Stocking European eels (*Anguilla anguilla*) into coastal waters of the German Baltic Sea

Dissertation

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Declaration on oath

I hereby declare in lieu of oath that I have written this dissertation independently and have not used any sources or aids other than those indicated.

Hamburg, 15.09.2023
Place, Date

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Datum der Disputation: 19. Februar 2024

For my sons, Henri & Felix

and for the love of my life, Björn

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Summary

Fish stocking is an important tool for fish stock management, for example, to reintroduce endangered fish species in certain areas, to support fisheries, or to promote recreational fishing. However, in terms of sustainable fisheries management combined with a holistic approach, complex mechanisms of action must be considered, such as the requirements of the target species, the timing of stocking, the characteristics of the biotope to be stocked, and the biocoenosis already present in it. The objective of the stocking must also be clearly defined so that fish stocking is carried out successfully and achieves the setting of the target - and does not have the opposite effect. A very important stocking fish species for fisheries is the European eel (*Anguilla anguilla*). Due to its unique and complex life cycle, large distribution range, and for commercial use, the eel has been stocked throughout Europe and beyond for nearly 200 years. However, the European eel population has been declining for many years and has stagnated at a persistently low level.

The purpose of this thesis is to shed light on some aspects of stocking eels in coastal waters, using the example of the eastern German Baltic Sea coast, which is not significantly influenced by the tide, and to clarify to what extent such a stocking strategy has an impact on the eel population and the local eel fishery. In order to be able to assess stocking strategies, it is of utmost importance to mark eels before stocking, for example to distinguish them from unstocked conspecifics and to identify possible differences between stocked and unstocked individuals. Furthermore, marked eels can be used to detect movement patterns, identify growth rates since stocking, and determine the percentage of stocked individuals in the local eel population. Based on these obtained results, the benefits for both the local fishery and the

conservation of the eel population can be assessed, contributing to the compliance with the EU Eel Regulation of 2007.

A chemical marker for eels that was widely used until 2017 is alizarin red S (ARS). However, the use of ARS for eel marking was prohibited in Germany due to various missing data regarding the toxicological potential because it was not possible to assess whether there could be a health risk to humans who regularly consume ARS marked eels. Primarily, it was essential that the bioaccumulation potential of ARS in eel muscle tissue could not be estimated to assess the uptake of ARS by human consumption.

Considering this, the first study of this thesis describes the development of a protocol to detect ARS in muscle tissue and simultaneously presents for the first time the results of this detection of ARS in fish. For this purpose, a total of 250 eels with body sizes ranging from 6 - 57 cm and different time points after the marking process (0 - 3 years) were examined. It could be shown that the highest ARS concentration of 6,056 µg kg^{-1} was detected in glass eels shortly after the marking process tagging and that after one year post marking the ARS content was below the detection limit of 8.9 µg kg^{-1} muscle tissue. Since eels at the eastern German Baltic Sea coast reach the minimum landing size of 50 cm after about three years, it can be assumed that the ingestion of ARS through the consumption of marked eels is unlikely.

Complementing the previously described importance of fish marking prior to stocking, chapter 2 of this thesis addresses the need of marking stocked pre-grown farmed eels in order to perform proper growth analyses. During their time in eel aquaculture facilities, eels are regularly sorted by size to counteract feeding pressure on smaller conspecifics. This procedure exerts a high level of stress on the animals, which can lead to the formation of so-called stress rings on the otoliths. These stress rings cannot be distinguished from the natural annual rings, which are formed, among other things, by the different food availability within a year, which has implications for the calculation of growth rates. The results showed that an age overestimation of up to seven years can occur, resulting in an underestimation of growth rate. This growth rate underestimation, in turn, has a significant impact on the estimated biomass of migrating silver eels (escapement), calculated by an age-based model and thus influences the model-based assessment of eel management measures.

The studies in chapters 3 and 4 present the results of the influence of a glass eel stocking experiment on the local eel population conducted between 2014 and 2016 in two stocking areas of the eastern German Baltic Sea coast. For later identification and comparison with unmarked conspecifics, the total of over 1.18 million glass eels (335 kg) were marked with ARS before stocking.

Fishery-independent eel monitoring was conducted in both stocking areas and in six other reference areas between 2009 and 2020 (chapter 3). An "enclosure" net specifically designed for non-tidal coastal waters was used as fishing gear to estimate yellow eel density over the study period, as well as before and after glass eel coastal stocking (Period I: 2009 - 2014; Period II: 2015 - 2020). As a result, a significantly increasing yellow eel density could be observed in a total of four reference areas (including both stocking areas). From this, it could be estimated that the local yellow eel stock size increased by about 180,000 eels in period II, bringing it to about 5 million yellow eels on the eastern German Baltic Sea coast. Furthermore, an attempt was made to estimate the stock development without the stocking effect under the assumption that stocked (mostly unmarked) eels of adjacent waters do not migrate into the study area. Under this assumption, the increase in local eel density may have been due to an increase in natural recruitment, although the influence of other stocking activities was probably rather underestimated.

In order to evaluate the influence of a glass eel coastal stocking on the local eel population in more detail and finally to assess whether such an eel stocking could function as a management option, the fourth chapter focuses on the recaptured stocked eels, which were caught from 2017 to 2019 (i.e., up to 5 years after the first stocking measure) at a total of 55 stations along the eastern German Baltic Sea coast. It was found that 19.3 % (n = 353) of all eels caught were ARS marked and located between 10 and 100 km from the stocking sites. It was also shown that with each additional stocking cohort, the proportion of stocked eels within an age class increased by approximately 10 %.

Furthermore, no particular disadvantage could be shown for stocked eels with respect to their health status compared to their non-marked conspecifics. On the contrary, significantly higher values were detected with respect to their fat content, condition factor and growth rate, which could be an advantage for the return migration in the silver eel stage to the spawning ground in the Sargasso Sea.

Zusammenfassung

Der Besatz von Fischen ist ein wichtiges Werkzeug des Fischbestandsmanagements, um beispielsweise gefährdete Fischarten in bestimmten Gebieten wieder anzusiedeln, die Fischerei zu unterstützen oder die Sportfischerei zu fördern. Im Sinne einer nachhaltigen fischereilichen Maßnahme in Verbindung mit einem ganzheitlichen Ansatz müssen jedoch komplexe Wirkmechanismen beachtet werden, wie z.B. die Lebensansprüche der Zielart, der Zeitpunkt des Besatzes, die Charakteristika des zu besetzenden Biotops und die darin bereits vorkommende Biozönose. Das Ziel des Besatzes muss zudem eindeutig definiert sein, damit Fischbesatz erfolgreich durchgeführt und zielführend ist – und nicht das Gegenteil bewirkt. Eine für die Fischerei sehr wichtige Besatzfischart ist der Europäische Aal (*Anguilla anguilla*). Durch seinen einzigartigen und komplexen Lebenszyklus, seinem großen Verbreitungsgebiet sowie die traditionelle und kommerzielle wirtschaftliche Nutzung wird der Aal bereits seit fast 200 Jahren in ganz Europa und darüber hinaus besetzt. Allerdings schrumpft die Aalpopulation seit vielen Jahren und stagniert auf einem anhaltend niedrigem Niveau.

Diese Arbeit soll einige Aspekte des Besatzes von Aalen in Küstengewässern am Beispiel der nicht wesentlich von der Tide beeinflussten östlichen deutschen Ostseeküste beleuchten und klären, inwiefern eine derartige Besatzstrategie einen Einfluss auf die Aalpopulation und die lokale Aalfischerei hat. Um Besatzstrategien beurteilen zu können, ist es von größter Wichtigkeit, die Aale vor dem Besatz zu markieren, um sie beispielsweise von nicht besetzten Artgenossen zu unterscheiden, um daraus folgend mögliche Unterschiede zwischen besetzten und nicht besetzten Artgenossen zu identifizieren. Des Weiteren können anhand von markierten Aalen deren Bewegungsmuster erkannt, Wachstumsraten seit Besatz identifiziert und der prozentuale Anteil von Besatztieren des lokalen Aalbestandes ermittelt werden.

Anhand dieser gewonnen Ergebnisse können der Nutzen sowohl für die lokale Fischerei als auch für den Erhalt der Aalpopulation beurteilt werden, was zur Erfüllung der EU-Aalverordnung von 2007 beiträgt.

Ein bis zum Jahre 2017 viel genutzter chemischer Markierungsstoff von Aalen ist Alizarin rot S (ARS). Allerdings wurde die Verwendung von ARS zur Aalmarkierung in Deutschland aufgrund von diversen fehlenden Daten hinsichtlich des toxikologischen Potenzials untersagt, weil nicht abgeschätzt werden konnte, ob eine gesundheitliche Gefahr beim Verzehr von ARS-markierten Aalen für den Konsumenten bestehen könnte. Wesentlich war, dass das Bioakkumulationspotenzial von ARS im Aalmuskelgewebe nicht abgeschätzt werden konnte, wodurch die Aufnahme von ARS durch den Verzehr beim Menschen nicht beurteilt werden konnte.

Vor diesem Hintergrund beschreibt die erste Studie dieser Arbeit die Entwicklung eines Protokolls zur Nachweisdetektion von ARS im Muskelgewebe und präsentiert gleichzeitig zum ersten Mal die Ergebnisse dieser Nachweisdetektion von ARS bei Fischen. Hierfür wurden insgesamt 250 Aale mit Körpergrößen von 6 – 57 cm sowie zu verschiedenen Zeitpunkten nach der Markierung (0 – 3 Jahre) untersucht. Es konnte gezeigt werden, dass die höchste ARS Konzentration von 6.056 µg kg^{-1} bei Glasaalen nach der Markierung nachgewiesen wurde und dass bereits nach nur einem Jahr nach der Markierung der ARS-Gehalt unterhalb der Nachweisgrenze von 8,9 µg kg^{-1} Muskelgewebe lag. Da an der deutsche Ostseeküste besetzte Aale frühstens nach drei Jahren eine vermarktungsfähige Größe von 50 cm erreichen, kann davon ausgegangen werden, dass die Aufnahme von ARS durch den Verzehr von markierten Aalen sehr unwahrscheinlich ist.

Ergänzend zur bereits beschriebenen Wichtigkeit von Fischmarkierung vor dem Besatz, thematisiert die Studie des 2. Kapitels die Notwenigkeit von Markierungen von besetzten vorgestreckten Aalen, um korrekte Wachstumsanalysen durchführen zu können. Während der Zeit in Aal-Aquakulturanlagen, werden Aale regelmäßig nach Größen sortiert, um den Fraßdruck auf kleinere Artgenossen entgegenzuwirken. Diese Prozedur übt einen hohen Stress auf die Tiere aus, wodurch sogenannte Stressringe auf den Otolithen entstehen können. Diese Stressringe können nicht von den natürlichen Jahresringen, welche unter anderem durch die unterschiedliche Nahrungsverfügbarkeit innerhalb eines Jahres entstehen, unterschieden werden, was Auswirkungen auf die Berechnung von Wachstumsraten hat. Die Ergebnisse zeigten, dass eine Altersüberschätzung von bis zu sieben Jahren stattfinden kann, was zu einer Unterschätzung der Wachstumsrate führte. Diese Wachstumsraten-unterschätzung wirkt sich wiederum erheblich auf die Gesamtbiomasse der abwandernden Blankaale aus, welche u.a. durch altersbasierte Bestandsmodelle berechnet werden, und beeinflusst somit die modellbasierte Beurteilung von Aalmanagementmaßnahmen.

Die Studien der Kapitel 3 und 4 präsentieren die Ergebnisse des Einflusses eines zwischen 2014 und 2016 durchgeführten Glasaalbesatzes in zwei Besatzgebieten der östlichen deutschen Ostseeküste auf den lokalen Aalbestand. Zur späteren Identifizierung und zum Vergleich mit nicht markierten Artgenossen wurden die insgesamt über 1.18 Mio. Glasaale (335 kg) vor Besatz mit ARS markiert.

In beiden Besatzgebieten und in sechs weiteren Referenzgebieten wurden zwischen 2009 bis 2020 ein fischereiunabhängiges Aalmonitoring durchgeführt (Kapitel 3). Als Fanggerät diente ein speziell für nicht-gezeitenabhängiges Küstengewässer entwickeltes ein Hektar großes „Enclosure“-Netz, um die Gelbaaldichte im Laufe der Studienzeit, und zwar vor und nach dem Glasaalküstenbesatz (Periode I: 2009 – 2014; Periode II: 2015 – 2020), abzuschätzen. Im Ergebnis konnte eine signifikant zunehmende Gelbaaldichte in insgesamt vier Referenzgebieten (inkl. beider Besatzgebiete) beobachtet werden. Daraus resultierend konnte abgeschätzt werden, dass die lokale Gelbaalbestandsgröße in der Periode II um ca. 180.000 Aale zugenommen hat und somit auf etwa 5 Mio. Gelbaalen an der östlichen deutschen Ostseeküste angestiegen ist. Des Weiteren wurde versucht, die Bestandsentwicklung ohne den Besatzeffekt zu schätzen unter der Annahme, dass besetzte (meist unmarkierte) Aale benachbarter Gewässer nicht in das Untersuchungsgebiet einwandern. Unter dieser Voraussetzung könnte der Anstieg der lokalen Aaldichte auf eine Zunahme der natürlichen Rekrutierung zurückgeführt werden, wobei der Einfluss weiterer Besatzmaßnahmen vermutlich eher unterschätzt wurde.

Um den Einfluss eines Glasaalküstenbesatz auf den lokalen Aalbestand noch detaillierter bewerten und schließlich beurteilen zu können, ob ein derartiger Aalbesatz als Managementoption fungieren könnte, fokussiert das vierte Kapitel auf die wiedergefangen besetzten Aale, welche von 2017 bis 2019 (also bis zu 5 Jahre nach der ersten Besatzmaßnahme) an insgesamt 55 Stationen entlang der östlichen deutschen Ostseeküste gefangen wurden. Es konnte festgestellt werden, dass 19,3 % (n = 353) aller gefangenen Aale eine ARS-Markierung aufwiesen und sich zwischen 10 und 100 km von den Besatzgebieten aufhielten. Außerdem konnte gezeigt werden, dass mit jeder weiteren Besatzkohorte der Anteil besetzten Aale innerhalb einer Altersklasse um etwa 10 % zunahm.

Des Weiteren konnten keine Nachteile bei besetzten Aalen bezüglich ihres Gesundheitszustandes im Vergleich zu den nicht besetzten Artgenossen nachgewiesen werden. Vielmehr wurden signifikant höhere Werte bezüglich ihres Fettgehalts, dem Konditionsfaktor und den Wachstumsraten nachgewiesen, was sich für die Rückwanderung im Blankaalstadium zum Laichplatz in der Sargassosee als Vorteil ergeben könnte.

General Introduction

The concept of stocking fish

Stocking is the single or repeated introduction of fish into any kind of water (e.g., rivers, lakes, or marine waters) where the species in question either exists naturally or existed in the past and thus serves to reintroduce native species into waters where they have either been overfished and/or are unable to reproduce (Baer *et al.*, 2007).

There are different forms of fish stocking with different objectives, which can be divided into four main categories of focus (e.g., Cowx, 1994; Cowx, 1999; Cowx *et al.*, 2012; Arlinghaus, 2017): Firstly, stocking can be used as a purely restauration or reintroduction measure for species, that extinct or disappeared in a particular area until the natural occurrence of the target species is established (i.e., self-reproductive stock). Secondly, it can be applied as a settlement measurement of native species in newly created habitat also until the target species is established. Thirdly, as compensatory measure when high removal losses occur due to fishing or predation. For this, the natural occurrence of a particular species must exist in principle, but the population cannot be maintained without supporting measures. And fourthly, conservation measurements when natural recruitment is minimal or absent, but good environmental habitat conditions exist for the target species in certain or all development stages.

A prerequisite for stocking or translocating fish is that the carrying capacity (CC) of the target environment has not yet been reached, which can be defined as the maximum density, biomass or number of organisms of a (fish) species or a group of different (fish) species that a habitat can maintain (Dhondt, 1988). Within this concept, CC of a habitat is considered to be achieved when there is no longer a relationship between growth rate and either biomass or density of a stock or population (Aprahamian, 2000). Thus, as long as there is a correlation between the population growth and its biomass, it is assumed that the number of available resources (e.g., food or space) of a particular habitat are not fully utilized and that therefore population size can be artificially increased (i.e., by stocking) without harming the target species. However, CC of a habitat is not a fixed space or size but can change due to various biotic and abiotic factors, allowing stocking itself to alter the CC of an environment.

In fisheries management and modelling, for example, CC is one of the most important parameters (Acou *et al.*, 2011) because it is used in the formulas to calculate the maximum sustainable yields (MSY), which is defined as "*the highest theoretical equilibrium yield that can be continuously taken on average from a stock under existing average environmental conditions without significantly affecting the reproduction process*" according to Article 4 of the Common Fisheries Policy of the EU (Regulation (EU) 1380/2014)). Originally, MSY was set at 50 % of CC, which is the phase when the maximum growth rate of a fish population occurred (e.g., Tsikliras and Froese, 2019; Figure 1). Nowadays, CC is considered to be about 30 % of the stock size, depending on the species (Thorpe *et al.*, 2015) and is important for the calculations of annual catch quotas of various economically important fish species.

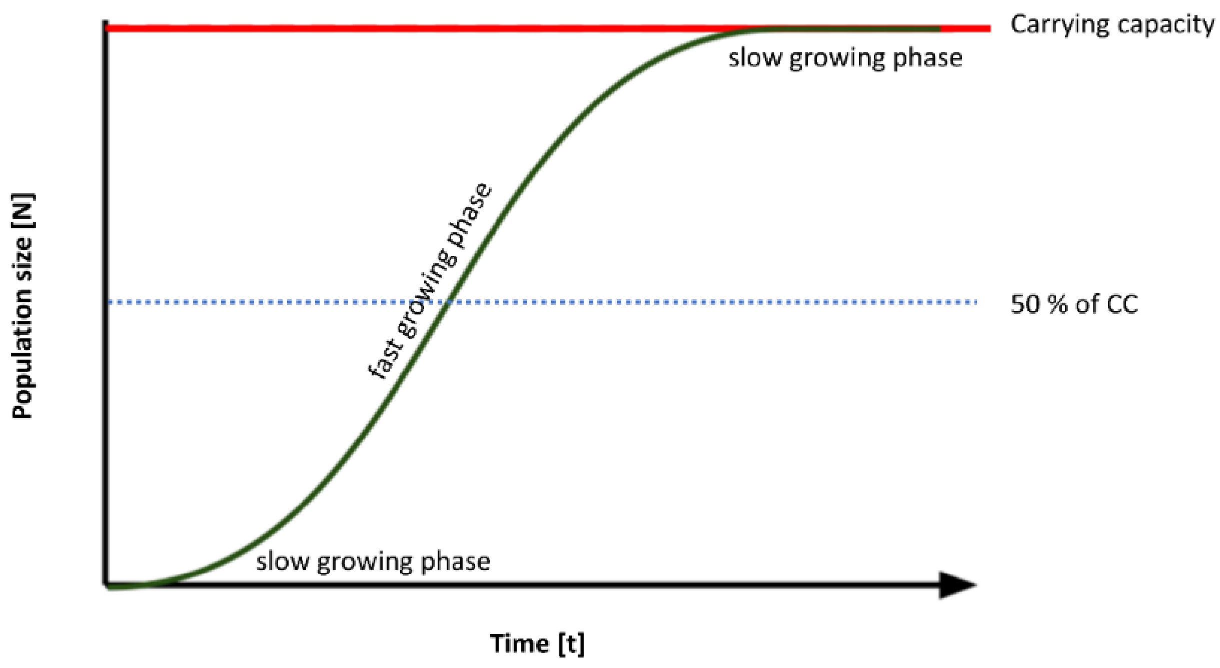

Figure 1: The relation of population growth and carrying capacity (CC) (Modified from Tsikliras and Froese, 2019).

In addition to the objectives of fish stocking, there are several ways to carry out fish translocation, which mainly depends on the species. The location of stocking may be different from the donor habitat (e.g., European eel) or may take place in the exact same habitat (e.g., salmonids), depending not only on the fish species but also on the developmental stage of the target species. This circumstance can result in several thousand kilometers between the donor water and the target water, which can create logistical challenges for stocking operations (Dekker and Beaulaton, 2016). Furthermore, between catching and stocking, various procedures are carried out with the fishes. For example, for some fish species the parents can be stripped, so that fertilization, hatching, and growing up to the desired size, weight, and/or stage of development is carried out artificially (white fish, Eckmann *et al.*, 2007). On the contrary, for some fish species, such as the European eel, artificial breeding is still not possible on a commercial scale so stocking is reliant on wild catches (ICES, 2016c). In principle, stocking of smaller fishes might be advantageous because it is assumed that they have not yet become accustomed to the conditions in the hatchery and can therefore still adapt to natural environmental conditions, while, in turn, the mortality of small fish must expected to be higher (Lorenzen, 2000).

A key tool of stocking measures is the success control, so that managers can evaluate whether the applied strategy has contributed to the achievement of management objectives. For this purpose, it is essential that stocking measures are accompanied by a monitoring program and that the fish are appropriately marked prior to stocking so that they can be clearly identified and examined. A variety of techniques are available for marking stocked fish, but those that can be applied to many animals at once and are relatively easy to identify have proven to be particularly suitable. Of particular importance are fluorescent markers (e.g., alizarin red S (ARS) or oxytetracycline (OTC)) that can be applied as an immersion bath and later visualized by preparation of the otoliths (Warren-Myers *et al.*, 2018).

Stocking of European eels

The European eel (*Anguilla anguilla* (Linneaeus, 1758)) is a facultative catadromous fish species that occurs in several coastal and freshwater aquatic habitats of the north east Atlantic including the North Sea, the Baltic Sea and the Mediterranean Sea (Tesch, 2003) and spawns in the Sargasso Sea in the Atlantic Ocean (Schmidt, 1922). The newly hatched leptocephalus larvae drift with ocean currents (Gulf Stream) to the continental shelf of Europe and North Africa, where they develop into glass eels (cf. Figure 2a) and enter the various aquatic systems of Europe, North Africa and even some parts of West Asia (Froese and Pauly, 2023). The growth phase known as the “yellow eel” stage (cf. Figure 2c) can occur in marine, brackish, or fresh waters before eels metamorphose into the final "silver eel" stage and then migrate a distance of up to 10,000 km back to their spawning ground (Righton *et al.*, 2016). Once in the spawning habitat, the adult eels probably reproduce only once and then die (Tsukamoto and Kuroki, 2014). However, since the discovery of spawning habitat in the Sargasso Sea more than 100 years ago (Schmidt, 1922), neither silver eels nor eel eggs have been discovered at this site.

The stocking of European eels is a special case compared to other common fish species for a few reasons: Breeding of European eels in captivity is still not feasible without immense efforts and only under strict laboratory conditions (Jéhannet *et al.*, 2021). From this it follows that stocking is entirely reliant on wild catches, which is related to the question of the so-called "net benefit" defined as "*where the stocking results in 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, 2016c). Also, the European eel is a semelparous species which means that it reproduce only once in their life time given that fat reserves are limited and are sufficient to migrate back to their suspected breeding sites (e.g., van den Thillart *et al.*, 2004). In this context it has to be noted, that eels can reach ages of 20 years and more (ICES, 2009b, 2011), so the generation cycle extends over decades making the stock restoration difficult and a very lengthy task. Moreover, the European eel stock is panmictic and the genetic structure of the population was found to be more or less homogenous (Palm *et al.*, 2009). Thus, unlike in other species that reproduce in their donor habitat, the source of the individuals might be of relative minor importance because there are no autochthonous subpopulations.

Stocking of European eels has been carried out for over 180 years. According to Miller (1870, cited from Dekker and Beaulaton, 2016), the first recorded stocking of *A. anguilla* was conducted in France in 1840, where these fish species were translocated from waters with high abundances (e.g., France, Spain; England, and Portugal) to water bodies in other countries and further inland. The goal of eel stocking has changed greatly in Europe since the first eel stocking experiments because, contrary to expectations, no new populations became established in the stocking waters (at that time neither the location of the spawning ground nor the life cycle of the European eel was known). Since the beginning of eel stocking almost two hundred years ago, the stock situation has changed considerably. While in the 1950s *A. anguilla* was the most frequently recorded species in inland catches in Europe and accounted for about 5 % of the total catch (Dekker and Beaulaton, 2016), a steadily declining trend of eel stock in Europe was observed from the 1960s onwards (ICES Advice, 2022), although eel stocking continued (Dekker and Beaulaton, 2016).

Then, starting in the early 1980s, the European eel population collapsed (Québec Declaration of Concern, 2003). The glass eel abundance dropped down to far below 10 % compared to the previous reference levels (mean glass eel recruitment from 1960 to 1979), reaching a historical low level in 2011 (ICES, 2021a). In 1999, ICES already recommended to establish a recovery plan for the European eel population in order to reduce all fisheries and later all anthropogenic mortality in general as much as possible. The European Union (EU) reacted in 2007 and a adopted the Eel Regulation (EU (VO) 1100/2007), which thereby imposed member states to establish management plans for each identified eel management unit (EMU) until 2010 to achieve a minimum silver eel escapement target of 40 % biomass compared to pristine levels. Given that natural recruitment was low in most EMUs (currently there exist 81 EMUs; EU COM, 2020), many EU member states implemented massive stocking activities in their EU-notified management plans in order to fulfill the escapement target, which – including stocking from 2010 on – now had to be considered as legally binding (Ubl and Jennerich, 2008).

In Germany, a total of nine eel river basins (or EMUs) have been identified, which are congruent with the river basin districts defined according to the Water Framework Directive (EG, 2000). Most of these plans include stocking measures, besides monitoring and reduction of various identified mortality sources, to supplement local stocks that suffer from insufficient recruitment (European Council, 2007; EU COM, 2020; Fladung and Brämick, 2021). Given that European eels cannot be reproduced in captivity yet, 60 % of the annual glass eel catch (40 – 60 tons annually; ICES, 2018) in coastal waters of France, Portugal, England, and Spain are reallocated into other suitable waters with current low natural eel recruitment (ICES, 2016c).

Research needs and knowledge gaps in eel stocking

Particularly against the background of the critical state of the population of the European eel and the fact that eel stocking merely corresponds to a redistribution of natural recruits, special attention must be paid to success control. In view of the very high numbers of stocked eels, mass marking must be possible for efficient success control. Furthermore, for this purpose a marking substance must be used that is compatible with both species' protection and utilization requirements, i.e., that neither harms the eel itself nor conflicts with human consumption. In the past, the substance alizarin red S (ARS) has been used very successfully, because eels of all life stages can be marked quickly and reliably (Simon and Dörner, 2005; Caraguel *et al.*, 2015) and the marking can be made visible with the aid of a fluorescence microscope without extensive preparation work (c.f. Kullmann *et al.*, 2018). The substance ARS was preferred for a long time due to its presumed low bioaccumulation potential, until in Germany the Federal Office for Risk Research (in German: Bundesinstitut für Risikobewertung = BfR) stated in an assessment that an accumulation and a health hazard associated with the consumption of these eels cannot be ruled out with sufficient certainty, so that the use for fish marking became impossible (BfR, 2017, 2019). At this point, there is an urgent need for research on the bioaccumulation potential and genotoxic effect of ARS, so that the use of this substance can be continued and eel stocking measures can again be subjected to a success control in the future, since other markers such as strontium or barium chloride, although equally safe to use, pose considerable technical requirements for detection by electron microscope (Wickström and Sjöberg, 2014). Also, oxytetracycline, which has been used successfully as a tetracycline in Baltic Sea explorers (Hüssy *et al.*, 2020), can be used to mark eels (Alcobendas *et al.*, 1991), but it has special disposal requirements due to its status as an antibiotic and is also perceived by the public as a rather less suitable marker.

There may also be other reasons why marking of stocked eels is necessary. For example, it is known that eels that are pre-grown for several months in aquaculture facilities until they reach a certain size or weight prior to stocking (cf. Figure 2b = farmed eels) are regularly exposed to stress there, which can lead to the age of these eels being overestimated (Simon *et al.*, 2017). This is primarily relevant because the biomass of migrating silver eels for German eel river basins (or EMUs) is estimated using the German Eel Model, which is centrally based on von Bertalanffy growth parameters (Oeberst and Fladung, 2012). An erroneous estimation of age in this context can lead to significantly altered parameters and a correspondingly erroneous calculation of silver eel biomass. Since farmed eels are of great importance in the implementation of stocking measures in many European countries (ICES, 2016c), it is of great importance that eel otolith readers can recognize the stress rings and that this could have an impact on the model-based estimation of the biomass of migrating silver eels, if applicable.

In Germany, for example, approximately 53.3 million farmed eels were stocked into the nine EMUs from 2011 to 2019, representing 34.6 % of all eel stocked during the same period (Fladung and Brämick, 2015; 2018a; 2021; cf. Figure 2), with only a small proportion of farmed eels used being marked (e.g., Simon *et al.*, 2013; Simon and Dörner, 2014; Thiel and Kullmann, 2019).

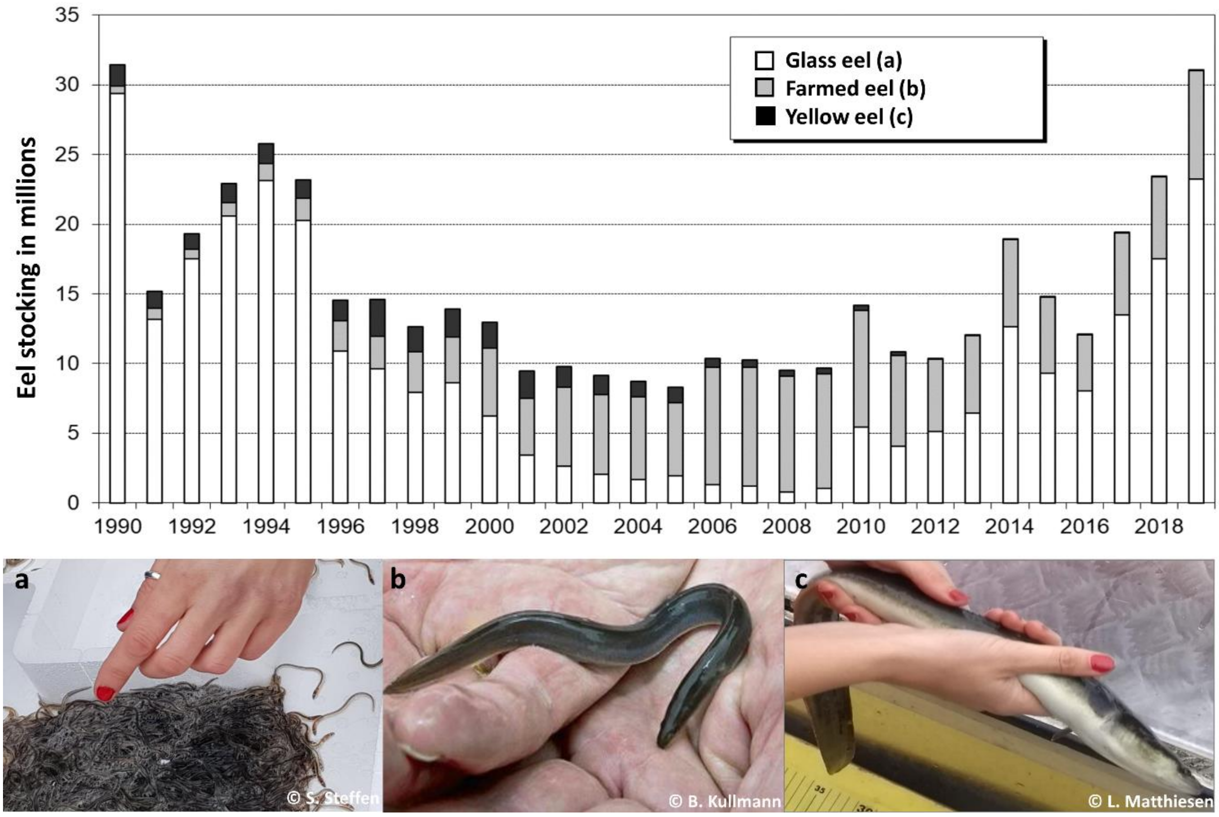

Figure 2: European eel stocking in Germany from 1990 to 2019 divided into glass eels (white bars & Figure a), farmed eels (grey bars & Figure b), and yellow eels (black bars & Figure c). (Modified from Fladung and Brämick, 2021).

Recent studies indicate that European eels do not necessarily migrate to inland/limnic waters, but that a significant proportion of eels remain in the marine environment without ever having been in freshwater: the European eel performs a facultative catadromous migration (Tsukamoto *et al.*, 1998; Marohn *et al.*, 2013). In the European eel, contrary to previous views, a pronounced phenotypic plasticity has been found (Vøllestad, 1992), which may have important implications for management. Thus, eels were most often stocked into limnic waters under the assumption that this would be suitable habitats for all individuals and in order to increase fishery yield there (Dekker and Beaulaton, 2016). Knowing that many eels naturally remain in coastal areas where they have advantages in terms of greater food availability and reduced parasitation, using coastal waters as a target environment for stocking activities could improve stocking efficiency (Dorow and Schaarschmidt, 2015). A final evaluation of the management option "coastal stocking" in terms of the EU Eel Regulation - i.e., the conservation and sustainable use of eel - is hindered by the fact that this has not been the focus of scientific research so far. However, since recruitment has only stabilized at a low level despite considerable efforts to achieve stocking targets, but a recovery in the form of an increase in recruitment is currently not observed (ICES, 2022), it might be necessary to explore further stocking options and apply them where appropriate.

Related to this, the relevant question is of whether the stocked eels differed from the possibly naturally immigrated individuals in terms of general fitness as well as growth (Dorow and Schaarschmidt, 2015). In order for stocking eels in coastal waters could contribute to increased recruitment, it must be ensured that the release of the fishes does not have a significantly negative effect on the local eel stock, which could negate the possibly positive effect. First of all, it is relevant that no diseases or parasites are introduced into the target water body by the release (Baer *et al.*, 2007), which makes it mandatory to check the health status of the eels before stocking. In this context, it is impressive that the swim bladder nematode *Anguillicola crassus*, originally native only to Asia and infesting Japanese eels, was significantly spread throughout Europe within a few years by stocking measures (Hartmann, 1993b). The same applies to the spread of viruses because here too it has already been demonstrated that these are spread by stocking measures (Kullmann *et al.*, 2017). Although the influence on eel reproduction has not been conclusively clarified for either parasites or viruses, which is already due to the extremely large distribution area and the mostly spatially limited research, the application of the precautionary principle should at least not encourage further spread by stocking measures.

A study on the very closely related American eel was observed that stocked eels had a significantly higher growth rate than naturally immigrated eels, which may be detrimental because fast-growing individuals mature faster, store fewer fat reserves, and may have corresponding difficulty reaching their spawning grounds (Stacey *et al.*, 2015). It is also known that eel habitat selection can have an influence on sexual determination (Davey and Jellyman, 2005) and this must be taken into account in the accompanying study for the evaluation of eel stocking measures, because eels show a pronounced sexual dimorphism with much smaller males (e.g., Davey and Jellyman, 2005) and precisely this would have a negative effect on the maintenance of an adequate, sustainable eel fishery, because males may not be able to reach a possibly legally introduced minimum size. In order to evaluate eel stocking measures, it is necessary to examine the translocated individuals after stocking to draw conclusions on whether the selected stocking strategy contributes to achieving the management objectives and to identify and implement any necessary adjustments. For all these questions, it is necessary to choose a before-and-after approach. Ideally, repeated studies on the eel stock must have been carried out before stocking begins, so that the influence of the stocking measures can be recorded comparatively.

