FII and FIII, 46 %), and silver eels (stage FIV, 2 %). For age estimation the otoliths of 86 eels were useable. Ages varied between 1 and 6 years with a mean of 2.8 ± 1.7 years (median age = 2). 12 ARS marked eels (TL = 28.8 ± 0.3 cm) were present within the total sample. 10 of them were identified as farmed eels (TL = 29.5 ± 2.8 cm) and 2 as glass eels (TL = 25.4 ± 0.3 cm). 100 % of them were in silvering stage I (undifferentiated). All marked eels belonged to age group 1.

Virus prevalence

The virus analysis by quantitative PCR revealed that 68 % (n = 68) of all examined eels from the four different stations in the Schlei fjord were infected with AngHV-1 (Table 10). There was a large variability in virus prevalence between stations. Eels caught at the stations Upper fjord, Lower fjord and Mouth showed an amount of virus positive individuals between 64 and 100 % while 72 % of eels at station Middle fjord were virus negative. In the small length

classes 20 – 30 cm (32 %) and >30 – 40 cm (52 %) the amount of virus negative eels was higher compared to eels in the middle length classes >40 – 50 cm (11 %) and >50 – 60 cm (20 %)(Figure 16). However, eels in the largest length class >60 cm showed a similar virus prevalence (33 %) than eels in the smallest length class (32 %). But in general, with increasing body length, eels in the Schlei fjord were significantly more often AngHV-1 positive (Figure 16c; T-test, t= -2.072, d.f. = 64.171, *P* = 0.0422). This pattern was roughly consistent within silvering stages but eels in stage FIII showed a virus prevalence (40 %) close to the level in stage I (44 %)(Figure 16b). In migration stage (FIV) virus prevalence was 100 % (n = 2). Within the ARS marked group (n = 12) virus was present in 83.3 % (n = 10) of all eels. One of the two glass eels was infected and 9 out of 10 farmed eels.

Virus load

In the DNA samples of 12 % (n = 8) of infected eels more than $1x10^4$ AGC were found, a virus load which could be considered as clinically relevant. Virus load seemed to be related to body length but in particular to silvering stage. Eels in silvering stages I and FII showed an amount of 6.9 % (n = 2) to 9.7 % (n = 3) individuals with a clinically relevant AGC whereby in stages FIII and FIV 16.7 (n = 1) and 100 % (n = 2) of eels were heavily infected with AgHV-1, respectively (Figure 16b). To exclude the probability of a random effect due to small sample

Table 10 Number (n) of investigated eels, amount of positive AngHV-1 isolations and amount thereof with clinically relevant virus load (AGC), total length (TL), range of *Anguillicoloides crassus* infestation, mean parasitation intensity (PInt), mean swim bladder index (SBI), and mean hepatosomatic index (HSI) in relation to sampling site and silvering stage

Sampling site	Silvering stage	*n*	AngHV 1 positive (% (*n*))	Clinically relevant AGC (% (*n*))[d]	TL ± SD (cm)	*n A. crassus* (min.–max.)	PInt (*n A. crassus* host^{-1})	SBI ± SD (%)	HSI ± SD (%)
Upper fjord	I	13[a]	100 (13)	15 (2)	32.7 ± 1.7	0–25	7.5 ± 8.4	14.7 ± 3.7	1.91 ± 0.44
	F II	11	100 (11)	18 (2)	51.1 ± 2.7	0–12	2.2 ± 4.1	12.8 ± 1.7	1.69 ± 0.27
	F III	0							
	F IV	1	100 (1)	100 (1)	69.1 ± NA	14–NA	14.0 ± NA	11.0 ± NA	1.05 ± NA
Middle fjord	I	13	8 (1)	0 (0)	31.3 ± 2.8	0–31	8.8 ± 8.8	14.7 ± 2.5	2.23 ± 0.36
	F II	7	57 (4)	0 (0)	50.8 ± 2.8	0–12	3.3 ± 5.6	13.5 ± 3.5	1.71 ± 0.29
	F III	5	40 (2)	0 (0)	63.1 ± 2.8	0–8	3.0 ± 3.5	12.1 ± 2.3	1.50 ± 0.41
	F IV	0							
Lower fjord	I	13[b]	31 (4)	0 (0)	31.9 ± 2.2	0–22	8.8 ± 7.5	14.7 ± 2.5	2.20 ± 0.29
	F II	9	100 (9)	11 (1)	50.3 ± 4.4	0–14	2.9 ± 5.3	12.5 ± 2.2	1.73 ± 0.32
	F III	2	100 (2)	50 (1)	59.1 ± 1.3	0–26	13.0 ± 18.4	11.4 ± 2.0	1.37 ± 0.27
	F IV	1	100 (1)	100 (1)	72.9 ± NA	25–NA	25.0 ± NA	12.7 ± NA	1.24 ± NA
Mouth	I	13[c]	85 (11)	0 (0)	27.3 ± 3.3	0–1	0.2 ± 0.4	14.0 ± 1.6	1.85 ± 0.38
	F II	9	78 (7)	0 (0)	49.8 ± 4.0	0–1	0.1 ± 0.3	14.7 ± 2.0	1.57 ± 0.22
	F III	3	67 (2)	0 (0)	62.2 ± 2.8	0–0	0.0 ± NA	13.7 ± 3.0	1.47 ± 0.13
	F IV	0							
Overall	I	52	56 (29)	7 (2)	30.8 ± 3.3	0–31	6.3 ± 7.8	14.5 ± 2.6	2.05 ± 0.40
	F II	36	86 (31)	10 (3)	50.5 ± 3.4	0–14	2.1 ± 4.3	13.3 ± 2.4	1.67 ± 0.27
	F III	10	60 (6)	17 (1)	61.8 ± 2.8	0–26	4.1 ± 8.2	12.4 ± 2.4	1.46 ± 0.30
	F IV	2	100 (2)	100 (2)	71.0 ± 2.7	14–25	19.5 ± 7.8	11.9 ± 1.2	1.15 ± 0.14
	Total	100	68 (68)	12 (8)					

SD, standard deviation; NA, not available; AngHV 1, *anguillid herpesvirus 1*; AGC, AngHV 1 genome copies 250 ng DNA^{-1}; ARS, alizarin red S.
[a]With $n = 3$ ARS marked farmed eels, 100% virus positive, 0% with clinically relevant AGC.
[b]With $n = 2$ ARS marked farmed eels, 50% virus positive, 0% with clinically relevant AGC.
[c]With $n = 5$ ARS marked farmed eels, 100% virus positive, 0% with clinically relevant AGC; and $n = 2$ ARS marked glass eels, 50% virus positive, 0% with clinically relevant AGC.
[d]Amount of virus-positive eels with clinically relevant virus load of $>1 \times 10^4$ AGC.

size in FIII and FIV, a GLM was fitted to predict probability of a clinically relevant AGC as function of total length (Figure 16d). The model showed an adequate fit for the input data (Hosmer-Lemeshow test, χ^2 = 6.758, d.f. = 8, *P* = 0.433) and overdispersion was not substantial (residual deviance divided by residual d.f. = 1.236). Model discrimination was acceptable (AUC = 0.741) and revealed a McFadden R^2 determination coefficient of 0.119. According to the model, predicted probability of clinically relevant AGC for eels in FIV with TL of 69.1 and 72.9 cm was 44.4 and 51.8 %, respectively. None of ARS marked individuals showed signs for a clinically relevant AngHV-1 load (loads << $1x10^4$ AGC 250 ng DNA^{-1}) and predicted probability was below 5 % (c.f. Figure 16d).

Anguillicoloides crassus

Prevalence of *A. crassus* was 50 % in eels from each of the stations with a mean intensity of 4.83 ± 7.29 *A. crassus* eel^{-1}. Parasitation intensity differed among sampling stations significantly (Kruskal-Wallis-test, H = 19.620, d.f. = 3, *P* < 0.0005) but only parasitation intensity at the station at the fjord opening (Mouth) was significant lower compared with all others (Nemeyi's post-hoc test, *P* < 0.05; Table 10). Within virus positive and negative eels, *A. crassus* prevalence was 39.5 % and 71.9 %, respectively. Eels that were virus negative showed with 6.9 ± 8.0 *A. crassus* eel^{-1} a significantly higher parasite load compared to virus positive eels with 3.9 ± 6.8 *A. crassus* eel^{-1} (Wilcoxon-test, w = 1436, *P* = 0.0063). Infected and non-infected eels, however, did not differ significantly in terms of SBI but AngHV-1 positive individuals showed significant higher HSI values (T-test, t = 2.944, d.f. = 66.246, *P* = 0.004). Among the group of virus positive eels occurs a significant positive correlation between parasitation intensity and virus load (Spearman's rank correlation, Rho = 0.245, *P* < 0.05) but parasite load was independent from TL (Spearman's rank correlation, Rho = -0.045, *P* > 0.05). In the

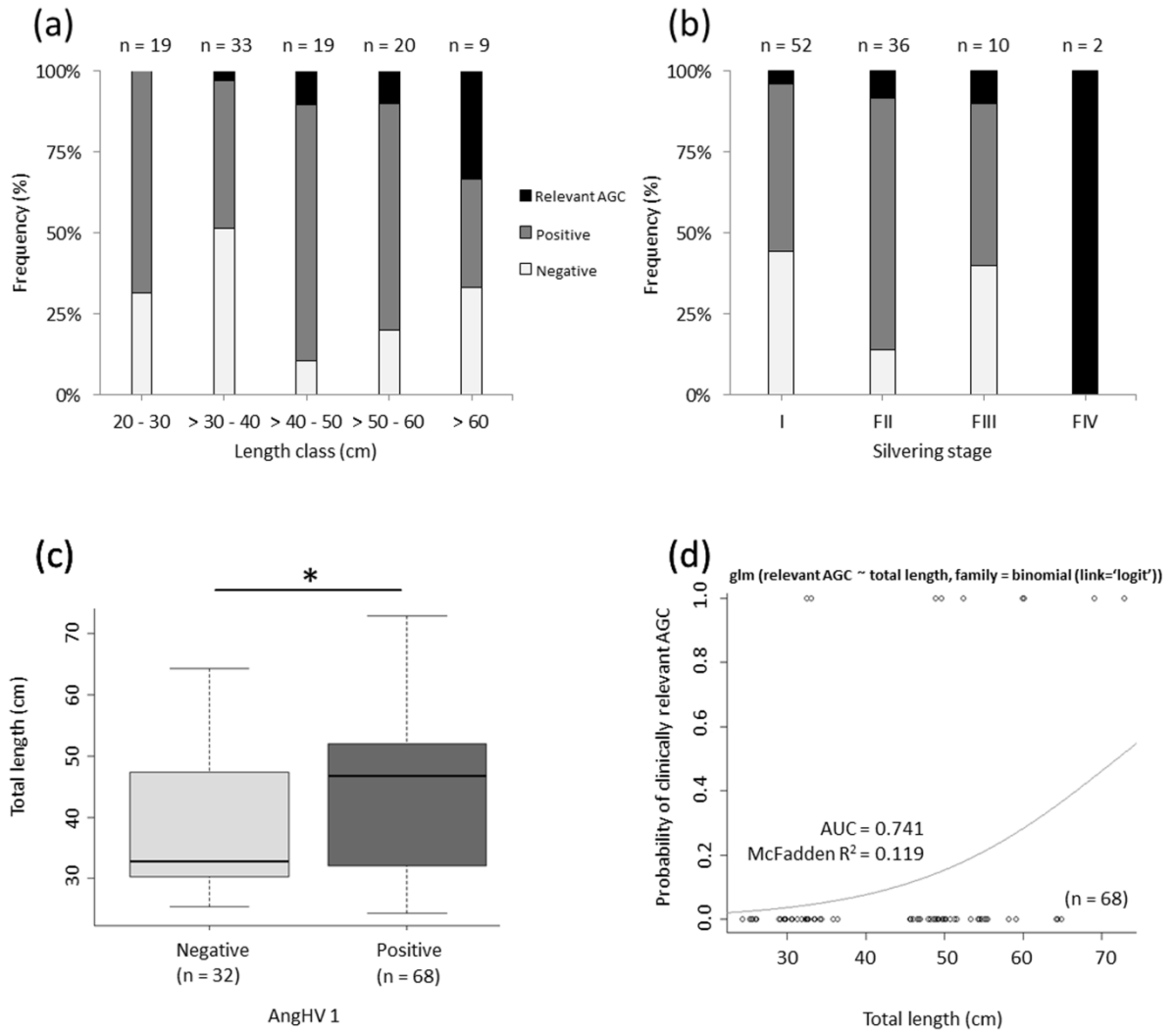

Figure 16 Frequency of AngHV-1 negative or positive individuals and with clinically relevant AGC (a) per length class and (b) in relation to the silvering stage (see also Table 4). (c) Boxplot of median total lengths of AngHV-1 negative or positive eels. The star indicates significant differences (T-test, t = -2.072, d.f. = 64.171, *P* = 0.0422), and (d) probability of clinically relevant AngHV-1 load by total length as fitted GLM (grey line).

ARS marked group (n = 12) an intensity of 4.6 ± 8.2 *A. crassus* eel^{-1} was found. Glass eels (n = 2) were not infected. This lead to an increase of mean parasitation in farmed eels to 5.5 ± 8.7 *A. crassus* eel^{-1}. For statistical analysis between marked and unmarked group only un-differentiated eels (silvering stage I, n = 52) were considered. Marked (n = 12) and unmarked (n = 30) eels did not differ significantly in terms of *A. crassus* load (T-test, t = - 0.853, d.f. = 17.403, *P* = 0.405).

Discussion

The risk of spreading diseases by stocking measures is well known but until now, only indirect evidence for anthropogenic spreading of AngHV-1 was found (e.g. ICES, 2009a; van Beurden *et al.*, 2012). Van Beurden *et al.* (2012) analysed glass eels from eel farms for the purpose of farming and (later) stocking and found >50 % risk of infection with AngHV-1. And van Ginneken *et al.* (2004) investigated farmed eels from several Dutch farms that fatten glass eels for stocking purposes and found AngHV-1 positive batches. Hence, the *eel herpesvirus* is present in many eel farms and a very high risk of infection can be considered for farmed eels. But, to the author's knowledge, this is the first time that stocking of demonstrably AngHV-1 infected eels could be directly documented. Eels are marked with ARS, so the infected stocking cohorts can be monitored in detail over time in the future. By now, the results of this study (68 % AngHV-1 prevalence) enables the conclusion that the hypothesis of a high infection rate is true (Figure 16 a and b) which is unlikely the result of only two contaminated stocking charges (Table 7). Ages between 1 and 6 years suggest that all examined eels did not enter the Schlei fjord before stocking activities started in 2010, thus all eels for this study were possibly stocked recruits. In this regard, Jakob *et al.* (2009b) were able to show via PCR that in 2006, 4 years before the stocking started in 2010, no adult eel (n = 30, TL = 71. 5 ± 7.4 cm) in the Schlei fjord (caught in June at station Mouth) was infected with AngHV-1. They discussed a minor temperature effect on detection sensitivity in their study, because slightly lower water temperatures in June (compared to August in this study) may have led to lower levels of virus DNA in eels, which might had masked virus detection. Although water temperatures in August might be higher than in June, eels are adapted to warm water (Tesch and Thorpe, 2003), hence, Mid-European summer conditions cannot be considered as stressful for eels. It can be assumed that differences between 2006 (0 % virus pos-

itive) and 2016 (68 % virus positive) are unlikely explicable only because of a virus detection error caused by slight differences in water temperature. Particularly, because the PCR method is known to be highly sensitive for detection of latent viruses (Bandín *et al.*, 2014; Hanson *et al.*, 2016; van Beurden *et al.*, 2016). This leads to the conclusion that AngHV-1 was absent in 2006 and the *eel herpesvirus* was introduced by stocking measures, and, despite its known infectivity (Hangalapura *et al.*, 2007; ICES, 2009a; Haenen *et al.*, 2012), stocking of infected eels took place very likely also before 2015.

Lager eels were significantly more often infected with AngHV-1 than smaller individuals (Figure 16c) which was consistent with findings in the Albufera Lake in Spain (Bandín *et al.*, 2014). This is probably a consequence of the glass eels stocking measures in the Schlei fjord (Table 7) or still occurring natural recruitment in the Albufera Lake (Esteve and Alcaide, 2009). Though glass eels cannot be considered per se as healthy (van Beurden *et al.*, 2012; UK Country Report 2016, 2016), however, they are less likely infected with AngHV-1 than farmed eels. But the risk of contagion of former healthy eels increases, when residence time (with associated growth) in a water body with a high abundance of virus positive eels increases.

The threshold value for consideration of a clinically relevant virus load is based on observations during routine virus diagnostics that a virus load of 10^4 genome copies of AngHV-1 and higher was found in eels showing clinical symptoms such as haemorrhages in skin and fins. This is in accordance with findings in other species likes common carp, *Cyprinus carpio* L., (infected with *cyprinid herpesvirus 3*; (Adamek *et al.*, 2014). The modelled probability of a clinically relevant virus load (Figure 16 d) was fitted adequately as a function of individual length but in fact, it is more a function of age or indirectly of silvering (maturation). Since silvering is strongly correlated with length increment (Durif *et al.*, 2005; Durif *et al.*, 2009),

the GLM based on body length was admissible. The model clearly showed the presence of a size effect, which is in accordance with the fact that all eels in development stage (FIV) revealed a clinically relevant AngHV-1 load. This indicates that silvering itself is stressful and can induce a disease outbreak while the exhausting long distance migration from the Schlei fjord to the spawning ground in the Sargasso Sea (ca. 7500 km apart) did not even started. Virus infected eels showed a significant lower mean parasite load than non-infected eels which might be a result of virus induced lethargy and related lack of appetite (Hangalapura *et al.*, 2007; EFSA, 2008; Haenen *et al.*, 2009; Lepa and Siwicki, 2012; van Beurden *et al.*, 2012). Because foraging is impaired, the risk of consuming prey loaded with *A. crassus* larvae must be decreased. Evidence for that is provided by significant higher HSI values of non-infected eels which can be interpreted as a better nutritional status. Though non-infected eels showed higher parasitation intensities, no statistical effect on swim bladder was found. This was observed previously by Haenen *et al.* (2010) and might be interpreted as adaptation to the parasite.

Within virus positive eels a significant positive correlation between virus and *A. crassus* load was found. Parasite load was significantly lower at station Mouth compared to all other stations, and no clinically relevant virus load was observed while virus prevalence in eels of stages I (85 %), FII (78 %), and FIII (67 %) was high. In accordance all eels with a clinically relevant virus load were found at stations Upper and Lower fjord where virus prevalence and parasite load were highest (Table 10). Inconsistently, at station Middle fjord parasite load was not statistically different from stations Upper and Lower fjord but no eel showed a clinically relevant virus load which might be a consequence of lower virus prevalence (I, 8 %; FII, 57 %; FIII, 40 %). However, the general pattern of higher virus load at higher parasites loads was statistically verifiable. Since this was independent from individual length and a relevant

virus load is a result of stress, this pattern provides additional evidence that *A. crassus* is an important stressor for eels (e.g. Sures *et al.*, 2001), especially in conjunction with viruses as previously hypothesized (Haenen *et al.*, 2010; Haenen *et al.*, 2012). A basic assumption was that all ARS marked farmed eels in the Schlei fjord were infected with AngHV-1 (Table 7). In fact, only 9 out of 10 were found to be virus positive. It was previously shown that the *eel herpesvirus* can form a persistent stage in its host (van Nieuwstadt *et al.*, 2001) which is typical for members of the family Alloherpesviridae (Hanson *et al.*, 2016). But results of this study might enable the conclusion that either some individuals exhibit a natural resistance against infection or some individuals might fully recover from virus infection. This suggests that immunization of eels for stocking purposes against AngHV-1 using e.g. recombinant vaccine is a promising option, however, no such antiviral treatment is commercially available yet (Hanson *et al.*, 2016). To draw a conclusion for individuals identified as stocked glass eels (n = 2) is impossible because the initial health status is unknown.