Objectives of this thesis

The population of the European eel has been in a worrying state since 1999, stagnating at a low level despite considerable efforts. In order to support the population and to preserve a sustainable eel fishery, in 2007 the EU Eel Regulation was adopted, in which several stock supporting and regulatory measures were established. One of these measures is stocking of eels, which has been conducted primarily in inland waters. This work, however, will focus on technical aspects of stocking success control and the alternative of stocking eels into coastal waters, as the facultative catadromous life cycle of eels offers this often neglected but promising management option given that eels grow faster and are in better conditions in marine waters. Marking of stocked eels is an important tool to assess the success of stocking programs. Despite years of implementation and experience with eel stocking, an important open question remained regarding consumer protection when using the common chemically marker alizarin red S (ARS). The use of ARS has been prohibited in Germany in 2017, because a health risk by consuming eels could not be ruled out with sufficient certainty. Therefore, the bioaccumulation potential of ARS has been investigated in this study for the very first time to derive information of a potential ARS uptake by consumers to potentially enable the resumption of marking eels with ARS prior to stocking. The present work also addresses the difficulties encountered when frequently used farmed eels (individuals, that were fattened in aquaculture facilities) were not marked prior to stocking. During the farming process eels are exposed to stress (e.g., several size gradings) which is known to produce rings on otoliths. Because these stress-induced additional rings (stress rings) on otoliths might interfere with ageing and stock assessment it has been investigated and quantify if and to which extend these stress rings can be distinguished from normal growth rings (annuli) and how this affects the currently used standard German eel Model. Moreover, the results of a long-term data series from 12 years of fishery-independent eel monitoring in the coastal waters of the eastern German Baltic Sea were analyzed. The main focus was on the trend of yellow eel stock size before and after a coastal glass eel stocking that took place in the study area between 2014 and 2016. Additionally, an attempt was made to estimate the stock trend without the stocking effect under certain defined preconditions. Also, the movement patterns, health status, and growth performance of glass eels stocked in coastal waters of the eastern German Baltic Sea from 2014 to 2016 has been investigated. Since all translocated glass eels were marked with ARS prior to stocking, comparisons could be made between the stocked and non-ARS marked conspecifics. The results obtained from this study will be used to assess whether a coastal glass eel stocking could be a valuable management option to support the local eel stock and thus to simultaneously maintain a sustainable, economically successful local eel fishery.

The scientific aspects of this work not only serve to increase knowledge, but also contribute to eel management in order to fulfill the tasks of the EU Eel Regulation on eel conservation and eel fishery management.

Chapter 1 – Evaluation of the bioaccumulation potential of alizarin red S in fish muscle tissue using the European eel as a model

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Abstract

For fish stock management and large-scale stocking programs, the chemical substance alizarin red S (ARS) is an important tool to mark fish permanently. Equally, for the IUCN red list species European eel (*Anguilla anguilla*), ARS is proven to be the most promising option for mass marking. ARS binds to calcified structures (i.e., bones and otoliths) and can be detected using a fluorescence microscope. Despite the frequent application of ARS, not only for eels but also for fish in general, until today, no study has evaluated its bioaccumulation potential. Therefore, the German Federal Risk Assessment Authority was unable to classify ARS as harmless because of a potential risk to consumers' health. Using the technique of liquid chromatography mass spectrometry, an ARS detection protocol was developed and the bioaccumulation potential of ARS in European eel muscle tissue was estimated. A detection limit of 8.9 µg kg^{-1} could be reached by optimizing the detection method in fish muscle tissue. In the current study, 250 eels between 6 and 57 cm of total length have been analyzed for ARS between 0 day and 3 years after the marking process. The highest concentration of ARS (6056 µg kg^{-1}) was observed immediately after marking in the smallest length class. Only 1 year after the marking procedure, the ARS concentration was below detection limit. A new method for ARS detection in fish muscle tissue, followed by utilization on marked eels, was able to show that the bioaccumulation of ARS in edible fish muscle was highly unlikely.

Introduction

The marking of fish is an important technique for fisheries scientists used to collect information from individual behavior up to population dynamic levels (Nielsen, 1992). The chemicals are often incorporated into bones and calcified structures like fish otoliths (ear stones) which allow fish batch identification and age determination (Beamish and McFarlane, 1983; Warren-Myers *et al.*, 2018). Principally, there is a variety of substances that can be irreversibly combined with otoliths without disproportionate stress for the fish. Some of these include tetracyclines, alizarin compounds, calcein, salts such as strontium and barium chloride, stable isotopes, and rare earth elements (Warren-Myers *et al.*, 2018). Having a marker which can be easily detected would be an asset to stock monitoring and standard mass marking within the framework of large-scale fish management programs. This favors fluorochromes (e.g., alizarin red S) over other agents due to the simple detectability via standard fluorescence microscopy instead of the need for electron microscopy (e.g., Wickström and Sjöberg, 2014) or laser ablation (e.g., Marohn *et al.*, 2013).

A typical case where mass marking and detection efficiency represent a relevant factor comprises the management of the European eel *Anguilla anguilla* (Linnaeus, 1758). The panmictic eel population is in poor condition because, in comparison to its origin average population level between 1960 and 1979 levels, the annual recruitment of glass eels in 2018 was 2.1 % in the North Sea and 10.1 % elsewhere in Europe (ICES, 2018). In view of the declining stock situation, the European Union (EU) adopted a regulation in 2007 to establish measures for the recovery of the European eel stock (European Council, 2007). In order to achieve the silver eel escapement target, the EU member states have been requested to set up eel management plans. In Germany, most of these plans include stocking measures, besides monitoring and reduction of various identified mortality sources, to supplement local stocks that suffer from insufficient recruitment (European Council, 2007; Fladung and Brämick, 2018b).

Given that European eels cannot be reproduced in captivity yet, 60 % of the annual glass eel catch (40 – 60 tons annually) (ICES, 2018) in coastal waters of France, Portugal, England, and

Spain is reallocated into other waters with current low natural eel recruitment (ICES, 2016c). The juvenile individuals are either stocked immediately as glass eels or as so-called farmed eels after a farming period of 2 months to 7 months in commercial fish farms, when the body length between 15 and 20 cm has been reached (Nielsen and Prouzet, 2008; Nielsen, 1992; Angelidis *et al.*, 2005; Pedersen and Rasmussen, 2016). In Germany alone, for instance, approximately 10 to 20 million eels are being used as stocking material on an annual basis (Fladung and Brämick, 2018b).

In order to discriminate the stocked eels from natural recruits, the International Council for the Exploration of the Sea (ICES) recommended mandatory chemical marking of all stocked individuals for the evaluation of a potential net benefit of these conservation activities (ICES, 2011, 2016c). Furthermore, Kullmann *et al.* (2018) demonstrated that marking of farmed eels is also necessary in order to avoid an age-reading error due to stress-related annulus-like rings on otoliths.

The chemical substance 1,2-dihydroxyanthracinone-3-sulfonic acid (Figure 3), better known as alizarin red S (ARS), turned out to be the most promising option for standard mass marking due to its ease of application and detection and low costs, and it was found to have no negative impact on survival or growth rate of marked European eels (Caraguel *et al.*, 2015; Kullmann and Neukamm *et al.*, 2017; Kullmann and Thiel, 2019).

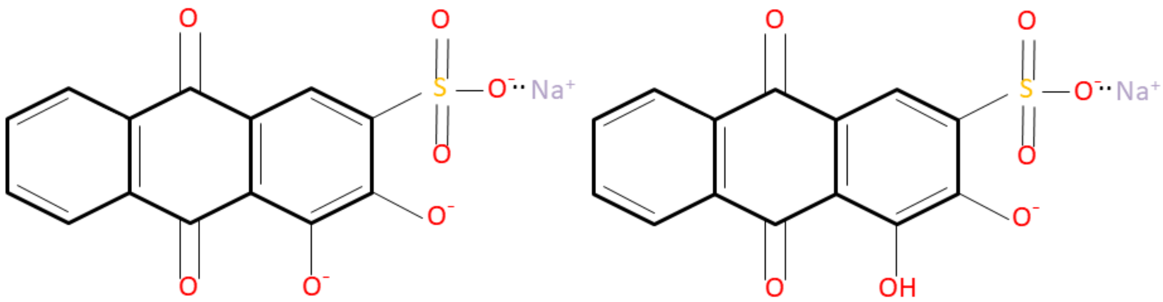


Figure 3: Chemical structure of alizarin red S (ARS).

However, available analytical literature dealing with ARS focused on its UV-vis properties for detection (Abdulrahman and Basavaiah, 2014) or applicability as reporter reagent (Springsteen and Wang, 2001) only. Accordingly, no studies on the bioaccumulation potential of ARS in fish species are available. Referring this lack of data, in 2017, the German Federal Risk Assessment (BfR) was interested in finding out whether marking eels with ARS could pose a risk to the health of consumers (Schendel *et al.*, 2018). The BfR concluded that a risk assessment regarding ARS marking in edible eel muscle tissue was not feasible, because of poor exposure data to evaluate its toxicity (Schendel *et al.*, 2018). In addition, it was found that there was no available data for ARS concentration in eel muscles or in muscle tissue of other comparable fish species at all.

The commonly used concentration of ARS to successfully mark young eels is 150 mg L^{-1} when using an exposure time of 3 h for glass eels or 9 h for farmed eels (Simon and Dörner, 2005; Kullmann and Neukamm *et al.*, 2017; Warren-Myers *et al.*, 2018). It is unknown how much ARS is absorbed by glass or farmed eels during the marking process. However, ARS has a small octanol-water partition coefficient (log KOW) ranging from − 2.76 (Rashid-Doubell and Horobin, 1993) to − 1.78 (Anastassiades *et al.*, 2017). It is therefore of hydrophilic nature, and due to the high fat content of eels of up to 30 % in average (e.g., Larsson *et al.*, 1990; Ahlgren *et al.*, 1994), the bioaccumulation potential of ARS in eel muscle tissue is expected to be rather low.

To the author's knowledge, no study has ever evaluated the bioaccumulation potential of ARS before. Consequently, it is unclear whether ARS marked eels pose a potential risk to consumers' health. In addition, the actual risk for consumers regarding side effects caused by ingesting ARS is also unknown (Schendel *et al.*, 2018). In this context, the present study focused on the following objectives: first, the development of a new method for the detection of ARS and the estimation of the bioaccumulation potential of ARS in muscle tissue using the example of the European eel; second, to study ARS exposure levels with age and length of the eels; and third, to find out if ARS is detectable in recaptured eels that have been initially marked and released as glass or farmed eels.

Material and methods

ARS marking of glass and farmed eels

For this study, a total number of 250 eels were analyzed for ARS concentration in their muscle tissue. European eels were marked with ARS applied as an immersion bath at different life stages during five different marking events between 2014 and 2016 (Table 1). In four out of five marking events, eels were marked in the glass eel developmental stage (ARS nos. 1 – 4). The fifth marking event was conducted with farmed eels (ARS no. 5) that were between 2 and 7 months older than glass eels due to their aquaculture period as already described in the "Introduction." However, regardless of the eel development phase during the marking events, the same ARS concentration of 150 mg L^{-1} was always used.

Table 1: Details of the different marking events of glass and farmed eels with alizarin red S (ARS).

No. of marking event	Date of marking event	Life stage	Type & size of marking tank	Concentration of ARS-solution [mg/L]	Volume ARS-solution per tank [L]	Eel mass/tank [kg]	Amount of ARS [g]/ tank	ARS concentration [g/kg eel]	Exposure time [h]	Stocked	Reference
ARS #1	March 2014	Glass eel	Fish transport bag, 70 L	150	30	1	4.5	4.50	3 – 4	Yes	(Dorow and Schaarschmidt, 2015)
ARS #2	March 2015	Glass eel	Fish transport bag, 70 L	150	30	1	4.5	4.50	3 – 4	Yes	(Dorow and Schaarschmidt, 2015)
ARS #3	March 2016	Glass eel	Fish transport bag, 70 L	150	30	1	4.5	4.50	3 – 4	Yes	(Dorow and Schaarschmidt, 2015)
ARS #4	May 2016	Glass eel	Tank, 300 x 145 cm	150	1500	60	225	3.75	3	No	(Kullmann and Hempel *et al.*, 2018)
ARS #5	July 2016	Farmed eel	Tank, 300 x 145 cm	150*	3500	235	525	2.24	9	No	(Kullmann and Adamek *et al.*, 2017)

** Buffered with 150 mg tris(hydroxymethyl)aminomethane L^{-1}*

For the first three marking events (ARS nos. 1 – 3), always 1 kg glass eel was placed in a 70 L fish transport bag which contained 30 L ARS solution, as described by Dorow and Schaarschmidt (Dorow and Schaarschmidt, 2015) and Kullmann and Thiel (Kullmann and Thiel, 2019). In total, the glass eels stayed in the ARS solution between 3 and 4 h and the amount of ARS concentration per kg eel was 4.5 g (see Table 1). After marking, glass eels were stocked at two different stocking sites (Salzhaff: 54° 04′ 06.7″ N, 11° 34′ 36.8″ E; Peenestrom: 53° 56′ 46.8″ N, 13° 54′ 29.7″ E) of the eastern German Baltic Sea coast, Mecklenburg-Western Pomerania. In 2017, 3 years after the first stocking event, eels were captured at both stocking sites and examined for ARS marking as described in “Identification by ARS mark and aging of recaptured eels.”

A further marking took place in May 2016, using approximately 60 kg glass eels per rectangular tank (ARS no. 4). Each tank contained a volume of 1500 L ARS solution, and in total, 225 g ARS was used for the marking procedure (Kullmann and Hempel *et al.*, 2018). Thus, for every 1 kg glass eel, 3.75 g ARS was used, with an exposure time of 3 h (Table 1). As described by Kullmann *et al.* (2018), 80 glass eels were sacrificed immediately after marking and stored as described above, while another subsample of 59 eels was held back for 14 days in observation tanks before being sacrificed and stored at − 20 °C.

The final marking event (ARS no. 5) was conducted in July 2016, and a total number of 940 kg farmed eels were placed in four different 3500 L tanks. In this case, exposure time was 9 h and 525 g ARS was added to the tanks in each case. Thus, 2.2 g ARS was used for every 1 kg farmed eel. After the marking procedure, a subsample of 30 farmed individuals was euthanized and deep frozen while 38 farmed eels were held back for 60 days in observation tanks before euthanasia as described by Kullmann *et al.* (2017).

Identification by ARS mark and aging of recaptured eels

The sagitta otoliths were used for both mark identification and age analysis. After weighing and measuring the glass eels (n = 139), they were decapitated. Using two needles, the otoliths were removed and stored dry in 1.5 mL tubes. Otoliths were positioned convexly embedded in Crystalbond 509 (Buehler, Esslingen, Germany) on a glass slide and polished as described by Kullmann *et al.* (2018). The thin section preparation was checked for an ARS mark using a light microscope (Leica DM2500; Wetzlar, Germany) equipped with a light filter for a wavelength range of 530 nm to 580 nm and a light source (CoolLED pE-300-W). The marking success was 100 % (2018).

In case off armed (n = 68) and recaptured (n = 43) eels both sagitta otoliths were extracted by a longitudinal dissection of the head. The otoliths were transversally cracked and glued with the cut surface showing down on glass sides, and without further polishing effort (2018), a yellow glowing band appears if the otolith was ARS marked.

In addition, for recaptured eels, the second otolith was prepared for age estimation by “crack and burn” protocol and aging recommendation according to ICES (ICES, 2009b, 2011) as well. Ages are presented as the number of annuli (winter rings). The ARS ring was considered to be age 0 (Kullmann and Thiel, 2018). The age of glass eels is still unknown; however, according to several otolith structure analysis and drift models, European eel migration from the Sargasso Sea to the continents might take between 6 months and more than 2 years (e.g., Bonhommeau *et al.*, 2009; Bonhommeau *et al.*, 2010; Westerberg *et al.*, 2017).

Tissue sample preparation

In order to develop and validate a method for measuring the ARS concentration and to detect ARS in eel muscle tissue, approximately 15 g of muscle tissue was removed. In recaptured eels, muscle tissue was cut out from the height of the tip of the caudal fin to the beginning of the dorsal fin from both body sides. In case of glass and farmed eels, the whole individual including the skeleton was used, with exception of the head. For farmed eels, always three to four eels from the same group (i.e., 0 day or 60 days after marking) were pooled together until the minimum sample weight was reached. Due to the low weight of glass eels, all individuals were pooled in two groups according to the time elapsed since marking (i.e., 0 day and 14 days). All tissue samples of glass and farmed eels were obtained from leftover samples from previous studies by Kullmann et al. (2017; 2018).

Design of method development and validation

Materials and reagents

Acetonitrile and methanol have been purchased from LGC Standards (Wesel, Germany) in LC-MS grade. LC-MS grade water was bought from VWR (Philadelphia, USA). Triethylamine and formic acid were purchased from Merck (Darmstadt, Germany), and ammonium formate was delivered by Honeywell (Charlotte, USA). ARS was bought from Carl Roth (Karlsruhe, Germany) whereas nicarbazin and 2.4-D (D3), used as isotopically labeled (IL), were purchased from LGC Standards (Wesel, Germany). For lack of commercially available IL ARS standard, alternative IL standards reknown in pesticide analysis were applied. Both IL standards have retention times with the final method close to the target analyte and are measured in the same measurement mode ESI negative. For sample extraction, 5 mL tubes with stainless steel beads from Biozym (Hessisch Oldendorf, Germany) were used in the homogenization system Benchmark

BeadBlaster 24 (Edison, USA). Centrifugation was carried out with an EBA 20 centrifuge from Hettich (Tuttlingen, Germany) and a Varifuge 3.0 R from Heraeus (Hanau, Germany). Sample extracts were transferred into 2-mL vials from Wicom (Heppenheim, Germany).

LC and MS conditions

Chromatographic separation was carried out on an Agilent 1290 LC system equipped with a G4226A autosampler, G4220A pump, and G1316C column oven. Detection of the analytes was done by an Agilent 6495 Triple Quad with a G1958-65138 ion source. For eluent A, water was set to pH 11, adjusted with 3 mM triethylamine. Acetonitrile was used as eluent B, and the following gradient was applied for both optimization and sample measurements: Eluent A was set to 95 % and decreased to 90 % within 5 min. It was then reduced to 0 % between 5 and 8 min and finally maintained at 0 % until 12 min. Afterwards, the gradient was set back to the starting conditions of 95 % within 2.5 min and held for reconditioning till 25 min.

Separation was done on a Waters Acquity UPLC BEH C18, 2.1 mm × 150 mm, 1.7 µm. The temperature of the column oven was set to 35 °C. Source parameters of the MS were 120 °C for gas temperature, 15 L min^{-1} for gas flow, 35 psi for the nebulizer, 375 °C for a sheath gas heater, and 12 L min^{-1} for sheath gas flow, with a capillary voltage of 3000 V and a fragmentor voltage of380 V for all analytes. For the detection of ARS in multiple reaction monitoring (MRM) in ESI negative mode, the following MRM transitions were optimized. Collision energy (CE) and cell acceleration voltage (CAV) are stated in brackets behind each MRM transition: 318.8 m/z → 238.9 m/z (CE = 33 V, CAV = 1 V), 318.8 m/z → 255.0 m/z (CE = 33 V, CAV = 1 V), 318.8 m/z → 210.9 m/z (CE = 45 V, CAV = 1 V) for ARS, 301.1 m/z → 137.0 m/z (CE = 11 V, CAV = 3 V), 301.1 m/z → 107.0 m/z (CE = 42 V, CAV = 3 V), and 301.1 m/z → 45.9 m/z (CE = 64 V, CAV = 2 V) for the internal standard nicarbazin and 221.0 m/z → 163.0m/z (CE = 9 V,CAV = 3

V) and 219.0 m/z → 161.0 m/z (CE = 9 V, CAV = 3 V) for the internal standard 2.4-D (D3) were optimized. The first transition was used for quantification and the other two for identification. As further identification point, the ion ratios of the transitions were used. Compared to the procedural calibration, a tolerance of 30 % was accepted. The LC-MS/MS system was operated with MassHunter©, version B 07.01 (Agilent, Santa Clara, USA).

Sample preparation procedure

Samples were homogenized using a Grindomix 300 (Retsch, Hahn, Germany) at room temperature and stored frozen until ready for sample preparation. For the extraction, 1 g of the homogenized sample was weighed into a 5 mL tube, and 10 µL of a 10 ng μL^{-1} internal standard solution and 2.5 mL acetonitrile were added to the sample. Extraction was carried out in the homogenizer for 1 min. Afterwards, the samples were centrifuged at 4000 rpm for 10 min, and 150 µL of the supernatant was transferred into LC vials and diluted with 300 µL acetonitrile. After dilution, a slight precipitation was observed. Hence, the sample was centrifuged again at 6000 rpm for 3 min and the supernatant was transferred again into vials for measurements with LC-MS/MS. For quantification, a procedural calibration was prepared by spiking blank matrix prior to extraction. Final extracts were measured within 3 days of sample preparation. The procedural internal standard was used to monitor extraction efficiency and measurement only; results were not corrected for recovery of internal standard.

Method optimization and validation

To optimize the chromatographic separation and detection of ARS in eel muscle tissue, four analytical columns at two different pH values were investigated. These include Dionex IonPac

(1), Kinetex (2), Zorbax (3), and Acquity (4). An ARS standard in methanol with a concentration of 0.1 ng µL^{-1} was prepared and used to assess peak resolution and shape.

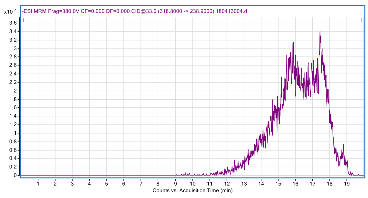
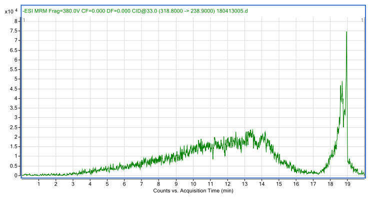
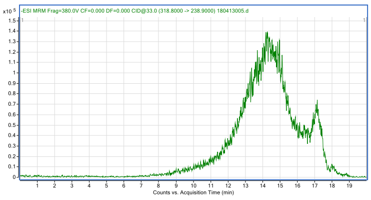
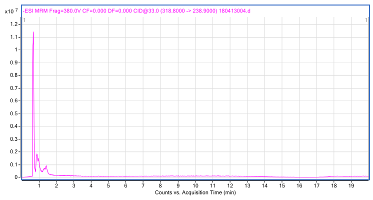
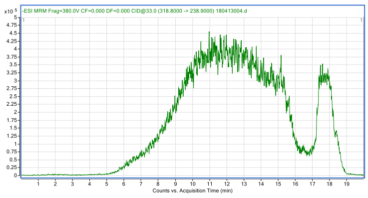
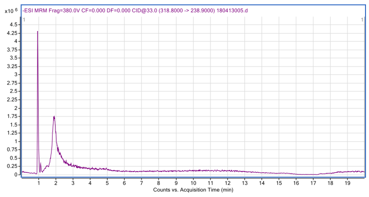
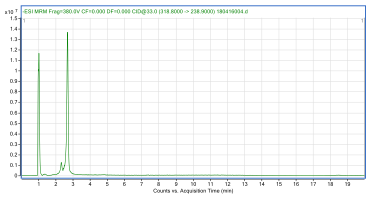
Depending on the pH value, ARS may be present in different ionic species exhibiting different retention times. Therefore, two different pH values for eluent A were investigated. A high pH value of 11, adjusted with 3 mM triethylamine, has been applied for the quantification of different dyes before (Fuh and Chia, 2002; Souto, 2010). A low pH value of 3.7, adjusted with a formic acid/ammonium formate buffer is often applied in residue analysis in food and feeding stuff (e.g., Anastassiades *et al.*, 2017).

To optimize the sample preparation procedure, parameters with potential significant effect on extraction efficiency were investigated using a design of experiment (DoE) approach (Wee *et al.*, 2010). The factors sample matrix, volume of extraction solvent, extraction time, and ARS spike level were tested using a Box-Behnken design with factor levels varied according to Table 2. The experimental setup included 27 experiment runs with three central points. For extraction solvent, highly polar solvents were tested due to the high polarity of ARS. Due to environmental considerations, small sample weight and small organic solvent volume were to be used throughout. Therefore, the homogenization system with a maximum of 5 mL extraction volume was investigated. Whole glass eels, muscle tissues of recaptured eels, and eel livers were supposed to be analyzed; therefore, all three matrices were tested for significant differences in extraction efficiency. For extraction efficiency experiments, blank tissue from each matrix was used and spiked with a known concentration of ARS prior to extraction.

For method validation, the optimized sample preparation procedure was used to extract the blank matrix eel muscle, spiked with known ARS concentrations. Stability under various conditions was investigated within a procedural calibration with equidistant calibration level

(DIN, 2008). Limit of detection (LOD), limit of quantification (LOQ), relative standard deviation (RSD), and correlation coefficient R2 were derived from these data.

Table 2: Summary of tested chromatographic conditions.

		Observation in acquired chromatogram	
No.	Analytical column	pH value 3.7	pH value 11
(1)	Dionex IonPac AS11, 2x250 mm, 13 µm with an IonPac AG11 2x50 mm (Dionex, Sunnyvale, USA))	 No satisfying peaks	 No satisfying peaks
(2)	Kinetex C18, 2.1x100 mm, 2.6 µm (Phenomenex, Torrance, USA)	 No satisfying peaks	 No satisfying peaks
(3)	Zorbax Eclipse C18, 2.1x150 mm, 3.5 µm (Agilent, Santa Clara, USA)	 No satisfying peaks	 Good beak shape, low and steady baseline (see Figure 4A)
(4)	Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm (Waters, Milford, USA)	Not further tested	 Good beak shape, low and steady baseline (see Figure 4B)

Statistical analysis

For the method optimization, statistical evaluation was done with minitab© 18.1 (Minitab GmbH, Munich, Germany).

For the model, statistical analysis of the different eel parameters and comparison of ARS concentration in eel muscle tissue related to time and sample weight were carried out in the software R (R Core Team, 2016). Significance was considered when the probability of error was below 5 % ($\alpha < 0.05$). Data distribution was tested for normality or non-normality using the Shapiro-Wilk test. Depending on the result, the Fligner-Killeen test or Bartlett test for homoscedasticity was performed. If data distribution was non-normal and homoscedasticity was not satisfied, the Kruskal-Wallis H test for n > 2 groups (χ^2) or Wilcoxon test for n = 2 groups (W) was applied. An analysis of variance (ANOVA) for n > 2 groups (F) was performed if data was normally distributed and homoscedasticity was satisfied.

Results

Method optimization

Measurements at low pH did not show suitable chromatography with any of the columns tested. The columns Acquity and Zorbax, however, gave clear and separated peak shapes with low baseline at the high pH level (Figure 4A and 2B). The occurrence of two peaks for ARS in a single chromatogram is due to the presence of two ARS species at the specific pH value. They elute with different retention times as shown in Figure 4. The Acquity column showed best peak shapes with narrow peak widths and higher intensities and, therefore, was used for chromatographic separation. For quantification, both peaks were integrated and sum of response was further processed. In Figure 3, the chemical structures of both ARS forms are shown. Peak splitting is a phenomenon to be avoided in analytical measurement as reproducibility of the splitting combined with different ionization response factors of the different species may cause serious drawbacks in quantification. However, spiked samples were analyzed over a timeline of several weeks. As shown by the correlation coefficient (Table 3) derived from the procedural calibration and a relative standard deviation of 10.8 % after multiple injections, the splitting of the peak itself can be considered reproducible.

Table 3: Method validation criteria and performance data achieved with the optimized sample preparation procedure.

Analyte	LOD [µg kg $^{-1}$]	LOQ [µg kg $^{-1}$]	RSD [%]	R^2
ARS	8.9	28.3	5.4	0.9965

To keep the sample preparation procedure fast and simple, a dispersive liquid extraction method was used as starting point for method development within this study. Therefore, acetonitrile, methanol, and water were tested as extraction solvents. To determine the influence of the extraction solvent and other extraction parameters, a DoE approach was used to develop and optimize the extraction method.

For a full factorial design, 243 experiment runs would have been necessary to investigate five factors tested for sample extraction at three levels. Applying a fractional factorial design based on Box-Behnken, only 27 experiment runs had to be conducted, e.g., 11 % still acquiring all data necessary for statistically confirmed conclusions. DoE approaches also reveal factor interactions that cannot be disclosed by the classical one factor at a time approach (Wee *et al.*, 2010).

Evaluation of the obtained DoE measurement data showed no significant effects for the sample matrix, encoded as block (p = 0.138). Therefore, no individual validation and calibration curves need to be conducted, but a generic calibration with eel muscle may be used for any of the three tested matrices. The volume of extraction solvent, evaluated after correction with the associated multiplier, was not significant ($p = 0.960$). Neither was the extraction time with $p = 0.907$. However, 2.5 mL as extraction solvent were chosen to not dilute the sample more than necessary, but still keeping enough volume for comfortable sample handling. As was to be expected, analyte concentration showed high significance ($p = 0.001$). The factor log KOW of extraction solvent showed significance for the quadratic term ($p = 0.015$). Figure 5 shows the response surface model for the two significant factors: log KOW of extraction solvent and analyte concentration. Highest responses were obtained at the intermediate log KOW value, regardless of the spiked analyte concentration. The calculated log KOW optimum to achieve the highest response after extraction was at − 0.38. Therefore, acetonitrile was applied as extraction solvent, with a log KOW value of − 0.34 closest to the calculated optimum. No significant interactions between factors were observed, and no other quadratic correlations were estimated except for the log KOW value.

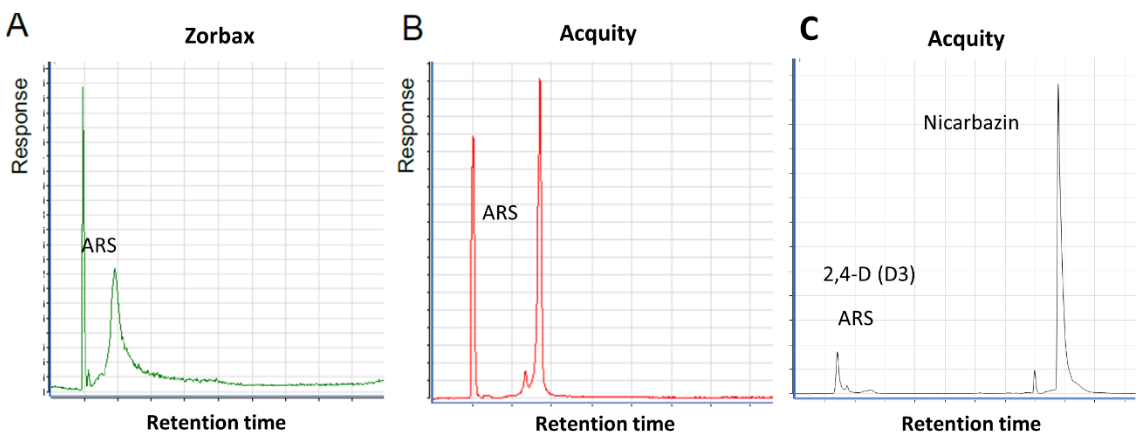


Figure 4: Extracted MRM chromatogram ofalizarin red S at pH 11 on the Zorbax (A)and Acquity (B) columns showing two ARS species with different retention times and including (C) internal standards (2.4-D (D3) peak covered by ARS peak).

Furthermore, column conditioning was investigated, showing higher and steadier peak intensity if column conditioning was applied. A spiked and extracted sample was injected and measured with and without column conditioning, 10 times each. The RSD of the peak intensity with applied column conditioning was 10.8 %, while the RSD without column conditioning was 12.4 %. However, an even stronger impact could be observed for the peak intensity itself. With column conditioning, the peak intensity was 2.7 times greater than without column conditioning. This effect is due to matrix eluting after the target peak. Without column conditioning, the matrix will elute during the run time of the next sample. This causes irreproducible ion suppression, affecting both peak intensity itself and relative standard deviation.

Method validation

Method performance parameters for the optimized sample preparation procedure measured as described above are shown in Table 4. At and above the limit of detection, signal-to-noise ratio of the second and third most intense transitions was 15.7 and 15.1, respectively. The ratios of both transitions to the most intense varied by 6.5 % and 5.7 % over the whole procedural calibration. Hence, both transitions were applicable for identification at the limits of detection. However, the limits of detection have been calculated with regard to the most intense transition. The limit of detection (8.9 µg kg^{-1}) and limit of quantification (28.3 µg kg^{-1}; K = 3) were derived from equidistant calibration level ranging from 10 to 100 µg kg^{-1} according to DIN [36]. Relative standard deviation was 5.4 %, calculated by the same procedure for the concentration range of 10 µg kg^{-1} to 100 µg kg^{-1}, as well as R2 of 0.9965.

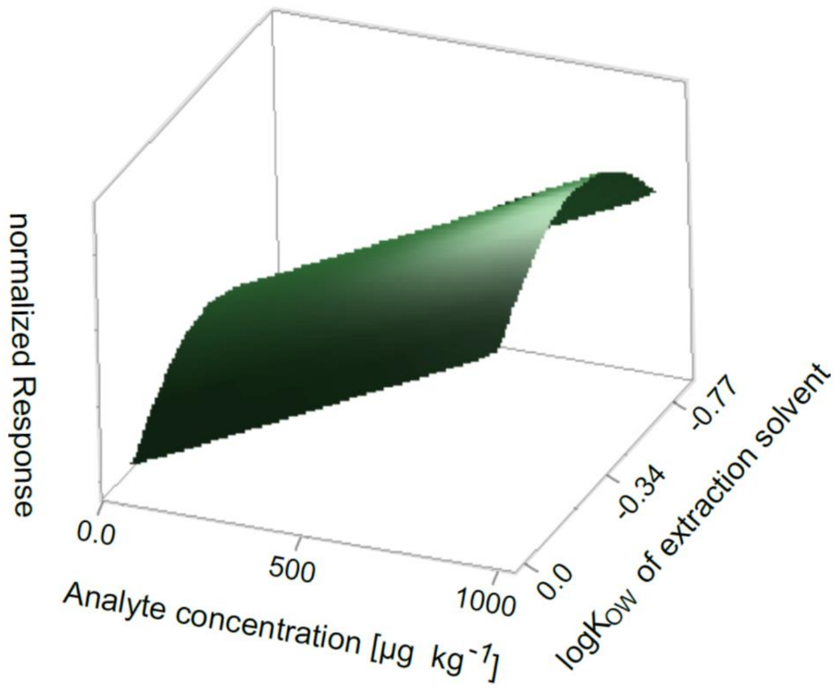


Figure 5: Response surface model for measured alizarin Red S concentrations in regard to a log KOW value of extraction solvent and spiked analyte concentration.

Table 4: Codes and values of experimental range of five factors for Box-Behnken design.

Factor	Coded level		
	-1	0	1
Block: sample matrix	Glass eel	eel muscle	eel liver
Analyte concentration [µg/kg]	1	500.5	1000
Volume of extraction solvent [mL]	1	2.5	4
logKow value of extraction solvent	0	- 0.34	- 0.77
Extraction time [min]	1	3	5

Factor sample matrix has been encoded as block

Ageing of recaptured eels

Age was estimated for 43 recaptured eels, which were sampled for the detection of ARS in muscle tissues. The individuals could be allocated to the age groups (AGs) 1, 2, and 3, whereby the most common with 31 individuals (72.1 %) was AG 3, while AG 1 was represented with only 2.3 %. Due to this heterogeneous ratio of the different AGs, all recaptured eels were divided into four different groups (recaptured eels = RE1 – RE4) with a defined total length range (TLR) of 10 cm, starting with 20 cm and ending with 59.9 cm.

The two smallest groups of 0 – 9.9 cm and 10 – 19.9 cm were exclusively made up of glass or farmed eels, because there were no recaptured eels in these TLRs. Furthermore, these two groups were once again separated into further subgroups to measure the ARS concentration directly after marking and after a time period of 14 days (glass eel = GL2) and 60 days (farmed eels = FE2), respectively. According to this division, eight different groups were created (Table 4). The statistical evaluation showed that the four groups of recaptured eels (RE1 – RE4) differed significantly in terms of total length ($F = 395.7$; $p < 0.001$), weight ($F = 140.2$; $p < 0.001$), and time after marking or age ($\chi^2 = 15.232$; d.f. = 3; $p < 0.01$). The groups of glass and farmed eels were not included in the statistical tests, as only the mean values were available.

ARS concentration in eel muscle tissue

The concentration of ARS was estimated using 250 different eels, pooled to 64 samples. They were divided into 8 different groups according to time and size. The highest ARS concentration with a value of 6056.75 µg kg^{-1} was detected in glass eels directly after marking (Figure 6 GE1). Already after 2 weeks, a clear decrease of the ARS values to 36.02 µg kg^{-1} was observed; hence, the concentration of ARS has been decreased by a factor of 168 within 2 weeks. A similar decrease occurred in the case of farmed eels (Figure 6; FE1 and FE2). Also here, a clearly lower ARS concentration of 1772.99 µg kg^{-1} was determined directly after the marking and, within 60 days, it decreased significantly to 14.64 µg kg^{-1} ($W = 90$; $p < 0.001$). No ARS could be detected in the four groups of recaptured eels (Figure 6; RE1 – RE4). Based on the estimated detection limit of 8.9 µg kg^{-1}, the ARS concentration for these four groups is below LOD; therefore, no statistical tests could be performed.

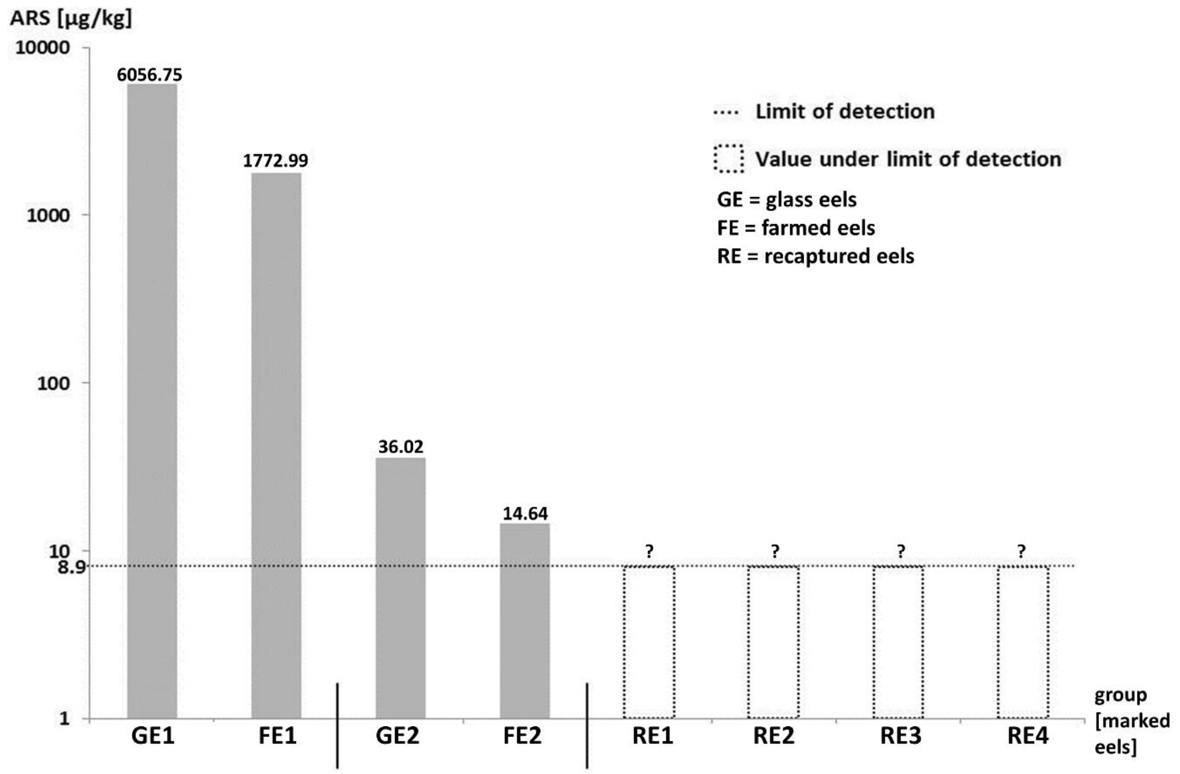


Figure 6: Mean alizarin red S (ARS) concentration (µg kg^{-1}) of pooled glass (GE1 and GE2), farmed (FE1 and FE2), and recaptured (RE1 – RE4) eels.

Discussion

The aims of the present study were to develop and validate a novel method for ARS concentration measurement in fish tissue and investigate for the first time the ARS bioaccumulation potential in European eels. Therefore, the tissues of ARS marked-glass, farmed, and on-grown eels (of up to 3 years after the marking event) have been used to establish a LC-MS/MS protocol with an ARS detection limit of 8.9 µg kg^{-1}. The detection method was used to demonstrate that the ARS concentration in all recaptured eels was below detection limit, which leads to the conclusions that (i) the bioaccumulation potential of ARS is very low in eel muscle tissue and (ii) ARS most likely poses a negligible, if any, risk to consumers of marked eels. With regard to the low octanol water partition coefficient of ARS (Rashid-Doubell and Horobin, 1993; Anastassiades *et al.*, 2017), enrichment in eel muscle was expected to be low. Given that ARS has been used to mark a variety of other fish species in different life stages among coregonids (e.g., Eckmann *et al.*, 1998; Eckmann, 2003), salmonids (e.g., (Baer and Rösch, 2008; CAUDRON and CHAMPIGNEULLE, 2009), flat fish (e.g., (Lagardère *et al.*, 2000; Liu *et al.*, 2009), and cyprinids (Beckman and Schulz, 1996), our developed method could be used as a standard detection technique on such research question because it is not matrix-specific but rather universally applicable.

For determination of ARS concentration in eel, an extraction procedure for sample preparation followed by LC-MS/MS detection was investigated. A DoE approach has been applied to enhance method optimization. The DoE approach enables scientist to simultaneously investigate various factors that might have a significant influence onto the result (Wee *et al.*, 2010). Maximum information about the overall system may be acquired by conducting the smallest, still statistically acceptable number of experiments. All data necessary for statistically confirmed conclusions is still acquired with a reduced design. Furthermore, DoE approaches also reveal factor interactions that cannot be disclosed by the classical one factor at a time approach (Klein, 2014).

To establish a method for the detection of ARS, two different buffers at a high and low pH values were tested. Testing further buffers and, at different pH values, might disclose conditions yielding one ARS species and combining the split response in one peak. So far, muscle and livers of adult eels, as well as the entire body of glass and farmed eels, have been analyzed. Considering the fact that ARS binds to bone structure, this matrix could provide further interesting results.

With the detection limit of 8.9 µg kg^{-1}, a supposed acute reference dose (ARfD) of up to 100 times lower than for some highly toxic pesticides can be covered. The ARfD indicates the level that can be consumed once without adverse effects. Assuming a 200 g portion size (Anonymus, 2010a) for an ARS marked eel muscle, a person weighing 70 kg might take up 0.025 µg ARS per kg bodyweight. This is equal to a total of 1.78 µg ARS, if ARS concentration was at LOD. Hence, an ARfD of 0.025 µg ARS per kg bodyweight would not be exceeded by eating marked and recaptured eel. For comparison, the pesticide omethoate, not approved within the EU, has an ARfD of 2 µg kg^{-1} bodyweight (ESFA, 2006). Therefore, any ARfD higher than 0.025 µg kg^{-1} indicating less toxicity could be ensured with the developed method.

Table 5: Numbers (n), total length (TL), body weight (BW) and time after marking of glass and farmed eel, number of pooled samples for Alizarin red S (ARS) concentration analysis (n) and recaptured eels divided according to different groups and total length ranges (TLR).

Groups	**TLR (cm)**	**Min & Max TL [cm]**	**Mean TL ± SD [cm]**	**Min & max BW [g]**	**Mean BW ± SD [g]**	**Min & max time after marking**	**Mean time after marking**	**Number of sampled eels [n]**	**Number of samples for ARS concentration analysis after pooling [n]**
GE1	0 – 9.9	n.v	6.74 ± 0.15	n.v	0.24 ± 0.07	0	0	80	1
GE2	0 – 9.9	n.v	6.74 ± 0.15	n.v	0.24 ± 0.07	14 days	14 days	59	1
FE1	10 – 19.9	n.v.	16.17 ± 0.71	n.v.	6.60	0	0	30	1
FE2	10 – 19.9	n.v.	16.17 ± 0.71	n.v.	6.60	60 days	60 days	38	1
RE1	20 – 29.9	25.5 – 29.5	27.92 ± 1.34	28.9 – 48.9	39.63 ± 8.32	1 – 3	2.17 ± 0.68 years	6	6
RE2	30 – 39.9	30.0 – 39.5	35.50 ± 2.95	47.9 – 151.4	91.37 ± 28.50	2 – 3	2.53 ± 0.50 years	17	17
RE3	40 – 49.9	40.0 – 49.0	45.29 ± 2.52	122.4 – 275,4	192.26 ± 44.03	3	3 years	14	14
RE4	50 – 59.9	50.0 – 57.0	54.17 ± 2.32	221.1 – 505.1	344.75 ± 85.04	3	3 years	6	6

n.v. no value

With this novel detection method, we were able to investigate for the first time the ARS concentration in freshly marked glass (n = 139) and farmed eels (n = 68) and additionally in recaptured adult eels (n = 43) within a time period of 2 weeks to 3 years after marking. As expected, the highest ARS concentration of 6056.75 µg kg^{-1} was determined in the glass eels immediately after the marking (Figure 6). It should be considered, however, that the result could be explained by the use of the whole individual including all calcified structures (without head). The presented ARS content, therefore, cannot be differentiated between bones, organs, scales, and muscles which is also the case for the other glass and farmed eel samples (i.e., GE2, FE1, and FE2). Nevertheless, a 168 times decreased concentration down to 36.02 µg kg^{-1} ARS in glass eels was observed after a time period of 2 weeks after marking. This indicates that the majority of the ARS was not bound to the skeleton or other calcified structures (otoliths). A similar trend was observed for farmed eels whereby the initial value of 1772.99 µg kg^{-1} ARS decreased significantly to 14.64 µg kg^{-1}, which is 121 times lower than the ARS value 60 days before. Accordingly, the study results clearly indicate that the ARS concentration decreases shortly after the marking process (glass and farmed eels). The lowering of the ARS concentration in the tissues shortly after marking might be caused by metabolic processes or eel growth.

Furthermore, considering these results, it is not surprising that the ARS concentration in recaptured eels (samples RE1 – RE4) is below the detection limit of 8.9 µg kg^{-1}. Thus, ARS is no longer detectable from a minimum body length of 25.5 cm and weight of 28.9 g and a time period after marking of 1 year (Figure 7). At least four explanations could be given for this result: First, the smallest group of recaptured eels (RE1) have increased in mean size by 4 times (from 6.74 to 27.92 cm); second, those eels have increased in weight by 165 times (from 0.24 to 39.63 g); third, marking was at least 2.17 years ago (see Table 5); and fourth, in recaptured eels, only the muscle tissue was examined for ARS. In addition, this study revealed that ARS marked glass eels reached the legal minimum size limit of 50 cm (Anonymus, 2017) earliest after a period of 3 years (Table 5) when stocked in coastal waters.

Despite Europe-wide efforts in eel management, the stock remains in a perilous state. Due to the vast distribution area of this semelparous species, a lack of knowledge of the actual spawning stock biomass (SSB) in the Sargasso Sea, and a variety of other uncertainties, the European eel stock might be in a state of dispensation, which is a strongly reduced recruitment at low SSB (Åström and Dekker, 2007). Glass eels, therefore, are a scarce resource and should be used prudently for stocking measures, at best mandatorily flanked by a success control. Here, ARS can play a key role due to its easy application, low costs, insignificant impact on growth or survival (Wickström and Sjöberg, 2014; ICES, 2016c), and — as shown in this study for the very first time — a negligible bioaccumulation potential. Hence, ARS marking could be integrated in eel management plans across Europe for the evaluation of a net benefit of conservation stocking activities defined as a higher total silver eel escapement compared to a scenario without the translocation of recruits (ICES, 2016c).

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Compliance with ethical standards

Prior to the study, the responsible Animal Protection Authority (State Office for Agriculture, Food Safety and Fisheries, Thierfelderstraße 18, 18059 Rostock, Germany) of the state Mecklenburg-Western Pomerania was contacted. Based on the consultation procedure, it was established that the present study complies with the Animal Welfare Regulation according to the national guidelines (Law on Animal Welfare §7a paragraph (2)3).

Conflict of interest

The authors declare that they have no conflict of interest.

Chapter 2 - Age-based stock assessment of the European eel (*Anguilla anguilla*) is heavily biased by stocking of unmarked farmed eels

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Abstract

Stocking of farmed eels is a commonly used management measure across Europe, and partly Asia, to supplement local stocks that chronically suffer from poor recruitment. During the farming process, increased growth and stress-related annulus-like rings are formed, which have been hypothesised to bias ageing and growth estimation. 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 estimates of total biomass, 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 otoliths can also be used for investigations on otolith chemistry, and microstructure analyses (Begg *et al.*, 2005; Campana, 2005; Sandin *et al.*, 2005).

The age structure of a fish population and associated cohort analyses are crucial for stock assessments, estimation of total allowable catches (TACs) 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 (e.g., 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, 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, 2023). 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 has ever been published for this stock (ICES, 2017a). ICES adopted the precautionary approach following the guideline to keep “all anthropogenic impacts as close to zero as possible “because of a severe recruitment decline since the 1970’s (ICES, 2016a) and the major uncertainties in the data (ICES, 2017b).

With the objective to create a comprehensive data resource for a reliable stock assessment, the European Commission requested EU member states to include European eel into the large-scale Data Collection Framework (EU COM, 2009), which 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 (2017c) reviewed data requirements for a reliable stock assessment, and age was identified as key parameter especially for the estimation of 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, that the annuli formation pattern differs markedly among 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).

ICES made efforts to standardize the ageing technique because ageing techniques for the European eel differ remarkably between countries, and recommended the "crack and burn" protocol. In addition, ICES provided advice for the interpretation of the winter ring structures (annuli) (ICES, 2009b, 2011). Stocking measures with farmed eels – an important management option in many eel management plans across Europe and partly Asia (Shiraishi and Crook, 2015; ICES, 2016c; Kaifu *et al.*, 2018) – is a potential source of biased age readings in *A. anguilla* and probably *A. japonica* Temminck & Schlegel 1846 because the stress-related formation of wintering-like zones on otoliths was hypothesised to interfere with ageing and subsequent growth estimations (Deelder, 1981; 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, 2016b). During the farming process, eels are subject to stress caused by multiple size gradings (Knights, 1987; Kamstra, 1993; Angelidis *et al.*, 2005) and additionally can be – deliberately or accidentally – infected with the eel herpesvirus (Kullmann and Adamek *et al.*, 2017) which may also lead to stress-related ring structures on otoliths (e.g., 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, 2009b) 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 for the European eel. We analysed blind readings from otoliths of recaptured alizarin red S (ARS) marked recruits previously reared in aquaculture facilities to quantify the possible error caused by false discrimination of stress rings from true annuli. Furthermore, we examined the effects of this error on population estimates in a simple age-based cohort model.

Materials and methods

Study sites and stocking measures

The eels used in the study were obtained from three brackish water bodies in northern Germany (Figure 7), the Kiel Canal (KC), the Schlei fjord and the German Baltic coast. The KC connects the North and Baltic Seas 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 ha. It is ca. 2–3 m 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 10–20 and a mean depth of 15–20m at the Kiel fjord.

From 2006, approximately 12 t of farmed eels with mean body mass between 4.6 and 8.4 g have been stocked in the KC. From 2009 onwards 45.5 % of farmed eels have been marked with ARS prior to stocking (Kullmann and Neukamm *et al.*, 2017) Taken together, the Schlei fjord as well as the western German Baltic Sea coast has received 3 t of ARS marked farmed eels between 2015 and 2016.

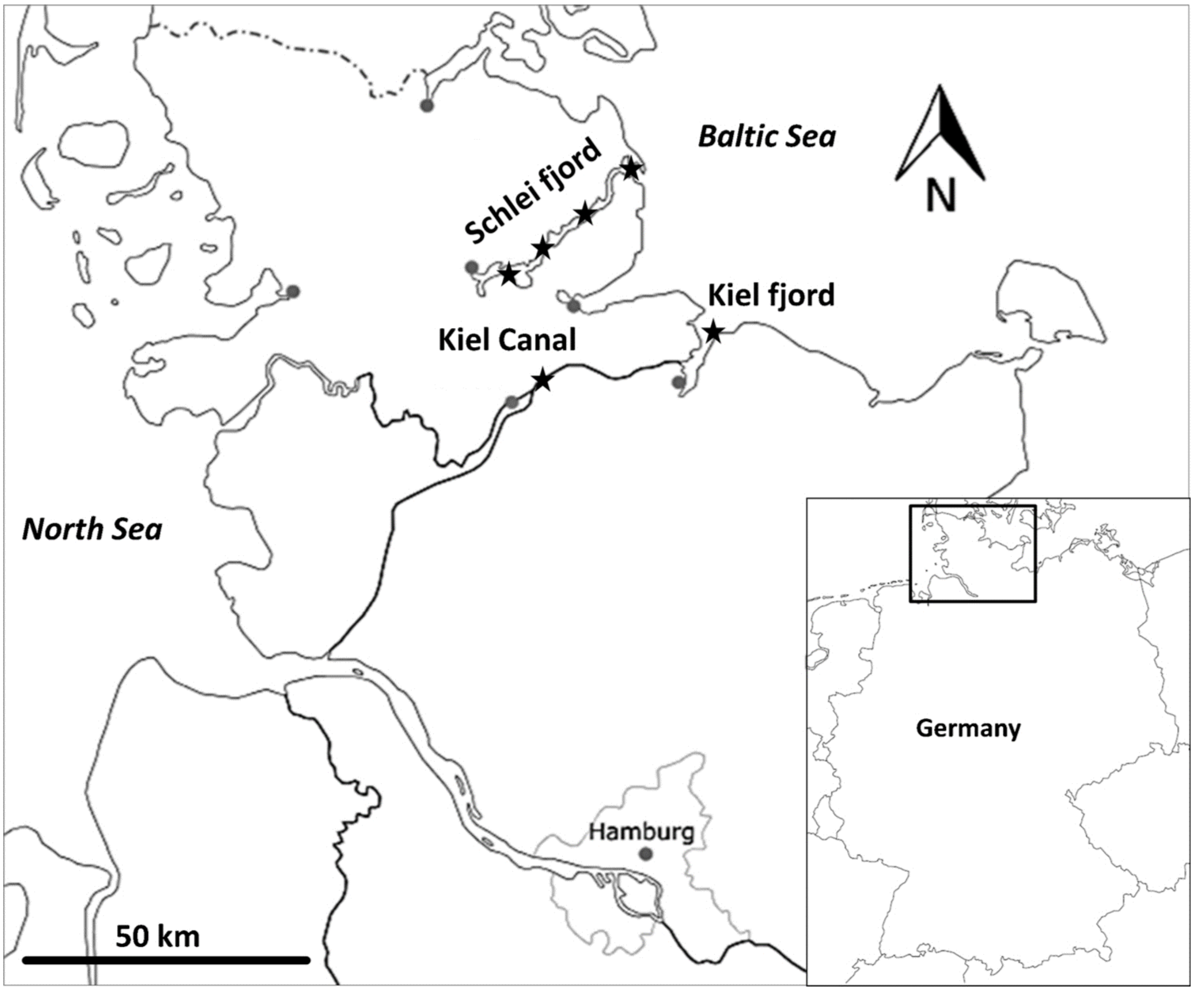

Figure 7: Overview map of the sampling locations (stars) at the western German Baltic Sea coast and the Kiel Canal.

ARS mark identification, ageing, and growth estimations

A total of 100 eels from the Schlei fjord, the Kiel-Canal, and Kiel fjord were used for this study (

Table *6*). 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 pE-300-W) and UV filters for specific wavelengths (530–580 nm). If an ARS mark was visible, the longest diameter was digitally measured (Figure 8 1a–3a), using the programme "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, 2009b).

Table 6: Numbers (n), total length (TL), and body weight (BW) of recaptured farmed eels with an ARS mark from the different sampling locations. The sampling period was between 18/06/2016 and 19/08/2017.

Location	n	TL ± SD (mm)	TL range (mm)	BW ± SD (g)	BW range (g)
Kiel fjord	15	298 ± 55	219–349	44 ± 23	16 – 66
Kiel Canal	45	511 ± 42	450–610	220 ± 88	124 - 397
Schlei fjord	40	325 ± 57	256–435	59 ± 29	23 – 131
Total	100	409 ± 106	219–610	131 ± 95	16 – 397

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

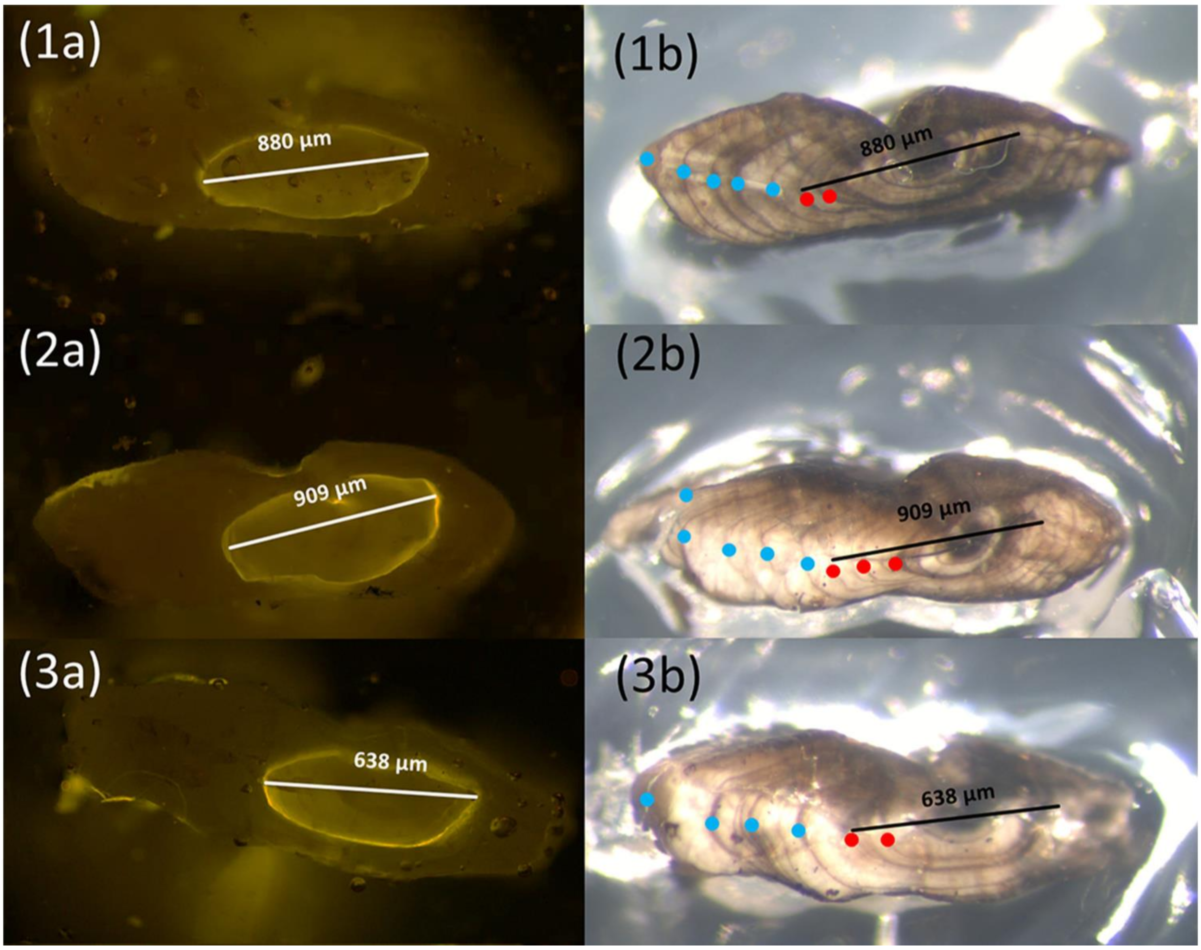

Figure 8: Eel otoliths prepared for the ARS mark identification under fluorescent light (1a–3a) and the same otoliths burned for the purpose of age estimation (1b–3b). Red dots indicate what could be mistaken as annuli, but are actually inside the mark ring shown by the black bar. Blue dots show the 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 annuli (ICES, 2009b). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Blind reading trial

A photograph was taken at each stage of the double prepared otolith process to standardize the reading procedure among 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 can estimate the age 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 sampling location and sampling date. This approach enabled the subsequent discrimination between potential annuli outside the ARS ring and definite stress rings inside the fluorescent mark (Figure 8 1b–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 (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 (Figure 9) and corrected by averaged individual number of rings inside the ARS mark (c.f. Figure 9). Additionally, length ($L\infty$) and weight ($W \infty$) growth was calculated with the averaged estimated and corrected age using the von Bertalanffy growth function (VBGF) as reviewed in Beverton and Holt (1957) and Ricker (1975):

$$TL_0 = L_\infty [1 - e^{-k(0-t_0)}]$$

and

$$W_0 = W_\infty [1 - e^{-k(0-t_0)}]^3$$

where $L\infty$ and $W\infty$ are the calculated asymptotic maximum TL and BW at age t (n annuli), k ($year^{-1}$) is the growth coefficient and t0 is the abscissa intercept. The growth functions were fitted according to Ogle (2018) using nonlinear least- squares estimates via the nls function in R (R Core Team, 2016). Because all investigated eels are known to be stocked pre-grown recruits the ordinate intercept was fixed at TL0 = 171 mm and W0 = 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.

Table 7: 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)[a]	Corrected mean age (n annuli)[b]	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 (%)[c]
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.
[a] Averaged number of counted rings inside and outside the ARS mark rings (cf. Fig. 8 1b-3b) rounded down.
[b] Estimated age minus the average number of counted inside the ARS mark (cf. Fig. 8 1b-3b, blue dots) rounded down.
[c] Percent increase compared to the estimated growth rate.

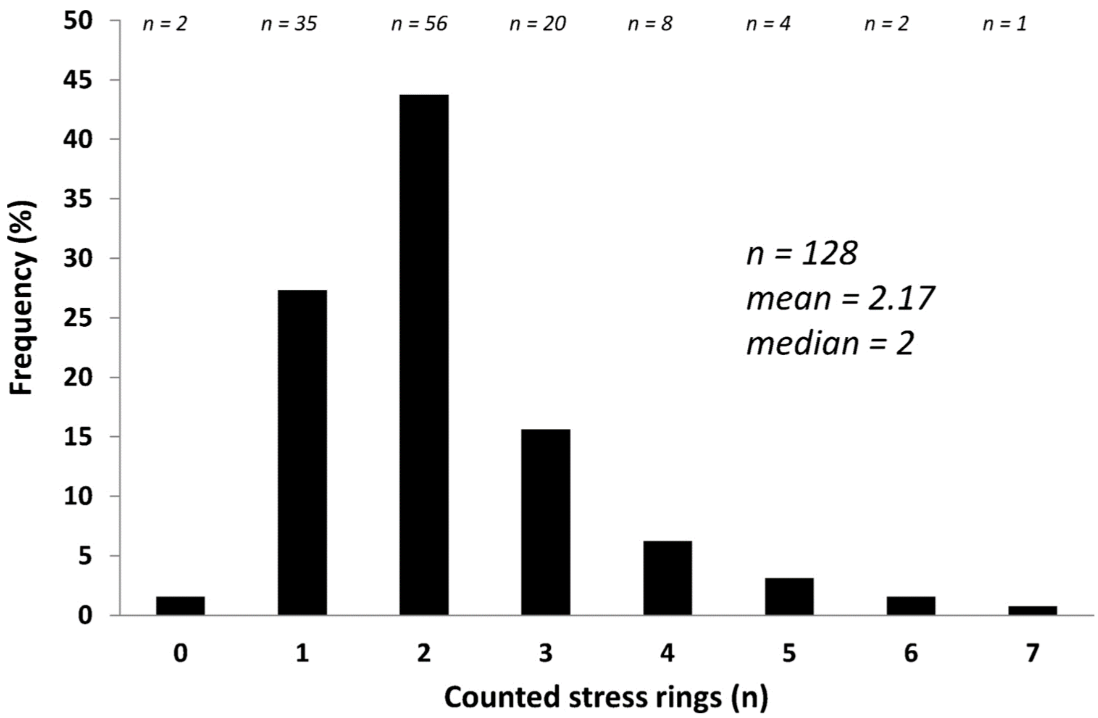

Figure 9: Pooled frequency and number of counted stress rings inside the alizarin red S mark during the blind reading trials. The readers agreed on the readability of 64 otoliths (i.e., n = 128).

Statistics and modelling

R was used for the statistical analysis and the significance level for all tests was $\alpha = 0.05$. The Wilcoxon test was used to test for significant differences from paired differences of 0 between the estimated and corrected age (Campana *et al.*, 1995). To calculate the effect of the IRH on estimates of the stock biomass, we followed the approach of Pohlmann *et al.* (2016), using the corrected and uncorrected growth function. Briefly, the procedure is based on the virtual population analysis (VPA; 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 Bevacqua *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 using the respective VBGF and the length-weight relationship as $W = 0.003*TL^{3.040}$ (n = 100 eels; c.f. Table *6*). For the sake of simplicity, we assumed that all individuals are recruited in age-group 0 (n = 1×106) 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 > 450 mm though the majority of silvering occurs at length > 600 mm; 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 7). Readers agreed on the age in 45.3 % of the analyzed 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 9 and Figure 10a), and between zero and nine annuli outside the mark (Figure 10b). 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. In general, the pooled estimated age was significantly higher than the pooled corrected age (Wilcoxon test, W = 0, $P < 0.001$). The deviance between readers tended to be higher when the estimated age increased and there was a significant positive correlation between the estimated age and the number of counted stress rings (Spearman rank correlation, $\rho = 0.516$, $P < 0.0001$; Figure 10c).

Considering only the counted number of rings inside the ARS mark, the readings differed significantly between readers (Wilcoxon test, W = 23, $P < 0.001$). The R^2 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 10a). Focusing on the number of rings outside the fluorescence mark, age readings differed slightly, but not significantly (Wilcoxon test, W = 30, $P = 0.059$). Moreover, by disregarding the stress rings inside the ARS mark, the R^2 value increased by a factor of 2.24 (Figure 10b).

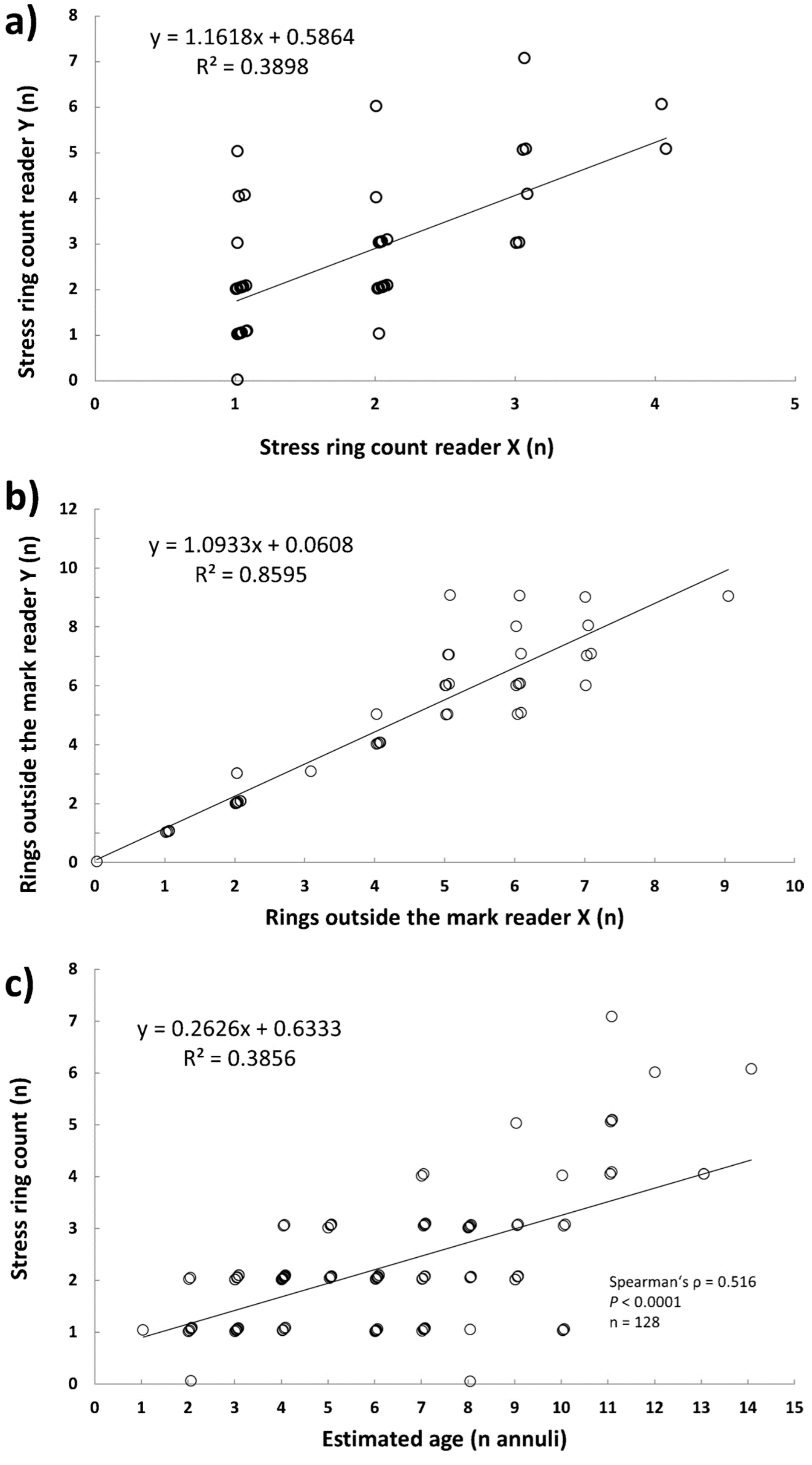

Figure 10: (a) Comparative presentation of the number of counted stress ring (i.e., rings inside the alizarin red S mark) of the two blind readers and (b) number of identified rings outside the mark. c) Significantly positive correlation between the estimated age and the number of counted stress rings inside the ARS mark.