This study is limited to deductions of prevalence and severity of *anguillid herpesvirus 1* infections in the Schlei fjord. But it is known that there are at least two other viruses – *Eel virus European* (EVE) and *Eel virus European X* (EVEX) – impacting the stock of the European eel and are especially important in aquaculture (van Ginneken *et al.*, 2005; EFSA, 2008; Haenen *et al.*, 2012; van Beurden *et al.*, 2012; ICES, 2016e). EVEX dramatically impairs endurance while EVE is related with serious renal damage (van Ginneken *et al.*, 2004; van Ginneken e*t al.*, 2005). Van Beurden *et al.* (2012) found evidence that eels from aquaculture often show double infections with AngHV-1 and EVEX or AngHV-1 and EVE which has to be taken into account for disease screenings prior to stocking. But also single infections with EVE and EVEX are possible, hence, 32 % of investigated eels in this study were negative for AngHV-1 but might be hosting other viruses.

The eel stock of the Schlei fjord was found to be heavily contaminated with AngHV-1 and that there is an obvious coherence with stocking activities. Although the effect of AngHV-1 on the ability of eels to spawn in the Sargasso Sea is still unknown, it is recommended to apply the precautionary approach and avoid stocking of diseased eels. Such an approach requires (i) an obligatory virus screening for glass and farmed eels prior to stocking including AngHV-1, EVE, and EVEX, (ii) a long-term diseases monitoring, and (iii) a stock-monitoring programme.

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Chapter V Bigger is better in eel stocking measures?

Bigger is better in eel stocking measures? Comparison of growth performance, body condition, and benefit-cost ratio of simultaneously stocked glass and farmed eels in a brackish fjord

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Abstract

The recruitment of the European eel stock has collapsed and the stock is in a perilous state compared to the reference period between 1960 and 1979. Despite extensive European Union wide stocking efforts towards a stock recovery and a self-reproducing stock, recruitment stagnates at historical low levels. The aim of this study was to compare the most commonly used stocking forms (glass and farmed eels) in terms of their growth performance, body condition, and benefit-cost ratio to test whether stocking efficiency can be increased by the choice of the stocking form. Therefore, glass eels (117 kg) and farmed eels (1040 kg) were purchased in a cost ratio of 1:1 and then marked chemically with alizarin red S prior to stocking in a brackish Baltic Sea fjord. Two years after stocking, farmed eels (374 ± 36 mm; 86.9 ± 25.8 g) showed a significantly higher total length (TL) and body weight (W) than stocked glass eels (323 ± 39 mm; 56.8 ± 25.0 g). Moreover, within age group 2, no statistically differ-

ences in the specific growth rates for length and weight were found between stocking forms indicating that the initial advantage in TL and W of farmed recruits is likely to persist. Derived from the recapture ratio, the mortality of age 2 glass eels was 3.9 times higher than in farmed eels indicating a higher benefit-cost ratio for farmed recruits to refill local eel stocks more efficiently. However, the farmed recruits in this study have been found to be infected with the *anguillid herpesvirus 1* which negates the conservation claim specified by the EU regulation.

Introduction

In 2003, eel specialists from all around the globe jointly published their findings that all three commercially most important eel species *Anguilla anguilla*, *A. rostrata*, and *A. japonica* revealed an obvious synchronic recruitment collapse (Québec Declaration of Concern, 2003) with minimum levels in the early 2010's (ICES, 2017c). The European Union recognized this dramatic trend and adopted a regulation in 2007 (EC, 2007) which requests its member states to establish eel management plans (EMP) and to take countermeasures that encompass the recovery of the stock of the European eel *A. anguilla*. A key objective of all EMPs is the sustainable attainment of a minimum silver eel escapement biomass of 40 % compared to estimated pristine levels. However, despite ten years of Europe-wide management the eel stock stagnates in a perilous state (Dekker, 2016; ICES, 2016d, 2017c). Hence management failed but also external factors (e.g. climate change, trophic interaction, depensation, and habitat loss) are suspected causes for the lack of recovery (Åström and Dekker, 2007).

In defiance of many uncertainties, a conservation measure of high relevance is 'stocking' which is also proposed by the EU. Natural recruits are caught and redistributed to waters

with current low recruitment. However, since the artificial eel seed production is still not feasible, this approach is entirely reliant on wild glass eels catches thus mortalities during catch and transport but also after stocking are highly relevant for the evaluation of management measures.

There is evidence that mortality of recruits can be density-dependant (Vøllestad and Jonsson, 1988; Leo and Gatto, 1996; ICES, 2000; Acou *et al.*, 2011; Bevacqua *et al.*, 2011; ICES, 2016e). The reallocation of natural eel recruits as a conservation measure, therefore, aims at the reduction of the natural mortality by redistribution of those eels that exceed the carrying capacity of the donor habitat. This means in general that eels are caught in waters with relative high natural recruitment (coast of Spain, Portugal, France, Great Britain) and transported to areas of current low abundances (e.g. Baltic Sea riparian states). ICES (2016e) recently defined that a net benefit of stocking measures exists, if this approach leads to a higher silver escapement biomass compared to a scenario, where no action has been taken. In this regard, Brämick *et al.* (2016) presented evidence that stocking is a key management tool to achieve the defined silver eel escapement target in a local inland river system with low natural recruitment at present. Furthermore, on a local level, evidence was found for a long-term net profit from of stocking measures (Wickström *et al.*, 1996). This is, however, still no evidence that the anthropogenic induced increase of a local silver eel biomass actually leads to an absolute increase for the panmictic European stock as whole (ICES, 2016e).

ICES (2016a) advised since 2000 that no fishery and since 2003 no anthropogenic mortality in general, should increase natural mortality and went further for 2017 to the advice that “all anthropogenic impacts” should be reduced to a minimum (ICES, 2017a). Stocking, however, is not an exclusively nature conservation measure but has also an economic aspect is thus also intended to enable the sustainable use of the eel stock (EC No 1100/2007). In order to

ensure that eel stocking is not an end in itself, e.g. ICES (2008) strongly recommends chemical marking of all stocked recruits to allow traceability through all life stages and thus estimations about the potential contribution of stocked recruits to future recruitment.

Notwithstanding the underlying objective of a stocking programme – rather stable fishing yields and/or number of potential spawners – the choice of the stocking material is crucial in any case. The most common stocking forms are glass eels (ca. 5.4 – 9.2 cm long young unpigmented recently caught for the purpose of stocking) and farmed eels (ca. 15 – 20 cm long elvers on-grown from glass eels in aquaculture facilities).

It was previously shown that smaller eels might be in advantage over larger eels because of better and continuous growth performances but also higher yields per recruit (Simon and Dörner, 2014; Pedersen and Rasmussen, 2016). However, most stocking studies were conducted either under inland, freshwater conditions only (Pedersen, 2000; Pedersen, 2009; Simon *et al.*, 2013a; Simon and Dörner, 2014), the small eels were farmed for several weeks as well before stocking (Pedersen, 2009; Pedersen and Rasmussen, 2016), or only one stocking form was investigated allowing only indirect comparison between studies (Wickström, 1986; Andersson *et al.*, 1991; Bisgaard and Pedersen, 1991; Pedersen, 1998, 2000). Moreover, the selection of the recipient habitat is also of major importance, whereby especially costal habitats revealed a high suitability as recipient water also because of lower parasite loads, higher growth rates, and better body conditions compared to eels in fresh water (Edeline *et al.*, 2005; Melià *et al.*, 2006; Lin *et al.*, 2007; Jakob *et al.*, 2009a; Marohn *et al.*, 2013; Simon *et al.*, 2013b).

The aim of this study was to compare simultaneously stocked glass and farmed eels in a brackish water system with regard to growth performance, body condition, and benefit-cost ratio after the first two years in the recipient brackish waterbody. Higher recapture rates for

farmed eels but better growth performances, and body conditions were hypothesized for glass eels.

Material and Methods

Study area

The study area was the Schlei fjord located in northern Germany which covers an area of 5460 ha (54.595976 °N, 9.852501 °E; Figure 17). It is a narrow brackish inlet of the Baltic Sea coast, which is characterized by a salinity gradient decreasing from ca. 18 - 20 at the opening (east) to ca. 3 – 5 at the innermost station (west). The mean water depth is ca. 2 – 3 m and the mean water temperature is between 11 and 12 °C (LANU, 2001). The mean Secchi depth during the winter is 0.9 and 1.5 m decreasing in summer times to 0.5 to 1.2 m, and eutrophic nitrogen (ammonia and nitrate) concentrations of > 1 mg L^{-1} can be found on average (LANU, 2001).

Stocking material, chemical marking, and health status

Between March 2015 and July 2016 a total of 117 kg of glass eels (approximately 351000 individuals) and 1040 kg of farmed eels (approximately 156000 individuals) were scattered all over the Schlei fjord by local fishermen (Table 11). This corresponds to a numerical proportion at date of stocking of roughly 2.3:1 (glass eels to farmed eels) and a purchase cost ratio of 1:1. This approach enables relative conclusions about the benefit-cost ratio, which would be equivalent at an approximately identical recapture frequency.

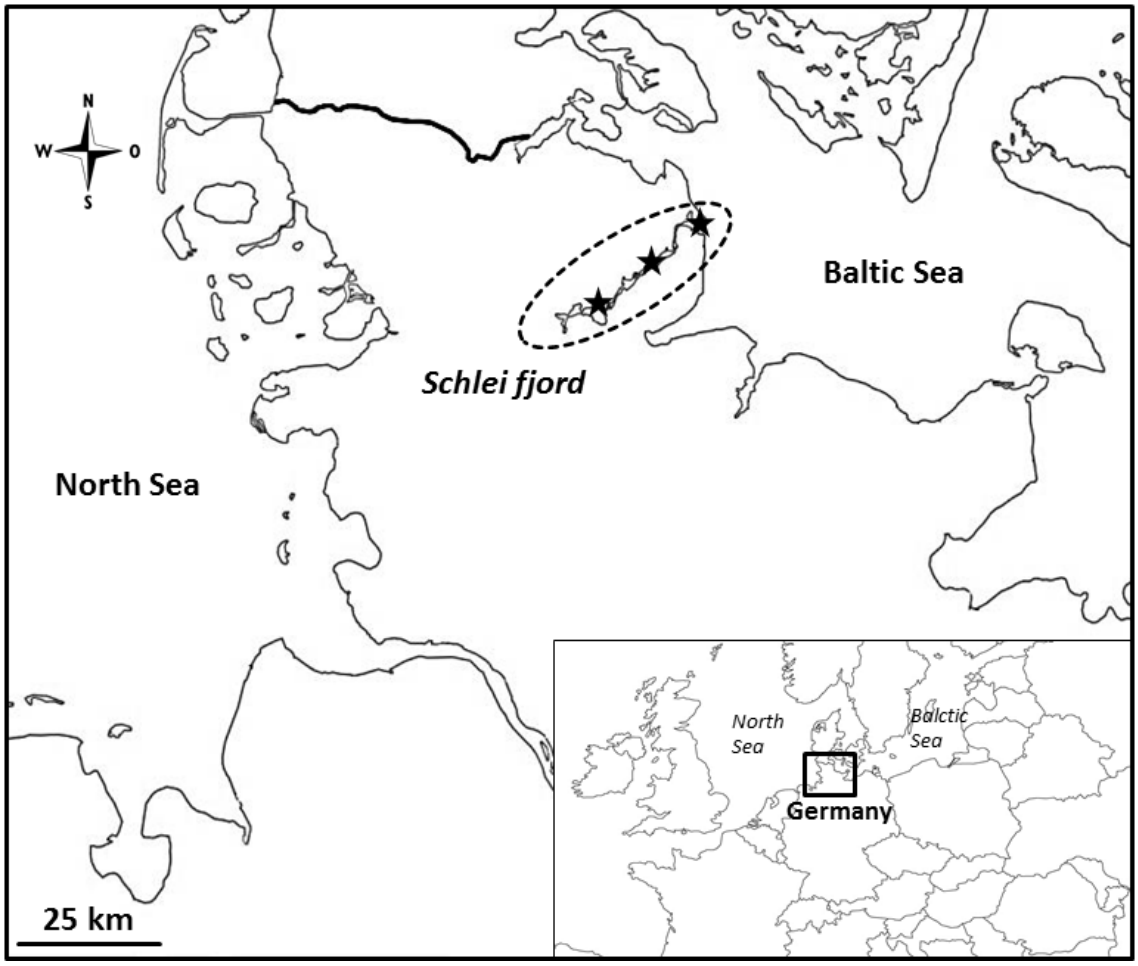

Figure 17 Overview map and location of the Schlei fjord at the western German Baltic Sea coast. The stars indicate approximate stocking and sampling locations within the Schlei fjord.

Table 11 Date, stocking form, total mass, mean total length (*TL*), mean body weight (W) and numbers of all known stocking measures with ARS marked eels in the Schlei fjord between 2015 and 2016.

Stocking date	Stocking form	Total mass (kg)	*TL* ± SD (mm)	W ± SD (g)	Numbers* (1×10^3)
13 March 2015	E_G	57	65 ± 3	0.22 ± 0.05	171
29 July 2015	E_F	500	183 ± 18	7.57 ± 2.74	75
14 April 2016	E_G	60	67 ± 3	0.24 ± 0.05	180
13 July 2016	E_F	540	157 ± 21	5.67 ± 1.78	81

E_G, stocked as glass eels; E_F, stocked as farmed eel; ARS, alizarin red S

* Assuming approximately 3000 glass eels (Tesch and Thrope, 2003) and 150 farmed eels (c.f. Angelidis *et al.,* 2012) per kilogramme

The glass eels were imported from England and the farmed eels have been raised in commercial eel farms, whereby used glass eels originated from France. Before stocking the entire stocking material was chemically marked with alizarin red S (ARS; Kullmann *et al.*, 2017b; Kullmann *et al.*, 2018). The marking induced mortality was consistently low (< 1.0 %) and marking success at 100 % throughout. Subsequently conducted virus screenings of the farmed stocking material in 2015 and 2016 revealed that both cohorts were found to be positive for the *anguillid herpesvirus 1* (Kullmann *et al.*, 2017a). The health status of stocked glass eels is not known at date of stocking but on-grown marked glass eels have been found to be infected with AngHV-1 (Kullmann *et al.* 2017a). Because an unknown amount of unmarked eels were simultaneously stocked in adjacent waters, unmarked eels could not be considered as natural recruits in any case.

Morphometrical measurements and otolith preparation

For this study a total number of 1005 eels were caught in 2016 and 2017 in the Schlei fjord at various stations (Figure 17; Table 12). Eels have been purchased from commercial longline fisheries and additionally fyke nets with a mesh-size of 5 mm at the cod end were operated to account for the low catchability of stocked glass eels in the first year after stocking (Bevacqua *et al.*, 2009). Eels were sacrificed and deep frozen at ca. -20 °C. After thawing the total length (TL) in mm, body weight (W) in g, and liver mass in g were measured and sagittal otoliths were removed by longitudinal dissections of the head, cleaned and stored in plastic tubes for further preparation. Sex was determined according to Tesch and Thorpe (2003). One otolith was used for cohort assignment by preparation according to the “crack and burn” protocol as recommended by ICES (2009c, 2011b). The annuli (winter rings) were counted and ageing reference date was the individual stocking date arising from the number

of annuli (Table 11). The age is presented as number of winter rings (annuli) or days post stocking (dps). For ARS mark detection, the second otolith was cracked on a transversal plane, embedded in thermoplastic wax with the cut surface down (Crystalbond, Buehler®) and ground to the primordium as described by Simon *et al.* (2013). These thin section preparations were checked for an ARS mark using a light microscope (Leica DM 2500) equipped with a light source (CoolLED *p*E-300-W) and a light filter for wavelengths between 530 and 580 nm. The ARS mark appears as glowing band that was defined as age zero. The ARS mark is identical to the 'zero band' in glass eels and discernable closer to the core than in farmed eels (Figure 18).

Calculation of body condition and growth performance

The body condition using Fulton's condition factor (K) and nutritional status using the hepatosomatic index (HSI) of the stocked recruits was described by calculating K and HSI after Ricker (1975) and Bolger and Connolly (1989) as follows:

$K = W \times TL^{-3} \times 100$

and

$HSI\ (\%) = LM \times W^{-1}$

Whereby TL is the total length in cm, W the body weight in g, and LM the liver mass in g.

The mean annual growth rate (MAG) was calculated as the total length or weight at catch minus total length or weight at date of stocking (Table 11) divided by the respective number of annuli.

Table 12 Investigated eels for this study caught in 2016 and 2017 in the Schlei fjord

Year	Sample size (n)	*TL* ± SD (mm)	*TL* range (mm)	W ± SD (g)	W range (g)	Sampling period
2016	580	336 ± 60	184 – 499	71.1 ± 42.0	9.4 – 253.7	02 June – 28 September
2017	425	374 ± 56	210 – 565	88.3 ± 37.6	12.6 – 247.3	12 May – 15 September

TL, total length; W, body weight; SD, standard deviation

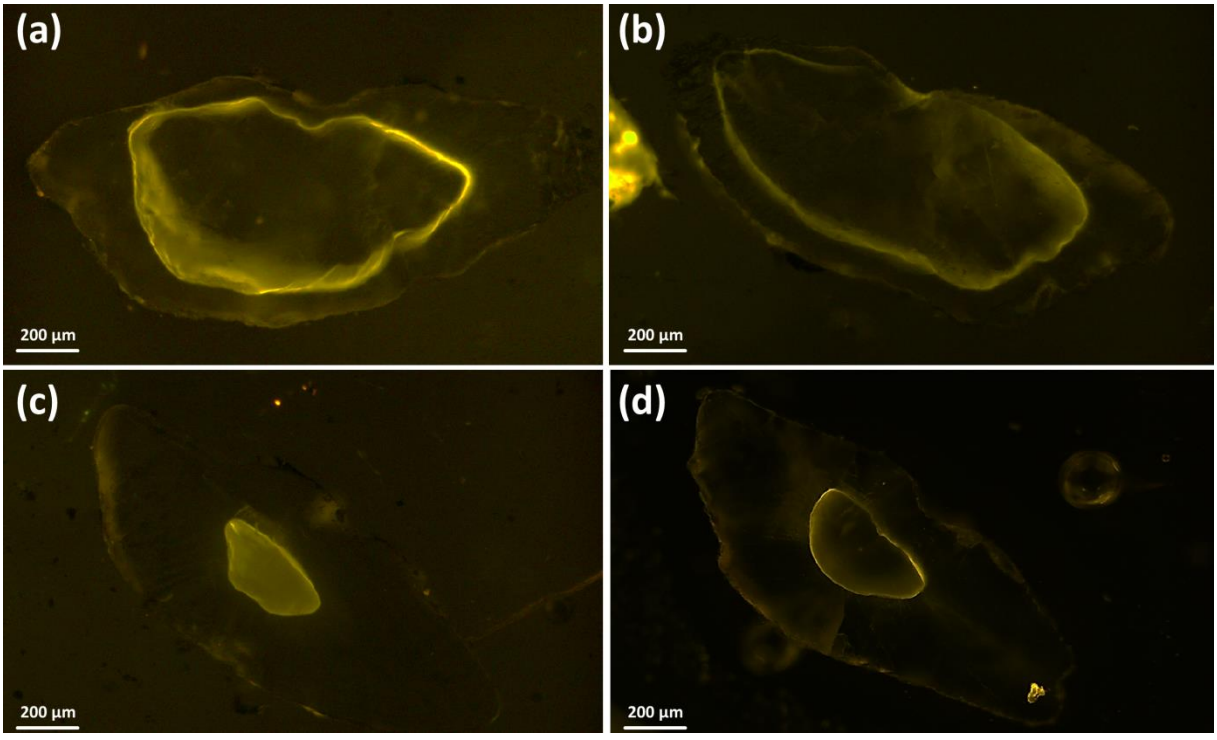

Figure 18 Examples of otolith thin section preparations of recaptured eels stocked as farmed eels (a and b) or as glass eels (c and d). The ARS ring in glass eels is conspicuous closer to the core than in farmed eels.