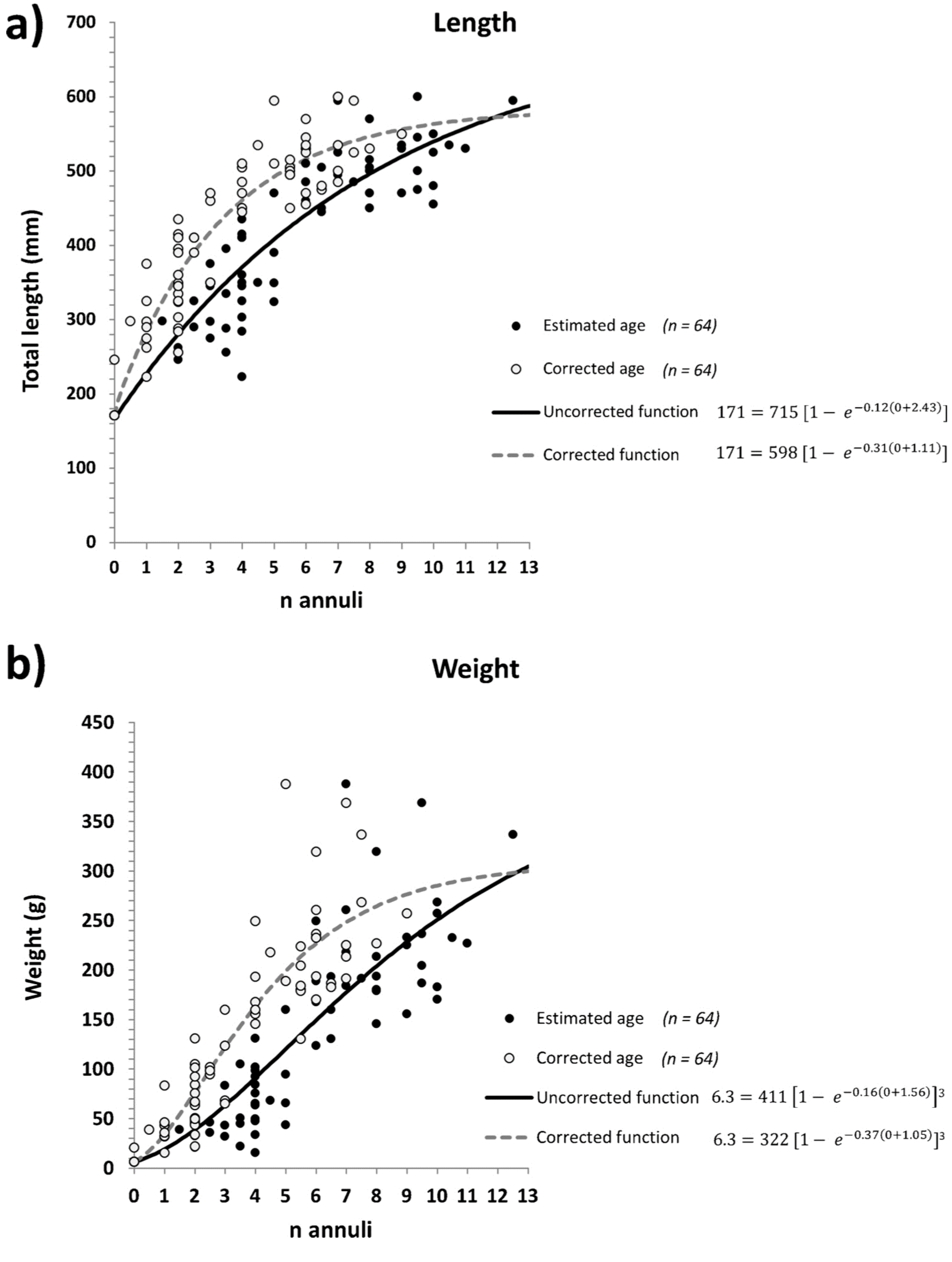

Figure 11: (a) Fitted von Bertalanffy growth function (VBGF) for the total length using the estimated and corrected mean age and (b) the VBGF for weight respectively.

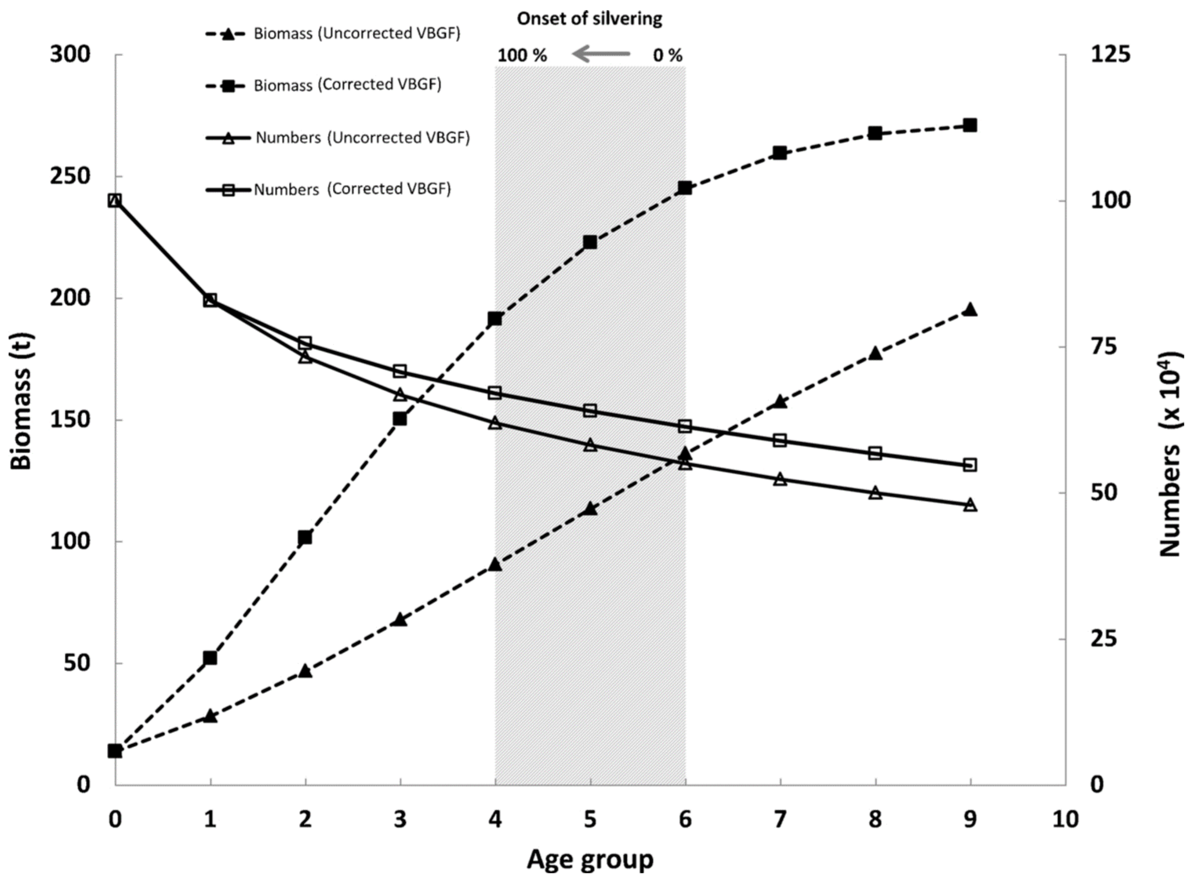

Figure 12: Output for biomass and number of recruits per cohort by using the corrected and uncorrected von Bertalanffy growth function (VBGF) of this study (Figure 11a) as input data for the age-based escapement model according to Pohlmann et al. (2016). The grey bar indicates the earliest onset of silvering (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.

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 7). 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 Ttest, $t = -6.5356$, 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 7), 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 *(*$L\infty$) of 715 mm (95 % confidence interval (CI): 612–790 mm) by a growth coefficient (k) of 0.12 ± 0.03 year−1 (Figure 11a). After age correction, $L\infty$ decreased by 16 % to 598 mm (95 % CI: 526–641 mm), while the growth coefficient increased to 0.31 ± 0.04 year^{-1} (i.e., 158 % higher). The uncorrected growth function for weight revealed an asymptotic weight *(*$W\infty$) of 411 g (95 % CI: 347–487 g) (by a k of 0.16 ± 0.08 year^{-1}). After age correction, $W\infty$ decreased by 22 % to 322 g (95 % CI: 253–366 g) and the growth coefficient increased by 131 % to 0.37 ± 0.09 year^{-1} (c.f. Figure 11b).

Impact on modelled biomass and number of recruits per age-group

The estimated biomass of age class 1 increased by 85 % compared to the initial model (0 % farmed eels in the stock) without substantial changes in numbers (Figure 12). The most marked underestimation of biomass was observed for the age-3 cohorts, with a maximum underestimation of 129 % assuming 100 % farmed eels in the stock. Thereafter, with increasing age, the estimated change in biomass decreases to a 47 % 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 120 % and 8 % 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. 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 9) and then used to exemplify the underestimation of the growth rates which were up to 106 ± 78 % higher after age correction (Table 7).

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 corrected age readings did not differ significantly. 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 10a and 10b). 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 9).

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. However, the methodology was also used by ICES (2009b, 2011) for comparative interreader calibration analysis, and is therefore considered appropriate. 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 readability between readers, further ensuring the quality of readings. In any case, potential uncertainties (e.g., due to 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.

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; ICES, 2017a). A tagging study on European hake has revealed a twofold underestimation 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 (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, it was demonstrated that stock assessment approaches that 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 using an age-based model

(Pohlmann *et al.*, 2016); Figure 12). This further adds to the previously described problems in the application of age-based models due to the phenotypic plasticity in eel body growth (Leo and Gatto, 1995; Melià *et al.*, 2006). Based on our results, the underestimation of growth led to 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 12). Consequently, stocking of unmarked farmed eels introduces bias in ALKs, and it can be concluded that, without knowledge of the IRH, the observed error is likely to affect, for example, estimates of silver eel escapement in age-based stock assessment models. It should be noted, however, that the degree of uncertainty will depend on the structure of the 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 considered (Figure 10c). This necessitates detailed information about the amount of stocked recruits per age class 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 age in farmed eels. It was shown that this 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, 2016b, 2016c), the IRH must be available to otolith readers 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 supplement local stocks (Shiraishi and Crook, 2015; Kaifu *et al.*, 2018). 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). Identification of the recruitment history should be possible or 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 such as the EU DCF (e.g., (ALCOBENDAS *et al.*, 1991; Simon and Dörner, 2005; Wickström and Sjöberg, 2014; Caraguel *et al.*, 2015; Kullmann and Neukamm *et al.*, 2017; Kullmann and Hempel *et al.*, 2018). This leaves the use of standard fluorescent microscopy as a common and wide spread technology to allow multi-national research collaborations including, for example, 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.

Conflict of interest

The authors have no conflict of interest to declare.

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Chapter 3 - Yellow eel (*Anguilla anguilla*) density trends along the German part of the southern Baltic Sea between 2009 and 2020

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Abstract

According to the recruitment indices provided by ICES for the years 2011–2021, the natural recruitment of the European eel remains at low level. However, fishery-dependent data series indicate an increasing trend in the southern Baltic Sea within recent years. In the light of the pan-European conservation efforts, the question is whether fishery-independent monitoring approaches can verify this positive trend. To monitor yellow eel density, a 1 ha enclosure system for non-tidal coastal waters was used in eight reference areas along the German part of the southern Baltic Sea (Federal State Mecklenburg-Western Pomerania) from 2009 to 2020. Changes in presence rate, mean yellow eel density and total length distribution were investigated. Additionally, the total stock size of yellow eels and the annual settlement rate of juvenile eels were estimated. Overall, the enclosure data indicate a positive trend in yellow eel stock size in recent years. An increasing mean yellow eel density was observed in five out of eight reference areas sampled. The density change was linked to an increased number of juvenile eels as evidenced by a significant change in the total length distribution. The mean annual settlement rate of juvenile eels was estimated as 13.2 eels of age class 0 + ha^{-1} $year^{-1}$ in 2012–2017. Besides the increased settlement rate, other mechanisms of population dynamics or conservation measures might have contributed the observed positive trend in yellow eel densities.

Introduction

As a facultative catadromous species, the European eel *(Anguilla anguilla,* (L.)) is found in various aquatic habitats from the Atlantic Ocean, the North Sea, the Baltic Sea, and the Mediterranean (Tesch, 2003; van Ginneken, Vincent J. T. and Maes, 2005). Mature silver eels migrate to the spawning area, which is assumed to be located in the Sargasso Sea (Tesch, 2003). During the complex life cycle, the eel stock is threatened by various pressures throughout the continental part of its life cycle, including fishing, habitat loss, hydropower use, parasitism and pollution effects (Feunteun, 2002; Jacoby and Gollock, 2014). Additionally, changing oceanic conditions might have negatively affected the eel stock in various ways (Friedland *et al.*, 2007; Bonhommeau *et al.*, 2008). Since the late 1970s, the recruitment of the European eel has declined severely (ICES, 2021a). Currently, the overall recruitment into the North Sea region is below 2 % and below 8 % in "Elsewhere Europe" compared to the pre-1980 level (ICES, 2021a). Due to the decline of the European eel, the International Council for the Exploration of the Sea (ICES) considers the species as "outside safe biological limits". In response to the critical state of stock, the European Commission adopted the Eel Regulation in 2007 (European Council, 2007). EU member states were obligated to establish eel conservation plans for their river basin districts (Eel Management Units, EMU) and to examine their outcomes (European Council, 2007). Without the implementation of management plans, eel fisheries should have been closed in these EMUs (European Council, 2007). Moreover, the European eel was listed in Appendix II by CITES in 2009 to control its international trade.

To develop effective management strategies for eel conservation, it is crucial to generate robust monitoring data by considering the full range of aquatic habitats settled by eels. As a facultative catadromous species, eels settle in marine, brackish as well as freshwater habitats during their sedentary continental life phase (Tesch, 2003; Daverat *et al.*, 2006; ICES, 2009a). The yellow eel, named as such for its color, is the continental life stage, which inhabits freshwater as well as transitional and coastal waters. The majority of monitoring activities occurs in freshwater habitats, but data on stock size and temporal trends of yellow eels inhabiting coastal waters are largely lacking (ICES, 2009). Furthermore, quantifying the proportion of eels living in coastal waters to the total European eel stock remains a major challenge (ICES, 2009a). Given the methodological shortcomings of available assessment approaches (ICES, 2009a), a 1 ha enclosure system approach specific to the yellow eel was developed (Ubl and Dorow, 2015). The enclosure approach was derived from the sedentary and bottom-dwelling lifestyle of yellow eels, which is associated with a small home range and predominantly nocturnal foraging behavior (Walker *et al.*, 2014; Verhelst *et al.*, 2017).

The present study has two major objectives: Firstly, the generated enclosure data should allow detecting the most recent trend development of the yellow eel stock in coastal waters of the southern Baltic Sea between 2009 and 2020. According to the ICES North Sea recruitment index, the settlement into the Baltic Sea remained at low level for more than 15 years (ICES, 2021a). Therefore, it was hypothesized that the obtained enclosure data would not show evidence of positive stock trends as reflected by parameters such as yellow eel presence rate, mean yellow eel density, or yellow eel length distribution. Second, as the specific catchability of the enclosure is known for yellow eels (Dorow *et al.*, 2018; Dorow *et al.*, 2020), the data thus collected allow estimates of the size of the yellow eel stock as well as the annual recruitment rate. Accordingly, this study provides basic population parameters for yellow eels in non-tidal coastal waters using a fishery-independent monitoring approach. This could therefore particularly promote eel stock assessment in the Baltic Sea as well as contribute to the understanding of eel stock dynamics in coastal waters in general.

Material and methods

Study area

The study area encompasses the East German part of the southern Baltic Sea (Figure 13), which is located in the federal state of Mecklenburg-Western Pomerania (MWP). The coastline of MWP is around 1945 km long with a total area (three nautical miles limit) of 365,520 ha. The main part of the area belongs to the EMU Warnow/Peene (337,800 ha), but a small part pertains to EMU Oder (27,720 ha). The coastal area is divided into inner lagoons and open coastal waters (Winkler *et al.*, 2007). Inner lagoons together with sheltered estuaries have a total size of 171,120 ha. Open coastal waters are the exposed areas within the three nautical miles limit with a total size of 194,400 ha, where about 40 % (78,334 ha) of the area lies west and 60 % (116,066 ha) east of the Darss-Sill (Figure 13). European eel is found throughout the coastal area of MWP (Maßnahmen zur Überwinterung der Satzkarpfen, 1963; Winkler *et al.*, 2007).

The commercial eel fishery in coastal waters of MWP has a long history and is still of commercial relevance for the area´s multispecies coastal fishery (Dorow *et al.*, 2017). Since the early 1970 s, annual eel landings declined continuously (Dorow *et al.*, 2017). In the period from 2010 to 2020, mean annual eel harvest was around 43 t, with a minimum of 32 t in 2016 (LALLF, 2021b). Despite the unchanged low annual recruitment rate in the North Sea region (ICES, 2021a) and the decline in registered full-time and part-time fishers in the study area (mostly individually operated fisheries; (LALLF, 2021a), annual eel landings have increased after 2016 with a mean annual eel harvest (2017–2020) of 48 t in recent years (LALLF, 2021b). The positive commercial eel harvest trend is supported by fishery-dependent catch per unit effort (CPUE) data showing higher catch rates for undersized and legal caught yellow eels in recent years (Dorow *et al.*, 2021).

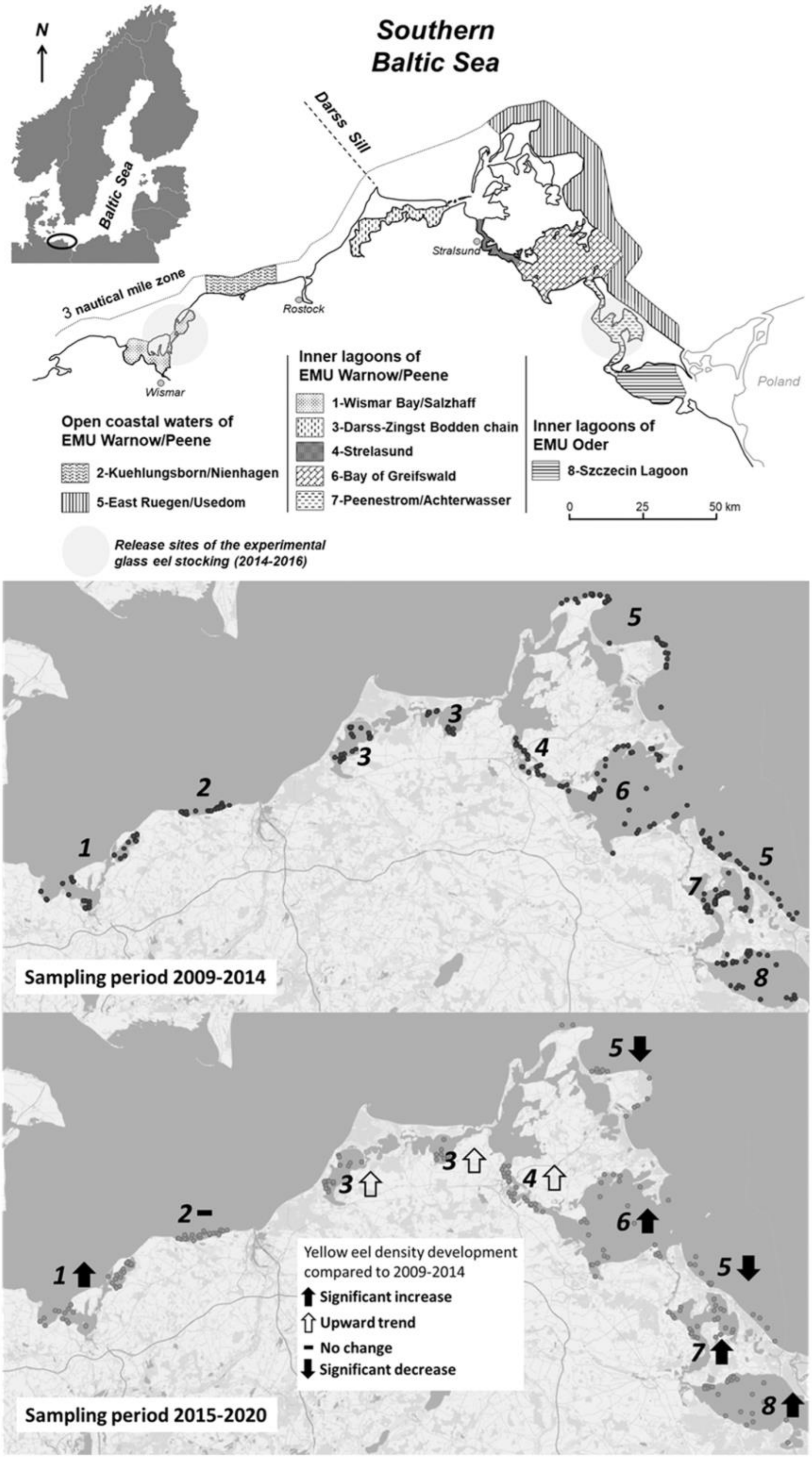

Figure 13: Sampled reference areas in which the enclosure system was fished, indicated is the affiliation of the reference areas to the EMU Warnow/Peene or EMU Oder, the release sites of the experimental stocking experiment are highlighted, randomly selected sampling stations along the Baltic Sea coastline of Mecklenburg-Western Pomerania shown as dots for the sampling period 1 (2009–2014) and period 2 (2015–2020), numbers indicate the eight sampled reference areas (from west to east, 1-Wismar Bay/ Salzhaff, 2-Kuehlungsborn/Nienhagen, 3-Darss-Zingst Bodden chain, 4-Strelasund, 5-East Ruegen/Usedom, 6- Bay of Greifswald, 7-Peenestrom/Achterwasser, 8-Szczecin Lagoon), for period (2015-2020) the density change is indicated in accordance to Table 9.

Various eel management efforts have been implemented in the study area. The minimum size limit has been raised from 45 to 50 cm in 2009. Starting in 2018, a three-month seasonal closure (November to January) was introduced for commercial fisheries. To assess the effect of translocating glass eels into coastal waters, a stocking experiment with alizarin red marked fishes was conducted in the inner coastal waters Wismar Bay/Salzhaff and Peenestrom/Achterwasser (Dorow and Schaarschmidt, 2014; Kullmann *et al.*, 2020; Buck and Kullmann, 2021). From 2014 until 2016, each stocking area (Figure 13) received annually 60 kg glass eel (in total 120 kg per year, with a mean weight of 0.3 g per glass eel), which is equivalent to approximately 400,000 released 0 + fishes per year.

Sampled reference areas

Along the coastline of MWP, eight reference areas were sampled between 2009 and 2020 (Figure 13). The selected areas can be assigned to open coastal waters (2-Kuehlungsborn/Nienhagen, 5-East Ruegen/ Usedom) and inner lagoons (1-Wismar Bay, 3-Darss-Zingst Bodden chain, 4-Strelasund, 6-Bay of Greifswald, 7-Peenestrom/Achterwasser, 8-Szczecin Lagoon). Due to similar habitat characteristics, the comparatively large geographic area "East Ruegen/Usedom" was defined.

All selected reference areas reflect the diversity of available growing habitats for yellow eels along the coast of MWP. For example, a salinity gradient exists with generally higher practical salinity unite (PSU) values in western and lower values in eastern parts of the study area (Winkler *et al.*, 2007). The salinity in open coastal waters ranges from 11 PSU (Kuehlungsborn/Nienhagen) to 6 PSU (East Ruegen/Usedom). Depending on the geographic location, the PSU of the inner lagoons likewise varies between 11 PSU (Wismar Bay/Salzhaff) and 2 PSU (Szczecin Lagoon). The overall mean fishing depth was 4.5 m (Table 8). Depending on the reference area (Table 8), the mean fishing depth ranged between 2.3 m (Darss-Zingst Bodden chain) and 7.5 m (Northeast Ruegen/Usedom). Moreover, the reference areas differ in their average Secchi depth or primary sediment type (see (Ubl and Dorow, 2015) for details).

The sampling stations were randomly chosen within the reference areas based on a stratified sampling design (Ubl and Dorow, 2015). In general, it was aimed, to realize 6 sampling stations per year in each reference area. In 2015 and 2016, only the reference area Wismar Bay/Salzhaff was sampled in course of the evaluation of the yellow eel specific enclosure catch efficiency (Dorow *et al.*, 2018; Dorow *et al.*, 2020). These data were partly considered in this study. In 2014, only the reference areas East Ruegen/Usedom, Bay of Greifswald, Peenestrom/Achterwasser and Szczecin Lagoon were sampled to increase the sampling stations for these areas.

Table 8: Mean fishing depth in the selected reference areas; number of sampled stations and yellow eel presence rate (% of sampling stations with at least one harvested yellow eel) separated for the two time periods; differences in presence rate between the two periods were tested with a χ2-test, stars indicate significant differences (p < 0.05).

	Mean fishing depth (m ± SD)	Number of fished stations 2009–2014	Yellow eel presence rate (%) 2009–2014	Number of fished stations 2015–2020	Yellow eel presence rate (%) 2015–2020	χ^2 –test-value
All reference areas	4.5 ± 2.5	304	74.7	211	84.4	6.96 *
1-Wismar Bay/ Salzhaff	3.5 ± 1.8	31	80.6	40	92.5	2.22
2-Kuehlungsborn/ Nienhagen	7.4 ± 2.2	32	93.8	24	75.0	3.94 *
3-Darss-Zingst Bodden chain	2.3 ± 0.7	32	78.1	24	75.0	0.08
4-Strelasund	3.2 ± 0.8	31	58.1	25	72.0	1.17
5-Northeast Ruegen/ Usedom	7.5 ± 2.1	70	74.3	24	62.5	1.21
6-Bay of Greifswald	4.6 ± 1.8	40	42.5	25	92.0	15.93 *
7-Peenestrom/ Achterwasser	3.1 ± 0.8	37	94.6	25	100	1.40
8-Szczecin Lagoon	2.5 ± 06	30	80.0	24	100	5.40 *

Calculated parameters

For each eel, the place of harvest (net corner or fyke net chain), the silvering index (Durif *et al.*, 2005; Durif *et al.*, 2009) using the parameters TL, weight, eye diameter, and pectoral fin length were documented. The silvering index was used to separate between mature silver eels and resident yellow eels. As migrating silver eels could originate from areas outside from the sampled reference areas, they were excluded from further analysis. Only the silvering stages I, FII and FIII were considered as resident yellow eels. Assuming an equal yellow eel distribution in the reference areas, the overall yellow eel number (TL > 36 cm) was calculated by multiplying the corrected mean densities and the water surface of each reference area.

According to the experimental glass eel stocking (conducted in 2014–2016), otoliths of eels harvested in 2017, 2018, and 2019 were checked for alizarin red staining by using a fluorescent microscope to separate between stocked eels and natural recruits. For both stocking areas (Wismar Bay/Salzhaff, Peenestrom/Achterwasser), the average proportion of marked eels and the yellow eel density without stocked eels was calculated. The potential effect of translocated fishes on the yellow eel stock was assessed by comparing the densities of period 1 (2009–2014) with densities of period 2 (2017–2019).

Based on the TL, the age of the yellow eels was assessed using the formula given in Simon *et al.* (2013), which is specific for the region. Since the total eel number of each age group is known, the mean density of every age class was calculated. The average annual settlement rate was estimated as number of 0 + fishes ha−1 year−1, assuming that only age class 0 + fishes settle coastal waters of the study area and that natural mortality alone acts until age class 3 +. Annual natural mortality rates were based on Bevacqua *et al.* (2011). The study area specific Bertalanffy growth function shown in Simon *et al.* (2013), an assumed low eel density level, and an annual average water temperature of 10.1 °C were used as input parameters. The natural mortality over the period from age class 0 + to 3 + corresponded to a factor of 2.03 (mortality rate from 0 + to 1 + = 33.1 %; from 1 + to 2 + = 17 % and from 2 + to 3 + = 11.3 %). The total number of settled juvenile eels were estimated by multiplying settlement rate with the water surface assuming that the current stock was solely made of females as suggested by sex determination of eels (> 90 % female yellow eels) in the study area (unpublished data).

To summarize, the following parameter variations were analyzed for the stock of yellow eels (TL > 36 cm): i) presence rate, ii) density, iii) mean TL, iv) age distribution, and v) annual settlement rate of juvenile eels based on the density of age class 3 +. In doing so, the monitoring period was divided in two 6-years' time spans with period 1 covering the years 2009–2014 and period 2 the years 2015–2020, ensuring enough sampling stations (N > 20) for each reference area and period. To estimate the overall annual recruitment for the EMU Warnow/Peene (this recruitment does not cover the freshwater part of the stock) in period 1 and 2, the annual settlement rate per ha was multiplied with the overall size of EMU Warnow/Peene. The number of stocked eels was removed from the total coastal recruitment in the EMU Warnow/Peene for period 2. For these analyses, it was simplified assumed, that the yellow eel stock consists solely of the coastal resident type (Daverat *et al.*, 2006) and that there is no migration between coastal and inland areas.

Statistical analysis

All statistical analyses were performed using SPSS 22. Mean values are shown together with the standard deviation (SD). To test for significant differences between both periods, a t-test was applied in case of variance homogeneity. If variance failed the homogeneity test (Levene-Test), the normal distribution was tested (Shapiro-Wilk-Test). If a normal distribution was detected, a t-test for unequal variances was used, otherwise (non-normal distributed) a non-parametric Mann-Whitney-test was applied. A $\chi2$-test was chosen for categorical data. For all tests, the significance level was set at $p < 0.05$. A bootstrapping approach (N = 5000 samples per reference area and period) was used to calculate the mean ± SD and the 95 % confidence interval for the point estimate of the corrected yellow eel density and the yellow eel stock size. For each reference area, the catch coefficient (Dorow *et al.*, 2020) and the observed yellow eel density were drawn with replacement from the calculated uncertainty intervals.

Results

Presence rate

Overall, 515 stations were sampled within the study period between 2009 and 2020 (Table 8). The overall number of stations as well as the number of sampling stations in each of the eight reference areas varied from 24 to 70 stations (Table 8). During the study, yellow eels were detected in all reference areas and at least one yellow eel was recorded in 405 of the 515 (78.1 %) monitoring stations with a significant increasing trend in the presence rate in recent years (Table 8). The presence rate increased significantly in two reference areas, namely Greifswalder Bodden and Szczecin Lagoon (Table 8). In the reference areas Wismar Bay, Darss-Zingst Bodden chain, Strelasund, Eest Ruegen/ Usedom and Peenestrom/Achterwasser the presence rate remained stable and in the reference area Kuehlungsborn/Nienhagen East, the presence rate decreased significantly (Table 8).

Yellow eel density

A total of 4,007 eels were caught. Only 204 (5.1 %) eels were staged as silver eels, most were classified as yellow eels (N = 3,775; 94.9 %). For 28 individuals, classification using the Durif-Index could not be made due to lack of data. For these eels, the silver or yellow eel stage were assigned using visual criteria. Most yellow eels (66 %) were caught with the fyke net chains and a smaller proportion (34 %) were caught with the corner fykes.

A range of 0–107 yellow eels ha^{-1} were observed. To account for potential bias due to high-density observations, two stations (0.4 %) with densities greater than 50 eels ha^{-1} were excluded from further analysis. Referring to the complete monitoring, an average density of 5.5 ± 9.8 eels ha^{-1} was observed with a significant increasing trend in recent years (Table 9). Depending on the reference area, the average yellow eel density varied between 1.3 ± 1.9 and 16.2 ± 11.3 yellow eels ha^{-1} (Table 9). The yellow eel density increased significantly in four reference areas, namely Wismar Bay/Salzhaff, Bay of Greifswald, Peenestrom/Achterwasser, Szczecin Lagoon. In two of eight reference areas (Darss-Zingst Bodden chain, Strelasund) an upward trend was observed and in the Kühlungsborn/Nienhagen area the density remained stable (Table 9). For the reference area East Ruegen/Usedom a significant decrease of the yellow eel density was detected (Table 9).

Table 9: Observed yellow eel density (TL > 36 cm) shown for the whole sampling period as well as for the two selected time periods, significant differences are indicated by a star ($p < 0.05$); density without stocked eels (TL > 36 cm, monitoring years 2017–2019) was 7.1 eels ha^{-1} in both stocking areas (Wismar Bay/Salzhaff and Peenestrom/Achterwasser).

Name of reference areas	Mean yellow eel density ± SD (eels*ha^{-1}) 2009–2014	Mean yellow eel density ± SD (eels*ha^{-1}) 2015–2020	U-test value	Yellow eel density trend evaluation
All reference areas	4.4 ± 6.9	7.1 ± 8.7	24,827.5 *	significant increase
1-Wismar Bay/ Salzhaff	2.4 ± 3.5	7.9 ± 7.7	301.5 *	significant increase
2-Kuehlungsborn/ Nienhagen	5.7 ± 5.6	5.2. ± 7.3	291.0	no change
3-Darss-Zingst Bodden chain	3.3 ± 3.3	5.0 ± 6.5	353.5	upward trend
4-Strelasund	1.4 ± 1.9	2.8 ± 3.7	299.0	upward trend
5-Northeast Ruegen/ Usedom	8.0 ± 11.3	2.2 ± 3.6	567.0 *	significant decrease
6-Bay of Greifswald	1.3 ± 2.6	3.3 ± 3.0	213.5 *	significant increase
7-Peenestrom/ Achterwasser	4.9 ± 4.5	14.0 ± 11.3	184.0 *	significant increase
8-Szczecin Lagoon	4.1 ± 5.9	16.2 ± 11.3	95.0 *	significant increase

Table 10: Calculation of the yellow eel stock size, given as total number of yellow eels (TL > 36 cm) based on the corrected mean yellow eel density, accounting for the sampled reference areas and both periods; means ± SD and CI intervals were calculated using a bootstrap approach with 5000 replicates.

	Area (ha)	Corrected mean density ± SD (eel ha^{-1}) 2009–2014	Corrected mean density ± SD (eel ha^{-1}) 2015–2020	Yellow eel stock 2009–2014 ± SD	Yellow eel stock 2015–2020 ± SD
Open coastal waters					
2-Kühlungsborn/ Nienhagen	78,344	15.3 ± 4.2	14.0 ± 5.5	1,202,345 ± 328,242	1,095,954 ± 432,952
5-Northeast Ruegen/Usedom	116,066	21.9 ± 5,9	6.0 ± 2.7	2,536,367 ± 686,469	698,531 ± 307,905
Inner lagoons					
1-Wismar Bay	16,890	6.6 ± 2.3	21.5 ± 5.5	111,593 ± 39,659	358,341 ± 992,867
3-Darss-Zingst Bodden Chain	19,680	8.9 ± 2.5	13.7 ± 5.0	174,831 ± 49,472	268,814 ± 97,529
4-Strelasund	6460	3.7 ± 1.3	7.6 ± 2.8	23,757 ± 8364	49,122 ± 18,356
6-Bay of Greifswald	83,980	3.5 ± 1.5	8.9 ± 2.6	295,193 ± 126,301	746,670 ± 218,886
7-Peenestrom/ Achterwasser	16,390	13.2 ± 3.4	38.0 ± 10.1	216,436 ± 55,435	623,618 ± 164,870
8-Szczecin Lagoon	27,720	11.4 ± 4.2	44.1 ± 11.2	315,614 ± 115,110	1,222,363 ± 310,958
Sum (with 95% CI range)	365,520			4,876,095 (2,649,440–8,064,840)	5,063,685 (2,493,283–8,649,464)

Using the total size of the reference areas and observed mean densities in combination combined with the specific capture coefficient for yellow eels *(*> 36 cm), the yellow eel stock size (TL > 36 cm) for the coastal waters of MWP was estimated (Table 10). Based on the monitoring data for period 1 (2009–2014), the stock size was about 4.88 million yellow eels (TL > 36 cm, 95 % confidence interval = 2.65 – 8.06 million yellow eels). Indicated by density changes, the stock size has increased by about 180,000 eels in period 2, resulting in total stock number of approximately 5.06 million yellow eels (TL > 36 cm, 95 % confidence interval = 2.49 – 8.65 million yellow eels).

In the stocking areas Wismar Bay/Salzhaff and Peenestrom/Achterwasser an average density of 11.4 ± 9.3 and 12.5 ± 10.3 yellow eels ha^{-1}, respectively, were detected from 2017 to 2019. The exclusion of all marked (stocked) eels (TL > 36 cm) resulted in a mean density of 7.1 yellow eel ha^{-1} for both areas. The proportion of stocked eels was 37.8 ± 32.3 % in the Wismar Bay/Salzhaff and 43.4 ± 23.4 % in the Peenestrom/Achterwasser. A comparison of the mean density value of 7.1 yellow eels ha^{-1} (without stocked eel) with the mean density observed for both areas in period 1 (Table 9), indicate an increased density in recent years even without stocking. This comparison confirmed the general observed trend of increased yellow eel densities in the study area.

Mean TL distribution

The TL measured from 3,775 yellow eels ranged from 18 to 94 cm with an average TL of 45.6 ± 11.2 cm. Yellow eels < 36 cm accounted for 21.1 % of the yellow eel harvest. Referring to all reference areas, the proportion of yellow eels below 36 cm significantly increased ($\chi 2 = 92.9$, $p < 0.05$) within in the course of the study (period 1 = 13.9 %; period 2 = 26.8 %). Taking into account the size-selectivity of the enclosure, the overall yellow eel TL (> 36 cm) was 49.4 ± 9.4 cm with an average weight of 247.6 ± 179.2 g. The mean TL decreased significantly (U = 741,059.5, $p < 0.05$), from 52.1 ± 9.8 cm in period in period 1 to 46.7 ± 8.1 cm in period 2 (Figure 14). Likewise, the mean weight significantly (U = 825,653.0, $p < 0.05$) dropped from 288.0 ± 203.1 g in 2009–2014 to 209.2 ± 143.1 g in 2015–2020. A significant decrease of the mean TL was detected in five of eight reference areas (Kuehlungsborn/Nienhagen, Strelasund, East Ruegen/Usedom, Peenestrom/ Achterwasser, Szczecin Lagoon). In the reference areas Wismar Bay/ Salzhaff, Darss-Zingst Bodden chain and Bay of Greifswald, no significant change of the yellow eel mean TL was observed (Figure 14).

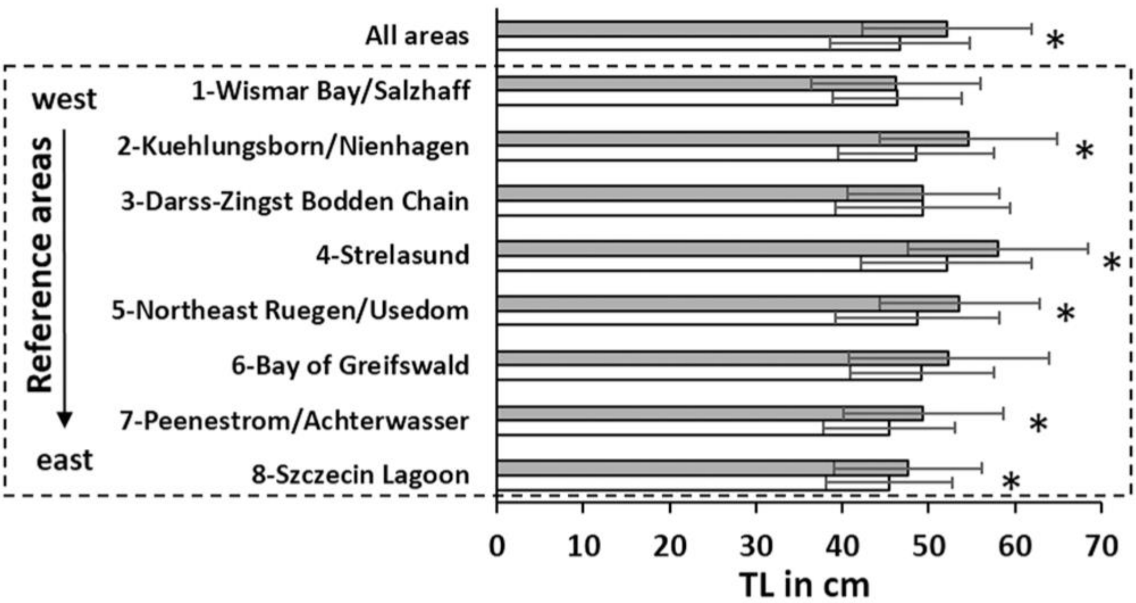

Figure 14: Mean yellow eel TL (TL > 36 cm), gray bars show the time period 1 (2009–2014) and white bars the time period 2 (2015–2020); error bars display SD, stars indicate significant differences (U-test, significance level p < 0.05) in the mean TL between the two time periods.

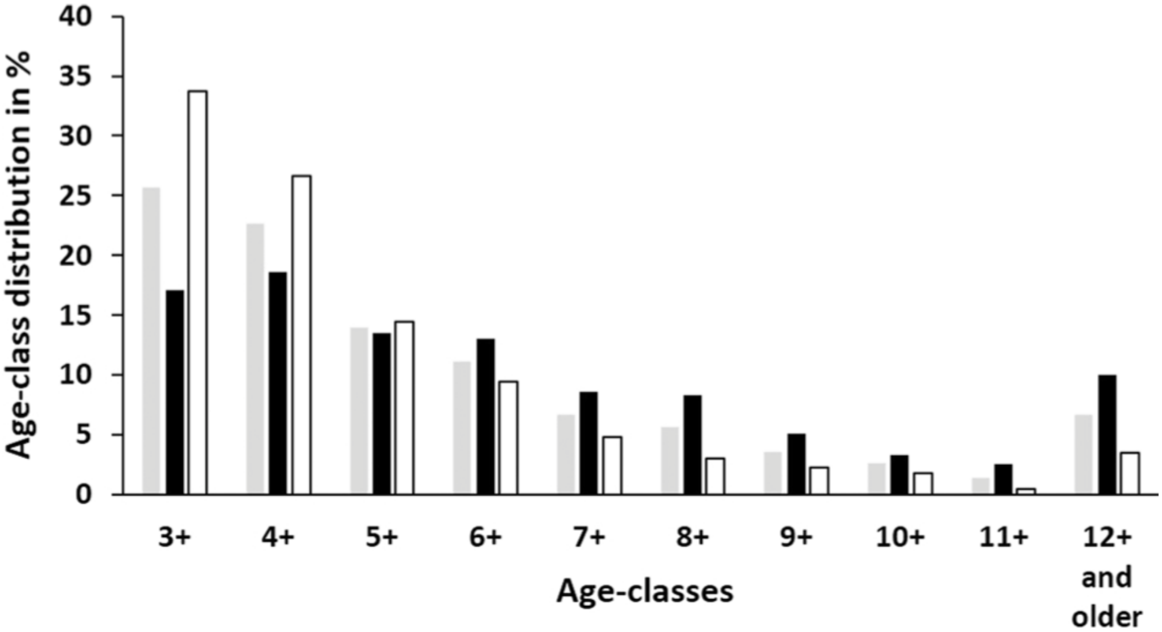

Figure 15: Age class frequency distribution of yellow eels (TL > 36 cm), gray bars indicate the overall time period, black bars reflect the period 1 (2009–2014) and white bars show the time period 2 (2015–2020).

Age distribution

Within the whole study period (2009–2020), the dominant age class of yellow eels were 3 + (25.7 %) and 4 + (22.7 %), accounting for around 48 % of all age classes (Figure 15). Comparing the age class distribution between period 1 and 2 (Figure 15), a significant change ($\chi 2 = 261.1$, $p < 0.05$) in the age class distribution was detected with an increased proportion of younger age classes in recent years.

Annual settlement rate

The mean density of eels of age class 3 + for period 1 was 0.84 eels ha^{-1}, afterwards the 3 + yellow eel density increased to 2.51 eels ha^{-1} in period 2. The application of capture coefficient and the natural mortality rate resulted in an annual settlement rate of 4.4 eels of age class 0 + ha^{-1} $year^{-1}$ in the years 2006–2011, which is the basis for the observed density of 3 + yellow eels in period 1. Reflected by the density change of age class 3 +, the estimated settlement rate increased to 13.2 eels of age class 0 + ha^{-1} $year^{-1}$ (2012–2017). Referring to the total size of the coastal waters of MWP, the total annual settlement sums up to around 1.62 million eels of age class 0 + in the years 2006–2011 and increased to 4.84 million eels of age class 0 + in the years 2012–2017. Accounting for the size of the EMU Warnow/Peene (337,800 ha) and considering the glass eel stocking measures, the natural part of annual coastal settlement corresponds to around 4.1 million eels of age class 0 + in the years 2014–2016.

Discussion

Marine and coastal waters represent important habitats for eels during their continental life phase (ICES, 2009a). Fishery-independent time series or assessment methods are mostly lacking for coastal habitats, resulting in considerable knowledge gaps regarding the quantification of the contribution of the eels settling in marine and coastal waters to the total eel stock. Within the study period 2009–2020, an enclosure approach was used to monitor the density of yellow eels in the coastal waters of the southern Baltic Sea (MWP). The observed data provided detailed insights into the yellow eel stock, like e.g., the density trend or shifts in the length distribution. Furthermore, the current stock size of yellow eels and the annual settlement rate were estimated. Regarding the various derivable stock parameters, the continuous application of the enclosure approach (Ubl and Dorow, 2015) proved to be a sensitive monitoring tool specific to yellow eels in non-tidal coastal waters.

Overall, the detailed fishery-independent enclosure data provided evidence for significant change in the yellow eel stock along the southern part of the eastern German Baltic Sea between 2009 and 2020. Yellow eels were detected in all reference areas. Significant higher presence rates of yellow eels as well as significant higher yellow eel densities were detected in recent years. These observations are further underpinned by fishery-dependent CPUE data, as significant higher CPUE rates were documented for undersized and legal retainable yellow eels after 2016 compared to the period before (Dorow *et al.*, 2021). Moreover, despite decreasing number of coastal fishers in the study region, annual eel landings increased since 2016 (LALLF, 2021a, 2021b), additionally confirming the specific trend of yellow eels in the study area as evidenced by the enclosure-based monitoring data in this study. The positive trend observed for the yellow eel stock in the eastern German Baltic Sea, based on fishery-independent (this study) and fishery-dependent data (Dorow *et al.*, 2021), corroborates the positive trend of the ICES yellow eel index in recent years compared to 2009–2010 (ICES, 2021a).

The number of naturally immigrating juvenile eels might primarily drive the development of the regional abundance of yellow eels in the study area. According to ICES (2021a), the North Sea glass eel recruitment series remained more or less constant at low level since the year 2000. Referring to the time period 2010–2020 the average annual recruitment corresponds to less than 2 % of the average reference level from 1960 to 1979 (ICES, 2021a). Given this consistently low recruitment within the last 20 years, the density of yellow eels should show a similar trend. However, the increased densities of yellow eels associated with a change in the mean TL distribution or the increased proportion of eels less than 36 cm indicate a stronger settlement in the study area than the ICES North Sea index suggests.

The positive development of settlement in the study area is further confirmed by fishery-based CPUE data (Dorow *et al.*, 2021). Based on various time series, an upward trend in settlement since 2011 was also detected in Sweden (Dekker *et al.*, 2021). These differing trends between local observation (this study and (Dekker *et al.*, 2021) and the ICES recruitment index lead to uncertainties in the assessment of the current recruitment. Multiple times series are considered in ICES North Sea recruitment index calculation, differing in sampling methods, the sampled habitat and the targeted developmental stage of eels (ICES, 2019, 2021a). For example, only a minor part of the considered time series is based on monitoring activities conducted in marine or coastal waters, most samplings take place in transitional (brackish) or freshwater areas (ICES, 2019). Furthermore, the stations are not evenly distributed geographically (ICES, 2019, 2021a) as most studies are conducted in Belgium, the Netherlands, British Isles and the west coast of Denmark. Only a few data series originate from the Kattegat, Skagerrak, or the Baltic Sea itself. Further, the data series considered in the North Sea index differ with respect to their duration. Accordingly, the derivation of the historic recruitment level is based on a few time series, most of which are located in the southern North Sea and to a lesser degree in the Baltic Sea. Given these constraints, the ICES North Sea recruitment index (ICES, 2021a) is subject to uncertainties and might therefore underestimate the settlement in coastal waters, which might explain the differing trends between the ICES North Sea recruitment index and the enclosure based settlement calculation. Therefore, we suggest adapting the calculation of the ICES glass eel recruitment index. Instead of allocation of the available recruitment series into the areas "Elsewhere Europe" or "North Sea", the glass eel recruitment index should also reflect the immigration route from marine to inland waters. Given the uncertainties in the assessment of the annual settlement, we further recommend to integrate the presented enclosure data into the dataset used for the calculation of the ICES North Sea recruitment index or the ICES yellow eel index.

The challenge of a representative recruitment assessment can be further illustrated by the differing settlement trends between inland and coastal areas in the study area. Since 2005, the immigration of juvenile eels is monitored at three inland sampling sites located close to the Baltic Sea (distance between 1 km and 10 km) in the study area (Frankowski *et al.*, 2018). In each year, a mix of immigrating glass eels and elvers were observed. In contrast to the shown substantial increased settlement rate in coastal waters, no similar significant increase in immigrating juvenile eels was found at these three monitoring stations within the same period (Frankowski *et al.*, 2018). Accordingly, this observation underlines the need for an improved recruitment monitoring with a stronger consideration of the habitat settled by the respective developmental stage. The difference in the rate of settlement in coastal waters and the recruitment observed in nearby inland waters raises the question of which mechanism primarily drive the immigration of juvenile eels into inland waters draining into the southern Baltic Sea. Westerberg (1998) summarized the timing and underlying oceanic factors for the immigration of eels from the North Sea to the Baltic Sea. On a smaller geographical scale, it should be investigated, in which degree density-dependent mechanism (Feunteun *et al.*, 2003; Lasne *et al.*, 2008), environmental factors (Feunteun *et al.*, 2003), or genetically predefined ecotypes (as shown for the American eel *A. rostrata,* Pavey *et al.*, 2015) determine the immigration dynamics at a low population status.

Yellow eel densities derived from the enclosure data were found to differ at relatively small regional scale. Beside the annual settlement rate, various other regional factors could cause the observed spatial variance of the presence rate or the mean densities. Possible explanations might relate to habitat preferences of eels (Laffaille *et al.*, 2004) or varying eel specific carrying capacities of different habitat types (see Acou *et al.*, 2011). Interactions, such as competition for food, space, or predation impacts, could also affect the spatial distribution of eels (Bevacqua and Andrello *et al.*, 2011).

Previous studies showed that various environmental parameters might influence the spatial organization of yellow eels in freshwater (e. g., Laffaille *et al.*, 2003; Domingos *et al.*, 2006; ACOU *et al.*, 2011). Laffaille *et al.* (2003) described a shift in localization of eel size classes that occurs based on microhabitat characteristics. Larger size classes are located in deeper habitats and/or with less vegetation and were more widespread than smaller size classes (Laffaille *et al.*, 2003; Laffaille *et al.*, 2004). For coastal habitats, Christoffersen *et al.* (2018) showed that juvenile eels prefer specific substrates. The extent to which such habitat-related parameter explains the observed density differences could be investigated in the future studies using the environmental data for each sampled station by considering the current low settlement rate in the study region.

Density-dependent mechanisms are known to contribute to variations in the yellow eel density (Acou *et al.*, 2011; Andersson *et al.*, 2012; Christoffersen *et al.*, 2018). The survival rate and the proportion of female to male eels increase at low or decreasing population densities (Bevacqua and Andrello *et al.*, 2011; Bevacqua *et al.*, 2019). To that effect, compensatory density dependence can cause, for example, stable silver eel escapement rates despite varying annual recruitment rates (Lobón-Cerviá and Iglesias, 2008). As the current natural settlement of juvenile eels can be assumed to be at a low level in the study area, the observed densities of yellow eels might be hardly influenced by density-dependent mechanisms (Bevacqua and Andrello *et al.*, 2011; Bevacqua and Melià *et al.*, 2011). However, density-dependent mechanisms might have contributed to the observed current sex ratio, where a low settlement rate results in a female dominated yellow eel stock. In contrast, in the early 1960 s, the proportion of female eels varied between 20 % and 70 % in the study area (Schlumpberger *et al.*, 1964).

The observed changes in the yellow eel stock might also be influenced by eel conservation efforts including eel stocking or eel-specific fishing regulations. Poland, for example, released juvenile on-grown eels into the polish part of the Szczecin Lagoon (T. Nermer, personal communication). These stocking activities might have added to an unknown degree to the detected positive yellow eel stock trend in the German part of the Szczecin Lagoon in recent years. Furthermore, between 2014 and 2016 stocking with glass eels was conducted in two reference areas, namely Wismar Bay/Salzhaff and Peenestrom/Achterwasser (Dorow and Schaarschmidt, 2014). Recaptures indicated that a major proportion of the stocked eels remained in or close to the stocked areas (Buck and Kullmann, 2021) and thus had partly contributed to the increased density in these areas after 2015. The area Wismar Bay/-Salzhaff could also have benefited from stocking activities in coastal waters in the neighboring state

Schleswig-Holstein (Thiel and Kullmann, 2019). However, the calculated number of natural recruits exceeds that of stocked fish by about an order of magnitude, reflected by the localized contribution of the latter. Moreover, positive density trends were observed also in non-stocked reference areas. Excluding stocked eels for contrasting the density differences of 2009–2014 with 2017–2019 underlines, that stocking had an additive effect on the overall increased settlement in both stocked areas. Additionally, raising the minimum size limit in 2009 could have positively influenced the yellow eel abundance by lowering the overall fishing mortality.

The area East Ruegen/Usedom represented an exception from the overall positive trend, since only in this area a downward trend in presence rate and density was observed. If we assume that settlement of juvenile eels was similar to other areas covered in this study, then this suggests, that local factors might explain this trend. For example, locally increased predation by birds and mammals could result in higher natural mortality rates or active displacement. The foraging by fish-eating birds and mammals could also lower the catchability of yellow eels. Likewise, the growing grey seal *(Halichoerus grypus)* population around the area East Ruegen/Usedom (Galatius *et al.*, 2020) and the grown cormorant *(Phalacrocorax carbo)* population (increased from approximately 3.000 to around 11.000 breeding pairs within three decades 1990–2020; (LUNG MV, 2020) might have added to an unknown degree to the observed trend of yellow eel densities. As both species predate on eel (Ostman *et al.*, 2013; Scharff-Olsen *et al.*, 2019, 2019; Tverin *et al.*, 2019), increasing population sizes of both predators might therefore increasingly threaten the surrounding eel population. The increased predatory pressure by both species might have induced further behavioral changes of eels, which in turn might have altered the capture probability for passive fishing gears like the enclosure. The lower water turbidity of the area East Ruegen/Usedom, if compared to the nearby areas Bay of Greifswald and Peenestrom/Achterwasser (Ubl and Dorow, 2015), might additionally increase the predation risk of bottom-dwelling fish species like eel by the opportunistic grey seals or cormorants. Higher grey seals number could also have added to the decrease in the presence rate in the area Kuehlungsborn/Nienhagen. Therefore, the densities and presence rates of yellow eels given for the areas East Ruegen/Usedom and Kuehlungsborn/Nienhagen should be seen as conservative estimates.

Various simplifying assumptions were made which need to be controlled in future investigations. For example, it was assumed that the fishing depth did not influence the enclosure catchability as well as the boundary net efficiency. If future studies provide evidence that depth affects the enclosure catchability, this should be considered in the calculation of the stock size of yellow eels. Further, the assumption that the studied stock of yellow eels is composed solely of the coastal resident types requires a verification. Investigating the life-history trait of eels in the study area by micro-chemical otolith analysis (Daverat *et al.*, 2006; Shiao *et al.*, 2006) could help to discover the proportion of the coastal resident type and to reveal other possible life-history types in the study region. For example, it could be possible that a certain percentage eel, stocked as on-grown fish in neighboring inland waters migrate into coastal waters and settle there for the rest of the continental life-phase. However, the development of the eel stock in inland waters of the EMU Warnow/Peene (Fladung and Brämick, 2021) suggests, that this proportion should be relatively low. Additionally, other national (Thiel and Kullmann, 2019) and international stocking activities in Poland (T. Nermer, personal communication), Sweden, or Finland (Wickström and Sjöberg, 2014) might have added to the observed trend of the yellow eel stock. Such short or long distances immigration events adding to the regional coastal eel stock could be evaluated by the mentioned micro-chemical otolith analysis and/or in combination with mass marking of eels before stocking.

However, the cumulative stocking effort (number of stocked fish) in the southern Baltic Sea, Kattegat, and Skagerrak is not nearly enough to produce regional yellow eel density changes as presented herein.

Conclusion

One major challenge for the assessment of the eel stock in non-tidal coastal waters, like the Baltic Sea, is the lack of a suitable methods, which allows the sampling of all relevant habitat types. 12 years of monitoring together with an evaluation of the specific catchability for yellow eels (Dorow *et al.*, 2019, 2020) have proven that the enclosure approach is sensitive enough to observe changes of the yellow eel density in non-tidal coastal waters. The obtained enclosure data can be used to reconcile the outputs (e.g., yellow eel stock size) of the utilized population models or to control the model input parameters (e.g., annual settlement rate). The detected differences in densities between the reference areas should further be integrated in the development of an area specific assessment of the eel stock and should be taken into account for future eel management strategies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Chapter 4 - Dispersal, body condition, and growth performance of stocked European glass eels (*Anguilla anguilla*) into coastal waters of the eastern German Baltic Sea

This chapter is not intended for any publication in the current version, but appears exclusively in this dissertation.

Abstract

The recruitment of the European eel (*Anguilla anguilla*) population has been collapsed and stagnates at historically low levels since the late 1970s despite various stock-supporting efforts. In 2007, the EU member states were requested to elaborate eel management plans to ensure a minimum escapement biomass of at least 40 % compared to the pristine level. Most eel management plans have solely considered stocking measures in inland waters. To determine if stocking is also a management option in non-tidal coastal waters, a stocking experiment with glass eels has been conducted in the eastern German Baltic Sea. In three consecutive years, 335 kg glass eels have been stocked between 2014 and 2016. To allow discrimination of stocked from natural recruits after recapture, all stocked eels were chemically marked with Alizarin red S (ARS). Between 2017 and 2019, a total number of 1829 eels have been captured, of which 19.3 % (n = 353) were ARS marked and were recaptured within 10 to 100 km of the stocking sites in a period of up to five years after the initial stocking. Moreover, stocked eels were found to have significantly higher values in terms of fat content, k-factor, total length per year, and specific growth rates. It was also observed that per

additional stocking cohort, the proportion of eels stocked within an age class increased by approximately 10 %. The results of this study provide evidence, that glass eel stocking in non-tidal coastal waters can serve as a valuable management option to rebuild the stock, increase silver eel escapement and maintain a sustainable and economically successful local eel fishery, which is of general interest for eel managers across the eel range of distribution.

Introduction

The recruitment of the European eel (*Anguilla anguilla* (Linnaeus 1758)) population has declined rapidly since the late 1970s compared to the reference level between 1960 and 1979 and remains at very low levels (ICES Advice, 2020, 2021, 2022). Current data of the International Council for the Exploration of the Sea (ICES) published that the glass eel recruitment in 2021 was 0.6 % in the index area "*North Sea*" and 5.5 % in the index area "*Elsewhere Europe*" (ICES Advice, 2022). Besides that, *A. anguilla* has been listed as a "critically endangered species" on the International Union for Conservation Nature (IUCN) Red List of threatened species (Pike *et al.*, 2020) and eels also, were listed on CITES appendix II allowing trade only after harmlessness assessment (CITES, 2022), both ongoing since 2008.

As a consequence of this recruitment decline, the European Union (EU) adopted the EU Regulation No. 1100/2007 to establish measures for the recovery of the European eel stock (European Council, 2007). As a result, all EU member states have been committed to elaborate eel management plans (EMP) and were requested to identify the natural habitats for the European eel in their respective territories. Subsequently, a specific eel management plan (EMP) was prepared for the different eel management units (EMU). The main objective of these management plans is an escapement of silver eels (the migratory stage, when European eels migrate to their spawning habitat in the Sargasso Sea) of 40 % of the biomass that most likely would have been observed compared to an anthropogenically unaffected stock. Various measures are listed for this purpose, such as reducing commercial and recreational fishing, controlling predators, and, one of the most important and wide spread management measurement, stocking (i.e., translocation of natural recruits) of juvenile eels in water bodies with currently low natural recruitment (European Council, 2007). In Germany, for example, eel stocking has been carried out for more than 180 years and was mainly limited to inland waters (Dekker and Beaulaton, 2016). However, inland waters today can represent a great challenge as a nursery habitat for eels, because they are not accessible without extensive human assistance due to numerous migration obstacles (for example dams and hydropower plants) and eels might not be able to escape from these waters (Brämick *et al.*, 2016).

According to (Ubl and Jennerich, 2008), the coastal waters of Mecklenburg-Vorpommern (MV) at the eastern German Baltic Sea serve also as a suitable nursery and feeding habitat for juvenile eels as well as for migrating silver eels and thus represent an important habitat for European eels.

Stock-supporting stocking measures were carried out in MV exclusively in inland waters until 2014 (Dorow *et al.*, 2017), although coastal waters have been shown to provide important habitat for eels and are considered in the local EMUs (Anonymus, 2008). In addition, eels that remain in coastal waters during their growth phase have been observed to show higher growth rates (Lin *et al.*, 2007; Simon *et al.*, 2013) and lower rates of infestation with the swim bladder parasite *Anguillicola crassus* (Jakob, 2009; Jakob and Hanel *et al.*, 2009) compared to inland waters.

Since it has not yet been clarified whether stocking measures in coastal waters of the eastern German Baltic Sea can have a positive effect on the recruitment a large-scale glass eel stocking experiment was conducted (Dorow and Schaarschmidt, 2014; Dorow, 2015; Wichmann, 2018; Buck and Kullmann, 2021).

In order to distinguish stocked glass eels from potentially naturally immigrated conspecifics, all eels were marked with the chemical marker Alizarin red S (ARS) immediately before stocking (Dorow and Schaarschmidt, 2014). ARS is a fluorescent marker that binds irreversible to bones and calcified structures (e.g., fish otoliths) and can be identified years later using a fluorescence microscope (Puchtler *et al.*, 1969; Caraguel *et al.*, 2015). Furthermore, marking eels with ARS has no negative impact on European eels (Simon *et al.*, 2009) and no difference in length after up to seven months has been demonstrated between marked and unmarked eels (Caraguel *et al.*, 2015).

The general objective of this mark and recapture study was to optimize the practice of eel stocking as a relevant management option in non-tidal coastal waters using the eastern German Baltic Sea coast as an example. To assess the effect of glass eel stocking on the local eel population, a total of 335 kg of European glass eels haven been chemically marked and stocked in two different stocking areas in three consecutive years. Eels were recaptured over a period of 3 to 5 years after stocking to assess (i) the dispersal patterns, (ii) the amount of stocked eels in the local eel stock per cohort, and (iii) the body condition by comparing relevant fitness parameters (i.e., growth, development stage, body condition, fat content) between stocked (marked) and considered natural (un-marked) recruits. This study thereby is a contribution to the so-called net-benefit discussion, i.e., whether translocation of eels from parts of Europe with relatively high to relatively low densities can lead an overall benefit for the eel population in terms of increasing recruitment.

Material and methods

Alizarin red S marking of glass eels and stocking areas

The Alizarin red S (ARS) marking procedures of 335 kg European glass eel (120 kg each in 2014 and 2015; 115 kg in 2016; LALLF, 2023a) were performed from 2014 to 2016 (Dorow and Schaarschmidt, 2014; Dorow and Schaarschmidt, 2015; Buck and Kullmann, 2021). In each year (always in March) approximately 1 kg of glass eel were placed in a 70 L fish transport bag which contained 30 L ARS solution (150 g ARS L^{-1}), as described by Dorow & Schaarschmidt (2015). In total, the glass eels have been kept in the ARS solution for 3 - 4 with an ARS concentration of 4.5 g per kg of glass eel.

The duration of the ARS marking process was used to transport the glass eels to the stocking areas, namely Wismar Bay/Salzhaff (54°04'06.7"N, 11°34'36.8"E) and Peenestrom/Achterwasser (53°56'46.8"N, 13°54'29.7"E), which are located in the eastern German Baltic Sea coast (Dorow and Schaarschmidt, 2015; Kullmann *et al.*, 2020; Buck and Kullmann, 2021). The two stocking areas, based on their respective salinity conditions, belong to two different main types of inner coastal waters of the German Baltic Sea. While the stocking area Wismar Bay/Salzhaff belongs to the mesohaline coastal waters, Peenestrom/Achterwasser is an oligohaline coastal water (Schernewski and Wielgat, 2004).

A subsample of stocked glass eels (approx. 100 – 200 eels) were taken in all three stocking years. Glass eels were euthanized with an overdose of clove oil (1 mL L^{-1}) and have been kept in 99 % ethanol (personal communication from M. Dorow, LFA). Eels were stored in Kautex containers and kept in a refrigerator until further investigations. Randomly selected glass eels (n = 10) were taken from each Kautex containers to verify the marking success, which was 100 % on otoliths (procedure as described by Kullmann *et al.*, 2020).

Study area and sampling effort

The study area was the eastern German Baltic Sea coast, which belongs to the federal state of MV (Figure 16). The coast has a length of approx. 1945 km (Anonymus, 2010b) and an area of about 365,520 ha (three nautical miles zone; Schubert and Klein, 2021). This area is characterized by a salinity gradient increasing from east to west of about 7 PSU to approximately 16 PSU which is mainly influenced by the Darss sill (IOW, 2023; Herlemann *et al.*, 2011).

The recapture was embedded in an already existing project of the Institute of Fisheries of the LFA MWP, in which the coastal area was divided into 8 reference areas starting in 2009 (Dorow *et al.*, 2023). The sampling gear used was a yellow eel specific enclosure net system for non-tidal coastal waters consisting of fyke net chains and an encircling boundary net, which is described in detail in the studies of Ubl & Dorow (2015) and Dorow *et al.* (2023). For this study, sampling took place from May 2017 to October 2019. Six different stations per reference area that were randomly selected by a stratified sampling design (Ubl and Dorow, 2015). However, either not all sampling could be done as planned (Dorow *et al.*, 2023) or there were no eels in the net system. Between 2017 and 2019, a total of 49 stations could be sampled according the sampling plan described above and at which eels could be caught. The sample stations at which no eels were caught are not considered further in this study.

Furthermore, six supplemental eel samples were purchased from commercial coastal eel fishermen in 2018 and 2019, which raised the number of samples to 55 in the current study. For the catches, the coastal fishermen used commercial eel traps with a legal minimum mesh size of 25 mm according to the Coastal Fisheries Regulation § 15 – mesh sizes (KüFischV M-V, 2006).

Morphometric parameter and indexes

For the all analysis of this study, eels were thawed and the following parameters were directly taken in this order: First, fat content was measured with a fat meter according to manufactures instructions (Distell fish fat meter, Model FFM-692), followed by determination of body weight (BW) in g, total length (TL) in cm, as well the pectoral fin length (PFL), and the eye diameter length (ED, both in mm). Then the abdominal cavity was opened with an incision from the anus to the throat to examine the liver and swim bladder. Liver was removed and weighed in g. Last, the swim bladder length (SBL) was measured in mm and subsequently opened to detect the presence of the swim bladder nematode *Anguillicola crassus*. In case, *A. crassus* were in the swim bladder, all parasites were carefully removed and counted.

With the parameters described aboded the following indexes were calculated:

Based on the TL and BW the Fulton´s condition factor K ($K = 100 \times BW \times TL^{-3}$; Ricker, 1975) was calculated, BW and Liver weight (LW) were used to determine the hepatosomatic index: (HSI [%] = LW x 100 / BW (Ricker, 1975; Bolger and Connolly, 1989)), and SBL and TL were applied to estimate the swim bladder index (SBI [%] = SBL x 100 / TL (Palstra *et al.*, 2007)). If *A. crassus* was present, parasite infestation (*A. crassus* (n) per swim bladder) was assessed.

To determine the developmental stages of eels according to Durif *et al.* (2005; 2009), the parameters TL, mean PFL, and mean ED in mm were used and substituted into the following formula for all possible stages (I, F II, F III, F IV, F V, and M II):

$S_i = c_i + w_{i1} \times X_1 + w_{i2} \times X_{i2} + \ldots w_{in} \times X_n.$

The highest calculated Si value represented the corresponding developmental stage.

For the specific growth rate (SGR), TL at stocking (ARS marked eels) or at start of growing phase on coastal waters (not ARS marked eels) and TL at catching time in cm were used and calculated per year (t = individual age per eel). For that purpose, the following formular was taken according to Busacker *et al.* (1990) and Lugert *et al.* (2016):

SGR [%] = (ln (TL at catch) – ln (TL at stocking))/ $\triangle$t) x 100

ARS identification & ageing

ARS mark identification and age estimation was performed with the sagitta otoliths. They were removed by a longitudinal head dissection, purified, and stored in 1.5 mL tubes for further processing.

For ageing, the otolith was transversally cracked and burned for about 25 seconds according to ICES (2009b, 2011). Then the broken edge of the otolith was carefully placed on a glass slide, so that the annuli or dark winter rings could be counted with a binocular. The number of annuli represent the age of the eel.

For identification of the ARS marking, the second otolith was also cut transversely and embedded in Crystal Bond 509 (Bühler, Esslingen, Germany) on a glass slide with the cut surface facing downwards. When the otolith was marked with ARS, a bright yellow ring at the zero band (Kullmann and Thiel, 2018) showed up under a Leica microscope (DM 2500, Wetzlar, Germany) with a light source (CoolLED pE-300-W) and a light filter of λ 530 ± 10 nm.

Statistical analysis

For statistical analysis of the different eel parameters and the comparison of ARS marked and not marked eels the software R was used (R Core Team, 2022). Significance between the tested groups was assumed when the probability of error was < 5% ($p < 0.05$). First, the Shapiro-Wilk test (SWT) was used to test the data for a normal distribution. If the SWT showed a normal distribution, the Bartlett-Test was used, and in if the SWT showed a significant deviation from normal distribution, the Fligner-Killeen-Test was executed to test the groups for homoscedasticity. In case data were normal distributed and the variances of groups were considered equal, the Two-sample t-test (t) for 2 groups or an ANOVA (F) for > 2 groups was used to determine statistical differences. In case, the results demonstrated variances heterogeneity, the Wilcoxon-test (W) was applied. Moreover, for comparison, the presence of ARS marked and not ARS marked eels as well as the different development stages of eels were examined for significant differences using the chi-square homogeneity test (χ^2). In case of group sizes < 5 within the χ^2 test, the Fisher´s exact test was applied retrospectively. The overview map of the study area including the locations of the marking place and the sampling stations, where ARS marked eels were caught was created with QGIS (QGIS Development Team, 2019).