The specific growth rate (SGR) was separately estimated for glass and farmed eels as percentage increase of total length (SGR_L) per day (Busacker *et al.*, 1990; Simon *et al.*, 2013b; Lugert *et al.*, 2016) as follows to account for the different dates of stocking (i.e. glass eels in spring and farmed eels in summer):

$$SGR_L\ (\%\ day^{-1}) = \frac{ln(L_{@catch}) - ln(L_i)}{\Delta\ t}\ \text{x}\ 100$$

$L_{@catch}$ is the individual total length in mm at date of catch and L_i represents mean total length in mm at date of stocking (for re-captured eels at age = 1 annulus) or the mean total length in mm from recaptured eels in the previous year (for re-captured eels at age = 2 annuli). And Δt is the individual age in days post stocking (for age = 1 annulus) or the individual age in days post stocking minus the mean age in days post stocking from recaptured eels in the previous year (for age = 2 annuli). The *SGR* for the increase of the W (SGR_W) was estimated accordingly.

Additionally, the absolute growth rate (AGR) was calculated as individual daily increase of length (AGR_L) and body weight (AGR_W) per day (Lugert *et al.*, 2016) separately for each age group and stocking form as:

$$AGR_L\ (mm\ day^{-1}) = \frac{L_{@catch} - L_i}{\Delta t}$$

The AGR for weight (AGR_W) was estimated accordingly.

Statistical analysis

For the statistical analysis the software environment R was used (R Core Team, 2017). A significant difference between groups was considered when the probability of error was below 5 per cent ($\alpha < 0.05$). Data distribution was checked for normality using the Shapiro-Wilk test (SWT). Depending on the result homoscedasticity was verified by the Bartlett test (SWT, $P > 0.05$) or the Fligner-Killeen test (SWT, $P < 0.05$). Because assumption of homoscedasticity

was never met, the non-parametric Wilcoxon test for pairwise multiple comparisons (WTPMC) was used with the Bonferroni correction of the P-value to control type I error inflation (Abdi, 2007).

Results

Recaptured eels, relative mortality, and benefit-cost ratio

A total number of 169 ARS marked eels comprising 60 individuals stocked as glass eels and 109 individuals stocked as farmed eels have been recaptured in the Schlei fjord since 2016 (Table 13). This corresponds to a total numerical proportion of 1:1.8 (glass eels to farmed eels). Taking into account only eels in age group 1, the proportion changes to 1:2.0 (33 glass eels to 65 farmed eels) and to 1:1.6 (27 glass eels to 44 farmed eels) in age group 2. At a given initial ratio of 2.3:1, the mortality of age-2 glass eels was 3.9 times higher than in age-2 farmed eels. The recapture frequency for glass eels was thus far below the purchase cost threshold equivalent value of 50 % indicating a higher benefit-cost ratio for farmed recruits (Figure 19).

Sexual differentiation

In age group 1, 66.7 % (n = 22) of the individuals stocked as glass eels were undifferentiated, 33.3 % (n = 11) already differentiated to females and none into a male eel. One year later, 7.4 % (n = 2) of the recaptured glass eels were found to be still undifferentiated, the majority of 74.1 % (n = 20) developed into females and 18.5 % (n = 5) turned into males.

In farmed eels at the age of 1, 69.2 % (n = 45) of all recaptured individuals were undifferentiated, 30.8 % (n = 20) already differentiated into females and none into males. At the age of

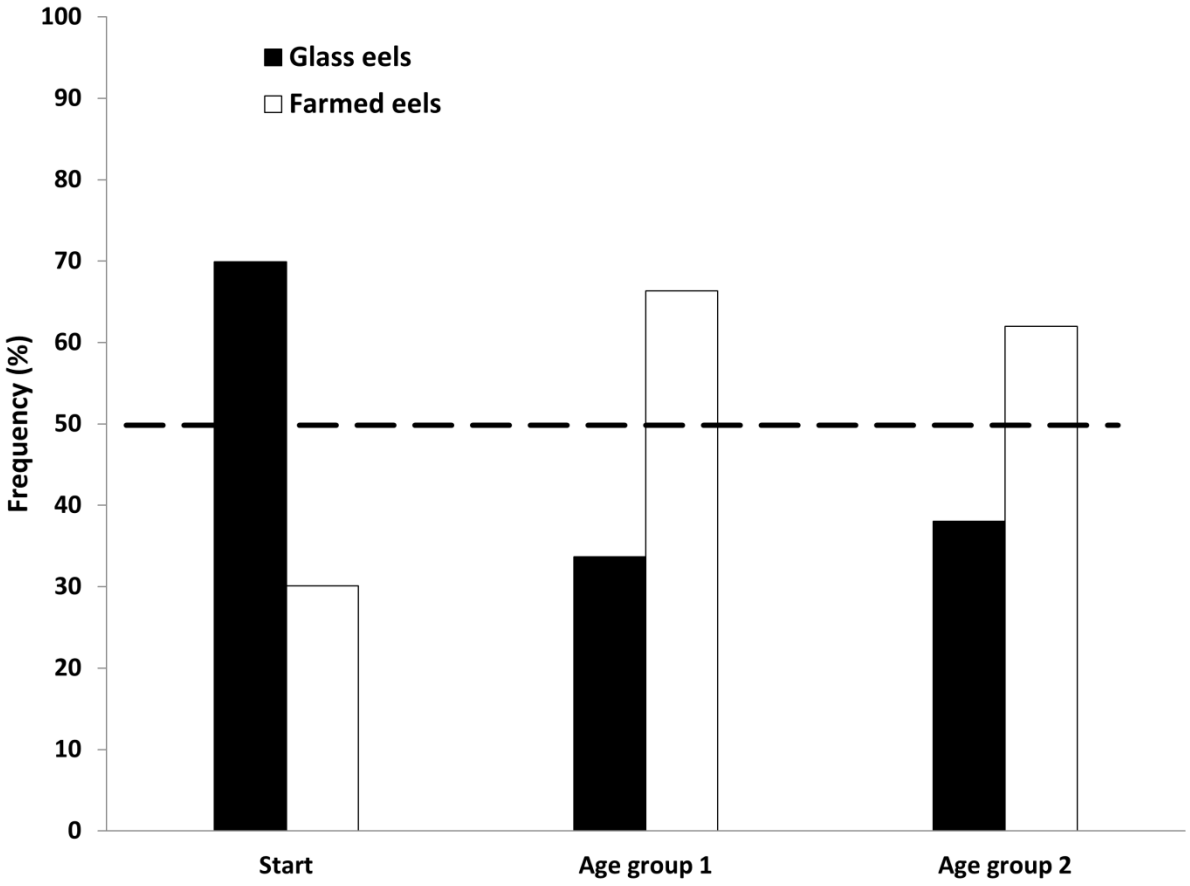

Figure 19 Frequency of glass and farmed eels at date of stocking (Start) and within recaptured sample at the age of 1 and 2. The dashed line indicates the purchase cost threshold equivalent value of 50%.

2, all recaptured farmed recruits have differentiated either into females (95.5 % (n = 42)) or males (4.5 % (n = 2)).

Body condition

Condition factor K was significantly lower in age-0 glass eels (K = 0.091 ± 0.012) than in same age farmed recruits (K = 0.120 ± 0.022; Table 13). Stocking forms in age group 1 (glass eels: 0.158 ± 0.013; farmed eels: 0.153 ± 0.031) and age group 2 (glass eels: K = 0.161 ± 0.026; farmed eels: K = 0.124 ± 0.060) did not differ significantly.

Table 13 Comparison of growth performance and body condition of recaptured eels stocked as glass or farmed eels in the first two years after stocking.

	Age group (n annuli)			
	Glass eels		Farmed eels	
	1	2	1	2
n	33	27	65	44
n sex (♂/♀/U)	(0/11/22)	(5/20/2)	(0/20/45)	(2/42/0)
Mean age (dps)	539 ± 17	886 ± 19	397 ± 20	731 ± 25
TL (mm)	$255 \pm 25^{a*}$	$323 \pm 39^{b**}$	$281 \pm 31^{y*}$	$374 \pm 36^{z**}$
W (g)	$27.2 \pm 9.2^{a*}$	$56.8 \pm 25.0^{b**}$	$36.7 \pm 13.3^{y*}$	$86.9 \pm 25.8^{z**}$
K	0.158 ± 0.013^{a}	0.161 ± 0.026^{b}	0.153 ± 0.031^{y}	0.124 ± 0.060^{z}
HSI (%)	1.682 ± 0.321^{a}	1.833 ± 0.402^{a}	1.873 ± 0.450^{y}	2.054 ± 0.433^{y}
SGR_L (% day $^{-1}$)	$0.248 \pm 0.022^{a*}$	0.067 ± 0.033^{b}	$0.130 \pm 0.032^{y*}$	0.085 ± 0.031^{z}
SGR_W (% day $^{-1}$)	$0.856 \pm 0.074^{a*}$	0.189 ± 0.108^{b}	$0.409 \pm 0.109^{y*}$	0.246 ± 0.091^{z}
AGR_L (mm day^{-1})	$0.349 \pm 0.052^{a*}$	0.197 ± 0.111^{b}	$0.281 \pm 0.113^{y*}$	0.134 ± 0.053^{z}
AGR_W (g day^{-1})	$0.050 \pm 0.018^{a*}$	$0.064 \pm 0.028^{a**}$	$0.076 \pm 0.035^{y*}$	$0.151 \pm 0.076^{z**}$

n, number; TL, total length; SD, standard deviation; W, body weight; SGRL, specific growth rate for the total length; SGRW, specific growth rate for the body weight; AGRL, absolute growth rate for the total length; AGRW, absolute growth rate for the body weight; K, condition factor; HSI, hepatosomatic index; ♂, male; ♀, female; U, undifferentiated; dps, days post stocking; §, mean across all years; $^{a\text{-}b}$, different subscript letters indicate significantly differences within glass eels separately for each parameter (WTPMC, $P < 0.001$) ;$^{y\text{-}z}$, different subscript letters indicate significantly differences within farmed eels separately for each parameter (WTPMC, $P < 0.001$). *Indicates significant differences between stocking forms for comparisons within age group 1 (WTPMC, $P < 0.001$); **Indicates significant differences between stocking forms for comparisons within age group 2 (WTPMC, $P < 0.001$).

The HSI of glass eels (age-1: 1.682 ± 0.321 %; age-2: 1.833 ± 0.402 %) and farmed eels (age-1: 1.873 ± 0.450 %; age-2: 2.054 ± 0.433 %) was not significantly different within stocking forms or between respective age groups (Table 13).

Body condition

Condition factor K was significantly lower in age-0 glass eels (K = 0.091 ± 0.012) than in same age farmed recruits (K = 0.120 ± 0.022; Table 13). Stocking forms in age group 1 (glass eels: 0.158 ± 0.013; farmed eels: 0.153 ± 0.031) and age group 2 (glass eels: K = 0.161 ± 0.026; farmed eels: K = 0.124 ± 0.060) did not differ significantly.

The HSI of glass eels (age-1: 1.682 ± 0.321 %; age-2: 1.833 ± 0.402 %) and farmed eels (age-1: 1.873 ± 0.450 %; age-2: 2.054 ± 0.433 %) was not significantly different within stocking forms or between respective age groups (Table 13).

Absolute and annual increment of length and weight

At date of stocking, glass eels showed a mean TL and W of 66 ± 4 mm and 0.2 ± 0.1 g. TL increased significantly after one and two years at liberty to 255 ± 25 mm and 323 ± 39 mm, respectively (Figure 20a). This equals a mean annual growth (MAG) rate of 188 ± 26 mm and 128 ± 19 mm in the first and second year after stocking (Figure 22a). The body weight increased significantly to 27.2 ± 9.2 g (age = 1) and further to 323 ± 39 g (age = 2)(Figure 21 a) which corresponds to a MAG rate of 26.9 ± 9.2 g and 28.3 ± 12.5 g $year^{-1}$, respectively (Figure 22b).

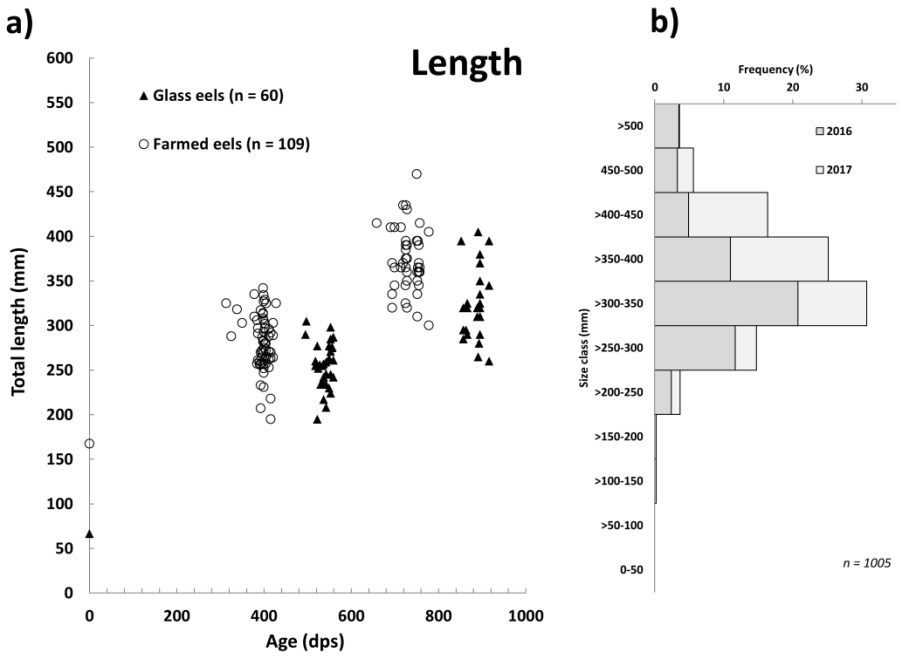

Figure 20 a) The absolute length increment of stocked glass and farmed eels in the Schlei fjord whereby the age is presented in days post stocking (dps). b) The coverage and frequency of the investigated length range in the whole sample.

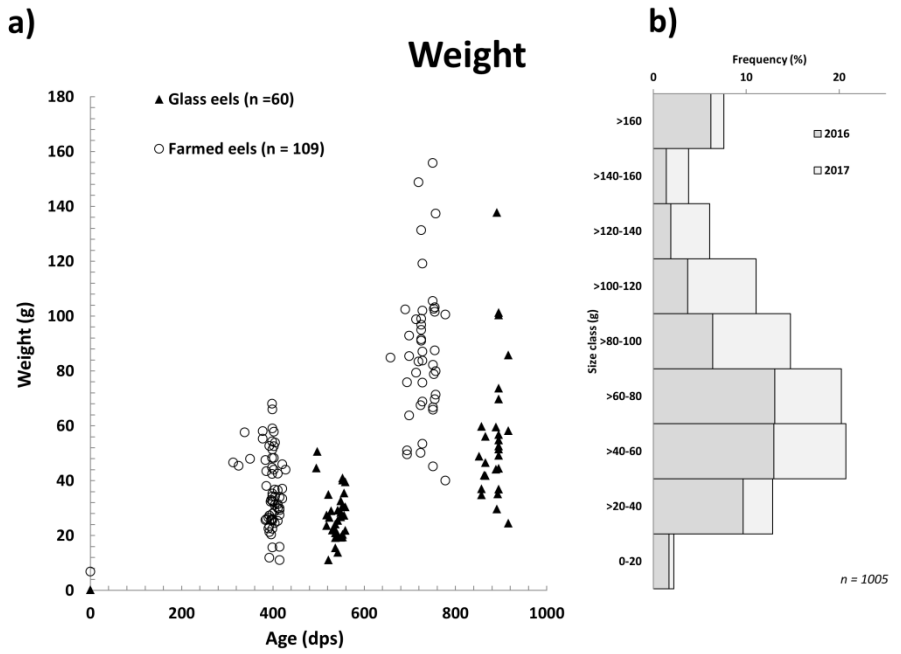

Figure 21 a) The absolute weight increment of stocked glass and farmed eels in the Schlei fjord whereby the age is presented in days post stocking (dps). b) The coverage and frequency of the investigated weight range in the whole sample.

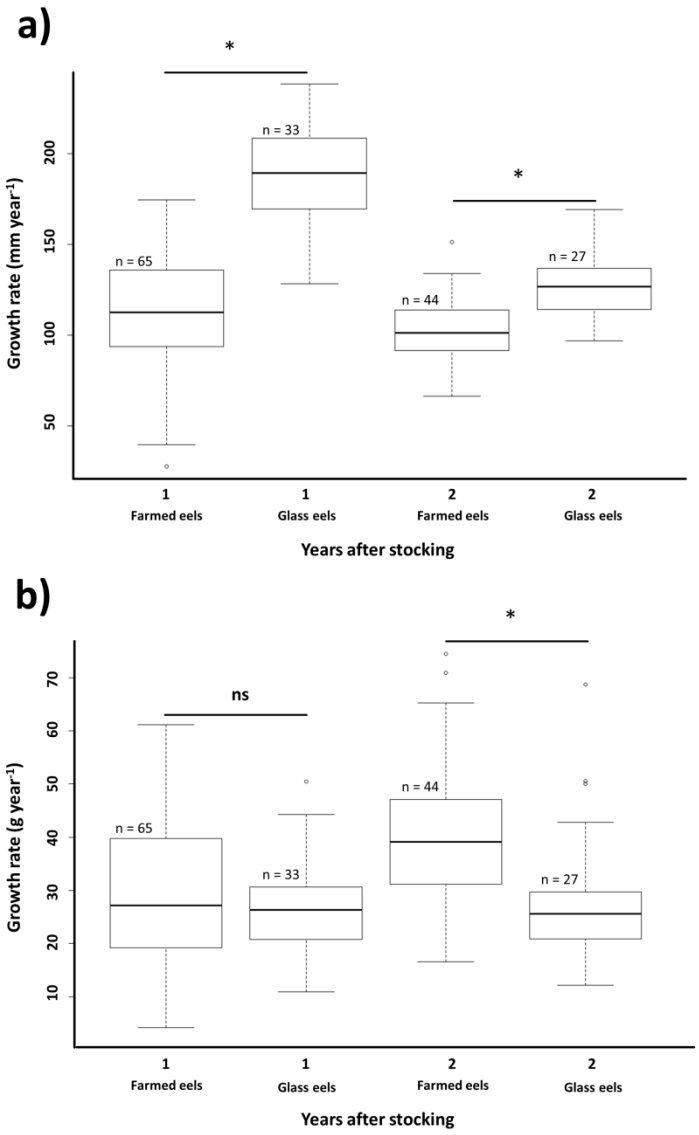

Figure 22 Annual growth rates for length (a) and weight (b) for the recaptured glass and farmed eels per age group. Stars indicate significant differences (WTPMC, $P < 0.001$). ns, not significant.