Results

Overview of coastal eels from 2017 to 2019 and dispersal of marked eels

For this study, a total number of 1829 European eels were caught at 55 different stations between 2017 and 2019 along the eastern German Baltic Sea coast (Figure 16).

At a total of 34 (61.8 %) of the 55 stations stocked and marked eels could be identified. In addition, stocked eels were most frequently caught across all stations in the first sampling year at 28 % (Table 11). The average total length (TL) of all captured eels was 43.12 ± 10.12

cm with a range of 18 - 88.5 cm and the body weight (BW) ranged from 9.1 - 1287.9 g with an average value of 175.96 ± 152.71 g (Table 11).

The ARS analysis showed that 19.3 % of the captured eels (n = 353) were ARS marked, and could thus be assigned to the coastal glass eels stocking from 2014 to 2016. In a total of 1455 (79.6 %), no marking was detected, resulting in a ratio of 1 : 4.1 marked to not marked eels. ARS identification was not possible for the remaining eels (1.2 %, n = 21).

Table 11: Overview of eel catches from 2017 to 2019 with number of sample stations and eels [n], mean total length (TL), body weight (BW) (± standard deviation (SD)), the range of values as well as the frequency and the number [n] of Alizarin red S (ARS) marked eels [%, n] by years.

Year	**Sample Station [n]**	**Caught eels [n]**	**Ø TL ± SD [cm]**	**TL min. – max. [cm]**	**Ø BW ± SD [g]**	**BW min. – max. [g]**	**Sample station with ARS marked eels [%(n)]**	**ARS marked eels [%(n)]**
2017	17	400	42.89 ± 10.17	18 – 79,5	179.66 ± 162.85	9.4 – 1185.4	58.8 (10)	28 (112)
2018	23	727	43.28 ± 10.5	19.5 – 88.5	182.63 ± 166.04	13.2 – 1287.9	43.5 (13)	16.78 (122)
2019	15	702	43.66 ± 10.23	19 - 77	176.21 ± 142.88	9.1 – 957.5	73.3 (11)	16.95 (119)
all years	55	1829	43.12 ± 10.12	18 - 88,5	175.96 ± 152.71	9,1 – 1287.9	61.8 (34)	19.3 (353)

When comparing the dispersal patterns of stocked (ARS marked) eels, it could be observed that in the first sampling years in 2017 (one to three years after the first coastal glass eel stocking) marked eels have been recaught up to approximately 10 km from both stocking areas (Figure 16, yellow points), while in the final sampling year of 2019, marked conspecifics were detected at an estimated distance of up to 100 km from the stocking areas (Figure 16, green points). Simultaneously, it was found in 2017, that 71.4 % (n = 80) of all marked eels have been recaptured within the stocking areas, while two years later the number of marked individuals within the stocking areas decreased to 40.3 % (n = 48).

Age and health status of the local European eel stock

The ages of all caught eels were estimated and ranged from 1 to 20 years. Based on stocking years 2014 - 2016 and sampled years from 2017 to 2019, the age of stocked eels ranged from 1 - 5 years, with a mean age of 3.22 ± 0.8 years, while the average age of eels without an ARS marking was significantly higher at 4.07 ± 1.75 years ($W = 124945$, $p < 0.01$; Table 12). In order to assess the health status of the European eel stock at the eastern German Baltic Sea coast, eel-specific parameters and indices, such as fat content or the hepatosomatic index (HSI), as well as the presence of the swim bladder parasite *Anguillicola crassus (A. crassus)* and the parasitation rate were analyzed and additionally compared between ARS marked and not marked individuals. It was shown that the group of ARS marked eels had both a significantly higher condition factor ($W = 124945$, $p < 0.01$) and a significant higher fat content ($W = 181382$, $p < 0.05$) compared to the group of not ARS marked conspecifics.

Examination of the infestation of the swim bladder by *A. crassus* revealed that not ARS marked eels (50.2 %) were on average slightly more often infected, but no significant difference was found ($\chi^2 = 0.27897$, d.f. = 1, $p > 0.05$). In addition, the average number of parasites per swim bladder and the swim bladder index (SBI) were also higher in not ARS marked eels (6.41 ± 7.91 *A. crassus* per swim bladder, SBI = 14.40 ± 3.19), but again no significance could be demonstrated in both cases (*A. crassus* per swim bladder: $W = 45671$, $p > 0.05$; SBI: $t = 1.5863$, d.f. = 229, $p > 0.05$; Table 12).

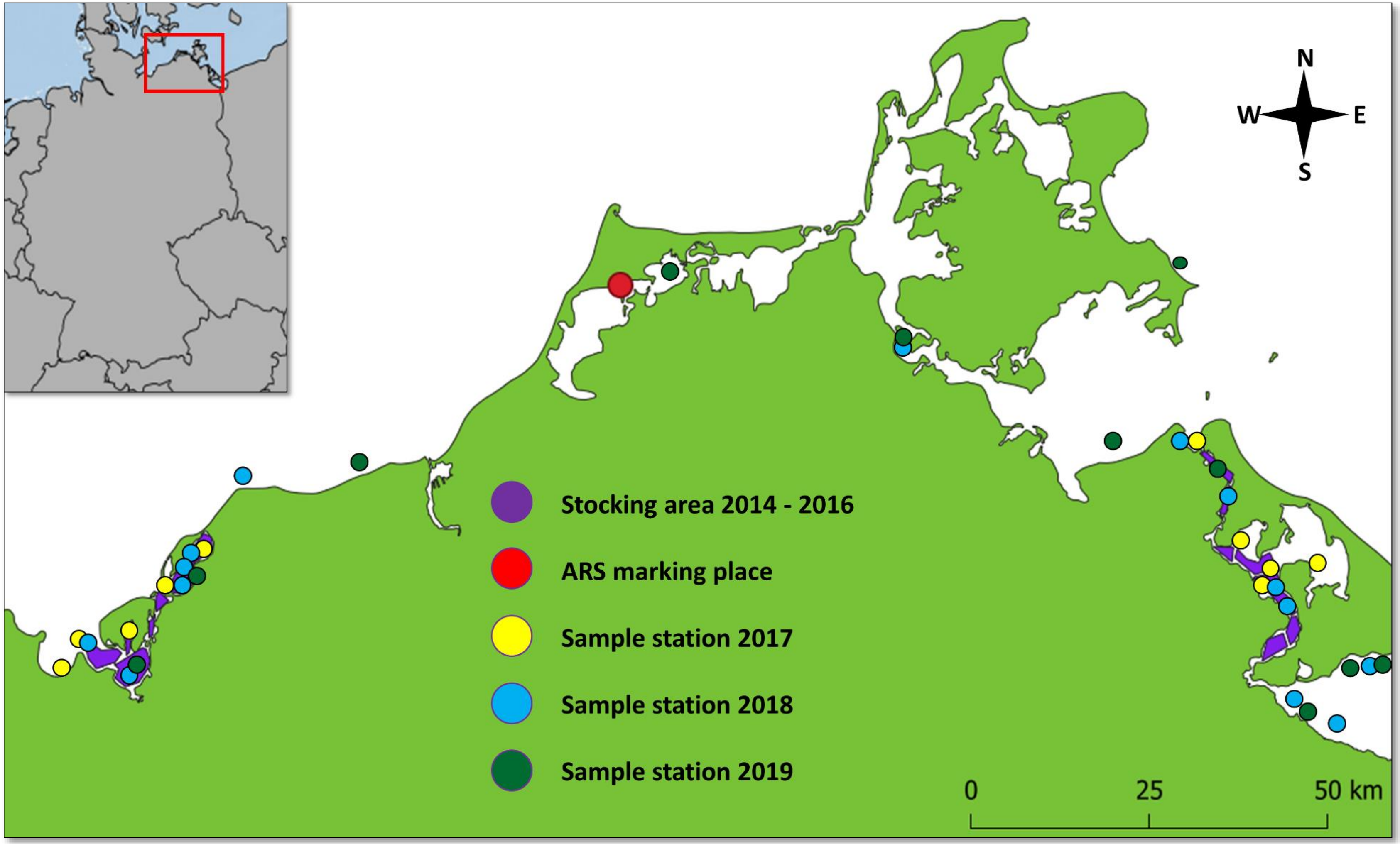


Figure 16: Overview map of study area showing the stocking areas (purple areas), ARS marking place (red points) and all sample stations in 2017 (yellow points), 2018 (blue points), and 2019 (green points) where marked and stocked eels (n = 353) were caught.

Table 12: Number of estimated eels (n), mean, standard derivation (SD) and range of age [years], hepatosomatic index (HSI), fat content [%], and swim bladder index [%], frequency and number of infected eels by A. crassus [%, n] as well as the mean, SD, and range of A. crassus per swim bladder divided by ARS marked and not ARS marked eels. Star symbolizes a significant difference between the groups compared with an indication of the statistical test used.

	ARS marked eels	**Not ARS marked eels**	**Statistical test**
N	353	1455	
Age ± SD [years] and age range (min-max) [year]	3.22 ± 0.8* (1-5)	4.07 ± 1.75* (1-20)	Wilcoxon-test; W = 124945, p < 2.2e-16
Fulton´s condition factor ± SD and range (min-max)	0.186 ± 0.03* (0.026- 0.425)	0.180 ± 0.03* (0.016- 1.804)	Wilcoxon-test; W = 286351, p < 0.0007869
HIS ± SD and range (min-max)	1.90 ± 0.098 (0.85- 15.78)	2.0 ± 1.56 (0.18- 25.37)	Wilcoxon-test; W = 146663, p = 0.3034
Fat content ± SD and range (min-max) [%]	18.09 ± 5.8* (7.20- 38.4)	17.28 ± 6.1* (7.20- 38.1)	Wilcoxon-test; W = 181382, p = 0.01825
Infected eels by *A. crassus* [%(n)]	48.3 % (145)	50.2 % (669)	X-squared = 0.27897, d.f. = 1, p = 0.5974
A. crassus per swim bladder ± SD and range (min-max) [n]	6.03 ± 6.65 (1-37)	6.41 ± 7.91 (1-83)	Wilcoxon-test; W = 45671, p = 0.2665
SBI ± SD and range (min-max) [%]	14.40 ± 3.19 (3.39- 22.73)	13.60 ± 3.40 (5.07-25.61)	Two sample t-test; t = 1.5863, d.f. = 229, p = 0.114

Proportion of stocked eels in the cohorts of 1 - 5 years

For a cohort-based analysis to estimate the possible impact of the experimental glass eel stocking on the local eel stock of the eastern German Baltic Sea coast, the proportion of ARS marked and not ARS marked eels for the age groups (AG) 1 – 5 were compared separately. All eels with an estimated age of six and more have been excluded from further analysis. Furthermore, only eels for which both age estimation and ARS identification was possible were considered for this analysis, which leads to a number of 305 ARS marked and 1001 not ARS marked eels.

The three stocked cohorts (from 2014 to 2016) have been sampled from 2017 to 2019 so that AG 1 and 5 comprised one stocking cohort (1-year-old eels = 2017; 5-year-old eels = 2019), AG 2 and 4 included two stocking cohorts (2-year-old eels = 2017 and 2018; 3-year-old eels = 2018 and 2019) and AG 3 contained all three glass eel stocking cohorts (Figure 17).

In total, 23.4 % of the considered eels (AG 1 – 5 years) showed an ARS marking. The amount of stocked eels ranged from 8.3 to 32.5 % with the highest value in AG 3, which is influenced by all glass eel stocking cohorts and the lowest value in AG 1.

Statistical analysis revealed, that there is a significant difference between all five AG in terms of the amount of ARS marked and not ARS marked eels ($\chi^2 = 50.615$, d.f. = 4, $p < 0.01$), but when comparing the AG 2 and 4, which were both influenced by a double stocking cohort, no significant difference regarding the presence of stocked eels could be observed ($\chi^2 = 0.29197$, d.f. = 1, $p > 0.05$). Comparison of AG 1 and 5 was considered impossible because of low amount of data in AG 1. Overall, it can be observed that the proportion of stocked eels increases on average by 10 % for each additional stocking cohort that affects an AG (mean amount of stocked eels of AG 1 and 5 = 10.15 ± 1.85; mean amount of AG 2 and 4 = 21.95 ± 5.35; Figure 17).

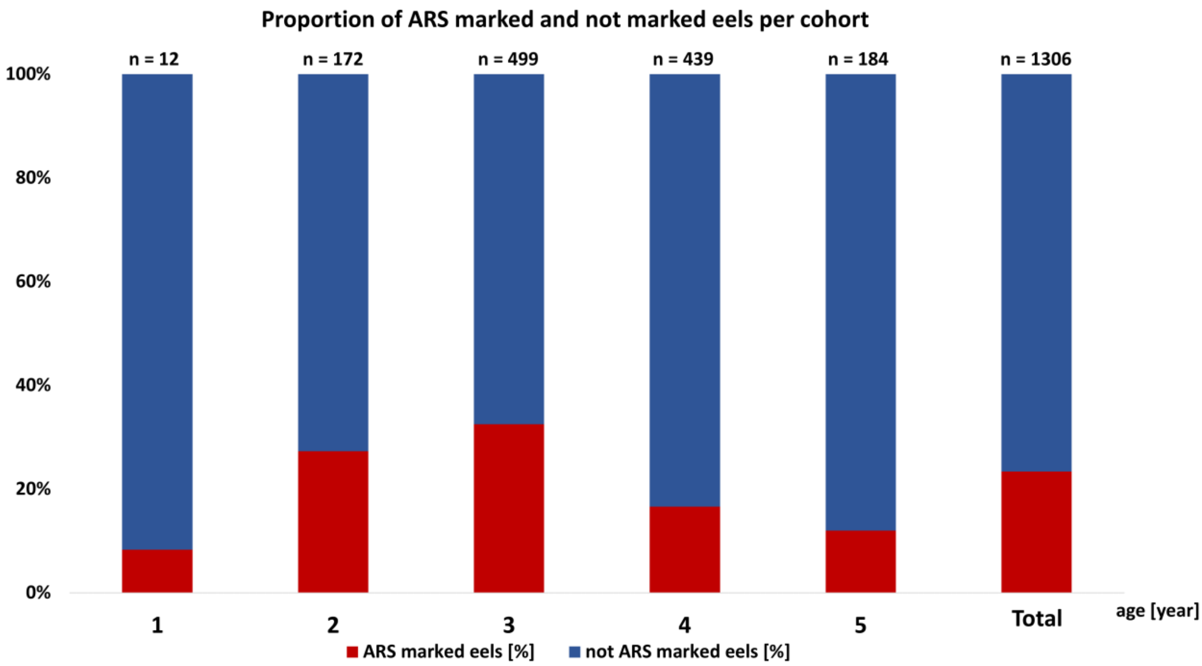


Figure 17: Presence of ARS marked (red) and not ARS marked eels (blue) by AG from 1 to 5 years.

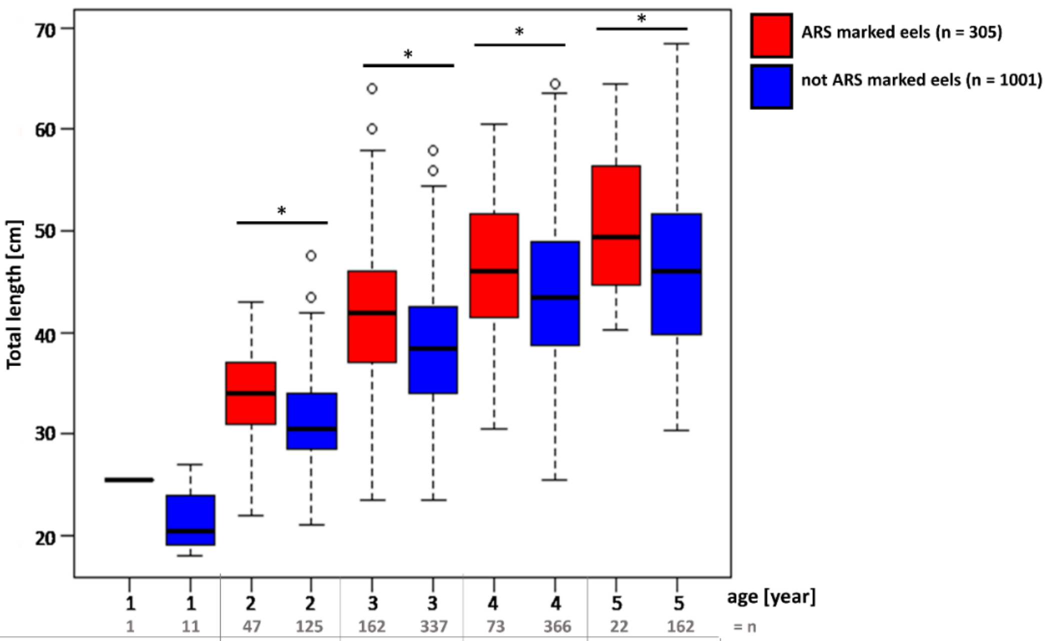
Growth comparison of ARS marked and not ARS marked eels

For growth analysis of the local eel stock of the eastern German Baltic Sea, first the separate AG from 1- 5 years of marked and not marked eels were compared in relation to TL (Figure 18). The 1-year-old eels have not been considered because of the small sample size ($n = 1$). It was found for all remaining AG, that stocked eels were significantly longer than the not ARS marked conspecifics (AG 2: $W = 4078$, $p < 0.05$; AG 3: $W = 34034$, $p < 0.05$; AG 4: $t = 2.6602$, $p < 0.05$; AG 5: $W = 1851$, $p < 0.05$).

In addition, besides the analysis of TL per age AG, the mean specific growth rate (SGR) of TL per years and for the hole study period were calculated and compared between ARS marked and not ARS marked eels. Findings show, that over a five-year period, the mean SGR was significantly higher with a value of 41.23 ± 3.27 % in marked eels, than the mean SGR (39.17 ± 3.54 %) of not ARS marked conspecifics ($t = 2.6205$, d.f. = 183, $p < 0.01$). Moreover, in the AG of 2 – 4 years, a significant difference between the two groups was also detected in each year

(AG 2: t = 3.2147, d.f. = 171, $p < 0.05$; AG 3: t = 4.808, d.f. = 509, $p < 0.01$; AG 4: W = 15965, $p < 0.01$). In the AG 1, no significant could be demonstrated, as not enough data were available for a statistical comparison.

Furthermore, it could be observed that the course of the annual mean SGR was quite identical in ARS marked and not ARS marked eels. Values were highest in the first two years from 119.1 to 136.7 % and decreased in the following period from values between 81.76 % and 39.17 % mean SGR per year (Figure 18).



ARS marked eels:	136.7 ± 0	81.76 ± 6.77*	61.34 ± 5.58*	48.82 ± 3.95*	41.23 ± 3.27*
not ARS marked eels:	119.1 ± 13.5	78.01 ± 6.87*	58.83 ± 5.51*	47.45 ± 4.37*	39.17 ± 3.54*

SGR [%]

Figure 18: Comparison of total length [cm] per age [years] of ARS marked (red) and not ARS marked eels (blue) from 1 to 5 years as well as the specific growth rate (SGR [%]) divided by ARS marked and not ARS marked eels. The star indicates significant difference (Modified from Buck & Kullmann, 2021).

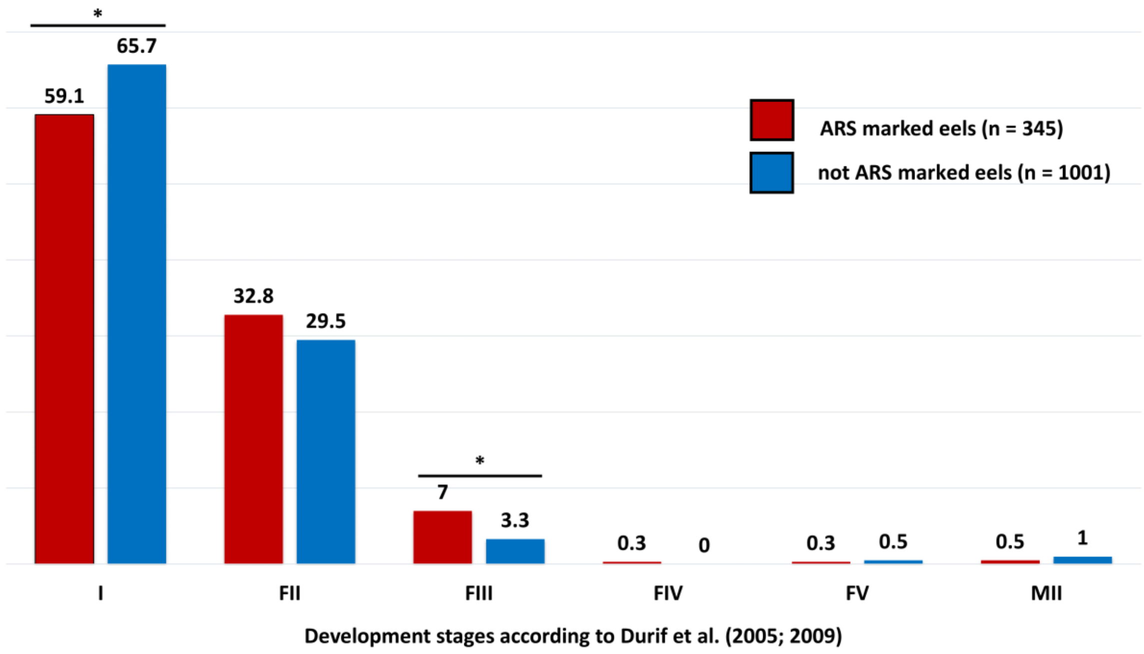


Figure 19: Amount (%) of eels per development stages for ARS marked (red) and not marked (blue) eels of the AG 1 – 5 years.

Development stages of ARS marked and not ARS marked eels of age classes 1 - 5 years

For the classification into development stages according to Durif *et al.* (2005; 2009) the AG 1 – 5 years (n = 1,346 eels) were considered. It was found that the majority of eels were grouped into the stage I with 65.7 % for ARS marked and 59.1 % not ARS marked eels, respectively, followed by the stage's F II (ARS marked eels = 32.8 %; not ARS marked eels = 29.5 %) and F III (ARS marked eels = 7 %; not ARS marked eels = 3.3 %). In stages F IV, F V, and M II presence of eels varied from 0 to 1 %. (Figure 19).

Overall, it was observed in both groups that significantly fewer eels were caught at higher development stages ($\chi^2 = 14.435$, d.f. = 5, $p < 0.05$; Fisher's exact test = $p < 0.05$). Further analysis showed, that the amount of ARS marked and not ARS marked eels differed significantly for eel at stage I ($\chi^2 = 4.5764$, d.f. = 1, $p < 0.05$) and F III ($\chi^2 = 7.5956$, d.f. = 1, p < = 0.01). In case of the two development stages that differ significantly, twice the proportion of ARS marked eels (7 %) was observed in the more development stage F III compared to not ARS marked eels (Figure 19).

Discussion

The aim of the present study was to assess the effect of glass eel stocking in coastal non-tidal waters of MV in order to derive conclusions for an effective eel stock management that contributes to the recovery of the eel stock both locally and as whole. This includes the aspect of maintaining a sustainable and economically successful local eel fishery in accordance with the EU Eel Regulation. In order to answer this question, the dispersal of stocked eels was examined over a time period of 3-to-5-year period after coastal stocking. Moreover, the proportion of stocked eels within the coastal stock was determined and additionally relevant fitness parameters such as the health status, growth performance, and development were studied and compared with unmarked conspecifics.

In summary, the results showed that approximately one fifth (19.3 %, n = 353) of all captured European eels for this study originated from the glass eel stocking between 2014 and 2016 (ARS identification on otoliths). Within a period of up to five years after the first glass eel stocking event, it was observed that ARS marked (stocked) eels moved further away from the stocking areas from year to year in a radius of about 10 (2017) to 100 km (2019). At the same time, the annual catches of marked eels inside the stocking areas decreased from 71.4 % to 40.3 % over time. Stocked eels have been shown to be advantaged in terms of the condition factor k and the fat content compared to not stocked conspecifics while, in turn, no difference was found between the two groups with regard to other fitness parameters (such as *A. crassus* infestation). It was also shown that the amount of stocked eels increased on average by 10 % for each additional stocking cohort with 32.5 % in age group 3 (note that age group three comprised of three stocking cohorts). The development stages of marked and not marked eels differed at stage I and F III of ages from 1 to 5 years, although twice as many stocked eels were observed at the higher stage of development.

In terms of the spatial dispersal pattern, which is based on annual recapture data of marked eels per monitoring station, it appeared that stocked eels rather stayed close to the stocking site (71.4 % of stocked and recaptured eels remain in both stocking area within the first sampling year) and have spread over time along the coast of up to 100 km away from the site of release. Similar observations of a relative stocking site fidelity have been published in previous studies for European eels (Hanel, 2009) and Japanese eels *Anguilla japonica* (Itakura *et al.*, 2017; Itakura *et al.*, 2018) in fresh water habitats.

For a better estimation of the dispersal of coastal stocked eels in future studies, the adjacent waters/connected water bodies should be monitored to recapture eels that either left the coastal habitat after stocking or moved between different habitats. Also, elemental analysis of otoliths (e.g., Strontium-Calcium ratio) could also be used to track movement patterns of stocked individuals (Tzeng *et al.*, 1997; Marohn *et al.*, 2011). Telemetry appears to be rather unsuitable given that stocked glass eels are likely too small or internal tagging.

This behavior of the site fidelity of the stocked eels leads to a higher density in the certain areas of the study area, which was already demonstrated by Dorow *et al.* (2023) and Buck and Kullmann (2021). Especially in the two stocking areas Wismar Bay/Salzhaff and Peenestrom/Achterwasser, a significant increase in yellow eel density have been detected since 2015 (Dorow *et al.*, 2023).

In analyzing the health status of stocked eels no negative influence of the stocking could be observed in recaught eels, because recaptured marked and unmarked eels did not differ significantly in the assessed fitness parameters (condition factor k, fat content, HIS, *A. crassus* infestation). This result is in line with previous observations where coastal stocking of young eels also took place in the German Baltic Sea. Kullmann & Thiel (2018) and Thiel & Kullmann (2019), compared simultaneously stocked glass and farmed eels over three years in coastal waters of the western German Baltic Sea coast and reported no difference in terms of *A. crassus* parasitation intensity, growth performance, HIS, and condition factor k. However, the infestation rate by *A. crassus* of stocked glass eels was 33 % in the study by Thiel and Kullmann (2019), which was much lower than the stocked glass eels in this study (48.3 %)

At least two explanations could be given for these findings: On the one hand, a higher parasite infestation of the swim bladder is a matter of time, since in the present study eels were able to became infected with *A. crassus* in a period of five years, whereas eels in the studies of Thiel & Kullmann (2019) and (Kullmann, 2018) had an infection possibility of only three years. Recorded by Moravec *et al.* (1994) the life circle duration of *A. crassus* is between 3 – 4 months (in laboratory conditions at 20 – 22 ° C and without paratenic hosts).

On the other hand, it was a matter of the different salinity levels of the various study locations (present study: between 8 – 17 PSU; Thiel & Kullmann (2019) and (Kullmann, 2018): 11 - 26 PSU (BSH, 2023). The last point is also supported by the fact that many studies have already shown that higher salinity has a negative effect on the survival probability of *A. crassus* larvae, making infection of eel swim bladder less likely (Charleroy *et al.*, 1989; Charleroy *et al.*, 1990; Nielsen, 1997; Kirk *et al.*, 2000; Lefebvre *et al.*, 2002; Hanel, 2009; Wysujack *et al.*, 2014). Kirk *et al.*, (2000) escribed that *A. crassus* larvae are capable of hatching in various salinities, but are infectious 10 times longer (80 days) in freshwater than in 100 % seawater (8 days) at 10 °C. It follows that both factors, time and salinity, have an influence on the infestation rate of *A. crassus* in eels.

In this study, some benefits in terms of significant higher values in the fat content and the condition factor k in stocked eels could detected. Provided that these higher values persist until the final development into silver eels, this could prove to be an advantage for stocked eels. In particular the fat reserves represent an important influence on the successful return of the silver eels to the spawning area (Larsson *et al.*, 1990; van Ginneken and van den Thillart, 2000; Belpaire *et al.*, 2009; Dainys *et al.*, 2017). However, only 0.3 % (n = 1) of stocked eels in this study have been developed into the silver eel stage (F V), illustrating that the duration of the study of 3 - 5 years after the first stocking was too short to conclusively assess what impact a glass eel coastal stocking has on the eel population or to what extent stocked European eels could contribute to the reproduction of the species. Due to the long growing phase of eels of at least 5 years and up to 50 years before they develop into silver eels (Dekker, 2000), the study period should be extended to many more years. One method of estimating earlier whether and how large an impact a coastal glass eel stocking could have on the eel population would be to stock marked eels only every second year in the same stocking areas. In subsequent years, annual variation between stocking and not stocking years could then be monitored and thus the effects of eel stocking on the eel population could be estimated.

One aspect that could be estimated from this study is the positive effect of glass eel stocking on the local eel stock and the local eel fishery. On the basis of the cohort-based analysis of eels of ages from 1 – 5 years it could be shown that the more years as a result of eels being stocked in the same area, the local proportion of stocked eels increases, by about 10 % per added stocking cohort. This resulted in the highest proportion of stocked eels in AG 3 (which was affected by three stocking cohorts) being just over 30 %, while for comparison in AGs 2 and 4 (which were both affected by a double stocking cohort) the proportion of stocked eels were about 20 %. This result confirms the observation on the study by Dorow *et al.* (2023), where an increasing of yellow eel density was proven since the beginning of the stocking measures in several areas along the coast the eastern German Baltic Sea coast. In addition to this, catch rates in AG 3 with n = 499 eels and AG 4 with n = 439 eels were particularly high compared to the other AGs, which has been confirmed by several local eel coastal fishermen in 2018 and 2019 (personal communication). The fact that many fewer eels were caught in AG 2 (n = 172) compared to AG 4 (both of which were influenced by a double stocking cohort) could be attributed to fishing gear, which had a mesh size of 10 mm (Ubl and Dorow, 2015; Dorow *et al.*, 2018; Dorow *et al.*, 2023).

Further results, which might have a positive impact on the local eel fisheries was the significant faster growth and the mean specific growth rate (41.23 ± 3.27 % per year) of stocked eels, which could be detected in almost all AG except of AG 1 (Figure 18).

This led to the fact that already three years after the first stocking event in 2014 eels have grown to the legal marketable minimum body size of 50 cm (LALLF, 2023b). Also, Lin *et al.* (2007) recorded, that lagoon stocked eels at ages of 5 to 8 years had a higher significant total length than unstocked conspecifics and postulated that these growth differences could be attributed to the saving of additional energy costs for the migration from the European arrival areas (e.g., coastal waters of France or Spain) to the nursery areas of the Baltic Sea. It is also conceivable that the timing of stocking in March, could result in higher growth rates of stocked eels, as the naturally recruits reach the coastal waters of the eastern German Baltic Sea later in the year between April and May (Tesch, 2003; Dorow and Frankowski, 2017; Simon *et al.*, 2017). This time interval between 1 and 2 months, stocked eels could use to adapt to various biotic and abiotic factors of the new habitat or by using the time to migrate to better suitable adjacent waters to subsequently begin feeding, which has a direct influence on growth rates (Paloheino and Dickie, 1984).

In conclusion, after up to 5 years following a coastal stocking of marked glass eels in non-tidal coastal waters of the eastern German Baltic Sea, no disadvantage could be identified in stocked eels in comparison to the non-stocked conspecifics on the coast in several fitness parameter such as parasite infestation of *A. crassus* or the HSI.

Rather, the stocked eels showed some advantages, such as values of condition factor k and fat content, which could have a positive effect on the eel population, as especially the higher fat content could increase the chance to reach the spawning ground in the Sargasso Sea. Furthermore, the proven faster growth rates of stocked eels, the increasing proportion local eel population due to stocked eels (up to 30 % of an age cohort) and the side fidelity to the stocking area were a benefit for the local eel fishery, because coastal stocking of glass eels leads to a higher eel density in certain areas and some of these individuals have reached a marketable size of 50 cm already three years after stocking. Therefore, based on the results presented, it can be concluded that the study area (non-tidal coastal area) is highly suitable for glass eel stocking and fortunately, the results of this project have already led to a further glass eel stocking of 120 kg in the eastern German Baltic Sea in 2022 (LALLF, 2023b).

However, the extent to which glass eel coastal stocking can add value to the net benefits discussion is nearly impossible to infer from this study, as very few stocked eels reached the silver eel stage during the study period (n = 1; 0.3 % of F V stage; Figure 19). This could be due to both the fishing gear used and the too short study period of 5 years, since the growing phase of European eels can reach a time period of up to 50 years (Dekker, 2000).

Nonetheless, coastal eel monitoring should continue with emphasis on the identification of stocked (tagged) eels, the duration of metamorphosis from stocked glass eel to silver eel, and the consequent proportion of silver eels that may contribute to reproduction of their species.

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General Discussion

Objectives and results in a nutshell

The objectives of the present work were to contribute to the improvement of eel stocking as a management option. Therefore, investigations were carried out with regard to the use of the substance alizarin red S (ARS), which has been used frequently in the past to chemically mark European eels in various life stages (chapter 1). The focus of the investigations was on analyses with reference to the bioaccumulation potential of ARS, since it was previously assumed only on the basis of a low diffusion coefficient that accumulation in the edible muscle tissue of eels would only occur in a very small circumstance and a health hazard from the consumption of marked eels was considered to be low. In addition, the importance of marking farmed eels for stock management were investigated because these eels are regularly exposed to stress, for example from handling, during the on-growing phase in eel aquaculture facilities and thus create stress rings on the otoliths used for age reading that in turn could have an effect on age determination (chapter 2). Blind readings were conducted to determine whether experienced eel otolith experts are able to distinguish stress rings on marked farmed eels from actual annual rings. Furthermore, any errors in age reading were attempted to be quantified and the influence on the cohort-based German eel stock model was investigated. The aim was to check whether stocking coastal waters could be an additional management option, since eels as a facultative catadromous migratory species do not necessarily migrate to limnetic waters, but a large part of the population remains in the marine area (chapter 3). To this end, a glass eel stocking experiment was conducted in the study area of the eastern German Baltic Sea coast. The effect of this stocking experiment was investigated using a before-and-after study design so that any changes in stock size could be related to the glass eel stocking. In the context of the experimental glass eel stocking, the aim was also to examine whether the stocked individuals differ in fitness parameters from conspecifics classified as natural immigrants, which is particularly relevant to the question of whether stocked eels can contribute to spawning biomass and recruitment (chapter 4).