The TL and W of the farmed recruits at age-0 were 170 ± 16 mm and 6.3 ± 1.9 g, respectively. Recaptured farmed eels showed significantly increased means of 281 ± 31 mm (MAG: 114 ± 31 mm $year^{-1}$) and 374 ± 36 mm (MAG: 103 ± 18 mm $year^{-1}$) in TL, and 36.7 ± 13.3 g (MAG: 29.8 ± 13.3 g $year^{-1}$) and 86.9 ± 25.8 g (MAG: 40.0 ± 12.9 g $year^{-1}$) in body weight after one and two years of growth, respectively.

At date of stocking, glass and farmed eels differed significantly in terms of both TL and W (Table 13). After one and two year at liberty, such significant differences persisted in TL and W (Table 13; c.f. Figure 20a Figure 21a). The MAG for length, however, was significantly higher for glass eels after one and two years after stocking (Figure 22a). No differences were found in the MAG for weight in the first year after stocking but it was significantly higher in farmed recruits within age group 2 (Figure 22b).

Specific growth rate

The SGR in glass eels was 0.248 ± 0.022 % day^{-1} for length and 0.856 ± 0.074 % day^{-1} for body weight in the first year and at a significantly decreased rate of 0.067 ± 0.033 % day^{-1} (SGR_L) and 0.189 ± 0.108 % day^{-1} (SGR_W) in the second year (Table 13). Age-1 farmed eels showed a SGR_L of 0.130 ± 0.032 % day^{-1} and a SGR_W of 0.409 ± 0.109 % day^{-1}. In the second year, the SGR of the farmed recruits significantly decreased for TL and W (SGR_L = 0.085 ± 0.031 % day^{-1}; SGR_W = 0.246 ± 0.091 % day^{-1}). In general, the SGR for length and weight was significantly higher for glass eels in the first year, whereas age-2 glass and farmed eels did not differ significantly in SGR (Table 13).

Absolute growth rate

Age-1 glass eels grew at an AGR of 0.349 ± 0.052 mm day^{-1} for length and 0.050 ± 0.018 g day^{-1} for weight while age-2 glass eels showed a significantly different increment of 0.197 ± 0.111 mm day^{-1} and 0.064 ± 0.028 g day^{-1} (Table 13). Respective same-age farmed eels showed rates of 0.281 ± 0.113 mm day^{-1} and 0.076 ± 0.035 g day^{-1} (age 1), and 0.134 ± 0.053 mm day^{-1} by 0.151 ± 0.076 g day^{-1} (age 2), respectively. The AGR for length was significantly higher in age-1 glass eels, while no differences were found between stocking forms in age

group 2. Recaptured farmed eels in age group 1 and 2 showed a significantly higher AGR for weight compared to respective same age group of glass eels (Table 13).

Discussion

This study was conducted to evaluate whether the choice of the stocking form might help to improve stocking measures to meet EU management objectives. Therefore, simultaneously stocked glass eels and farmed eels have been compared concerning their growth rates, body condition, and benefit-cost ratio.

Summarising, the present findings revealed that glass eels grew significantly faster in the first years after stocking, whereby the initially higher length and weight of farmed eels persisted (Table 13). Glass eels have lost their disadvantage over farmed eels in terms of the body condition (condition factor K) at the age of 1. The specific growth rates (length and weight) did not differ in age group 2 while the mean annual growth rate for body weight was significantly higher in age-2 farmed eels (Figure 22b). Farmed recruits have been recaptured more abundantly than glass eels indicating a higher benefit-cost ratio for pre-grown recruits (c.f. Figure 19, Figure 20a and b).

The use of a size selective fishing gear (i.e. fyke nets with 5 mm mesh size at the cod end) was unavoidable because the brackish water conditions in the Schlei fjord have excluded non-selective sampling methods such as electrofishing. Therefore, the sample was limited to eels equal or superior to 184 mm (Table 12) which have biased the presented growth of glass eels in the first year towards fast growing individuals. In contrast, the catchability of farmed eels was high shortly after stocking and glass eels at the age of 2 were acceptable represented in the sample (c.f. Figure 20a and b; c.f. Figure 21a and b). Therefore, reliable comparative conclusions about the relative mortality and growth performances referred on-

ly to age-2 individuals. Of importance for stocking programmes is that sexual differentiation in eels is known to be metagamic i.e. influenced by external factors like, especially, the population density (Davey and Jellyman, 2005). As experimentally shown, high densities in eel farms favour the development of males because their initial growth rate is higher than in females, which is a crucial advantage over conspecifics under these unnaturally high-density conditions (Holmgren and Mosegaard, 1996). The use of larger farmed eels (TL of 27 – 33 cm) for stocking purposes will lead to strongly skewed sex ratios because eels of this size class from farms have already been differentiated to males while smaller recruits of 15 to 26 cm TL still can develop also into females (Pedersen, 1998). In the present study, 95.5 % of all farmed eels at age 2 have differentiated into females while 18.4 % of the age-2 glass eels have also developed into males (c.f. Table 13). In this regard, indications have been found that coastal and estuarine habitats are more important growth habitats for males but at low percentages between 5 and 12 % of the population (Daverat and Tomas, 2006). Both investigated stocking forms have been found to match approximately these male development rates for near shore waters and are thus unlikely to interfere with the natural habitat specific sex ratio.

At the coast of Denmark and Sweden, stocked on-grown eels at the age of 2 and 3 have been observed at TL of 346 ± 36 mm (Pedersen, 1998) and 310 ± 13 mm to 357 ± 30 mm (Anderson *et al.*, 1991), hence comparable to the growth observed in the Schlei fjord. A stocking experiment in isolated lakes has revealed that age-2 glass eels showed total length between 119 ± 9 mm and 215 ± 18 mm, whereas same age farmed individuals showed a significantly higher total length of between 151 ± 19 and 278 ± 15 mm (Simon *et al.*, 2013a). There, in contrast to stocked glass eels, a discontinuous growth pattern was observed in farmed recruits, also explained by a lag phase in adaptation to natural prey. Moreover, after 4 years

stocked glass and farmed eels did not differ in total length any more, whereby farmed recruits occasionally showed negative growth (Simon *et al.*, 2013b). The present findings could not confirm such an unsteady growth performance for any group. Specific growth calculations instead revealed a synchronically growth pattern after two years at liberty which indicates that differences are likely to persist over time. The mean annual growth rate for length was consistently significantly higher for glass eels (Figure 22a), however, the MAG is biased towards an overestimation of the growth performance of glass eels, because of the time interval (ca. 142 days) between stocking of glass eels in spring and farmed eels in the summer (Table 11 and Table 13). The growth phase of glass eels was therefore considerable longer, whereby the SGR and AGR describe the growth performance more precisely on a daily basis which revealed that stocking forms differed significantly only in terms of AGR_W at the age of 2 (Table 13). This leads to the conclusion that there might be an advantage of farmed eels supported by the significantly higher MAG for weight of farmed recruits in age group 2 though likewise biased towards glass eels (Figure 22b).

Previously, smaller stocked eels have consistently been found to show higher survival rates compared to larger eels both farmed and wild (Simon *et al.*, 2013a; Simon and Dörner, 2014; Pedersen and Rasmussen, 2016; Dainys *et al.*, 2017). In the present study, the proportion of glass to farmed eels was approximately 2.3:1 and corresponded to a purchase cost ratio of 1:1 at date of stocking. If the mortality was equal in both groups, the approximately same ratio of 2.3:1 should be found in the sample (c.f. Figure 19). The ratio was found to be 1:1.6 (glass to farmed eels) in age group 2 which allows the conclusion that the relative mortality in glass eels was about 3.9 times higher than in farmed eels making on-grown eels more valuable in terms of yield expectations (c.f. Table 13). In contrast, Simon and Dörner (2014) observed substantially higher mortalities in farmed recruits compared to stocked glass eels and

speculated that pre-grown eels might be of low quality and might lack natural foraging behaviour (e.g. Huntingford and Adams, 2005). Additionally, yield per recruit calculations from a Danish fjord and experimental feeding approaches demonstrated advantages in survival of smaller farmed recruits (Pedersen and Rasmussen, 2016) and glass eels (Dainys *et al.*, 2017) over larger farmed eels. The findings of this study, however, indicate that the use of 6 - 8 g farmed eels in stocking measures might allow a more efficient use of financial resources compared to glass eels because more recruits have been generated per financial unit. This, on the one hand, is particularly relevant for the EU request of a sustainable use of the stock. On the other hand, the farmed stocking cohorts have been found to be positive for the persistent *eel herpesvirus* (AngHV-1; Kullmann *et al.*, 2017a) which contradicts the aim of long-term stock recovery because infected eels are unlikely to contribute to future recruitment (Haenen *et al.*, 2009; Haenen *et al.*, 2010; Haenen *et al.*, 2012). Glass eels, however, can also show various virus infections (van Beurden *et al.*, 2012) and, additionally, the risk of contagion after stocking is high because AngHV-1 is transferable by bath immersion (Hangalpura *et al.* 2007). Therefore, recaptured glass eels in the Schlei fjord can be considered as virus afflicted as well (c.f. Kullmann *et al.* 2017a) and the observed higher mortality might be explained by an elevated vulnerability of glass eels to subsequent AngHV-1 infections. Farmed recruits in turn are known to be less vulnerable for a repeated disease outbreak which is the reason for deliberate AngHV-1 infections of recruits in commercial eel farms (EFSA 2008).

This study is limited to comparative deductions about two stocking forms. Whether stocking leads to a net benefit (ICES, 2016e) for the stock as a whole remains an open question e.g. because the mortality during catch, transport, and the farming process for farmed and glass eels have been neglected due to unavailable information. It would be necessary, therefore, to monitor the entire supply chain in order to draw valid conclusions about a potential high-

er reproductive fitness of the whole stock. In this regard, the chemical marking of stocked recruits (Simon and Dörner, 2005; Caraguel *et al.*, 2015; Kullmann *et al.*, 2017b; Kullmann *et al.*, 2018) should be mandatory because fitness of hatchery reared fish might be decreased (e.g. salmonids often show a decreased predator avoidance behaviour; e.g. Einum and Fleming, 2001). Moreover, marking of stocked recruits will create a comprehensive data basis for multi-national research collaborations to address both the wide geographic distribution and the pronounced phenotypic plasticity of the European eel.

Summarising, after two years at liberty glass and farmed eels did not differ significantly in terms of specific growth rates but the initial significantly lead in total length and weight of farmed recruits was found to be persistent. In contrast to previous studies, relative survival was higher in farmed compared to glass eels even though the latter have been stocked in spring, hence the expected optimal time in the year (ICES, 2016e). Therefore, stocking efficiency might be increasable by stocking farmed eels later in the year during summer times. This deduction, however, is restricted to the use of eels which have undergone comprehensive disease screenings prior to stocking in order to avoid anthropogenic spreading of diseases.

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General Discussion

Benefit from this study for monitoring and stock assessment

Stocking measures have been proposed by the EU in order to achieve a stock recovery and the sustainable use of eels in EU waters (EC No 1100/2007) and at least 16 Member States have implemented stocking of eels within the framework of eel management plans (ICES, 2013). While evidence was presented that stocking can be essential to sustain a certain level of silver eel escapement on a local level in inland waters and to support the eel fishery (Righton and Walker, 2013; Brämick *et al.*, 2016) a proof of concept with respect to the conservation character of stocking has not yet been ascertained (e.g. Dekker and Beaulaton, 2016a). A major obstacle concerning the comprehensive evaluation of a possible net benefit from reallocating natural recruits into waters with currently low natural recruitment (referred to as stocking) was the long-term traceability of stocked recruits and the ease of detectability (ICES, 2016e). Hence, marking of stocked recruits is necessary, and, due to the extraordinary longevity of eels (e.g. Tesch and Thorpe, 2003), a decisive reason for the choice of a particular marker must be its durability. In this regard, only the chemical mass-marking of otoliths appears to be reasonable because these structures are considered as closed chemical systems showing negligible post-formational degeneration processes (Campana *et al.*, 1993; Campana, 1999). While previous studies have demonstrated the basic suitability of various chemical markers (e.g. oxytertracycline: Alcobendas et al., 1991; alizarin red S: Simon and Dörner, 2005; Strontium chloride: Wickström and Sjöberg, 2014) the description of actually handling large quantities of both glass and farmed eels has not been considered so far. Even though one study has already dealt with mass-marking of glass eels using alizarin red S (ARS), only water parameters have been described in detail, whereas handling details have

not been provided (Caraguel *et al*., 2015). A main objective of the present thesis was to establish efficient mass-marking procedures and, therefore, to make a contribution to a data basis that enables reliable conclusions about the effect of eel stocking on the reproductive fitness of the entire stock. A milestone towards the life-time traceability of whole stocking cohorts was the description of efficient chemical mass-marking procedures for the most commonly used stocking forms, that are glass and farmed eels, using ARS and, for glass eels only, also ARS in combination with strontium chloride ($SrCl_2$) (**Chapter I and II**). In particular, details about stocking densities, the used equipment (e.g. knotless nets) and tanks (e.g. fully drainable) have been provided in order to give guidance for the large-scale application.

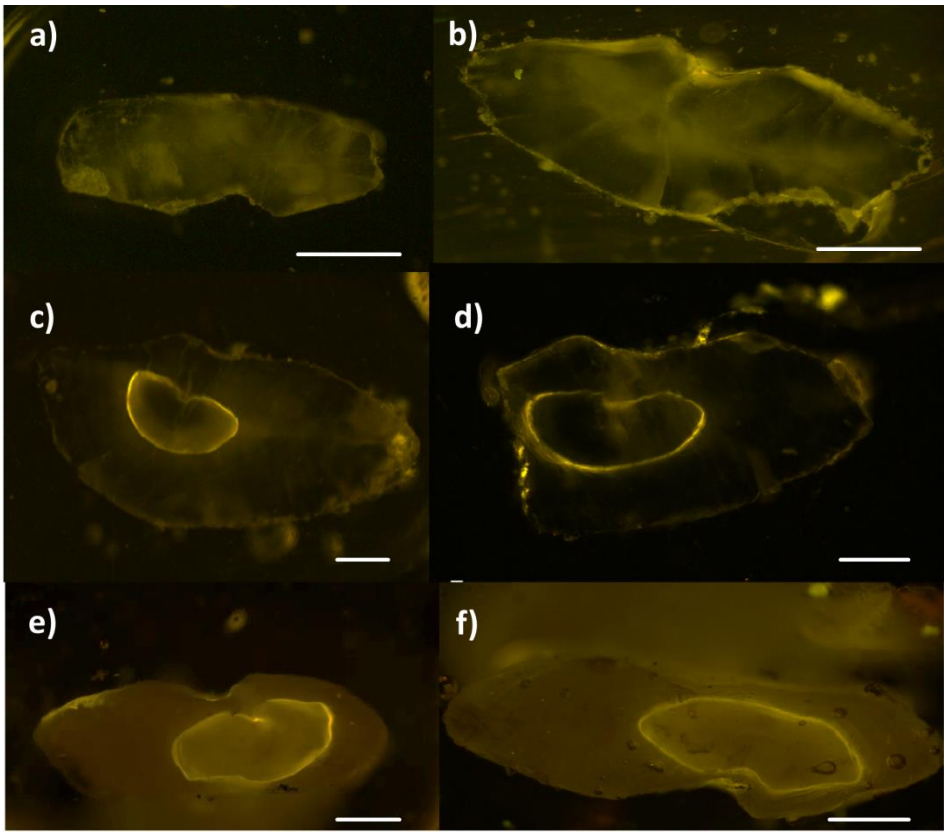

Figure 23 The cutsurface of unground *A. anguilla* otoliths showing no mark (a and b) and with clear alizarin red S marks (c - f). The position of the mark allows the dicrimination between marked glass (c and d) and farmd eels (e and f). All photographs have been taken under identical fluorescence microscope settings. The white bar equals 200 µm.

Additionally, information about the observed instantaneous mortality rates have been provided (**Chapter I:** Table 1 and Table 2**; Chapter II:** Table 4) which represent, apart from marking success, also crucial data for the assessment of a possible net benefit.

The detectability of marks deserves special attention since large-scale monitoring programmes, by definition, have to handle large numbers of eels. In this regard, for the detection of ARS, thin section preparation of sagitta otoliths was necessary (e.g. Simon *et al.*, 2013; Figure 18), which is a very time consuming process. This was also the case for $SrCl_2$ whereby additional technical effort is required by electron microscopically measurements (Wickström and Sjöberg, 2014). Substantial progress has been made by showing that ARS marks are visible on transversally cut otoliths without any further grinding or polishing effort (**Chapter III**; Figure 9). Furthermore, also the discrimination of the two stocking forms was shown to be possible without any further preparation (Figure 23), hence ARS has been identified as the optimal option for mass-marking of stocking cohorts and, importantly, ARS detection has now been made a practicable task for large-scale monitoring programmes such as the EU Data Collection Framework (DCF).

In addition, it has been demonstrated that the chemical marking of farmed recruits is even necessary in order to avoid an age reading error (**Chapter III**). It is indeed well known that ageing of 'cultured eels' (i.e. raised in aquaculture facilities) could be strongly biased by supernumerary zones (Deelder, 1981). Especially stress during the farming process, e.g. from size grading's (Kamstra, 1993; Angelidis *et al.*, 2012), is known to cause annuli-like rings on otoliths (Simon *et al.*, 2017). The present study, however, was the very first (i) to show that these farming-related stress rings cannot be distinguished from true annuli in blind readings and (ii) to quantify this bias using ARS marked otoliths from recaptured farmed eels. The further presented calculations are certainly valid for the available data set only. However, a

more universally valid conclusion is that the massive stocking of unmarked farmed eels, e.g. in German inland water bodies (Brämick *et al*., 2016), is likely to introduce significant bias in age-length keys (c.f. Figure 13a) and, therefore, impedes a reliable stock assessment particularly if escapement models fundamentally rely on the conversion from age to length and then to biomass via length-weight relationships.

Open questions concerning the chemical marking and future directive

The chemical marking process must be as harmless as possible and a low instantaneous mortality rate (i.e. of below 1 % of the total biomass) has been defined as target criterion (**Chapter II**). However, also delayed effects on survival have to be considered hence a random subsample including a control group must be held back for approximately two (glass eels) or eight (farmed eels) weeks under controlled conditions to assess (i) the marking success and (ii) potential short-term mortality. A considerable gap of knowledge exists concerning possible sub-lethal long-term effects that might affect marked recruits which is especially relevant in the context of conservation. On the one hand, Simon *et al*. (2009) observed ARS marked and unmarked glass eels over 192 days and found no statistically significant difference in survival or growth. On the other hand, mortality and length increment are not the only criteria for harmlessness. For instance, chronic sublethal effects on yolk sac larvae and eggs of Baltic cod *Gadus morhua* Linnaeus 1758 were found after exposure to alizarin complexone (Meyer *et al.*, 2012). Moreover, it is still unclear if chemicals such as ARS might accumulate in the liver, brain, muscle tissue or elsewhere in eels apart from the calcified structures potentially impacting the spawning migration or final maturation in the Sargasso Sea. However, since published studies on post marking effects found hardly any negative effect of the ARS treatment on growth or survival (**Chapter I**; Caraguel *et al.*, 2015; Josset *et al.*, 2016), ARS

can be considered as the best studied marker and is the most promising option for the development of a EU-wide harmonized mass-marking protocol. By contrast, mass-marking of eels with OTC was shown to be practicable (Alcobendas *et al.*, 1991), however, this substance is an antibiotic and therefore unrecommendable for standard mass-marking protocols within eel management plans particularly because of the risk of creating multi-resistant germs (e.g. Zheng and Zhou, 1999). Warren-Myres *et al*. (2018) recommend the use of $SrCl_2$ for standard mass-marking although the detection is comparably laborious and technically demanding.

Recommendations for alizarin red S standard mass-marking

The Working Group on Eels (WGEEL) of the International Council for the Exploration of the Sea (ICES) has repeatedly identified the marking of stocked recruits as crucial for the evaluation of a net benefit from stocking measures (e.g. ICES, 2009a, 2011a, 2016e). The use of ARS is hereby proposed for the implementation of a multinational research approach to establish a comprehensive data basis of stocked eels to assess their contribution to future recruitment. The following recommendations could be considered for the development of a harmonized mass-marking protocol that might be integrated in eel management plans or large-scale monitoring programmes:

i. Glass and farmed eels should be exposed 3 and 9 h to ARS, respectively. A concentration of 150 mg L^{-1} was repeatedly shown to result in clear marks on otoliths.
ii. Fully drainable tanks are recommended to reduce handling as much as possible during the separation of eels from the marking solution. The use of nets should be reduced as much as possible and knotless material should be preferred.

iii. The determination of mortality (e.g. in percent of the total biomass) after transport, after the marking process, and after transport to the stocking site is proposed in order to identify critical interim steps.

iv. In order to avoid additional stress (e.g. gas bubble diseases from O_2 hypersaturation; Espmark *et al.*, 2010; Angelidis, 2011) the marking solution should be aerated rather than oxygenated.

v. For the assessment of the marking success a randomly taken representative sample (including a control group) should be held back for not less than 14 days and 60 days for glass and farmed individuals, respectively.

After recapture of marked individuals, the separation of stocking cohorts, which have been marked identically, is only possible by counting annuli on cracked and burnt otoliths (ICES, 2009c, 2011b). This might be improvable by combining ARS with other markers such as $SrCl_2$ and $BaCl_2$ (**Chapter I**; Wickström and Sjöberg, 2014). An alternating marking cycle might be used to mark e.g. three consecutive stocking cohorts uniquely (Figure 24). To account for the technical demanding $SrCl_2$ and $BaCl_2$ detection, all cohorts could be marked with ARS first to allow the quick separation of marked from unmarked eels, hence only a much smaller subsample would have been checked for an additional mark.

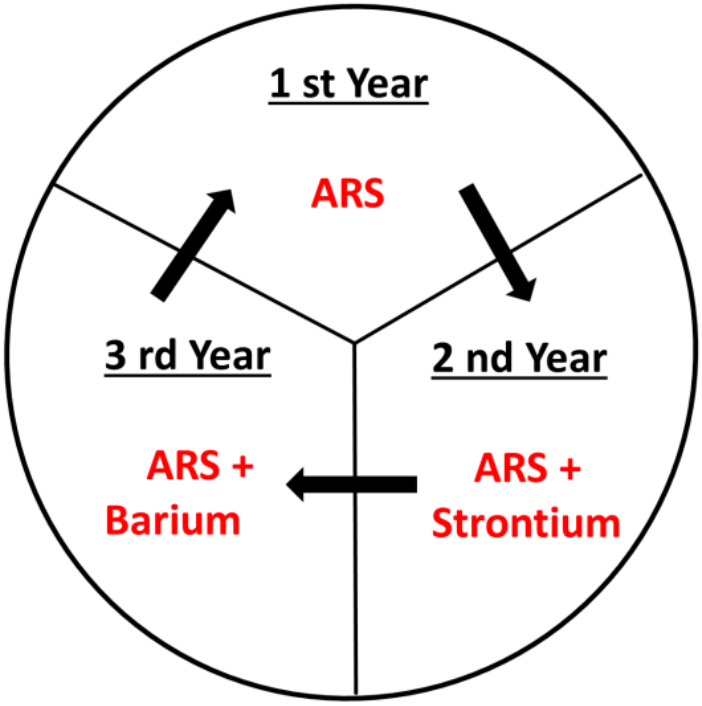

Figure 24 Alternating chemical marking scheme with ARS (alizarin red S), Barium and Strontium chloride in order to improve cohort assignment and ageing related growth estimations.

Spreading of diseases and biosecurity

The biosecurity is an important but oftentimes neglected part of stocking measures (e.g. Cowx, 1999; Haenen *et al.*, 2012). For instance, the swim bladder parasite *Anguillicola crassus* Kuwahara, Niimi & Itagaki 1974, originally native to Asia and hosted by the Japanese eel only, has spread over Europe in the early 1980's and a coherence with live eel imports and stocking measures was hypothesised soon (Peters and Hartmann, 1986). By contrast, viruses such as the *Eel virus European* (EVE), *Eel virus European X* (EVEX), and the a*nguillid herpesvirus-1* (AngHV-1) are known to be native to wild European eel stocks (Bandín *et al.*, 2014). However, these viruses play an important role in eel aquaculture (Haenen *et al.*, 2002) and the deliberate infection of newly arrived glass eels in eel farms (EFSA, 2008; ICES, 2009a) is common and misleadingly called "vaccination" while treated eels are in fact disease-carriers.

The anthropogenic-induced dissemination of viruses has been assumed as a certainty (ICES, 2009a), however, the first direct evidence that stocking measures with on-grown eels from aquaculture facilities actually contribute to the spread of disease was shown in this study for the first time (**Chapter IV**).

Sweden, in the matter of disease containment, has proven to be a good example with a rigorous implementation of conservation goals. In 2017, a total of 965 kg of glass eels – approximately 3 million individuals – were imported from France into a Swedish quarantine facility and were found to be positive for the virus EVEX (ICES, 2017e). Instead of being used for stocking purposes, the whole batch has been destroyed. It might be counter-intuitive why this is in accordance with a species protection plan in the first place. However, EVEX is suspected to play an important role in the recruitment decline because it seriously impairs swimming endurance of infected silver eels (van Ginneken *et al.*, 2005). EVEX was found in eel batches for the purposes of stocking (van Ginneken *et al.*, 2004), hence the high prevalence across Europe is potentially explained by uncontrolled stocking measures. Also other viruses such as AngHV-1 (**Chapter IV**) and EVE are known to be infectious diseases that can be found on a regular basis in eels from aquaculture facilities but as well in newly arriving glass eels (van Beurden *et al.*, 2012). Sweden's decision, therefore, to accept the short term loss of 3 million recruits on the one hand, prevented the anthropogenic dissemination of EVEX. And on the other hand, this practice lowered the overall pressure on the stock while the non-contaminated habitats remain suitable recipient waters for stocking measures and might produce potential spawners. This vividly demonstrates the importance of a quarantine phase in eel stocking measures in order to apply necessary counter-measures. In addition, virus-infected eels occasionally show no clinical signs of a disease, hence, a reliable diagnosis of viruses must be based on PCR assays (van Beurden *et al.*, 2016).

A situation as described in the Schlei fjord (**Chapter IV**) is the worst possible outcome of stocking measures and a management failure. The former AngHV-1 free fjord (Jakob *et al.*, 2009b) is now heavily contaminated while infected eels are unlikely to contribute to spawning (Haenen *et al.*, 2009). This virus can be spread through water, hence, even if the following cohorts are virus-free, a contamination is very likely making further stocking measures unreasonable from a conservation point of view.

Other threats and improvement potential of stocking measures

The translocation of natural recruits is a widely accepted management measure while a lack of recovery can still be observed. The absence of a positive effect on the recruitment, however, might not necessarily be explained by a general ineffectiveness but maybe by inefficiency and systematic errors within eel management plans. For example it was shown that the use of farmed recruits might increase the outcome of stocking measures because of a higher benefit-cost ratio and a better growth performance (**Chapter V**).

Rapid growth, however, could have also negative effects on the stock. For instance, stocked American glass eels (*Anguilla rostrata*) in the St. Lawrence River basin have been found to follow life history patterns (Couillard *et al.*, 2014; Stacey *et al.*, 2015). The glass eels have been caught alive at the south coast of Nova Scotia and New Brunswick, were marked with oxytetracycline and transferred far upstream in the St Lawrence River. Stocked recruits grew significantly faster than naturally recruited conspecifics, and the catch location (i.e. distance from the spawning ground in the Sargasso Sea) was identified as an indicator for their ability to grow. The growth rate is negatively correlated with total length at maturation and smaller eels reveal lower lipid contents than slow growing but finally larger individuals (Degani *et al.*, 1986; Andersson *et al.*, 1991). Hence, ‘fast growers’ might be incapable of arriving in the

Sargasso Sea when stocked far away from the donor habitat. Therefore, the source of eels for the purpose of stocking should be close to the recipient water i.e. stocking location (Couillard *et al.*, 2014; Stacey *et al.*, 2015). Having regard to this, marked recruits could not be compared to unmarked eels in the present study because the recruitment history of the latter is unknown (**Chapter V**). By contrast, for the comparison of the two stocking forms, the life history effect was considered as negligible because the origin of the used glass eels (England) and farmed eels (France) do not differ substantially in their distance to the Sargasso Sea (6000 to 6500 km). This pattern, however, in combination with the presence of genetically different eco-types also found in *A. rostrata* (Pavey *et al.*, 2015), should be considered in the reallocation of natural recruits.

The migration pattern of *A. rostrata* was found to be genetically directed (Pavey *et al.*, 2015). Stocked eels, in turn, cannot 'choose' their preferred growing habitat according to their genotype due to human intervention, and phenotypic plasticity might not compensate the genotype. Consequently, genotyping of recruits before stocking should be mandatory in order to avoid interferences with natural population characteristics. This, however, is very unlikely because of the associated high analytical efforts.

Furthermore, the future sex of eels is known to be metagamic i.e. determined by external environmental factors (Davey and Jellyman, 2005). Especially eel density and growth rate during the initial phase after the larval trans-Atlantic migration are key factors impacting sexual determination (e.g. Holmgren and Mosegaard, 1996; Huertas and Cerdà, 2006). This is of outstanding importance for eel stocking programmes because the recipient water must be selected carefully as improper stocking densities might lead to untypically skewed sex ratios and reduced reproductive potential of the stock as a whole.

Additionally, the high market value of *A. anguilla* makes it a valuable target species, thus there is a serious risk that managers might be driven by the credo 'the more eels the better'. Stocking effort might potentially be beyond the habitat carrying capacity because economically valuable species might be considered as more important than non-target taxa without monetary value. This might lead to heavily over-stocked river basins including coastal areas with unexpected consequences for other fish species or trophic interactions in general (Mazumder and Edmundson, 2002).

The migration and orientation ability of stocked individuals is also of crucial importance since stocked escapees are at least proportionally intended to contribute to spawning. Natural recruits migrate all the way from the Sargasso Sea to their growing habitat and have thus the possibility to imprint the route back. Stocked individuals that have been transported over long distances might therefore lack orientation with the consequence that their contribution to future recruitment is negligible (Westin, 1998). Stocked recruits in the Baltic Sea were found to struggle swimming towards the outlet (Kattegat) and an initial phase of disorientation in stocked silver eels was observed (Westin, 2003; Prigge *et al.*, 2013). In contrast, a comprehensive telemetry study in the Baltic Sea could find convincing proof of directed migration of both stocked and natural recruits (Westerberg *et al.*, 2014). This suggests that earth's magnetic field has influence on eels' orientation behaviour (Cresci *et al.*, 2017; Naisbett-Jones *et al.*, 2017).

The most important obstacles towards a recovery of the eel stock might be the commercial value, and the intention of the EU Commission to ensure the sustainable use of the stock in combination with the increasing need of renewable energy from e.g. hydropower (European Council, 2007; European Commission, 2009). In this regard, an individual-based model called "GenEveel" was used to theoretically demonstrate that anthropogenic induced mortality

(fishery- and hydropower-induced) might be amongst the most important pressures on the European eel stock (Mateo *et al.*, 2017). The glass eel fishery, though inducing the highest instantaneous mortality rates, was of secondary importance while silver eel fishery and losses at hydropower plants, in turn, appeared to be far more responsible for the low number of escaping silver eels. The effect of phenotypic plasticity as compensatory factor for migration obstacles and mass-mortality caused by glass eel fisheries was concluded. However, the practice of stocking i.e. the modifying of eel density and population characteristics should critically be reviewed since the effects of anthropogenic influence on the stock is still poorly understood (Mateo *et al.*, 2017).

Outlook and prospective study design

The comparability of the two investigated stocking strategies (glass and farmed eels) could be improved substantially if all stocked recruits would come from the same glass eel catch with known health status (Figure 25). This particular catch should be split into two equivalent groups. One half would be marked with ARS immediately after catch and before the release. The other group would be transferred into an eel farm for the purpose of farming under controlled conditions (food, temperature, light regime, veterinary care). Several size grading's every approximately 6 to 8 weeks are necessary in order to limit cannibalism due to large variability in individual growth performance (Angelidis *et al.*, 2012). After 200 to 240 days eels show a mean individual weight of 6 to 8 g and could be marked using ARS before stocking (**Chapter II**). The timing of the marking hereby is essential to enable discrimination between stocking forms with the aid of the mark position (Figure 18). The overall mortality could be estimated using the relative number of recaptured eels and potential differences in the growth performance would be explained only by the farming process and associated tim-

ing of the release. As the described approach is certainly a useful design for future studies, the already marked eels can facilitate new insights into the longevity of the ARS mark. The marked cohorts of this thesis can be followed over several years during routine stock monitoring samplings which might elucidate the durability of ARS on eel otoliths. This indeed is crucial knowledge for the quantification of the contribution of stocking measures to silver eel escapement and also, if possible in the future, spawning in the Sargasso Sea.

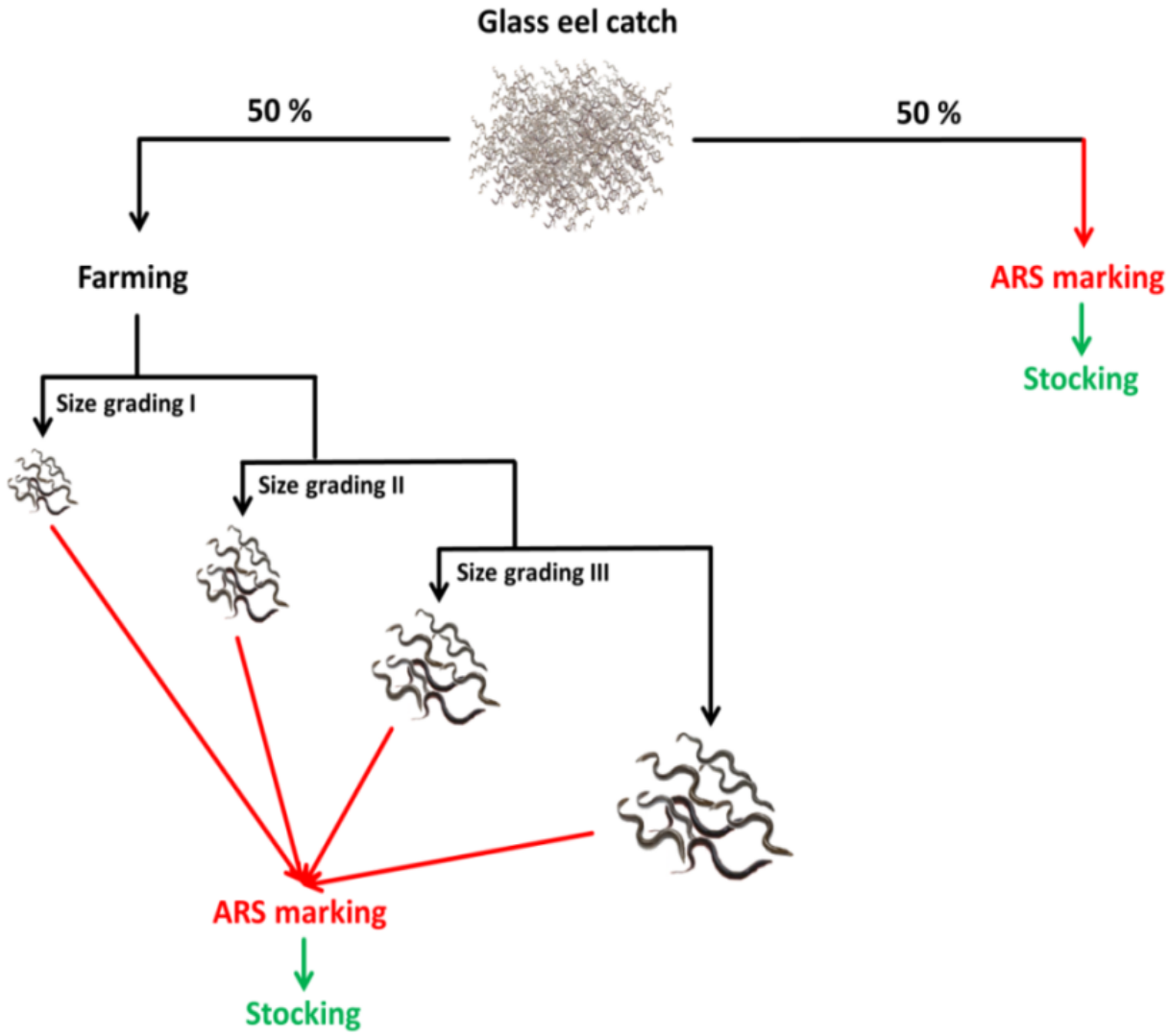

Figure 25 The flow chart shows the experimental approach that enables comparative deductions about different stocking strategies. A specific glass eel catch should be split into two equivalent groups. One half could be stocked immediately after the catch marked with alizarin red S (ARS) and the other half is used for farming purposes and later stocking at the same site. All size graded groups should be marked and stocked simultaneously. Note that more size grading's might be necessary to limit cannibalism (e.g. Angelidis *et al.,* 2012).

In the future, an internationally coordinated research project might initiate the first comprehensive mass-marking of the entire European stocking material in three consecutive years by using three similar but distinguishable markers and combinations (Figure 24). Eligible chemical substances are ARS, barium chloride dihydrate and strontium chloride hexahydrate. All are rather low risk compounds and recommended by ICES specialists for the purpose of marking *Anguilla anguilla* (ICES, 2011a, 2016e). The alternation would help to identify the cohorts and thus ageing related growth estimations. Additionally, natural recruitment and, accordingly, also the contribution of stocked eels to the silver eel escapement biomass could be quantified precisely.

The proposed scheme could also be used to apply recent findings from Pedersen *et al.* (2017) and Pedersen and Rasmussen (2016). Their findings suggest that there might be an optimal size (i.e. best growth performance and highest survival rate) of stocked recruits between the glass eel stage and a mean body weight of 3 g. Therefore, recruits could be farmed as described above and batches (e.g. glass, 1, 2, 3 g eels) could be uniquely marked and successively stocked (c.f. Figure 25). If the entire glass eels catch would be marked with ARS after catch, a fourth unique mark would be possible by using ARS again (i.e. two ARS rings will appear on the otoliths).

Lessons learned and final conclusion

The results of this thesis have confirmed the basic eligibility of farmed eels as stocking material within the framework of eel management plans as far as they have been marked chemically and the health status is irreproachable. In contrast to numerous other studies, the mortality of the used glass eels has been found to be fourfold higher and the initial lead of the farmed recruits in total length and body weight was observed to be persistent. The conclu-

sion that stocking of farmed eels efficiently improves the recovery of the European eel stock, however, would be premature since, on the one hand, there is still a wide range of uncertainty concerning the net benefit of stocking measures in the first place. And on the other hand, various risks are associated with stocking measures in general (e.g. life history effects or the corruption of the natural population structure), especially regarding the use of farmed recruits (e.g. dissemination of diseases or interference with ageing; **Chapter III and Chapter IV**).

Further research in the field of eel stocking is an indispensable necessity whereby the established mass-marking protocols presented in this thesis can be a basic element of international research collaborations and an important first step towards the sustainable management of the European eel stock.