In summary, it was determined that a viable ARS detection method could be developed with a detection threshold of 8.9 µg kg^{-1} ARS in eel muscle tissue. For this purpose, both glass and farmed eels were tested at different time intervals after marking (0 – 3 years after marking), and an overall decrease with increasing distance from marking was observed. In general, the results provided evidence that the bioaccumulation potential of ARS in eel muscle tissue is very low and a health hazard from consumption of individuals that have reached the minimum landing size (i.e., 50 cm in coastal waters of M-V; (LALLF, 2023b) can be excluded with a probability bordering on certainty. The studies on the otolith blind readings of stocked and marked eels have shown that even experienced eel otolith readers cannot distinguish stress from true growth/annual rings. Readings overestimated the age of eels by up to seven years, which had significant implications for model-based estimates of a simulated eel population. This result demonstrated that marking of farmed eels is essential, particularly for model-based estimates of silver eel escapement, when a significant portion of the population consist of stocked farmed eels. The glass eel coastal stocking experiment into areas of the eastern German Baltic Sea showed that the considered phases from 2009 to 2014 (without stocking) and 2015 to 2020 (with stocking) differed significantly in terms of eel density, with a significant increase of the local stock. It was concluded that stocking of glass eels in coastal waters is suitable to increase the local population and may be beneficial to maintain an economically successful eel fishery. Evidence was also found that the stock increase may also be due to an increase in natural recruitment, although the influence of stocking in neighboring areas may have played a decisive role here. The comparative studies of stocked and possibly naturally recruited eels in the study area showed that the stocked eels had significantly larger fat reserves than the not ARS marked eels and moreover, the stocked conspecifics grew significantly faster. On the one hand, this could be interpreted as beneficial to achieving management objectives; on the other hand, the higher fat reserves of the marked eels could be only a snapshot in time and a different picture could emerge at the time of the onset of spawning migration.

Using ARS for marking eels – more research is needed

The results of the present work have shown that in the context of stocking measures with farmed eels, it is necessary to mark them so that the observed error with respect to age reading can be corrected and a corresponding falsifying influence on a German eel model-based calculation of silver eel biomass can be taken into account (cf. chapter 2). Although such a systematic error of age estimation is rather not to be expected for unmarked stocked glass eels according to the current state of knowledge, a marking is also highly recommended here so that the proportion of stocked eels in the total stock can be determined and thus conclusions can be drawn on the efficiency of the stocking measures carried out. In this context, ARS is a suitable marker for the reasons already described, although discussions have arisen, particularly in Germany, as to whether the ARS residues that may still be present in the edible eel muscle tissue after years could pose a health risk to consumers (BfR, 2017).

On the basis of empirical values with substances having a structure comparable to ARS, the Federal Office for Risk Assessment (BfR, 2021) has assumed a 'Threshold of Toxicological

Concern' (TTC) for ARS of 0.0025 µg kg^{-1} body weight, which corresponds to a safe intake of 0.28 g eel per kg body weight day^{-1} at a determined detection limit of 8.9 µm kg^{-1} eel muscle tissue (chapter 1). An intake of ARS marked eel exceeding this limit could have a genotoxicological effect, which, according to the BfR, could be conceivable at least for a certain population group. According to the assumption of the BfR, an intake of ARS marked eels of 588 g per month or 7,056 g per year would be harmless for a human of about 70 kg. Given an average per capita fish consumption of 12.7 kg in 2021 (FIZ, 2022), with eel being insignificant in terms of consumption in Germany, accounting of less than 0.3 % of mean per capita consumption (i.e., on average less than 0.04 kg per capita per year), it can indeed be assumed that ARS marked eels do not pose a significant risk to human health in the general population. Nevertheless, in certain water bodies such as the Kiel Canal and the Elbe-Lübeck Canal (cf. Neukamm *et al.*, 2018), which have been regularly stocked with ARS marked eels for a number of years, it is quite possible that local fishermen and anglers could catch and consume larger quantities of ARS marked eels and be exposed to an increased risk. However, it is certainly the case that the detection limit of 8.9 µg kg^{-1} eel muscle tissue set by BfR (chapter 1) merely corresponds to the application of the precautionary approach, although Baer at al. (2021) were able to establish an optimized detection limit for ARS in fish muscle tissue of 6.9 µg kg^{-1} and further optimization appears possible, so that the actual ARS concentration in muscle tissue could possibly be significantly lower. Nevertheless, BfR also criticizes the method of the present work (chapter 1) and of Baer *et al.* (2021), which would not comply with the standards for the determination of additives in animal tissue and refers to '*guidance on the assessment of the safety of feed additives for the consumer*' (EFSA, 2017). According to this, further studies on the toxicological relevance of ARS are required using radioactive substances for any metabolites that may be produced as well as information on any chemical enrichment that may occur, which could also lead to an underestimation of the toxicological relevance of ARS (BfR, 2021). But according to the BfR (2021), it is of central importance for the future use of ARS that investigations are carried out to characterize the genotoxic potential in order to ensure that ARS does not develop a DNA-reactive mechanism of action and that a deliberate introduction of this substance into the food chain cannot have any negative effects on human health with sufficient certainty.

Effect of eel stocking measures

As explained in the general introduction of this work, an important prerequisite for the success of stocking measures is that the "carrying capacity" (CC) of a habitat has not yet been reached and that the abundance of one or more species can be positively influenced by human intervention. This requires very extensive and habitat-specific studies (cf. Acou *et al.*, 2011), which were not conducted for the study area of this work (chapters 3 and 4). Rather, it was generally assumed that the CC could not have been reached, because eel stocks and eel catches have declined sharply by the time EU eel management began in 2011 (Ubl and Jennerich, 2008), so that the habitat of the eastern German Baltic coast should still be able to accommodate eels. However, this assumption may not be correct because of an unoccupied ecological niche due to low abundance may be occupied by another species, which would then lead to stocking measures possibly remaining ineffective (Arlinghaus *et al.*, 2014).

As previous studies (e.g., Brämick *et al.*, 2016) have shown, the present work demonstrated that eel stocking measures can lead to an increase in the local eel population (chapter 3). However, one approach that has received little attention so far was to use open coastal waters for stocking instead of inland waters, so that stocked eels could disperse freely and, depending on preference, also migrate to inland waters to accommodate the repeatedly documented facultative catadromous life cycle.

An attempt was also made to show that the increase of the local eel population in the Baltic coast of Mecklenburg-Vorpommern (MV) could not be exclusively due to the implementation of stocking measures. For this purpose, the approximately 335 kg of glass eels stocked between 2014 and 2016 in the Baltic coast of MV were not included in the population trend analyses, which was possible due to the ARS marking. Even without the stocked eels, an increase in eel density could be detected. However, this result was based on the assumption that the studied coastal waters of MV were most likely not influenced by other stocking activities in neighboring waters (e.g., Thiel and Kullmann, 2019; cf. also data in Dorow *et al.,* 2023 on other stocking programs). Furthermore, all coastal eels were considered stationary and immigration from inland waters was basically excluded (Daverat *et al.*, 2006). In addition, it was concluded that these and other observations would suggest that natural recruitment in the Baltic coast of MV has increased, contrary to surveys in all other regions of Europe (ICES, 2022). However, a number of survey results are available that indicate that the assumptions of Dorow *et al.* (2023) are not accurate. First, Daverat *et al.* (2006) found quite high variability within the genes of eels studied, which is also consistent with the fundamentally very pronounced phenotypic plasticity of the European eel (Tsukamoto *et al.*, 1998; Enbody *et al.*, 2021). In addition, telemetric studies have shown that not only silver eels but also yellow eels can travel considerable distances of up to 49 km (e.g., McCleave, 1999). Therefore, it can rather be assumed that the stocked eels in adjacent waters (e.g., Schleswig-Holstein, Germany and Poland) did spread and massively influenced the results of Dorow *et al.* (2023). Against this background, one of the central conclusions of Dorow *et al.* (2023) must also be rejected: namely, that the increase in the local eel population would also be due to an increase in natural recruitment. Taking into account of the historically low ICES recruitment index, this appears to be a difficult assumption to sustain. Instead, the observed increase in eel density is most likely not attributable to an increase in natural recruitment, but - as some other studies show - to the extensive eel stocking that has been carried out for years in MV and in the Baltic Sea catchment area, which has led to an overall increase in the local eel population. ICES points out that the examined glass eel recruitment was at a very low level from 1980 to 2022. The current status of the glass eel recruitment index for the North Sea is 0.5 % "*as a percentage of the 1960-1979 geometric mean*" (ICES Advice, 2022). The calculation of the glass eel recruitment index is based on a total number of 57 glass eel time series for the advice for 2023 (26 for the North Sea from Norway, Sweden, Germany, Denmark, Netherlands, United Kingdom and Belgium). These time series currently provide the best possible estimate of European eel recruitment, taking into account different habitats, and needed to be considered much more comprehensively than to the local study by Dorow *et al.* (2023).

In addition, the calculation method by ICES is subject to an ongoing, transparent review process within the framework of the ICES WGEEL meetings and ICES workshops held specifically for this purpose (ICES, 2021b), so that a very high-quality standard is ensured. Although no separate recruitment index is calculated for the Baltic Sea, it may be considered unlikely that higher immigration of eels into the Baltic Sea can occur with such low recruitment in the North Sea. Frankowski *et al.* (2018) were also unable to demonstrate such an increase in the inland area of the EMU Warnow/Peene (where the study area of chapter 3 and 4 is located). It is also relevant that Dorow *et al.* (2023) did not take into account that since 2010, all EU Baltic Sea countries (Germany, Denmark, Estonia, Finland, Lithuania, Latvia, Poland, Sweden) have consistently carried out very extensive stocking measures as a part of the implementation of the EU Eel Regulation and that, in addition, the largest proportion of stocked European eels were not marked at all (ICES, 2021a).

Fitness of natural versus stocked recruits

It was observed that the stocked and marked glass eels had a higher growth rate than the eventual naturally immigrated eels (chapter 4). In fact, this has also been noted in other research on European eels (Lin *et al.*, 2007) as well as on American eels (Stacey *et al.*, 2015). This circumstance is fundamentally favorable to the goal of maintaining an eel fishery, as stocked eels may reach the minimum landing size of 50 cm (LALLF, 2023b) after less time than not stocked conspecifics (Figure 18; chapter 4) and thus recruit more quickly into the fishery. On the other hand, however, a significantly higher growth rate of stocked eels may be contrary to the aspect of contribution to net benefits (i.e., stocking increases recruitment of the stock), since total length at the time of silvering (onset of silver eel stage) is negatively correlated to growth rate (e.g., Larsson *et al.*, 1990), such that fast-growing eels remain smaller and have lower fat content, which is critical in determining whether eels can survive the debilitating return to the spawning area in the Sargasso Sea (Stacey *et al.*, 2015).

In connection with the implementation of eel stocking measures, which is also simply referred to as translocation (ICES, 2016c), it remains still unclear whether the translocated eels are able to find the way back to their spawning grounds, although they could not capture the outward route due to human influence. There is evidence that stocked eels, especially in the Baltic Sea, may indeed have difficulty finding the return path, which manifested in less directed swimming behavior (Prigge *et al.*, 2013; Sjöberg *et al.*, 2016). On the other hand, Westerberg *et al.* (2014) were able to prove that even stocked eels in the Baltic Sea do indeed purposefully head for the spawning area via the Skagerrak and Kattegat. Nevertheless, there are results on the orientation ability of stocked eels, which suggest that translocated eels might show a reduced reproductive fitness, that could lead to an immanent problem of eel stocking measures and is a consequence of the complex life cycle as well as the complicated reproductive biology with a spawning area that is several thousand kilometers away from the nursery areas. A problem is that the eels have to travel a further distance from the Baltic Sea than from the Western European areas to reach their spawning grounds. Swimming tunnel experiments showed that silver eels can have sufficient fat reserves to complete a 6,000 km migration and sufficient further reserves for final gonadal development to occur (van Ginneken and van den Thillart, 2000). However, the migration from the Baltic Sea area is again about 1,000 to 1,500 km longer compared to waters of western Europe if eels were to take the direct route (which is unlikely, cf. Righton *et al.*, 2016), reducing the likelihood that eels from the Baltic Sea can make a significant contribution to recruitment. One conclusion could be that eel stocking - whether with glass eels or farmed eels - in the Baltic Sea is suitable to increase the local eel population (cf. chapter 3), but a contribution of stocked eels to the net benefit is rather unlikely.

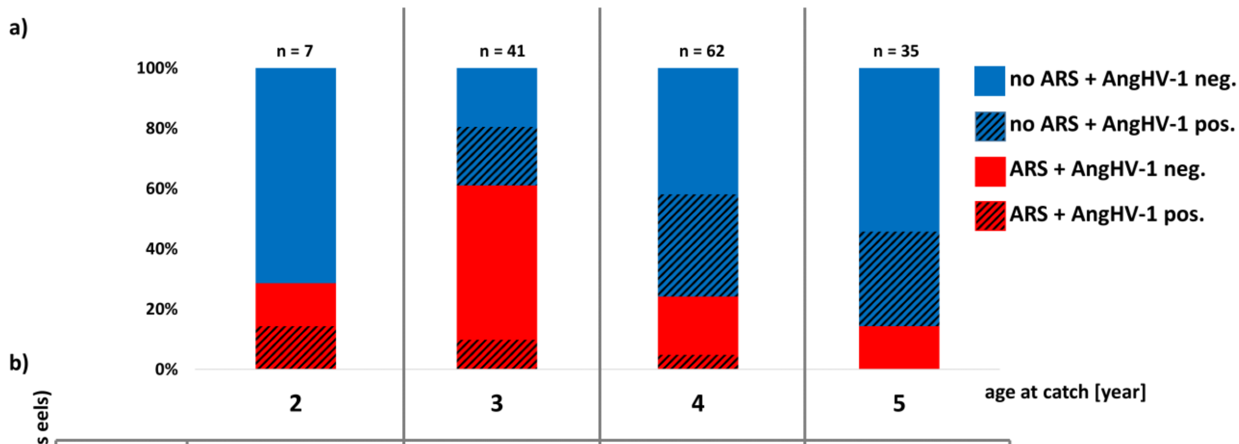
There is also the fundamental question of whether the glass eels used for experimental glass eel stocking (chapters 3 and 4) are sufficiently suitable for the Baltic Sea. ICES assumes that recruitment in the Baltic Sea does not take place at the glass eel stage at all, but exclusively at the yellow eel stage (ICES Advice, 2022), because natural immigration into the Baltic Sea via the Skagerrak and Kattegat takes so long that metamorphosis to the yellow eel stage should already be completed in almost all eels. Therefore, the eels that naturally occur in the Baltic coast are much larger than glass eels at the time of arrival and have significantly different requirements in terms of feeding preference. While glass eels typically consume primarily small planktonic or benthic organisms, larger eels become increasingly omnivorous and consume larger prey (Bardonnet and Riera, 2005; Denis *et al.*, 2022). Of importance here is whether the stocked glass eels had adequate food availabilities during the time of stocking, which can affect growth performance and mortality rates. It can therefore not be excluded that pre-grown farmed eels could have a higher value for stocking the coastal water of the Baltic Sea. In this regard, Thiel and Kullmann (2019) showed in a comparative study that stocked glass and farmed eels on the neighboring Schleswig-Holstein Baltic Sea coast that there were only minor differences between stocked glass eels and farmed eels with respect to different fitness parameters. However, probably all farmed eels were contaminated with the eel herpesvirus Anguillid herpesvirus 1 (Kullmann *et al.* 2017), which may have had an impact on the results and makes it necessary to consider the option of stocking the study area of the present study with farmed eels in the future, at least experimentally, because this stocking form corresponds much more closely to the natural conditions than the use of glass eels.

Eel stocking and the spread of diseases

One benefit of stocking programs in general is the need of strictly adhering to health regulations - either as part of good practice or to comply with legal standards - to prevent the anthropogenically induced spread of diseases. A well-documented case of what can happen without a disease prevention strategy is the spread of the swim bladder nematode *A. crassus* in the 1980s and 1990s. This parasite originally infected only the Japanese eel *Anguilla japonica* and had a relatively minor impact on the population (Egusa, 1979). International and uncontrolled trade of European eels and Japanese eels brought the parasite to Europe and it spread rapidly throughout the European continent (e.g., Peters and Hartmann, 1986;

Hartmann, 1993a). Within a few years, the nematode was present in almost all water bodies, most likely accelerated by anthropogenic activities (Paggi *et al.*, 1982; Peters and Hartmann, 1986; Taraschewski *et al.*, 1987; Belpaire *et al.*, 1989). Of particular importance for the rapid spread are overland transports by trucks, interrupted by frequent water changes, and also stocking activities with infected eels (e.g., Belpaire *et al.*, 1989). It is unclear if the *A. crassus* infection affects recruitment and it is known that many recruitment indices have already been in decline for several years at first occurrence of *A. crassus* (Kettle *et al.*, 2011). But a negative influence on the fitness of eels is due to the strongly negative effect on the physical condition of infected eels (e.g., more pronounced stress response to hypoxia, Gollock *et al.,* 2005), a reduced reproductivity can also be considered very likely. In addition to the spread of parasites through national and international stocking programs, several viruses can have a significant negative impact on both stocked fishes and conspecifics living in the stocking areas. Among others, the most common eel viruses are European virus eel (EVE), Europe eel virus X (EVEX), and Anguillid herpesvirus 1 (AngHV-1) (EFSA, 2008; Haenen *et al.*, 2012). The viruses EVE and EVEX (both rhabdovirus viruses (RNA viruses)) cause hemorrhage and anemia during migration and are thus suspected to have a negative impact on the escapement rate of silver eels and also could have a negative influence on reproduction (van Ginneken *et al.*, 2004; van Ginneken *et al.*, 2005; Haenen *et al.*, 2009).

Since the stocked glass eels in the present study were only visually inspected before stocking without performing the necessary virological tests (M. Dorow, pers. comm.), Kullmann *et al.* (2022) performed a subsequent virological examination (on possible infections with AngHV-1, EVE and EVEX) and were able to show that a proportion between 5.3 % and 37.4 % of the stocked glass eel cohorts from 2014 to 2016 were infected with AngHV-1 and thus contributed to the spread of the virus, which would suggest that all eels should be tested for possible virus infections prior to stocking measurements. EVE and EVEX were found only sporadically, which may indicate a comparatively low importance of these viruses in glass eel stocking. In particular, AngHV-1 can cause lesions and necrosis of the skin, liver, and gills in infected eels, which can lead to death of the fishes (Davidse *et al.*, 1999; Haenen *et al.*, 2002; Hangalapura *et al.*, 2007), or AngHV-1 infected eels remain asymptomatic but can act as carrier hosts (van Nieuwstadt *et al.*, 2001). Furthermore, Kullmann *et al.* (2022) found evidence that the virus prevalence in stocking fishes may be related to the duration of the glass eel supply chain given that arriving glass eels were found to be rather pathogen free (Delrez *et al.*, 2021). The longer the eels were kept in the catch area (France) prior to transport to the stocking area in the aquaculture facilities of the dealers, the higher the prevalence was, which would argue for keeping the intermediate holding period as short as possible. In addition, Kullmann *et al.* (2022) followed infected cohorts in the stocking water for five years (Figure 20), and AngHV-1 infected eels were subject to higher mortality than non-infected conspecifics.



	2	3	4	5
2014 (37.4 %)			X	X
2015 (5.3 %)		X	X	
2016 (5.3 %)	X	X		

Figure 20: AngHV-1 prevalence (black stripes) of marked (red) and not marked (blue) eels divided by age at catch from 2 to 5 years (a) and the reference (cross) to the appropriate stocking cohorts for each age group (b). Taken unchanged from Kullmann et al. (2022).

The authors concluded that stocking of infected eels could thus be detrimental to achieving management objectives. In addition, virological studies have also been conducted in neighboring waters and evidence has been found that uncontrolled eel stocking is a dispersal vector for AngHV-1 in particular. For example, Kullmann *et al.* (2017) showed that stocking of AngHV-1 infected farmed eels was detected in the Schlei Fjord, which is an adjacent water body of the western German Baltic Sea (west of the study area of chapters 3 and 4 of this thesis), in 2015 and 2016. Six years prior to the conducted stocking, the Schlei Fjord was still free of AngHV-1 infected eels (Jakob and Neuhaus *et al.*, 2009), while eight years later a virus prevalence of 68 % was detected (Kullmann *et al.*, 2017). Kempter *et al.* (2014) and Nguyen *et al.* (2017) also examined eels for the eel herpes virus, which came from the Szczecin Lagoon and Lake Dabie, respectively (both areas are connected to the study site of chapters 3 and 4) and found that 28.6 % and 58.3 % of the eels examined were AngHV-1 positive, respectively. Moreover, AngHV-1 infected eels were also stocked in Lübeck Bay in 2015 and 2016 (Thiel and Kullmann, 2019), so overall the stocking area of the present work was significantly affected by the stocking of infected eels.

Final conclusion and outlook

The results of the present thesis have provided evidence that the marker ARS has a relatively low bioaccumulation potential and can therefore certainly be considered as a standard chemically marker in the context of eel stocking measures (chapter 1). It has also been shown that this is even necessary when using farmed eels, so that an age reading error can be corrected and a corresponding misestimation of silver eel biomass can be avoided (chapter 2). However, the use of ARS is not currently feasible and alternative substances that are equally easy to use and detectable are not available. Therefore, future research should focus on determining the toxicological relevance and additionally fill existing knowledge gaps regarding the genotoxic potential of ARS. In addition, evidence has been found that eel stocking in coastal waters can be a suitable management option to increase the local eel population and thus achieve the escapement targets of eel management plans on the one hand and support the local eel fishery on the other hand. For the evaluation of the efficiency of eel stocking, and in particular, any associated potential net benefits, the possibilities of locally delimited research projects as in the context of the present thesis (cf. chapters 3 and 4) are rather unsuitable. Although an increase in the local eel population was observed, thus confirming the results of other research, the effect on recruitment as a whole is not detectable. Rather, no effect on recruitment has been observed despite the extensive implementation of massive stocking programs throughout Europe. Although a stagnation has occurred roughly since the start of EU-wide management in 2011, this may have other causes and cannot be clearly considered a management success.

One reason for the lack of increase in recruitment may be that the period of attempted EU-wide management was simply too short to have an effect due to the eel's long generation times combined with its longevity and long migrations. Accordingly, it is necessary to continue the management, but for the overall consideration of the benefits of eel stocking measures, the Europe-wide approach would have to be improved, which could be based on two interlocking strategies. First, it would be urgently necessary to mark the stocked eels at least temporarily (in the future, for example, with ARS), so that these cohorts can be clearly assigned to stocking measures. Second, a multi-year phase of stocking could be followed by an equally multi-year phase of inactivity, so that in a few years an influence on recruitment could be identified. However, this would require changes to the EU Eel Regulation as well as to the notified eel management plans, since the target of a 40 % silver eel escapement biomass, which is to be considered legally binding, is massively dependent on stocked recruits compared to the estimated relatively unaffected state by humans. However, this also seems very unlikely from a socio-economic point of view, because the eel stocks of the Baltic Sea region in particular are probably recruited almost exclusively from stocked eels and a failure to stock would pose an existential threat to the local eel fishery.

Another aspect that has received little attention so far, especially in eel stocking measures on the eastern German Baltic coast, is the role of viruses and the possible spread through the release of infected eels. Since it is now known that eels contaminated with the eel herpes virus have already been released in adjacent waters as well as in the study area itself, and that infected eels are probably subject to a higher mortality rate than uninfected eels (Kullmann *et al.*, 2022), it would be advisable, with a view to achieving the management objectives, to at least test the stocking fish for certain pathogens prior to release. The primary focus should be on at least AngHV-1, EVEX, and EVE because these viruses can persist and have a significant negative impact on the reproductive potential of infected eels.

Contribution of authors

Chapter 1 - Evaluation of the bioaccumulation potential of alizarin red S in fish muscle tissue using the European eel as a model

Laura Kullmann: Development of the study, conception of the study and sample design, labor work (collection of eel parameter, otolith preparation, age estimation, and eel tissue preparation), data analysis, wrote the original manuscript, manuscript submission to the journal.

Friederike Habedank: Supported the development of the study, design of method development and validation, labor work (optimization of chromatographic separation and detection of the marker in eel muscle tissue), parts of data analysis, partly wrote the original manuscript.

Björn Kullmann: Conception of the study and sample design, marking and stocking of glass and farmed eels, labor work (partly collected eel parameter), provided samples of marked farmed and glass eels, manuscript revision.

Eric Tollkühn: Design of method development and validation, labor work (optimization of chromatographic separation and detection of the marker in eel muscle tissue), manuscript revision.

Jens Frankowski and Malte Dorow: Funding acquisition, supported the development of the study, marking and stocking of glass eels, manuscript revision.

Ralf Thiel: Supported the designed and concept of the study, manuscript revision.

Confirmation of accuracy

Hamburg, 14.09.2023

Place, date

Prof. Dr. Christian Möllmann

Chapter 2 - Age-based stock assessment of the European eel (*Anguilla anguilla*) is heavily biased by stocking of unmarked farmed eels

Björn Kullmann: Development of the study, conception of the study and sample design, labor work (collection of eel parameter and otolith preparation), data analysis, wrote the original manuscript, manuscript submission to the journal.

Jan-Dag Pohlmann: Conducting blind readings for age estimation, wrote parts of the original manuscript, manuscript revision.

Marko Freese: Conducting blind readings for age estimation, manuscript revision.
Alexander Keth: supported the data analysis, manuscript revision.

Laura Kullmann (née Wichmann): Laboratory work (support the of eel parameter collection and otolith preparation), manuscript revision.

Rüdiger Neukamm: Development of the study, manuscript revision.
Ralf Thiel: Supported the designed and concept of the study, manuscript revision.

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Prof. Dr. Christian Möllmann

Chapter 3 - Yellow eel (*Anguilla anguilla*) density trends along the German part of the southern Baltic Sea between 2009 and 2020

Malte Dorow: Development of the study, conception of the study and sample design, data collection, formal analysis, funding acquisition, wrote the original manuscript, writing – review & editing.

Laura Kullmann: Funding acquisition, data collection (eel parameter, age estimation, marker identification), provision of data from the years 2017 to 2019, manuscript revision.

Melanie Buck: Data collection, manuscript revision.

Jens Frankowski: Funding acquisition, manuscript revision.

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Place, date

Prof. Dr. Christian Möllmann

Chapter 4 - Dispersal, body condition, and growth performance of stocked European glass eels (*Anguilla anguilla*) into coastal waters of the eastern German Baltic Sea

Laura Kullmann was the sole author of this article including data collection and interpretation.

Confirmation of accuracy

Hamburg, 14.09.2023
Place, date

Prof. Dr. Christian Möllmann

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References

Abdulrahman, S. A.M., and Basavaiah, K. 2014. Use of alizarin red S as a chromogenic agent for the colorimetric determination of dothiepin hydrochloride in pharmaceutical formulations. Journal of Saudi Chemical Society, 18: 107–114.

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. Available at http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2427.2010.02540.x/full.

Ahlgren, G., Blomqvist, P., Boberg, M., and Gustafsson, I.-B. 1994. Fatty acid content of the dorsal muscle-an indicator of fat quality in freshwater fish. Journal of Fish Biology, 45: 131–157.

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.

Anastassiades, M., Lehotay, S. J., Stajnbaher, D., and Schenck, F. J. 2017. Safety data sheet Carl Roth: https://www.carlroth.com/downloads/ sdb/de/3/SDB_3586_DE_DE.pdf. Access 30 Apr 2019.

Andersson, J., Florin, A.-B., and Petersson, E. 2012. Escapement of eel (*Anguilla anguilla*) in coastal areas in Sweden over a 50-year period. ICES Journal of Marine Science, 69: 991–999.

Angelidis, P., Pournara, I., and Photis, G. 2005. Glass eels (*Anguilla anguilla*) growth in a recirculating system. Mediterranean Marine Science, 6: 99–106.

Anonymus. 2008. Aalbewirtschaftungspläne der deutschen Länder zur Umsetzung der EG – Verordnung Nr. 1100/2007 des Rates vom 18. September 2007 mit Maßnahmen zur Wiederauffüllung des Bestands des Europäischen Aals für die Flusseinzugsgebiete Eider, Elbe, Ems, Maas, Oder, Rhein, Schlei/Trave, Warnow/Peene und Weser. Available at https://www.portal-fischerei.de/fileadmin/redaktion/dokumente/fischerei/Bund/Bestandsmanagement/Aalbewirtsc haftungsplaeneLaender.pdf.

Anonymus. 2010a. Belastung von wildlebenden Flussfischen mit Dioxiden und PCB. Aktualisierte Stellungnahme* Nr. 027/2010 des BfR vom 16. Juni 2010.: https://mobil.bfr.bund.de/ …/belastung_von_wildlebenden_flussfischen_mit_dioxinen_und_pcb.pdf. Access 08 May 2019.

Anonymus. 2010b. Regelwerk Küstenschutz Mecklenburg-Vorpommern: Küstenraum und Bemerssungsgrößen von Küstenschutzanlagen in M-V, 2 - 1 / 2010 Küstenlänge Mecklenburg-Vorpommern. 24 pp.

Anonymus. 2017. Coastal fisheries ordinance.: §4: https:// www.lav-mv.de/downloads/KueFVO_2017.pdf. Access 30 Apr 2019.

Aprahamian, M. W. 2000. The growth rate of eel in tributaries of the lower River Severn, England, and its relationship with stock size. Journal of Fish Biology, 56: 223–227. Available at https://onlinelibrary.wiley.com/doi/full/10.1111/j.1095-8649.2000.tb02100.x.

Arlinghaus, R. 2017. Nachhaltiges Management von Angelgewässern: Ein Praxisleitfaden. 234 pp.

Arlinghaus, R., Cyrus, E.-M., Eschbach, E., Fujitani, M., Hühn, D., Johnston, F., Pagel, T., *et al.* 2014. Hand in Hand für nachhaltigen Fischbesatz: Zehn Besatzfisch-Kernbotschaften aus fünf Jahren angelfischereilicher Forschung, Berlin. 56 pp. Available at https://www.fona.de/mediathek/pdf/Besatzfisch_broschuere.pdf (accessed 13 September 2018).

Åström, M., and Dekker, W. 2007. When will the eel recover? A full life-cycle model. ICES Journal of Marine Science, 64: 1491–1498. Available at https://academic.oup.com/icesjms/article-pdf/64/7/1491/2167778/fsm122.pdf.

Baer, J., and Rösch, R. 2008. Mass-marking of brown trout (Salmo trutta L.) larvae by alizarin: method and evaluation of stocking. Journal of Applied Ichthyology, 24: 44–49. Available at http://onlinelibrary.wiley.com/doi/10.1111/j.1439-0426.2007.01038.x/full.

Baer, J., Becke, C., Bentele, H. M., Habedank, F., and Brinker, A. 2021. Quantification of alizarin red S uptake in coregonid eggs after mass-marking. Journal of Fish Biology, 98: 895–897.

Baer, J., George, V., Hanfland, S., Lemcke, R., Meyer, L., and Zahn, S. 2007. Gute fachliche Praxis fischereilicher Besatzmaßnahmen. Schriftenreihe des Verbandes Deutscher Fischereiverwaltungsbeamter und Fischereiwissenschaftler e.V., 14: 158 pp. (accessed 22 September 2016).

Bardonnet, A., and Riera, P. 2005. Feeding of glass eels (*Anguilla anguilla*) in the course of their estuarine migration: New insights from stable isotope analysis. Estuarine, Coastal and Shelf Science, 63: 201–209.

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.

Beckman, D. W., and Schulz, R. G. 1996. A Simple Method for Marking Fish Otoliths with Alizarin Compounds. Transactions of the American Fisheries Society, 125: 146–149.

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. Available at http://www.publish.csiro.au/mf/pdf/MF05111.

Belpaire, C. G. J., Goemans, G., Geeraerts, C., Quataert, P., Parmentier, K., Hagel, P., and Boer, J. de. 2009. Decreasing eel stocks: Survival of the fattest? Ecology of Freshwater Fish, 18: 197–214 (accessed 25 January 2017).

Belpaire, C., Charleroy, D. de, Thomas, K., van Damme, P., and Ollevier, F. 1989. Effects of eel restocking on the distribution of the swimbladder nematode *Anguillicola crassus* in Flanders, Belgium. Journal of Applied Ichthyology, 5: 151–153. Available at http://onlinelibrary.wiley.com/doi/10.1111/j.1439-0426.1989.tb00486.x/pdf.

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 (accessed 2 July 2017).

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. Available at https://academic.oup.com/icesjms/article-pdf/64/5/981/1796373/fsm039.pdf.

Bevacqua, D., Andrello, M., Melià, P., Vincenzi, S., Leo, G. A. de, and Crivelli, A. J. 2011. Density-dependent and inter-specific interactions affecting European eel settlement in freshwater habitats. Hydrobiologia, 671: 259–265. Available at https://link.springer.com/content/pdf/10.1007%2Fs10750-011-0725-1.pdf.

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. Available at http://link.springer.com/content/pdf/10.1007%2Fs00442-010-1727-9.pdf.

Bevacqua, D., Melià, P., Schiavina, M., Crivelli, A. J., Leo, G. A. D., Gatto, M., and Durif, C. 2019. A demographic model for the conservation and management of the European eel: An application to a Mediterranean coastal lagoon. ICES Journal of Marine Science, 73: 101.

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. 1 online resource (533, [3].

BfR. 2017. Stellungnahme zur Aalmarkierung mit Alizarin-Rot S und Strontiumchlorid. Aalmarkierung mit Alizarin-Rot S und Strontiumchlorid: Bewertung vom 28. März 2017, 5-3520-01-9346217.

BfR. 2019. Stellungnahme zu möglichen gesundheitlichen Risiken durch Verwendung von Alizarinrot S als Farbstoff zur Markierung von Aalbeständen: Stellungnahme vom 28. Oktober 2019, 5-3520-01-9346217.

BfR. 2021. Markierung von Aalen mit Alizarinrot S: Zur Bewertung gesundheitlicher Risiken sind weitere Studien erforderlich: Stellungnahme 034/2021 des BfR vom 5. November 2021. 8 pp.

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. Available at http://onlinelibrary.wiley.com/doi/10.1111/are.12284/full.

Bolger, T., and Connolly, P. L. 1989. The selection of suitable indices for the measurement and analysis of fish condition, Dept. Zool., Univ. Col., Dublin, Belfield, Dublin 4. J. Fish Biol, 34: 171–182.

Bonhommeau, S., Blanke, B., TRÉGUIER, A.-M., Grima, N., RIVOT, E., VERMARD, Y., GREINER, E., *et al.* 2009. How fast can the European eel (*Anguilla anguilla*) larvae cross the Atlantic Ocean? Fisheries Oceanography, 18: 371–385.

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. Available at http://onlinelibrary.wiley.com/doi/10.1111/j.1467-2979.2010.00362.x/full.

Bonhommeau, S., CHASSOT, E., and RIVOT, E. 2008. Fluctuations in European eel (*Anguilla anguilla*) recruitment resulting from environmental changes in the Sargasso Sea. Fisheries Oceanography, 17: 32–44. Available at http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2419.2007.00453.x/full.

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: Journal du Conseil, 73: 91–100. Available at http://icesjms.oxfordjournals.org/content/73/1/91.full.

Buck, M., and Kullmann, L. 2021. Glasaalbesatz in Küstengewässern als Managementoption – Evaluierung eines großskaligen Besatzversuchs. 96 pp. Available at https://www.landwirtschaft-mv.de/static/LFA/Dateien/Hefte/MdLFA_JB_2020.pdf (accessed 3 February 2023).

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. 2005. Otolith science entering the 21st century. Marine and Freshwater Research, 56: 485–495. Available at http://www.publish.csiro.au/MF/pdf/MF04147.

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.

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 (accessed 31 March 2017).

Charleroy, D. de, Grisez, L., Thomas, K., Belpaire, C., and Ollevier, F. 1990. The life cycle of *Anguillicola crassus*. Diseases of Aquatic Organisms, 8: 77–84 (accessed 10 November 2016).

Charleroy, D. de, Thomas, K., Belpaire, C., and Ollevier, F. 1989. The viability of the free living larvae of *Anguillicola crassus*. Journal of Applied Ichthyology, 5: 154–156. Available at http://onlinelibrary.wiley.com/doi/10.1111/j.1439-0426.1989.tb00487.x/pdf.