References

Abdi, H. 2007. The Bonferonni and Šidák Corrections for Multiple Comparisons. *In* Encyclopedia of Measurement and Statistics, pp. 103–107. Ed. by N. Salkind. Sage Publications, Inc, 2455 Teller Road, Thousand Oaks California 91320, United States of America.

Acou, A., Rivot, E., van Gils, J. A.A.N., Legault, A., Ysnel, F., and Feunteun, E. 2011. Habitat carrying capacity is reached for the European eel in a small coastal catchment: evidence and implications for managing eel stocks. Freshwater Biology, 56: 952–968.

Adamek, M., Rakus, K. Ł., Brogden, G., Matras, M., Chyb, J., Hirono, I., Kondo, H., *et al.* 2014. Interaction between type I interferon and cyprinid herpesvirus 3 in two genetic lines of common carp Cyprinus carpio. Diseases of Aquatic Organisms, 111: 107–118.

Airoldi, L., and Beck, M. W. 2007. Loss, Status and Trends for Coastal Marine Habitats of Europe. *In* Oceanography and marine biology, 45, pp. 345–405. Ed. by R. N. Gibson, R. J. A. Atkinson, and J. D. M. Gordon. CRC Press, Boca Raton.

Alcobendas, M., Lecomte, F., Castanet, J., Meunier, F. J., Maire, P., and Holl, M. 1991. Technique de marquage en masse de civelles (*Anguilla anguilla* L.) par balnéation rapide dans le fluorochrome. Application au marquage à la tétracycline de 500 Kg de civelles. Bulletin Français de la Pêche et de la Pisciculture, 321: 43–54.

Andersson, J., Sandström, O., and Hansen, H.J.M. 1991. Elver (*Anguilla anguilla* L.) stockings in a Swedish thermal effluent-recaptures, growth and body condition. Journal of Applied Ichthyology, 7: 78–89.

Angelidis, P. 2011. Improved System to Normalize the Oxygen Distribution In the Tank Water In Aquaculture. *In* Proceedings of the International Conference on Information and Communication Technologies for Sustainable Agri-production and Environment (HAICTA 2011), Skiathos, 8-11 September, 2011, pp. 473–485. Ed. by m. Salampasis, and A. Matopoulos.

Angelidis, P., Pournara, I., and Photis, G. 2012. Glass eels (*Anguilla anguilla*) growth in a recirculating system. Mediterranean Marine Science, 6: 99–106.

Antunes, C., and Tesch, F.-W. 1997. A critical consideration of the metamorphosis zone when identifying daily rings in otoliths of European eel, *Anguilla anguilla* (L.). Ecology of Freshwater Fish, 6: 102–107.

Aoyama, J. 2009. Life History and Evolution of Migration in Catadromous Eels (Genus *Anguilla*). AquaBioScience Monographs, 2: 1-42.

Aoyama, J., Nishida, M., and Tsukamoto, K. 2001. Molecular phylogeny and evolution of the freshwater eel, genus *Anguilla*. Molecular Phylogenetics and Evolution, 20: 450–459.

Arai, K., Itakura, H., Yoneta, A., Yoshinaga, T., Shirotori, F., Kaifu, K., and Kimura, S. 2017. Discovering the dominance of the non-native European eel in the upper reaches of the Tone River system, Japan. Fisheries Science, 83: 735-742.

Armitage, J., Hewlett, N. R., Twigg, M., Lewin, N. C., Reading, A. J., Williams, C. F., Aprahamian, M., *et al.* 2014. Detection of *Herpesvirus anguillae* during two mortality investigations of wild European eel in England: implications for fishery management. Fisheries Management and Ecology, 21: 1–12.

Ashley, P. J. 2007. Fish welfare: Current issues in aquaculture. Applied Animal Behaviour Science, 104: 199–235.

Åström, M., and Dekker, W. 2007. When will the eel recover? A full life-cycle model. ICES Journal of Marine Science, 64: 1491–1498.

Bandín, I., Souto, S., Cutrín, J. M., López-Vázquez, C., Olveira, J. G., Esteve, C., Alcaide, E., *et al.* 2014. Presence of viruses in wild eels *Anguilla anguilla* L, from the Albufera Lake (Spain). Journal of fish diseases, 37: 597–607.

Bartley, D. M., Bondad-Reantaso, M. G., and Subasinghe, R. P. 2006. A risk analysis framework for aquatic animal health management in marine stock enhancement programmes. Restocking and Stock Enhancement of Coastal Fisheries Potential, Problems and ProgressSpecial Symposium, 7th Asian Fisheries Forum, 80: 28–36.

Beamish, R. J., and McFarlane, G. A. 1983. The Forgotten Requirement for Age Validation in Fisheries Biology. Transactions of the American Fisheries Society, 112: 735–743.

Begg, G. A., Campana, S. E., Fowler, A. J., and Suthers, I. M. 2005. Otolith research and application: Current directions in innovation and implementation. Marine and Freshwater Research, 56: 477–483.

Berg, R. 1985. Age determination of eels, *Anguilla anguilla* (L): comparison of field data with otolith ring patterns. Journal of Fish Biology, 26: 537–544.

Berkeley, S. A., Hixon, M. A., Larson, R. J., and Love, M. S. 2004. Fisheries Sustainability via Protection of Age Structure and Spatial Distribution of Fish Populations. Fisheries, 29: 23–32.

Bertignac, M., and Pontual, H. de. 2007. Consequences of bias in age estimation on assessment of the northern stock of European hake (*Merluccius merluccius*) and on management advice. ICES Journal of Marine Science, 64: 981–988.

Bevacqua, D., Leo, G. A. de, Gatto, M., and Melià, P. 2009. Size selectivity of fyke nets for European eel *Anguilla anguilla*. Journal of Fish Biology, 74: 2178–2186.

Bevacqua, D., Melià, P., Leo, G. A. D., and Gatto, M. 2011. Intra-specific scaling of natural mortality in fish: the paradigmatic case of the European eel. Oecologia, 165: 333–339.

Beverton, R. J. H., and Holt, S. J. 1957. On the dynamics of exploited fish populations. Fish and fisheries series, 11. Springer Science+Business, Dordrecht.

Bisgaard, J., and Pedersen, M. I. 1991. Mortality and growth of wild and introduced cultured eels (*Anguilla anguilla* (L.)) in a Danish stream, with special reference to a new tagging technique. Dana, 9: 57–69.

Boerrigter, J. G. J., Manuel, R., Bos, R., Roques, J. A. C., Spanings, T., Flik, G., and Vis, H. W. 2015. Recovery from transportation by road of farmed European eel (*Anguilla anguilla*). Aquaculture Research, 46: 1248–1260.

Bolger, T., and Connolly, P. L. 1989. The selection of suitable indices for the measurement and analysis of fish condition. Journal of Fish Biology, 34: 171–182.

Bonhommeau, S., Castonguay, M., Rivot, E., Sabatié, R., and Le Pape, O. 2010. The duration of migration of Atlantic Anguilla larvae. Fish and Fisheries, 11: 289–306.

Bonhommeau, S., Le Pape, O., Gascuel, D., Blanke, B., Tréguier, A.-M., Grima, N., Vermard, Y., *et al.* 2009. Estimates of the mortality and the duration of the trans-Atlantic migration of European eel *Anguilla anguilla* leptocephali using a particle tracking model. Journal of Fish Biology, 74: 1891–1914.

Bradford, M. J. 1991. Effects of Ageing Errors on Recruitment Time Series Estimated from Sequential Population Analysis. Canadian Journal of Fisheries and Aquatic Sciences, 48: 555–558.

Brämick, U., Fladung, E., and Simon, J. 2016. Stocking is essential to meet the silver eel escapement target in a river system with currently low natural recruitment. ICES Journal of Marine Science, 73: 91–100.

Brauner, C. J., Seidelin, M., Madsen, S. S., and Jensen, F. B. 2000. Effects of freshwater hyperoxia and hypercapnia and their influences on subsequent seawater transfer in Atlantic salmon (*Salmo salar*) smolts. Canadian Journal of Fisheries and Aquatic Sciences, 57: 2054–2064.

Busacker, G. P., Adelman, I. R., and Goolish, E. M. 1990. Growth. *In* Methods for Fish Biology, pp. 363–387. Ed. by C. B. Schreck, and P. B. Moyle, Bethesda, Md.

Campana, S. E. 1999. Chemistry and composition of fish otoliths: Pathways, mechanisms and applications. Marine Ecology Progress Series, 188: 263–297.

Campana, S. E. 2005. Otolith science entering the 21st century. Marine and Freshwater Research, 56: 485–495.

Campana, S. E., and Thorrold, S. R. 2001. Otoliths, increments, and elements: Keys to a comprehensive understanding of fish populations? Canadian Journal of Fisheries and Aquatic Sciences, 58: 30–38.

Campana, S. E., Annand, M. C., and McMillan, J. I. 1995. Graphical and Statistical Methods for Determining the Consistency of Age Determinations. Transactions of the American Fisheries Society, 124: 131–138.

Campana, S. E., Oxenford, H. A., and Smith, J. N. 1993. Radiochemical determination of longevity in flyingfish *Hirundichthys affinis* using Th-228/Ra-228. Marine Ecology Progress Series, 100: 211–219.

Caraguel, J.-M., Charrier, F., Mazel, V., and Feunteun, E. 2015. Mass marking of stocked European glass eels (*Anguilla anguilla*) with alizarin red S. Ecology of Freshwater Fish, 24: 435–442.

Caudron, A., and Champigneulle, A. 2009. Multiple marking of otoliths of brown trout, Salmo trutta L., with alizarin redS to compare efficiency of stocking of three early life stages. Fisheries Management and Ecology, 16: 219–224.

Couillard, C. M., Verreault, G., Dumont, P., Stanley, D., and Threader, R. W. 2014. Assessment of Fat Reserves Adequacy in the First Migrant Silver American Eels of a Large-Scale Stocking Experiment. North American Journal of Fisheries Management, 34: 802–813.

Cowx, I. G. 1999. An appraisal of stocking strategies in the light of developing country constraints. Fisheries Management and Ecology, 6: 21–34.

Cowx, I. G., ed. 2003. Interactions between fish and birds: Implications for management. Fishing News Books, Oxford. 376 pp.

Crean, S. R., Dick, J. T. A., Evans, D. W., Rosell, R. S., and Elwood, R. W. 2005. Survival of juvenile European eels (*Anguilla anguilla*), transferred among salinities, and developmental shifts in their salinity preference. Journal of Zoology, 266: 11–14.

Cresci, A., Paris, C. B., Durif, C. M. F., Shema, S., Bjelland, R. M., Skiftesvik, A. B., and Browman, H. I. 2017. Glass eels (*Anguilla anguilla*) have a magnetic compass linked to the tidal cycle. Science Advances, 3: e1602007.

Crook, V. 2010. Trade in *Anguilla* species, with a focus on recent trade in European Eel *A. anguilla*. TRAFFIC report prepared for the European.

Crook, V., and Nakamura, M. 2013. Glass eels: Assessing supply chain and market impacts of a CITES listing on *Anguilla* species. Traffic Bulletin, 25: 24-30.

Dainys, J., Gorfine, H., Šidagytė, E., Jakubavičiūtė, E., Kirka, M., Pūtys, Ž., and Ložys, L. 2017. Do young on-grown eels, *Anguilla anguilla* (Linnaeus, 1758), outperform glass eels after transition to a natural prey diet? Journal of Applied Ichthyology, 33: 361–365.

Daverat, F., and Tomas, J. 2006. Tactics and demographic attributes in the European eel *Anguilla anguilla* in the Gironde watershed, SW France. Marine Ecology Progress Series, 307: 247-257.

Davey, A. J. H., and Jellyman, D. J. 2005. Sex Determination in Freshwater Eels and Management Options for Manipulation of Sex. Reviews in Fish Biology and Fisheries, 15: 37–52.

Davidse, A., Haenen, O.L.M., Dijkstra, S. G., van Nieuwstadt, A. P., van der Vorst, T.J.K., Wagennaar, F., and Wellenberg, G. J. 1999. First isolation of herpesvirus of eel (*Herpesvirus anguillae*) in diseased European eel (*Anguilla anguilla* L.) in Europe. Bulletin of the European Association of Fish Pathologists, 19: 137–141.

Deelder, C. L. 1981. On the age and growth of cultured eels, *Anguilia anguilla* (Linnaeus, 1758). Aquaculture, 26: 13–22.

Degani, G., Hahamu, H., and Levanon, D. 1986. The relationship of eel *Anguilla anguilla* (L.) body size, lipid, protein, glucose, ash, moisture composition and enzyme activity (aldolase). Comparative Biochemistry and Physiology Part A: Physiology, 84: 739–745.

Dekker, W. 2000. The fractal geometry of the European eel stock. ICES Journal of Marine Science, 57: 109–121.

Dekker, W. 2003. Status of the European Eel Stock and Fisheries. Springer Japan. 237 pp.

Dekker, W. 2004. Slipping through our hands: Population dynamics of the European eel. PhD thesis, University of Amsterdam, Amsterdam.

Dekker, W. 2016. Management of the eel is slipping through our hands! Distribute control and orchestrate national protection. ICES Journal of Marine Science, 73: 2442-2452.

Dekker, W., and Beaulaton, L. 2016a. Climbing back up what slippery slope? Dynamics of the European eel stock and its management in historical perspective. ICES Journal of Marine Science, 73: 5–13.

Dekker, W., and Beaulaton, L. 2016b. Faire mieux que la nature?: The History of Eel Restocking in Europe. Environment and History, 22: 255–300.

Dekker, W., ed. 2002. Monitoring of glass eel recruitment. RIVO report, no. C007/02-WD. RIVO-Netherlands Institute of Fisheries Research, Ijmuiden.

Dekker, W., van Os, B., and van Willigen, J. 1998. Minimal and maximal size of eel. Bulletin Français de la Pêche et de la Pisciculture: 195–197.

Durif, C., Dufour, S., and Elie, P. 2005. The silvering process of *Anguilla anguilla*: a new classification from the yellow resident to the silver migrating stage. Journal of Fish Biology, 66: 1025–1043.

Durif, C., Guibert, A., and Elie, P. 2009. Morphological Discrimination of the Silvering Stages of the European Eel. *In* Eels at the edge: Science, status, and conservation concerns. American Fisheries Society symposium, 58, pp. 103–111. Ed. by J. M. Casselman, and D. K. Cairns. American Fisheries Society, Bethesda, Md.

Edeline, E., Dufour, S., and Elie, P. 2005. Role of glass eel salinity preference in the control of habitat selection and growth plasticity in *Anguilla anguilla*. Marine Ecology Progress Series, 304: 191–199.

EFSA. 2008. Animal welfare aspects of husbandry systems for farmed fish - European eel - Scientific Opinion of the Panel on Animal Health and Welfare. The EFSA Journal, 809: 1–18.

Egginton, S., and Johnston, I. A. 1984. Effects of acclimation temperature on routine metabolism muscle mitrochondrial volume density and capillary supply in the elver (*Anguilla anguilla* L.). Journal of Thermal Biology, 9: 165–170.

Einum, S., and Fleming, I. A. 2001. Implications of Stocking: Ecological Interactions Between Wild and Released Salmonids. Nordic Journal of Freshwater Research, 75: 56–70.

Eskild Kirkegaard, ed. 2010. European Eel and Aquaculture: DTU Aqua Report No 229-2010, 229-2010. DTU Aqua.

Espmark, Å. M., Hjelde, K., and Baerverfjord, G. 2010. Development of gas bubble disease in juvenile Atlantic salmon exposed to water supersaturated with oxygen. Aquaculture, 306: 198–204.

Esteve, C., and Alcaide, E. 2009. Influence of diseases on the wild eel stock: The case of Albufera Lake. Aquaculture, 289: 143–149.

EU COM. 2009. Commission Decision 2010/93/EU of 18 December 2009 adopting a multiannual Community programme for the collection, management and use of data in the fisheries sector for the period 2011-2013 (notified under document C(2009) 10121). Official Journal of the European Union, L41: 8–71.

European Commission. 2009. Directive 2009/28/EC of the European Parliament and of the Council of 23 April 2009 on the promotion of the use of energy from renewable sources and amending and subsequently repealing Directives 2001/77/EC and 2003/30/EC. Official Journal of the European Union, L140: 16-62.

European Commission. 2014. Report from the Commission to the Council and the European Parliament: On the outcome of the implementation of the Eel Management Plans, including an evaluation of the measures concerning restocking and of the evolution of market prices for eels less than 12 cm in length.

European Council. 2000. Directive 2000/60/EC of the European Parliamnet and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Official Journal of the European Union: L 327/1.

European Council. 2007. Council Regulation (EC) No 1100/2007 of 18 September 2007 establishing measures for the recovery of the stock of European eel. Official Journal of the European Union: L 248/17.

Feunteun, E. 2002. Management and restoration of European eel population (*Anguilla anguilla*): An impossible bargain. Ecological Engineering, 18: 575–591.

Fliedner, A., Lohmann, N., Rüdel, H., Teubner, D., Wellmitz, J., and Koschorreck, J. 2016. Current levels and trends of selected EU Water Framework Directive priority substances in freshwater fish from the German environmental specimen bank. Environmental Pollution, 216: 866–876.

Freese, M., Suhring, R., Marohn, L., Pohlmann, J.-D., Wolschke, H., Byer, J. D., Alaee, M., *et al.* 2017. Maternal transfer of dioxin-like compounds in artificially matured European eels. Environmental pollution, 227: 348–356.

Freese, M., Sühring, R., Pohlmann, J.-D., Wolschke, H., Magath, V., Ebinghaus, R., and Hanel, R. 2016. A question of origin: dioxin-like PCBs and their relevance in stock management of European eels. Ecotoxicology, 25: 41–55.

Froese, R., and Pauly, D. 2017. FishBase World Wide Web electronic publication. www.fishbase.org, (06/2017): Main Ref. 172 + 51442.

Fukuda, N., Kuroki, M., Shinoda, A., Yamada, Y., Okamura, A., Aoyama, J., and Tsukamoto, K. 2009. Influence of water temperature and feeding regime on otolith growth in *Anguilla japonica* glass

eels and elvers: Does otolith growth cease at low temperatures? Journal of Fish Biology, 74: 1915–1933.

Gollock, M. J., Kennedy, C. R., and Brown, J. A. 2005. European eels, *Anguilla anguilla* (L.), infected with *Anguillicola crassus* exhibit a more pronounced stress response to severe hypoxia than uninfected eels. Journal of fish diseases, 28: 429–436.

Greenwood, P. H., Rosen, D. E., Weitzman, S. H., and Myers, G. S. 1966. Phyletic Studies of Teleostean Fishes, with a provisional Classification of Living Forms. Bulletin of the American Museum of Natural History, 131: 339–456.

Gulland, J. A. 1965. Estimation of mortality rates: Annex to the Northeast Arctic working group report. ICES C.M. Doc. No. 3: 231–241.

Haenen, O. L. M., Dijkstra, S. G., van Tulden, P. W., Davidse, A., van Nieuwstadt, A. P., Wagnenaar, F., and Wellenberg, G. J. 2002. *Herpesvirus anguillae* (HVA) isolations from disease outbreak in cultured European eel, *Anguilla anguilla* in The Netherlands since 1996. Bulletin of the European Association of Fish Pathologists, 22: 247–257.

Haenen, O. L. M., Mladineo, I., Konecny, R., Yoshimizu, M., Groman, D., Muñoz, P., Saraiva, A., *et al.* 2012. Diseases of eels in an international perspective: Workshop on Eel Diseases at the 15th International Conference on Diseases of Fish and Shellfish, Split, Croatia, 2011. Bulletin of the European Association of Fish Pathologists, 32: 109–115.

Haenen, O., van Ginneken, V., Engelsma, M., and van den Thillart, G. 2009. Impact of Eel Viruses on Recruitment of European Eel. *In* Spawning migration of the European eel: Reproduction index, a useful tool for conservation management. Fish & fisheries series, v. 30, pp. 387–400. Ed. by G. van den Thillart, S. Dufour, and J. C. Rankin. Springer Science + Business Media, Dordrecht.

Haenen, O.L.M., Lehmann, J., Engelsma, M. Y., Stürenberg, F.-J., Roozenburg, I., Kerkhoff, S., and Breteler, J. K. 2010. The health status of European silver eels, *Anguilla anguilla*, in the Dutch River Rhine Watershed and Lake IJsselmeer. Aquaculture, 309: 15–24.

Hangalapura, B. N., Zwart, R., Engelsma, M. Y., and Haenen, O. L. M. 2007. Pathogenesis of *Herpesvirus anguillae* (HVA) in juvenile European eel *Anguilla anguilla* after infection by bath immersion. Diseases of Aquatic Organisms, 78: 13–22.

Hanson, L., Doszpoly, A., van Beurden, S. J., Viadanna, P., and Waltzek, T. 2016. Alloherpesviruses of fish. *In* Aquaculture virology, pp. 153–172. Ed. by F. S. B. Kibenge, and M. G. Godoy. Academic Press, London.

Hein, S., Voss, J., Poethke, H.-J., and Boris, S. 2007. Habitat suitability models for the conservation of thermophilic grasshoppers and bush crickets—simple or complex? Journal of Insect Conservation, 11: 221–240.

Heinsbroek, L. T. N. 1989. Preliminary investigations on husbandry, nutrition and growth of glass eels and elvers, *Anguilla anguilla* L. Aquaculture Research, 20: 119–127.

Hilborn, R., and Walters, C. J. 1992. Quantitative Fisheries Stock Assessment: Choice, Dynamics and Uncertainty. Chapman and Hall, London.

Hirt-Chabbert, J. A., Skalli, A., Young, O. A., and Gisbert, E. 2012. Effects of feeding stimulants on the feed consumption, growth and survival at glass eel and elver stages in the European eel (*Anguilla anguilla*). Aquaculture Nutrition, 18: 152–166.

Holmgren, K. 1996. Otolith growth scaling of the eel, *Anguilla anguilla* (L.), and back-calculation errors revealed from alizarin labelled otoliths. Nordic Journal of Freshwater Research, 72: 71–79.

Holmgren, K., and Mosegaard, H. 1996. Implications of individual growth status on the future sex of the European eel. Journal of Fish Biology, 49: 910–925.

Hosmer, D. W., and Lemeshow, S. 2000. Applied Logistic Regression, 2nd edn. John Wiley & Sons, Inc, Hoboken, NJ.

Huertas, M., and Cerdà, J. 2006. Stocking density at early developmental stages affects growth and sex ratio in the European eel (*Anguilla anguilla*). The Biological bulletin, 211: 286–296.

Huntingford, F., and Adams, C. 2005. Behavioural syndromes in farmed fish: Implications for production and welfare. Behaviour, 142: 1207–1221.

Hüssy, K., Radtke, K., Plikshs, M., Oeberst, R., Baranova, T., Krumme, U., Sjöberg, R., *et al.* 2016. Challenging ICES age estimation protocols: Lessons learned from the eastern Baltic cod stock. ICES Journal of Marine Science, 73: 2138–2149.

ICES. 1999. Report of the ICES Advisory Committee on Fishery Management, 1998: Copenhagen, 13-22 May 1998 Copenhagen, 20-29 October 1998. ICES Cooperative Research Report No. 229 Part 2.

ICES. 2000. Report of the EIFAC/ICES Working Group on Eels Silkeborg, Denmark 20–24 September 1999. ICES CM 2000/ACFM:6, 28 pp.

ICES. 2008. Report of the 2007 session of the Joint EIFAC/ICES Working Group on Eels Bordeaux, France, 3–7 September 2007. ICES CM 2007/ACFM: 23, 156 pp.

ICES. 2009a. Report of the 2008 session of the Joint EIFAC/ICES Working Group on Eels Leuven, Belgium, 3–9 September 2008. ICES CM 2008/ACOME: 15, 623 pp.

ICES. 2009b. Report of the Study Group on Anguillid Eels in Saline Waters (SGAESAW), 16–18 March 2009, Sackville, Canada; 3–5 September 2009, Gothenburg, Sweden. ICES CM/DFC:06, 183 pp.

ICES. 2009c. Workshop on Age Reading of European and American Eel (WKAREA) 20-24 April 2009 Bordeaux, France. ICES CM 2011/ACOM:48, 66 pp.

ICES. 2011a. Report of the Joint EIFAAC/ICES Working Group on Eels (WGEEL), Lisbon, Portugal, 5–9 September 2011. CM 2011/ACOM:18, 223 pp.

ICES. 2011b. Report of the Workshop on Age Reading of European and American Eel (WKAREA2) 22-24 March 2011 Bordeaux, France. ICES CM 2011/ACOM:43, 35 pp.

ICES. 2012. Report of the Workshop on Eel and Salmon DCF Data (WKESDCF) 3 – 6 July 2012 ICES HQ, Copenhagen, Denmark. ICES CM/ACOM:62, 67 pp.

ICES. 2013. Report of the Workshop on Evaluation Progress Eel Management Plans (WKEPEMP). CM 2013/ACOM:32, 757 pp.

ICES. 2015. Advice on fishing opportunities, catch, and effort Northeast Atlantic Ecoregions: European eel (*Anguilla anguilla*) throughout its natural range. ICES Advice 2015, Book 9: 1–5

ICES. 2016a. Advice on fishing opportunities, catch, and effort Northeast Atlantic: 9.3.8 European eel (*Anguilla anguilla*) throughout its natural range. ICES Advice 2016, Book 9: 1–6

ICES. 2016b. Report of the Joint EIFAAC/ICES/GFCM Working Group on Eel (WGEEL), 24 November–2 December 2015, Antalya, Turkey. ICES CM 2015/ACOM:18, 130 pp.

ICES. 2016c. Report of the Working Group on Eels (WGEEL), 15–22 September 2016, Cordoba, Spain. ICES CM 2016/ACOM:19, 107 pp.

ICES. 2016d. 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. ICES CM 2015/SSGEPD:20, 98 pp.

ICES. 2016e. Report of the Workshop on Eel Stocking (WKSTOCKEEL): 20-24 June 2016 Toomebridge, Nothern Ireland, UK. ICES CM 2016/SSGEPD:21, 75 pp.

ICES. 2017a. Advice on fishing opportunities, catch, and effort Ecoregions in the Northeast Atlantic: European eel (*Anguilla anguilla*) throughout its natural range. ICES Advice 2017, ele.2737.nea.

ICES. 2017b. ICES Advice on fishing opportunities, catch, and effort: Cod (*Gadus morhua*) in subdivisions 24–32, eastern Baltic stock (eastern Baltic Sea). ICES Advice 2017, cod.27.24-32.

ICES. 2017c. Report of the Joint EIFAAC/ICES/GFCM Working Group on Eels (WGEEL), 3–10 October 2017, Kavala, Greece. ICES CM 2017/ACOM:15, 99 pp.

ICES. 2017d. Report of the Workshop on Designing an Eel Data Call (WKEELDATA) 28 February–2 March 2017 Rennes, France. ICES CM 2017/SGIEOM:30, 38 pp.

ICES. 2017e. WGEEL Country Reports 2016/2017. EIFAAC/ICES/GFCM WGEEL REPORT 2017: 481 pp.

Iglesias, J., and Rodríguez-Ojea, G. 1997. The use of alizarin complexone for immersion marking of the otoliths of embryos and larvae of the turbot, *Scophthalmus maximus* (L.): Dosage and treatment time. Fisheries Management and Ecology, 4: 405–417.

Jacoby, D., and Gollock, M. 2014. IUCN Red List of Threatened Species 2014: e.T60344A45833138. http://dx.doi.org/10.2305/IUCN.UK.2014-1.RLTS.T60344A45833138.en.

Jakob, E. 2009. Monitoring of parasite and virus infections of the European eel, *Anguilla anguilla*, in northern Germany. PhD thesis, Christian-Albrechts-Universität, Kiel.

Jakob, E., Hanel, R., Klimpel, S., and Zumholz, K. 2009. Salinity dependence of parasite infestation in the European eel *Anguilla anguilla* in northern Germany. ICES Journal of Marine Science, 66: 358–366.

Jakob, E., Neuhaus, H., Steinhagen, D., Luckhardt, B., and Hanel, R. 2009. Monitoring of *Herpesvirus anguillae* (HVA) infections in European eel, *Anguilla anguilla* (L.), in northern Germany. Journal of fish diseases, 32: 557–561.

Josset, Q., Trancart, T., Mazel, V., Charrier, F., Frotté, L., Acou, A., and Feunteun, E. 2016. Pre-release processes influencing short-term mortality of glass eels in the French eel (*Anguilla anguilla*, Linnaeus 1758) stocking programme. ICES Journal of Marine Science, 73: 150–157.

Kaifu, K., Itakura, H., Amano, Y., Shirai, K., Yokouchi, K., Wakiya, R., Murakami-Sugihara, N., *et al.* 2018. Discrimination of wild and cultured Japanese eels based on otolith stable isotope ratios. ICES Journal of Marine Science, 75: 719–726.

Kamstra, A. 1993. The effect of size grading on individual growth in eel, *Anguilla anguilla*, measured by individual marking. Aquaculture, 112: 67–77

Kettle, A. J., Bakker, D. C. E., and Haines, K. 2008. Impact of the North Atlantic Oscillation on the trans-Atlantic migrations of the European eel (*Anguilla anguilla*). Journal of Geophysical Research: Biogeosciences, 113 (G3): 1-24.

King-Heiden, T. C., Mehta, V., Xiong, K. M., Lanham, K. A., Antkiewicz, D. S., Ganser, A., Heideman, W., *et al.* 2012. Reproductive and developmental toxicity of dioxin in fish. Molecular and Cellular Endocrinology, 1-2: 121–138.

Kirk, R. S. 2003. The impact of Anguillicola crassus on European eels. Fisheries Management and Ecology, 10: 385–394.

Knights, B. 1987. Agonistic behaviour and growth in the European eel, *Anguilla anguilla* L., in relation to warm-water aquaculture. Journal of Fish Biology, 31: 265–276.

Krueger, W. H., and Oliveira, K. 1999. Evidence for Environmental Sex Determination in the American eel, *Anguilla rostrata*. Environmental Biology of Fishes, 55: 381–389.

Kullmann, B., Adamek, M., Steinhagen, D., and Thiel, R. 2017. Anthropogenic spreading of anguillid herpesvirus 1 by stocking of infected farmed European eels, *Anguilla anguilla* (L.), in the Schlei fjord in northern Germany. Journal of fish diseases, 40: 1695–1706.

Kullmann, B., Hempel, M., and Thiel, R. 2018. Chemical marking of European glass eels *Anguilla anguilla* with alizarin red S and in combination with strontium: In situ evaluation of short-term salinity effects on survival and efficient mass-marking. Journal of Fish Biology, 92: 203–213.

Kullmann, B., Neukamm, R., and Thiel, R. 2017. Mass-marking of farmed European eels (*Anguilla anguilla* (Linnaeus, 1758)) with alizarin red S. Journal of Applied Ichthyology, 33: 914–917.

Kuroki, M., Marohn, L., Wysujack, K., Miller, M. J., Tsukamoto, K., and Hanel, R. 2017. Hatching time and larval growth of Atlantic eels in the Sargasso Sea. Marine Biology, 164: 118.

Lammens, E. H. R. R., Nie, H. W. d., Vijverberg, J., and van Densen, W. L. T. 1985. Resource Partitioning and Niche Shifts of Bream (*Abramis brama*) and Eel (*Anguilla anguilla*) Mediated by Predation of Smelt (*Osmerus eperlanus*) on *Daphnia hyalina*. Canadian Journal of Fisheries and Aquatic Sciences, 42: 1342–1351.

LANU. 2001. Ergebnisse langjähriger Wasseruntersuchungen in der Schlei: Eine Informations- und Planungsgrundlage (in German), Flintbek, 26 pp.

Lefebvre, F., Fazio, G., Palstra, A. P., Székely, C., and Crivelli, A. J. 2011. An evaluation of indices of gross pathology associated with the nematode *Anguillicoloides crassus* in eels. Journal of fish diseases, 34: 31–45.

Lele, S. R., Keim, J. L., and Solymos, P. 2016. ResourceSelection: Resource Selection (Probability) Functions for Use-Availability Data. R package version 0.2-6. http://CRAN.R-roject.org/package=ResourceSelection.

Leo, G. A. de, and Gatto, M. 1996. Trends in Vital Rates of the European Eel: Evidence for Density Dependence? Ecological Applications, 6: 1281–1294.

Lepa, A., and Siwicki, A. K. 2012. Fish herpesvirus diseases: A short review of current knowledge. Acta Veterinaria Brno, 81: 383–389.

Lievremont, M., Potus, J., and Guillou, B. 1982. Use of Alizarin Red S for Histochemical Staining of Ca2+ in the Mouse; Some Parameters of the Chemical Reaction in vitro. Cells Tissues Organs, 114: 268–280.

Lin, Y.-J., Ložys, L., Shiao, J.-C., Iizuka, Y., and Tzeng, W.-N. 2007. Growth differences between naturally recruited and stocked European eel *Anguilla anguilla* from different habitats in Lithuania. Journal of Fish Biology, 71: 1773–1787.

Lugert, V., Thaller, G., Tetens, J., Schulz, C., and Krieter, J. 2016. A review on fish growth calculation: multiple functions in fish production and their specific application. Reviews in Aquaculture, 8: 30–42.

Mangiafico, S. 2016. rcompanion: Functions to Support Extension Education Program Evaluation. R package version 1.1.3. https://CRAN.R-project.org/package=rcompanion.

Marohn, L., Hilge, V., Zumholz, K., Klügel, A., Anders, H., and Hanel, R. 2011. Temperature dependency of element incorporation into European eel (*Anguilla anguilla*) otoliths. Analytical and bioanalytical chemistry, 399: 2175–2184.

Marohn, L., Jakob, E., and Hanel, R. 2013. Implications of facultative catadromy in *Anguilla anguilla*. Does individual migratory behaviour influence eel spawner quality? Journal of Sea Research, 77: 100–106.

Marohn, L., Prigge, E., and Hanel, R. 2014. Escapement success of silver eels from a German river system is low compared to management-based estimates. Freshwater Biology, 59: 64–72.

Mateo, M., Lambert, P., Tétard, S., and Drouineau, H. 2017. Impacts that cause the highest direct mortality of individuals do not necessarily have the greatest influence on temperate eel escapement. Fisheries Research, 193: 51–59.

Mazumder, A., and Edmundson, J. A. 2002. Impact of fertilization and stocking on trophic interactions and growth of juvenile sockeye salmon (*Oncorhynchus nerka*). Canadian Journal of Fisheries and Aquatic Sciences, 59: 1361–1373.

McAllister, M. K., and Ianelli, J. N. 1997. Bayesian stock assessment using catch-age data and the sampling - importance rsampling algorithm. Canadian Journal of Fisheries and Aquatic Sciences, 54: 284–300.

McCleave, J. D. 2008. Contrasts between spawning times of *Anguilla* species estimated from larval sampling at sea and from otolith analysis of recruiting glass eels. Marine Biology, 155: 249.

Melià, P., Bevacqua, D., Crivelli, A. J., Panfili, J., Leo, G. A. de, and Gatto, M. 2006. Sex differentiation of the European eel in brackish and freshwater environments: A comparative analysis. Journal of Fish Biology, 69: 1228–1235.

Meyer, S., Sørensen, S. R., Peck, M. A., and Støttrup, J. G. 2012. Sublethal effects of alizarin complexone marking on Baltic cod (*Gadus morhua*) eggs and larvae. Aquaculture, 324-325: 158–164.

Midwood, J. D., Larsen, M. H., Aarestrup, K., and Cooke, S. J. 2016. Stress and food deprivation: linking physiological state to migration success in a teleost fish. The Journal of experimental biology, 219: 3712–3718.

Minegishi, Y., Aoyama, J., Inoue, J. G., Miya, M., Nishida, M., and Tsukamoto, K. 2005. Molecular phylogeny and evolution of the freshwater eels genus *Anguilla* based on the whole mitochondrial genome sequences. Molecular Phylogenetics and Evolution, 34: 134–146.

Naisbett-Jones, L. C., Putman, N. F., Stephenson, J. F., Ladak, S., and Young, K. A. 2017. A Magnetic Map Leads Juvenile European Eels to the Gulf Stream. Current Biology, 27: 1236-1240.

Nielsen, J. 1988. Marking and tagging methods applied to eel, *Anguilla anguilla* (L.). EIFAC Occasional Paper: 1–24.

Nielsen, T., and Prouzet, P. 2008. Capture-based aquaculture of the wild European eel (*Anguilla anguilla*). *In* Capture-based aquaculture: Global overview. FAO fisheries technical paper, 508, pp. 141–149. Ed. by A. Lovatelli, and P. F. Holthus. Food and Agriculture Organization of the United Nations, Rome.

Nijman, V. 2017. North Africa as a source for European eel following the 2010 EU CITES eel trade ban. Marine Policy, 85: 133–137.

Nzau Matondo, B., Benitez, J. P., Dierckx, A., Philippart, J. C., and Ovidio, M. 2017. Assessment of the Entering Stock, Migration Dynamics and Fish Pass Fidelity of European Eel in the Belgian Meuse River. River Research and Applications, 33: 292–301.

Oliveira, K. 1996. Field validation of annular growth rings in the American eel, *Anguilla rostrata*, using tetracycline-marked otoliths. Fishery Bulletin, 94: 186–189.

Pacariz, S., Westerberg, H., and Björk, G. 2014. Climate change and passive transport of European eel larvae. Ecology of Freshwater Fish, 23: 86–94.

Palstra, A. P., Heppener, D.F.M., van Ginneken, V.J.T., Székely, C., and van den Thillart, G.E.E.J.M. 2007. Swimming performance of silver eels is severely impaired by the swim-bladder parasite *Anguillicola crassus*. Journal of Experimental Marine Biology and Ecology, 352: 244–256.

Pankhurst, N. W. 1982. Relation of visual changes to the onset of sexual maturation in the European eel *Anguilla anguilla* (L.). Journal of Fish Biology, 21: 127–140.

Pankhurst, N. W., and Lythgoe, J. N. 1982. Structure and colour of the integument of the European eel *Anguilla anguilla* (L.). Journal of Fish Biology, 21: 279–296.

Pankhurst, N. W., and Sorensen, P. W. 1984. Degeneration of the alimentary tract in sexually maturing European *Anguilla anguilla* (L.) and American eels *Anguilla rostrata* (LeSueur). Canadian Journal of Zoology, 62: 1143–1149.

Pavey, S. A., Gaudin, J., Normandeau, E., Dionne, M., Castonguay, M., Audet, C., and Bernatchez, L. 2015. RAD Sequencing Highlights Polygenic Discrimination of Habitat Ecotypes in the Panmictic American Eel. Current Biology, 25: 1666–1671.

Pearsons, T. N., and Hopley, C. W. 1999. A Practical Approach for Assessing Ecological Risks Associated with Fish Stocking Programs. Fisheries, 24: 16–23.

Pedersen, M. I. 1998. Recapture rate, growth and sex of stocked cultured eels *Anguilla anguilla* (L.). Bulletin Français de la Pêche et de la Pisciculture, 349: 153–162.

Pedersen, M. I. 2000. Long-term survival and growth of stocked eel, *Anguilla anguilla* (L.), in a small eutrophic lake. Dana, 12: 71–76.

Pedersen, M. I. 2009. Does Stocking of Danish Lowland Streams with Elvers Increase European Eel Populations? American Fisheries Society Symposium, 58: 149–156.

Pedersen, M. I., and Rasmussen, G. H. 2016. Yield per recruit from stocking two different sizes of eel (*Anguilla anguilla*) in the brackish Roskilde Fjord. ICES Journal of Marine Science, 73: 158–164.

Pedersen, M. I., Jepsen, N., and Rasmussen, G. 2017. Survival and growth compared between wild and farmed eel stocked in freshwater ponds. Fisheries Research, 194: 112–116.

Peeler, E. J., and Feist, S. W. 2011. Human intervention in freshwater ecosystems drives disease emergence. Freshwater Biology, 56: 705–716.

Peters, G., and Hartmann, F. 1986. *Anguillicol*a, a parasitic nematode of the swim bladder spreading among eel populations in Europe. Diseases of Aquatic Organisms, 1: 229–230.

Pohlert, T. 2016. The Pairwise Multiple Comparison of Mean Ranks Package (PMCMR). R package, http://CRAN.R-project.org/package=PMCMR.

Pohlmann, J.-D., Freese, M., and Hanel, R. 2016. Minimum landing size in European eel fisheries management: limitations of simplistic management approaches in a semelparous species. ICES Journal of Marine Science, 73: 2509–2517.

Pontual, H. de, Groison, A. L., Piñeiro, C., and Bertignac, M. 2006. Evidence of underestimation of European hake growth in the Bay of Biscay, and its relationship with bias in the agreed method of age estimation. ICES Journal of Marine Science, 63: 1674–1681.

Prigge, E., Marohn, L., and Hanel, R. 2013. Tracking the migratory success of stocked European eels *Anguilla anguilla* in the Baltic Sea. Journal of Fish Biology, 82: 686–699.

Québec Declaration of Concern. 2003: Worldwide decline of eel resources necessitates immediate action. Fisheries, 28: 28-30.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/. Available at http://www.R-project.org/.

Reeves, S. A. 2003. A simulation study of the implications of age-reading errors for stock assessment and management advice. ICES Journal of Marine Science, 60: 314–328.

Richards, L. J., Schnute, J. T., Kronlund, A. R., and Beamish, R. J. 1992. Statistical Models for the Analysis of Ageing Error. Canadian Journal of Fisheries and Aquatic Sciences, 49: 1801–1815.

Ricker, W. E., ed. 1975. Computation and Interpretation of Biological Statistics of Fish Populations. Bulletin of the Fisheries Research Board of Canada, 191, Ottawa, Canada.

Righton, D., and Walker, A. M. 2013. Anguillids: Conserving a global fishery. Journal of Fish Biology, 83: 754–765.

Righton, D., Westerberg, H., Feunteun, E., Økland, F., Gargan, P., Amilhat, E., Metcalfe, J., *et al.* 2016. Empirical observations of the spawning migration of European eels: The long and dangerous road to the Sargasso Sea. Science Advances, 2: e1501694.

Robinet, T. T., and Feunteun, E. E. 2002. Sublethal Effects of Exposure to Chemical Compounds: A Cause for the Decline in Atlantic Eels? Ecotoxicology, 11: 265–277.

Sandin, S. A., Regetz, J., and Hamilton, S. L. 2005. Testing larval fish dispersal hypotheses using maximum likelihood analysis of otolith chemistry data. Marine and Freshwater Research, 56: 725–734.

Sano, M., Fukuda, H., and Sano, T. 1990. Isolation and characterization of a new herpesvirus from eel. *In* Pathology in Marine Science, pp. 15–31. Ed. by Frank O. Perkins, and Thomas C. Cheng. Academic Press, Inc., London.

Schmidt, J. 1922. The Breeding Places of the Eel. Philosophical Transactions of the Royal Society of London. Series B, Containing Papers of a Biological Character, 211: 179–208.

Shiraishi, H., and Crook, V. 2015. Eel Market Dynamics: An analysis of Anguilla production, trade and consumption in East Asia. TRAFFIC, Tokyo, Japan.

Simon, J. 2015. Age and growth of European eels (*Anguilla anguilla*) in the Elbe River system in Germany. Fisheries Research, 164: 278–285.

Simon, J., and Dörner, H. 2005. Marking the European eel with oxytetracycline, alizarin red and coded wire tags: An evaluation of methods. Journal of Fish Biology, 67: 1486–1491.

Simon, J., and Dörner, H. 2014. Survival and growth of European eels stocked as glass- and farm-sourced eels in five lakes in the first years after stocking. Ecology of Freshwater Fish, 23: 40–48.

Simon, J., Dörner, H., and Richter, C. 2009. Growth and mortality of European glass eel *Anguilla anguilla* marked with oxytetracycline and alizarin red. Journal of Fish Biology, 74: 289–295.

Simon, J., Dörner, H., Scott, R. D., Schreckenbach, K., and Knösche, R. 2013. Comparison of growth and condition of European eels stocked as glass and farm sourced eels in lakes in the first 4 years after stocking. Journal of Applied Ichthyology, 29: 323–330.

Simon, J., Dorow, M., Ubl, C., Frankowski, J., and Schaarschmidt, T. 2017. Validation of the age determination of eels (*Anguilla anguilla*) in coastal and inland waters of Mecklenburg-Vorpommern. *In* Mitteilungen der Landesforschungsanstalt für Landwirtschaft und Fischerei Mecklenburg-Vorpommern: Beiträge zum Aalmanagement, 58, pp. 89–101. Ed. by LfA, Gülzow.

Simon, J., Ubl, C., and Dorow, M. 2013. Growth of European eel *Anguilla anguilla* along the southern Baltic coast of Germany and implication for the eel management. Environmental Biology of Fishes, 96: 1073–1086.

Skov, C., Jepsen, N., Baktoft, H., Jansen, T., Pedersen, S., and Koed, A. 2014. Cormorant predation on PIT-tagged lake fish. Journal of Limnology, 73: 177–186.

Sørensen, S. R., Tomkiewicz, J., Munk, P., Butts, I. A.E., Nielsen, A., Lauesen, P., and Graver, C. 2016. Ontogeny and growth of early life stages of captive-bred European eel. Aquaculture, 456: 50–61.

Stacey, J. A., Pratt, T. C., Verreault, G., and Fox, M. G. 2015. A caution for conservation stocking as an approach for recovering Atlantic eels. Aquatic Conservation: Marine and Freshwater Ecosystems, 25: 569–580.

Støttrup, J. G., and Sparrevohn, C. R. 2007. Can stock enhancement enhance stocks? Proceedings of the Sixth International Symposium on Flatfish Ecology, Part IThe Sixth International Symposium on Flatfish Ecology, 57: 104–113.

Sühring, R., Byer, J., Freese, M., Pohlmann, J.-D., Wolschke, H., Möller, A., Hodson, P. V., *et al.* 2014. Brominated flame retardants and Dechloranes in European and American eels from glass to silver life stages. Chemosphere, 116: 104–111.

Sures, B., Knopf, K., and Kloas, W. 2001. Induction of stress by the swimbladder nematode *Anguillicola crassus* in European eels, *Anguilla anguilla*, after repeated experimental infection. Parasitology, 123: 179–184.

Teng, H.-Y., Lin, Y.-S., and Tzeng, C.-S. 2009. A New *Anguilla* Species and a Reanalysis of the Phylogeny of Freshwater Eels. Zoological Studies, 48*:* 808–822.

Tesch, F.-W., and Thorpe, J. E. 2003. The eel, 3rd edn. Blackwell Science, Oxford, UK.

Tseng, M.-C. 2016. Overview and Current Trends in Studies on the Evolution and Phylogeny of *Anguilla*. *In* Biology and Ecology of Anguillid Eels, pp. 21–35. Ed. by T. Arai. CRC Press, Boca Raton, FL.

Tsukamoto, K., Nakai, I., and Tesch, W.-V. 1998. Do all freshwater eels migrate? Nature, 396: 635–636.

Tzeng, W. N., Severin, K. P., and Wickström, H. 1997. Use of otolith microchemistry to investigate the environmental history of European eel *Anguilla anguilla*. Marine Ecology Progress Series, 149: 73–81.

UK Country Report 2016. 2016. Report on the eel stock, fishery and other impacts, in United Kingdom 2016. Joint EIFAAC/ICES/GFCM WGEEL REPORT 2016: 644-684.

van Beurden, S. J., Engelsma, M. Y., Roozenburg, I., Voorbergen-Laarman, M. A., van Tulden, P. W., Kerkhoff, S., van Nieuwstadt, A. P., *et al.* 2012. Viral diseases of wild and farmed European eel *Anguilla anguilla* with particular reference to the Netherlands. Diseases of Aquatic Organisms, 101: 69–86.

van Beurden, S. J., Voorbergen-Laarman, M. A., Roozenburg, I., Tellingen, J., Haenen, O. L. M., and Engelsma, M. Y. 2016. Development and validation of a real-time PCR assay for the detection of *anguillid herpesvirus 1*. Journal of fish diseases, 39: 95–104.

van den Thillart, G., Palstra, A. P., and van Ginneken, V. 2009. Energy Requirements of European Eel for Trans Atlantic Spawning Migration. *In* Spawning migration of the European eel: Reproduction index, a useful tool for conservation management. Fish & fisheries series, v. 30, pp. 179–199. Ed. by G. van den Thillart, S. Dufour, and J. C. Rankin. Springer Science + Business Media, Dordrecht.

van Ginneken, V., Antonissen, E., Muller, U. K., Booms, R., Eding, E., Verreth, J., and van den Thillart, G. 2005. Eel migration to the Sargasso: remarkably high swimming efficiency and low energy costs. The Journal of experimental biology, 208: 1329–1335.

van Ginneken, V., Ballieux, B., Willemze, R., Coldenhoff, K., Lentjes, E., Antonissen, E., Haenen, O., *et al.* 2005. Hematology patterns of migrating European eels and the role of EVEX virus. Comparative biochemistry and physiology. Toxicology & pharmacology: CBP, 140: 97–102.

van Ginneken, V.J.T., Haenen, O.L.M., Coldenhoff, K., Willemze, R., Antonissen, E., van Tulden, P. W., Dijkstra, S., *et al.* 2004. Presence of virus infections in Eel species from various geographic regions. Bulletin of the European Association of Fish Pathologists, 24: 268–272.

van Liefferinge, C., Dillen, A., Ide, C., Herrel, A., Belpaire, C., Mouton, A., Deckere, E. de, *et al.* 2012. The role of a freshwater tidal area with controlled reduced tide as feeding habitat for European eel (*Anguilla anguilla*, L.). Journal of Applied Ichthyology, 28: 572–581.

van Nieuwstadt, A. P., Dijkstra, S. G., and Haenen, O. L. M. 2001. Persistence of herpesvirus of eel *Herpesvirus anguillae* in farmed European eel *Anguilla anguilla*. Diseases of Aquatic Organisms, 45: 103–107.

Verhelst, P., Reubens, J., Pauwels, I., Buysse, D., Aelterman, B., van Hoey, S., Goethals, P., *et al.* 2017. Movement behaviour of large female yellow European eel (*Anguilla anguilla* L.) in a freshwater polder area. Ecology of Freshwater Fish, 371/372: 107.

Verreault, G., Dumont, P., Dussureault, J., and Tardif, R. 2010. First record of migrating silver American eels (*Anguilla rostrata*) in the St. Lawrence Estuary originating from a stocking program. Journal of Great Lakes Research, 36: 794–797.

Virtanen, P., and Isotupa, K. 1980. Staining properties of alizarin red S for growing bone in vitro. Acta anatomica, 108: 202–207.

Vøllestad, L. A., and Jonsson, B. 1988. A 13-Year Study of the Population Dynamics and Growth of the European Eel *Anguilla anguilla* in a Norwegian River: Evidence for Density-Dependent Mortality, and Development of a Model for Predicting Yield. The Journal of Animal Ecology, 57: 983–997.

Walker, A., Apostolaki, P., Pawson, M., Walton, J., Scutt-Phillips, J., and Martin, T. 2009. Developing guidelines for best practice in stocking eel for enhancement purposes: Cefas Project C3243 funded by the Fisheries Challenge Fund, Marine & Fisheries Agency, Defra, UK.

Warren-Myers, F., Dempster, T., and Swearer, S. E. 2018. Otolith mass marking techniques for aquaculture and restocking: Benefits and limitations. Reviews in Fish Biology and Fisheries. https://doi.org/10.1007/s11160-018-9515-4

Westerberg, H., Pacariz, S., Marohn, L., Fagerström, V., Wysujack, K., Miller, M. J., Freese, M., *et al.* 2017. Modeling the drift of European (*Anguilla anguilla*) and American (*Anguilla rostrata*) eel larvae during the year of spawning. Canadian Journal of Fisheries and Aquatic Sciences, 75: 224-234.

Westerberg, H., Sjöberg, N., Lagenfelt, I., Aarestrup, K., and Righton, D. 2014. Behaviour of stocked and naturally recruited European eels during migration. Marine Ecology Progress Series, 496: 145–157.

Westin, L. 1998. The spawning migration of European silver eel (*Anguilla anguilla* L.) with particular reference to stocked eel in the Baltic. Fisheries Research, 38: 257–270.

Westin, L. 2003. Migration failure in stocked eels *Anguilla anguilla*. Marine Ecology Progress Series, 254: 307–311.

Wickström, H. 1986. Growth of cultured eels stocked in two swedish lakes. Vie Mileu, 36: 273–277.

Wickström, H., and Sjöberg, N. B. 2014. Traceability of stocked eels - the Swedish approach. Ecology of Freshwater Fish, 23: 33–39.

Wickstrom, H., Westin, L., and Clevestam, P. 1996. The biological and economic yield from a long-term eel-stocking experiment. Ecology of Freshwater Fish, 5: 140–147.

Wilson, J. A., Vigliola, L., and Meekan, M. G. 2009. The back-calculation of size and growth from otoliths: Validation and comparison of models at an individual level. Journal of Experimental Marine Biology and Ecology, 368: 9–21.

Würtz, J., and Taraschewski, H. 2000. Histopathological changes in the swimbladder wall of the European eel *Anguilla anguilla* due to infections with *Anguillicola crassus*. Diseases of Aquatic Organisms, 39: 121–134.

Wysujack, K., Westerberg, H., Aarestrup, K., Trautner, J., Kurwie, T., Nagel, F., and Hanel, R. 2015. The migration behaviour of European silver eels (*Anguilla anguilla*) released in open ocean conditions. Marine and Freshwater Research, 66: 145–157.

Zheng, G., and Zhou, K. 1999. Drug resistance of *Aeromonas hydrophila* strains isolated from skin ulcer of *Anguilla anguilla*. Journal of Fishery Sciences of China, 6: 69–72.

Contribution of authors

Chapter I Mass-marking of glass eels

Björn Kullmann (BK) and Mattias Hempel (MH) designed the experimental setup and conducted the experiments under advice of Ralf Thiel (RT). BK was responsible of the practical implementation of the mass-marking procedure and was in charge during the laboratory work. The manuscript was written by BK as corresponding author. RT and MH reviewed the text and gave comments.

Confirmation of correctness

Place, date	Prof. Dr. Ralf Thiel

Chapter II Mass-marking of farmed eels

Rüdiger Neukamm (RN) invented the basic details of the marking procedure and Björn Kullmann (BK) was responsible for the mass-marking approaches in 2015 and 2016. The manuscript was written by BK as main author under advice of RN and Ralf Thiel.

Confirmation of correctness

Place, date	Prof. Dr. Ralf Thiel

Chapter III Biased age-based stock assessment

Björn Kullmann (BK) and Rüdiger Neukamm (RN) developed the objective of the study and BK designed the study concept with advice of Ralf Thiel (RT). BK was responsible for the sampling design, the laboratory work, otolith preparation, and data analysis. Laura Wichmann (LW) supported the laboratory work. Jan-Dag Pohlmann (JP) and Marko Freese (MF) were responsible for the blind readings. BK, and partly JP, wrote the manuscript under advice of all other authors.

Confirmation of correctness

Place, date	Prof. Dr. Ralf Thiel

Chapter IV Spreading of the *eel herpesvirus*

Björn Kullmann (BK) and Ralf Thiel (RT) were responsible for the conceptual planning of the sampling design. BK coordinated the sampling and the laboratory work under advice of Dieter Steinhagen (DS). Mikolaj Adamek (MA) conducted the virus isolation and provided virus diagnostic raw data. BK analysed the data, did the statistics and, as corresponding author, wrote the first version of the manuscript for submission with the collaboration of RT and MA. All authors critically reviewed the manuscript.

Confirmation of correctness

Place, date	Prof. Dr. Ralf Thiel

Chapter V Bigger is better in eel stocking measures?

Ralf Thiel (RT) and Björn Kullmann (BK) elaborated the project outline and the objectives of this paper. BK coordinated the sampling and was responsible for the sampling design, the laboratory work, data analysis, statistics, and wrote the manuscript. RT reviewed the draft and gave comments.

Confirmation of correctness

Place, date	Prof. Dr. Ralf Thiel

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Wenn ich auf den ersten Tag meiner Zeit als Doktorand zurückblicke, erinnere ich noch einen kurzen Moment der Euphorie gefolgt von einem etwas längeren Moment des Zweifelns, der so in etwa zwei Jahre angedauert hat. Wie soll all diese Arbeit nur zu schaffen sein? Nun, da am Ende alles geschrieben, geschliffen, gebrannt, ausgewertet, analysiert und bald gedruckt sein wird, kann ich es immer noch nicht genau sagen. Gerade am Anfang waren die Zeiten für mich keine leichten und ich habe gleich mehrere Male mit dem Gedanken gespielt, aufzuhören. Die ersten eineinhalb Jahre kann man in etwa so zusammenfassen: Zunächst der „Abschleim-Skandal“ bei der ersten Glasaalmarkierung, dann die – man muss es fast so nennen – Katastrophe mit den vielen toten Herpesvirus infizierten Farmaalen in der Schlei und dann noch das „radioaktive“ Strontium … Es war natürlich klar, dass die Promotion kein Spaziergang sein würde. Allerdings wurde ich anfangs regelmäßig eines besseren belehrt, dass es nicht noch schlimmer kommen könnte. Der Doktortitel war für mich in etwa so weit entfernt, wie die Sargassosee für die Aale.

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Die **Massenmarkierung** von Aalen ist im Grunde ganz einfach. Wasser einlaufen lassen, Alizarin dazu, Aale dazu, warten, Aale aus dem Bad nehmen und aussetzen. In der Praxis ist das sichere und schonende Handling allerdings eine Herausforderung, die ohne die Erfahrung und Hilfe von Fachleuten unmöglich ist. Ich möchte dem Leiter der Aalversandstelle des Deutschen Fischereiverbandes, **Arne Koops**, und seinen beiden Mitarbeiten **Lars Renken** und **Christian Oertel** meinen Dank ausdrücken und speziell bei **Jan Kemnitz** für seine große Gastfreundschaft und Hilfe bedanken.

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Abschließend möchte ich nicht unerwähnt lassen, dass für diese Arbeit eine große Zahl von Aalen geopfert wurden. Ich hoffe sehr, mit dieser Arbeit insgesamt einen **Beitrag zum Erhalt des Europäischen Aals** geleistet zu haben und dass kein Aal umsonst gestorben ist.

Das war AALES.