Christoffersen, M., Svendsen, J. C., Kuhn, J. A., Nielsen, A., Martjanova, A., and Støttrup, J. G. 2018. Benthic habitat selection in juvenile European eel *Anguilla anguilla*: Implications for coastal habitat management and restoration. Journal of Fish Biology, 93: 996–999.

CITES. 2022. CITES convenes international technical workshop on eels (*Anguilla* spp.) at Royal Botanic Gardens KEW, London, UK | CITES. Available at https://cites.org/eng/news/cites-convenes-international-technical-workshop-on-eels-anguilla-spp-at-royal-botanic-gardens-kew-london-uk_15052018 (accessed 2 March 2023).

Cowx, I. G. 1994. Stocking strategies. Fisheries Management and Ecology, 1: 15–30.

Cowx, I. G. 1999. An appraisal of stocking strategies in the light of developing country constraints. Fisheries Management and Ecology, 6: 21–34. Available at http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2400.1999.00139.x/full.

Cowx, I. G., Nunn, A. D., Harvey, J. P., and Noble, R.A.A. 2012. Guidelines for stocking of fish within designated natural heritage sites: Scottish Natural Heritage Commissioned Report No. 513. 120 pp. Available at http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.738.3232&rep=rep1&type=pdf (accessed 18 September 2018).

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. Available at http://onlinelibrary.wiley.com/doi/10.1111/jai.13347/full.

Daverat, F., Limburg, K. E., Thibault, I., Shiao, J. C., Dodson, J. J., Caron, F., Tzeng, W. N., *et al.* 2006. Phenotypic plasticity of habitat use by three temperate eel species, *Anguilla anguilla*, A. japonica and A. rostrata. Marine Ecology Progress Series, 308: 231–241.

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.

Dekker, W. 2000. The fractal geometry of the European eel stock. ICES Journal of Marine Science, 57: 109–121. Available at https://academic.oup.com/icesjms/article-pdf/57/1/109/1797991/57-1-109.pdf.

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

Dekker, W., van Gemert, R., Bryhn, A., Sjöberg, N., and Wickström, H. 2021. Assessment of the eel stock in Sweden, spring 2021 : fourth post-evaluation of the Swedish eel management: Scientific Report, Swedish University of Agricultural Science – Department of Aquatic Resources. pp. 49 plus appendix.

Delrez, N., Zhang, H., Lieffrig, F., Mélard, C., Farnir, F., Boutier, M., Donohoe, O., *et al.* 2021. European eel restocking programs based on wild-caught glass eels: Feasibility of quarantine stage compatible with implementation of prophylactic measures prior to scheduled reintroduction to the wild. Journal for Nature Conservation, 59: 125933.

Denis, J., Rabhi, K., Le Loc'h, F., Ben Rais Lasram, F., Boutin, K., Kazour, M., Diop, M., *et al.* 2022. Role of estuarine habitats for the feeding ecology of the European eel (*Anguilla anguilla* L.). PLOS ONE, 17: e0270348.

Dhondt, A. A. 1988. Carrying capacity: A confusing concept. Acta OEcologica, 9: 337–346. Available at https://www.researchgate.net/profile/andre-dhondt/publication/229192031_carrying_capacity_a_confusing_concept.

DIN. 2008. 32645:2008-11. Chemische Analytik - Nachweis-, Erfassungs- und Bestimmungsgrenze unter Wiederholbedingungen - Begriffe. Auswertung: Verfahren.: https://doi.org/10.31030/1465413.

Domingos, I., Costa, J. L., and COSTA, M. J. 2006. Factors determining length distribution and abundance of the European eel, *Anguilla anguilla*, in the River Mondego (Portugal). Freshwater Biology, 51: 2265–2281.

Dorow, M. 2015. Aalbesatzmaßnahmen im Bereich des Landes Mecklenburg-Vorpommern: Jahresbericht 2014. 123 pp.

Dorow, M., and Frankowski, J. 2017. Aalbestandsmonitoring im Binnen- und Küstenbereich und managementbegleitende Untersuchungen: Jahresbericht 2016. 98 pp.

Dorow, M., and Schaarschmidt, T. 2014. Effektivität von Aalbesatz in Küstengewässern – Vorstellung eines neuen wissenschaftlichen Projektes: In: Fischerei & Fischmarkt in Mecklenburg-Vorpommern, 14, pp. 41–42 (in German).

Dorow, M., and Schaarschmidt, T. 2015. Besatz mit Glasaalen in Küstengewässern 2015. Fischerei & Fischmarkt in Meckleburg-Vorpommern, 1: 64–65.

Dorow, M., Jünger, J., Frankowski, J., and Ubl, C. 2020. Application of a 3-pass removal experiment to assess the yellow eel specific capture efficiency of a 1-ha enclosure. Restocking and Stock Enhancement of Coastal FisheriesPotential, Problems and ProgressSpecial Symposium, 7th Asian Fisheries Forum, 221: 105409.

Dorow, M., Kullmann, L., Buck, M., and Frankowski, J. 2023. Yellow eel (*Anguilla anguilla*) density trends along the German part of the southern Baltic Sea between 2009 and 2020. Fisheries Research, 257: 106497.

Dorow, M., Lewin, W.-C., Lill, D., Ubl, C., and Frankowski, J. 2021. Using logbook-based catch-rate data to detect yellow eel population trends in the southern Baltic Sea. Fisheries Management and Ecology, 28: 564–572.

Dorow, M., Lill, D., and Ubl, C., eds. 2017. Die Aalfischerei in den Küstengewässern Mecklenburg-Vorpommerns zwischen 1955 und 2015: Beiträge zum Aalmanagement, 58, Gülzow. 116 pp. (accessed 31 May 2017).

Dorow, M., Schulz, S., Frankowski, J., and Ubl, C. 2018. Using a telemetry study to assess the boundary net efficiency of an enclosure system used for yellow eel density monitoring. Fisheries Management and Ecology, 164: 237 (accessed 27 August 2018).

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. Available at http://onlinelibrary.wiley.com/doi/10.1111/j.0022-1112.2005.00662.x/full.

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. xxvi, 460.

Eckmann, R. 2003. Alizarin marking of whitefish, Coregonus lavaretus otoliths during egg incubation. Fisheries Management and Ecology, 10: 233–239 (accessed 13 February 2017).

Eckmann, R., Czerkies, P., Helms, C., and Kleibs, K. 1998. Evaluating the effectiveness of stocking vendace (*Coregonus albula* (L.)) eleutheroembryos by alizarin marking of otoliths. Arch. Hydrobiol. Spec. Issues Advanc. Limnol., 50: 457–463 (accessed 18 October 2016).

Eckmann, R., Kugler, M., and Ruhlé, C. 2007. Evaluating the success of large-scale whitefish stocking at Lake Constance. Advances in limnology: 361–368.

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.

EFSA. 2017. European Food Safety Authority: Panel on Additives and Products or Substances used in Animal Feed (FEEDAP): Guidance on the assessment of the safety of feed additives for the consumer. EFSA Journal, 15.

EG. 2000. RICHTLINIE 2000/60/EG DES EUROPÄISCHEN PARLAMENTS UND DES RATES vom 23. Oktober 2000 zur Schaffung eines Ordnungsrahmens für Maßnahmen der Gemeinschaft im Bereich der Wasserpolitik: L 327/1. 72 pp.

Egusa, S. 1979. Notes on the culture of the European eel (*Anguilla anguilla*) in Japanese eel-farming ponds. Rapports et Procès-Verbaux du Conseil International pour l´Exploration de la Mer, 174: 51–58.

Enbody, E. D., Pettersson, M. E., Sprehn, C. G., Palm, S., Wickström, H., and Andersson, L. 2021. Ecological adaptation in European eels is based on phenotypic plasticity. Proceedings of the National Academy of Sciences, 118.

ESFA. 2006. Conclusion regarding the peer review of the pesticide risk assessment of the active substance dimethoate. EFSA Scientific Report.: https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2006.84r. Accessed 16 May 2019.

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 (accessed 8 May 2017).

EU COM. 2020. Evaluation of the Eel Regulation: Final Report. Available at, https://data.europa.eu/doi/10.2771/679816.

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 (accessed 8 September 2017).

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

Feunteun, E., Laffaille, P., Robinet, T., Briand, C., Baisez, A., Olivier, J.-M., and Acou, A. 2003. A Review of Upstream Migration and Movements in Inland Waters by Anguillid Eels: Toward a General Theory. Eel Biology. Springer Japan. 191-213.

FIZ. 2022. Fischwirtschaft: Daten und Fakten 2022. Available at https://www.fischinfo.de/images/broschueren/pdf/FIZ_DF_2022_fin.pdf (accessed 27 May 2023).

Fladung, E., and Brämick, U. 2015. Umsetzungsbericht 2015 zu den Aalbewirtschaftungsplänen der deutschen Länder 2008. 48 pp. Available at https://www.portal-fischerei.de/fileadmin/redaktion/dokumente/fischerei/Bund/Umsetzungsbericht_deutsche_Aalbewirtschaftungsplaene_2015.pdf (accessed 8 August 2017).

Fladung, E., and Brämick, U. 2018a. Umsetzungsbericht 2018 zu den Aalbewirtschaftungsplänen der deutschen Länder 2008. 62 pp.

Fladung, E., and Brämick, U. 2018b. Umsetzungsbericht 2018 zu den Aalbewirtschaftungsplänen der deutschen Länder 2008.: https://wwwportal-fischereide/fileadmin/redaktion/dokumente/fischerei/B und/Bestandsmanagemen t / Umsetzungsbericht_dt_AMP_2018pdf. Access 30 Apr 2019.

Fladung, E., and Brämick, U. 2021. Umsetzungsbericht 2021 zu den Aalbewirtschaftungsplänen der deutschen Länder 2008. 69 pp. Available at https://www.portal-fischerei.de/fileadmin/SITE_MASTER/content/Dokumente/Bund/Umsetzungsbericht_dt._AMP_2021.pdf.

Frankowski, J., Dorow, M., and Fehling, F. 2018. Aalbestandsmonitoring im Binnen- und Küstenbereich des Landes Mecklenburg-Vorpommern sowie managementbegeleitende Untersuchungen im Zuge der Umsetzung der Europ¨aischen Aalverordnung. Mecklenburg-Vorpommern Research Centre for Agriculture and Fisheries, Institut of Fisheries, Rostock, Scientific Report, p. 35.

Friedland, K. D., Miller, M. J., and Knights, B. 2007. Oceanic changes in the Sargasso Sea and declines in recruitment of the European eel. ICES Journal of Marine Science, 64: 519–530. Available at https://academic.oup.com/icesjms/article-pdf/64/3/519/2442108/fsm022.pdf.

Froese, R., and Pauly, D. 2023. FishBase World Wide Web electronic publication. www.fishbase.org, (06/2017): Main Ref. 172 + 51442.

Fuh, M.-R., and Chia, K.-J. 2002. Determination of sulphonated azo dyes in food by ion-pair liquid chromatography with photodiode array and electrospray mass spectrometry detection. Talanta, 56: 663–671.

Galatius, A., Teilmann, J., Dähne, M., Ahola, M., Westphal, L., Kyhn, L. A., Pawliczka, I., *et al.* 2020. Grey seal Halichoerus grypus recolonisation of the southern Baltic Sea, Danish Straits and Kattegat. Wildlife Biology, 2020: 1–10.

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. Available at http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2761.2005.00649.x/full.

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. Available at http://eprints.lib.hokudai.ac.jp/dspace/bitstream/2115/61073/1/proceesings67.pdf.

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. 1 online resource.

Hanel, R. 2009. Habitatselektion des Europäischen Aals: Endbericht 04HS065 (01.10.2005 - 28.02.2009): 78 pp. Available at https://service.ble.de/ptdb/index2.php?detail_id=7263&site_key=141&stichw=04HS065&zeilenzahl_zaehler=1#newContent (accessed 13 March 2019).

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.

Hartmann, F. 1993a. Untersuchungen zur Biologie, Epidemiologie und Schadwirkung von *Anguillicola crassus* Kuwahara, Niimi und Itagaki 1974 (Nematoda), einem blutsaugenden Parasiten in der Schwimmblase des europäischen Aals (*Anguilla anguilla* L.), IHF, Olbersweg 24, 22767 Hamburg, FRG. Dissertation: 1–142.

Hartmann, F. 1993b. Untersuchungen zur Biologie, Epidemiologie und Schadwirkung von *Anguillicola crassus* Kuwahara, Niimi und Itagaki 1974 (Nematoda), einem blutsaugenden Parasiten in der Schwimmblase des europäischen Aals (*Anguilla anguilla* L.). Dissertation, Universität Hamburg, Hamburg. 149 pp.

Herlemann, D. P. R., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J. J., and Andersson, A. F. 2011. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. The ISME Journal, 5: 1571–1579.

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

Hüssy, K., Casini, M., Haase, S., Hilvarsson, A., Horbowy, J., Krüger-Johnsen, M., Krumme, U., *et al.* 2020. Tagging Baltic Cod – TABACOD Eastern Baltic cod: Solving the ageing and stock assessment problems with combined state-of-the-art tagging methods: DTU Aqua Report no. 368-2020; https://tabacod.dtu.dk/ (accessed 23 May 2023).

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 Advice. 2020. European eel (*Anguilla anguilla*) throughout its natural range. 17 pp.

ICES Advice. 2021. European eel (*Anguilla anguilla*) throughout its natural range: ICES Advice on fishing opportunities, catch, and effort Ecoregions in the Northeast Atlantic: 1–17.

ICES Advice. 2022. European eel (*Anguilla anguilla*) throughout its natural range.

ICES. 2009a. 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. (accessed 4 July 2017).

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

ICES. 2011. 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. (accessed 8 May 2017).

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. (accessed 8 May 2017).

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 (accessed 17 February 2017).

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 Workshop on Eel Stocking (WKSTOCKEEL): 20-24 June 2016 Toomebridge, Nothern Ireland, UK. ICES CM 2016/SSGEPD, 21: 75 pp. (accessed 12 September 2016).

ICES. 2017a. ICES Advice on fishing opportunities, catch, and effort: Cod (*Gadus morhua*) in subdivisions 24–32, eastern Baltic stock (eastern Baltic Sea) (accessed 19 July 2017).

ICES. 2017b. 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. Available at http://ices.dk/sites/pub/Publication%20Reports/Expert%20Group%20Report/acom/2017/WGEEL/wgeel_2017.pdf (accessed 11 March 2017).

ICES. 2017c. 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. Available at http://ices.dk/sites/pub/Publication%20Reports/Expert%20Group%20Report/SSGIEOM/2017/WKEELDATA/wkeeldata_2017.pdf (accessed 24 August 2017).

ICES. 2018. Report of the Joint EIFAAC/ICES/GFCM Working Group on Eels (WGEEL), 5–12 September 2018, Gdańsk, Poland. ICES CM 2018/ACOM:15: 152 pp. (accessed 30 November 2018).

ICES. 2019. Joint EIFAAC/ICES/GFCM Working Group on Eels (WGEEL). ICES Sci. Rep. 1 (50), 177. https://doi.org/10.17895/ices.pub.5545.

ICES. 2021a. Joint EIFAAC/ICES/GFCM Working Group on Eels (WGEEL). ICES Scientific Reports. 3:85. 205 pp. https://doi.org/10.17895/ices.pub.8143.

ICES. 2021b. Workshop on the future of eel advice (WKFEA): ICES scientific report, ISSUE 13, Copenhagen. 74 pp.

ICES. 2022. Joint EIFAAC/ICES/GFCM Working Group on Eels (WGEEL), and Country Reports 2020–2021. Available at https://ices-library.figshare.com/articles/report/Joint_EIFAAC_ICES_GFCM_Working_Group_on_Eels_WGEEL_and_Country_Reports_2020_2021/18620876 (accessed 27 May 2023).

Itakura, H., Arai, K., Kaifu, K., Shirai, K., Yoneta, A., Miyake, Y., Secor, D. H., *et al.* 2018. Distribution of wild and stocked Japanese eels in the lower reaches of the Tone River catchment revealed by otolith stable-isotope ratios. Journal of Fish Biology, 93: 805–813 (accessed 12 March 2018).

Itakura, H., Miyake, Y., Kitagawa, T., and Kimura, S. 2017. Site fidelity, diel and seasonal activities of yellow-phase Japanese eels (*Anguilla japonica*) in a freshwater habitat as inferred from acoustic telemetry. Ecology of Freshwater Fish. Available at http://onlinelibrary.wiley.com/doi/10.1111/eff.12389/full.

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. Downloaded on 02 December 2016.

Jakob, E. 2009. Monitoring of parasite and virus infections of the European eel, *Anguilla anguilla*, in northern Germany. PhD thesis, Christian-Albrechts-Universität, Kiel. 115 pp.

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: Journal du Conseil, 66: 358–366. Available at http://icesjms.oxfordjournals.org/content/66/2/358.full.

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.

Jéhannet, P., Palstra, A. P., Heinsbroek, L. T. N., Kruijt, L., Dirks, R. P., Swinkels, W., and Komen, H. 2021. What Goes Wrong during Early Development of Artificially Reproduced European Eel *Anguilla anguilla*?: Clues from the Larval Transcriptome and Gene Expression Patterns. Animals, 11: 1710.

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: Journal du Conseil, 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. Available at https://academic.oup.com/icesjms/article-pdf/75/2/719/24531212/fsx173.pdf.

Kamstra, A. 1993. The effect of size grading on individual growth in eel, *Anguilla anguilla*, measured by individual marking. Aquaculture, 112: 67–77 (accessed 30 January 2017).

Kempter, J., Hofsoe, P., Panicz, R., and Bergmann, S. M. 2014. First detection of anguillid herpesvirus 1 (AngHV1) in European eels (*Anguilla anguilla*) and imported American eel (*Anguilla rostrata*) in Poland. Bulletin of the European Association of Fish Pathologists, 34: 87–94.

Kettle, A. J., Asbjørn Vøllestad, L., and Wibig, J. 2011. Where once the eel and the elephant were together: decline of the European eel because of changing hydrology in southwest Europe and northwest Africa? Fish and Fisheries, 12: 380–411. Available at http://onlinelibrary.wiley.com/doi/10.1111/j.1467-2979.2010.00400.x/full.

Kirk, R. S., Lewis, J. W., and Kennedy, C. R. 2000. Survival and transmission of *Anguillicola crassus* Kuwahara, Niimi and Itagaki, 1974 (Nematoda) in seawater eels. Parasitology, 120: 289–295. Available at https://eurekamag.com/research/047/500/047500563.php.

Klein, B. 2014. Versuchsplanung - DoE: Einführung in die Taguchi/Shainin-Methodik, 4th edn. De Gruyter, Berlin. 357 pp. Available at http://www.degruyter.com/search?f_0=isbnissn&q_0=9783110343847&searchTitles=true.

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. Available at http://onlinelibrary.wiley.com/doi/10.1111/j.1095-8649.1987.tb05230.x/pdf.

KüFischV M-V. 2006. Verordnung zur Ausübung der Fischerei in den Küstengewässern (Küstenfischereiverordnung - KüFVO M-V) Vom 28. November 2006. 36 pp.

Kullmann, B. 2018. Growth and condition of stocked glass and farmed eels in a brackish water system. Dissertation, Universität Hamburg, Hamburg. 153 pp. Available at http://ediss.sub.uni-hamburg.de/volltexte/2018/9337/.

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. Available at http://onlinelibrary.wiley.com/doi/10.1111/jfd.12637/full.

Kullmann, B., and Thiel, R. 2018. 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. Fisheries Research, 205: 132–140.

Kullmann, B., and Thiel, R. 2019. The chemical marking of eels - state of the art and application perspectives. *In* Eels Biology, Monitoring, Management, Culture and Exploitation: Proceedings of the First International Eel Science Symposium, pp. 172–187. Ed. by A. Don, and P. Coulson. 5m Publishing, Sheffield. 504 pp.

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. Available at http://onlinelibrary.wiley.com/doi/10.1111/jfb.13508/full.

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. Available at http://onlinelibrary.wiley.com/doi/10.1111/jai.13424/full.

Kullmann, B., Pohlmann, J.-D., Freese, M., Keth, A., Wichmann, L., Neukamm, R., and Thiel, R. 2018. Age-based stock assessment of the European eel (*Anguilla anguilla*) is heavily biased by stocking of unmarked farmed eels. Fisheries Research, 208: 258–266.

Kullmann, L., Adamek, M., Steinhagen, D., and Thiel, R. 2022. Glass eels and viruses – a lesson learnt from stocking the eastern German Baltic Sea coast. Transboundary and Emerging Diseases.

Kullmann, L., Habedank, F., Kullmann, B., Tollkühn, E., Frankowski, J., Dorow, M., and Thiel, R. 2020. Evaluation of the bioaccumulation potential of alizarin red S in fish muscle tissue using the European eel as a model. Analytical and bioanalytical chemistry.

Laffaille, P., Baisez, A., Rigaud, C., and Feunteun, E. 2004. Habitat preferences of different European eel size classes in a reclaimed marsh: A contribution to species and ecosystem conservation. Wetlands, 24: 642–651.

Laffaille, P., Feunteun, E., Baisez, A., Robinet, T., Acou, A., Legault, A., and Lek, S. 2003. Spatial organisation of European eel (*Anguilla anguilla* L.) in a small catchment. Ecology of Freshwater Fish, 12: 254–264.

Lagardère, F., Thibaudeau, K., and Anras, M. L. B. 2000. Feasibility of otolith markings in large juvenile turbot, *Scophthalmus maximus*, using immersion in alizarin-red S solutions. ICES Journal of Marine Science: Journal du Conseil, 57: 1175–1181. Available at http://icesjms.oxfordjournals.org/content/57/4/1175.full.pdf.

LALLF. 2021a. Landing statistic for coastal waters of Mecklenburg-Vorpommern. Available at https://www.lallf.de/fischerei/statistik/fangstatistik-kuestengewaess er/〉 (last accessed 10.12.2021.

LALLF. 2021b. Statistic of fulltime coastal fisheries: Available at 〈https://www.lallf.de/fi scherei/statistik/fischer-und-fahrzeuge/〉 (last accessed 10.12.2021).

LALLF. 2023a. Besatzmaßnahmen. Available at https://www.lallf.de/fischerei/fischereischutz/besatzmassnahmen/ (accessed 1 June 2023).

LALLF. 2023b. Mindestmaße und Schonzeiten der Fische in M-V. Available at https://www.lallf.de/fischerei/fischereischutz/mindestmasseschonzeiten/ (accessed 1 June 2023).

Larsson, P., Hamrin, S., and Okla, L. 1990. Fat content as a factor inducing migratory behavior in the eel (*Anguilla anguilla* L.) to the Sargasso Sea. Naturwissenschaften, 77: 488–490 (accessed 25 January 2017).

Lasne, E., Acou, A., Vila-Gispert, A., and Laffaille, P. 2008. European eel distribution and body condition in a river floodplain: Effect of longitudinal and lateral connectivity. Ecology of Freshwater Fish, 17: 567–576. Available at http://onlinelibrary.wiley.com/doi/10.1111/j.1600-0633.2008.00307.x/full.

Lefebvre, F., Contournet, P., and Crivelli, A. J. 2002. The health state of the eel swimbladder as a measure of parasite pressure by Anguillicola crassus. Parasitology, 124: 457–463. Available at https://www.cambridge.org/core/services/aopcambridge-core/content/view/1B1334DB6189457A0B81540B3BADB1D5/S0031182001001378a.pdf/div-class-title-the-health-state-of-the-eel-swimbladder-as-a-measure-of-parasite-pressure-by-span-class-italic-anguillicola-crassus-span-div.pdf.

Leo, G. A. de, and Gatto, M. 1995. A size and age-structured model of the European eel (*Anguilla anguilla* L.). Canadian Journal of Fisheries and Aquatic Sciences, 52: 1351–1367.

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. Available at http://onlinelibrary.wiley.com/doi/10.1111/j.1095-8649.2007.01642.x/full.

Liu, Q., Zhang, X. M., Zhang, P. D., and Nwafili, S. A. 2009. The use of alizarin red S and alizarin complexone for immersion marking Japanese flounder Paralichthys olivaceus (T.). Fisheries Research, 98: 67–74.

LOBÓN-CERVIÁ, J., and IGLESIAS, T. 2008. Long-term numerical changes and regulation in a river stock of European eel *Anguilla anguilla*. Freshwater Biology, 53: 1832–1844.

Lorenzen, K. 2000. Allometry of natural mortality as a basis for assessing optimal release size in fish-stocking programmes. Canadian Journal of Fisheries and Aquatic Sciences, 57: 2374–2381.

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. Available at http://onlinelibrary.wiley.com/doi/10.1111/raq.12071/full.

LUNG MV. 2020. Kormoranbericht Mecklenburg-Vorpommern 2020. Arbeitsbericht Landesamt für Umwelt, Naturschutz und Geologie MV, pp. 6. (in German).

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.

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 (accessed 8 May 2017).

McCleave, J. 1999. Movements of yellow- and silver-phase European eels (Anguilla anguillaL.) tracked in the western North Sea. ICES Journal of Marine Science, 56: 510–536.

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.

Merkblatt für die Binnenfischerei. 1963. Maßnahmen zur Überwinterung der Satzkarpfen: 3–26.

Moravec, F., Di Cave, D., Orecchia P., and Paggi, L. 1994. Experimental observations on the development of *Anguillicola crassus* (Nematoda: Dracunculoidea) in its definitive host, *Anguilla anguilla* (Pisces). Folia Parasitologica, 41: 138–148 (accessed 17 May 2019).

Neukamm, R., Hempel, M., Behrens, M., and Kullmann, B. 2018. Der Einfluss von Aalbesatzmaßnahmen auf die Bestandsstruk-tur in den Gewässersystemen Nord-Ostsee-Kanal und Elbe-Lübeck-Kanal Zwischenbericht 2017 Hegegemeinschaft: Zwischenbericht 2017. Available at https://www.schleswig-holstein.de/DE/Fachinhalte/F/fischerei/Downloads/zwischenberichtAalprojekt.pdf?__blob=publicationFile&v=2 (accessed 16 January 2019).

Nguyen, T. T., Jin, Y., Kiełpińska, J., Bergmann, S. M., Lenk, M., and Panicz, R. 2017. Detection of *Herpesvirus anguillae* (AngHV-1) in European eel *Anguilla anguilla* (L.) originating from northern Poland—assessment of suitability of selected diagnostic methods. Journal of Fish Diseases. Available at http://onlinelibrary.wiley.com/doi/10.1111/jfd.12689/full.

Nielsen, L. A. 1992. Methods of marking fish and shellfish. Special Publication / American Fisheries Society, 23. AFS, Bethesda. XIV, 208 s.

Nielsen, M. E. 1997. Infection status of the swimbladder worm, *Anguillicola crassus* in silver stage European eel, *Anguilla anguilla*, from three different habitats in Danish waters. Journal of Applied Ichthyology, 13: 195–196.

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. 298 pp.

Oeberst, R., and Fladung, E. 2012. German Eel Model (GEM II) for describing eel, *Anguilla anguilla* (L.), stock dynamics in the river Elbe system. Informationen aus der Fischereiforschung, 59: 9–17 (accessed 31 July 2017).

Ogle, D. H. 2018. FSA: Fisheries Stock Analysis. R package version 0.8.20.9000, https://github.com/droglenc/FSA.

Okamura, A., Yamada, Y., Yokouchi, K., Horie, N., Mikawa, N., Utoh, T., Tanaka, S., *et al.* 2007. A silvering index for the Japanese eel *Anguilla japonica*. Environmental Biology of Fishes, 80: 77–89.

Oliveira, K. 1996. Field validation of annular growth rings in the American eel, *Anguilla rostrata*, using tetracycline-marked otoliths. Fishery Bulletin, 94: 186–189 (accessed 8 August 2017).

Ostman, O., Boström, M. K., Bergström, U., Andersson, J., and Lunneryd, S.-G. 2013. Estimating competition between wildlife and humans-a case of cormorants and coastal fisheries in the Baltic Sea. PloS one, 8: e83763.

Paggi, L., Orecchia P., and Mattiucci, S. 1982. Sulla comparsa di Anguillicola australiensis Johnston e Mawson, 1940 (Dracuncoloidea: Anguillicolidae) in *Anguilla anguilla* del lago di Bracciano: Appearance of Anguillicola australiensis Johnston and Mawson, 1940 (Dracuncoloidea: Anguillicolidae) in *Anguilla anguilla* of Lake Bracciano. Parassitologia: 139–144. Available at https://pubmed.ncbi.nlm.nih.gov/6926929/ (accessed 15 May 2023).

Palm, S., Dannewitz, J., Prestegaard, T., and Wickström, H. 2009. Panmixia in European eel revisited: No genetic difference between maturing adults from southern and northern Europe. Heredity, 103: 82–89.

Paloheino, J. E., and Dickie, L. M. 1984. Food and growth of fishes. I. A growth curve derived from experimental date. J. Fish. Res. Bd. Can, 22: 521–542.

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.

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.

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: Journal du Conseil, 73: 158–164.

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 (accessed 11 July 2017).

Pike, C., Crook, V., and Gollock, M. 2020. *Anguilla anguilla*, European eel. 46 pp. Available at wwww.iucnredlist.org (accessed 10 February 2023).

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: Journal du Conseil, 73: 2509–2517. Available at http://icesjms.oxfordjournals.org/content/73/10/2509.full.

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. Available at https://academic.oup.com/icesjms/article-pdf/63/9/1674/7549391/63-9-1674.pdf.

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.

Puchtler, H., Sweat, F., Terry, M. S., and Conner, H. M. 1969. Investigation of staining, polarization and fluorescence-microscopic properties of myoendothelial cells. Journal of Microscopy, 89: 95–104.

QGIS Development Team. 2019. QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org.

Québec Declaration of Concern. 2003: Worldwide decline of eel resources necessitates immediate action. Fisheries, 28: 28–30 (accessed 2 June 2017).

R Core Team. 2016. 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/.

R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/: version 4.1.3.

Rashid-Doubell, F., and Horobin, R. W. 1993. Selection of Fluorescent Golgi Complex Probes Using Structure-Activity Relationship Models. *In* Biotechnology Applications of Microinjection, Microscopic Imaging, and Fluorescence, pp. 73–78. Ed. by P. H. Bach, C. H. Reynolds, J. M. Clark, J. Mottley, and P. L. Poole. Springer US; Imprint; Springer, Boston, MA. 1 online resource (266.

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. Available at https://academic.oup.com/icesjms/article-pdf/60/2/314/7548074/60-2-314.pdf.

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. 382 pp. (accessed 2 February 2017).

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. Available at http://advances.sciencemag.org/content/advances/2/10/e1501694.full.pdf.

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 (accessed 8 May 2017).

Scharff-Olsen, C. H., Galatius, A., Teilmann, J., Dietz, R., Andersen, S. M., Jarnit, S., Kroner, A.-M., *et al.* 2019. Diet of seals in the Baltic Sea region: A synthesis of published and new data from 1968 to 2013. ICES Journal of Marine Science, 76: 284–297.

Schendel, T., Jung, C., Lindtner, O., and Greiner, M. 2018. Guidelines for Uncertainty Analysis: Application of the respective Documents of EFSA and BfR for Exposure Assessments. EFSA Supporting Publications, 15: 84.

Schernewski, G., and Wielgat. 2004. Towards a Typology for the Baltic Sea.

Schlumpberger, W., Lauterbach, R., and Falk, K. 1964. Jahresfischereibericht 1963 – Teil 5: Innere Küstengew¨asser. Fisch. Forsch. 2, 135–163.

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.

Schubert, K., and Klein, M. 2021. Das Politiklexikon: Begriffe, Fakten, Zusammenhänge, 5th edn. J.H.W. Dietz Nachf. Bonn, Bonn. 13 pp.

Shiao, J. C., Ložys, L., Iizuka, Y., and Tzeng, W. N. 2006. Migratory patterns and contribution of stocking to the population of European eel in Lithuanian waters as indicated by otolith Sr:Ca ratios. Journal of Fish Biology, 69: 749–769. Available at http://onlinelibrary.wiley.com/doi/10.1111/j.1095-8649.2006.01147.x/full.

Shiraishi, H., and Crook, V. 2015. Eel Market Dynamics: An analysis of *Anguilla* production, trade and consumption in East Asia. TRAFFIC, Tokyo, Japan. 53 pp.

Simon, J. 2015. Age and growth of European eels (*Anguilla anguilla*) in the Elbe River system in Germany. Fisheries Research, 164: 278–285. Available at https://www.sciencedirect.com/science/article/pii/S0165783614003610/pdfft?md5=c7f1957f91 89298c7c39683bcdf748f4&pid=1-s2.0-S0165783614003610-main.pdf.

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. Available at http://onlinelibrary.wiley.com/doi/10.1111/eff.12050/full.

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., 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* Die Aalfischerei in den Küstengewässern Mecklenburg-Vorpommerns zwischen 1955 und 2015: Beiträge zum Aalmanagement, 58, pp. 89–101. Ed. by M. Dorow, D. Lill, and C. Ubl, Gülzow. 116 pp.

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.

Sjöberg, N. B., Wickström, H., Asp, A., and Petersson, E. 2016. Migration of eels tagged in the Baltic Sea and Lake Mälaren—in the context of the stocking question. Ecology of Freshwater Fish. Available at http://onlinelibrary.wiley.com/doi/10.1111/eff.12296/full.

Souto, C. 2010. Analysis ofearly synthetic dyes with HPLC-DAD-MS: an important database for analysis of colorants used in cultural heritage.: https://run.unl.pt/bitstream/10362/5656/1/Souto_2010.pdf. Accessed 30 Apr 2019.

Springsteen, G., and Wang, B. 2001. Alizarin Red S. as a general optical reporter for studying the binding of boronic acids with carbohydrates. Chemical Communications: 1608–1609.

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. Available at http://onlinelibrary.wiley.com/doi/10.1002/aqc.2498/full.

Tanaka, E. 2014. Stock assessment of Japanese eels using Japanese abundance indices. Fisheries Science, 80: 1129–1144.

Taraschewski, H. M. F., Lamah, T., and Anders, K. 1987. Distribution and morphology of two helminths recently introduced into European eel populations: *Anguillicola crassus* (Nematoda, Dracunculoidea) and Paratenuisentis ambiguus (Acanthocephala, Tenuisentidae). Dis. aquat. Org, 3: 167–176.

Tesch, F.-W. 2003. The eel, 3rd edn. Blackwell Science, Oxford, UK. 408 pp.

Thiel, R., and Kullmann, B. 2019. Abschlussbericht über das Vorhaben: Untersuchung zur möglichen Optimierung der Besatzstrategie und wissenschaftlichen Begleitung des Aalbesatzprogramms an der Ostseeküste Schleswig-Holsteins: 166 pp. Available at https://www.schleswig-holstein.de/DE/Fachinhalte/F/fischerei/Downloads/abschlussberichtAalprojekt.pdf?__blob=publicationFile&v=2 (accessed 29 April 2019).

Thorpe, R. B., Le Quesne, W. J. F., Luxford, F. Collie, J. S., and Jennings, S. 2015. Evaluation andmanagement implications of uncertainty in amultispecies size-structured model of population and community responses to fishing. Methods in Ecology and Evolution, 6: 49–58.

Tsikliras, A. C., and Froese, R. 2019. Maximum Sustainable Yield. *In* Encyclopedia of ecology, 108-115. Ed. by B. D. Fath. Elsevier. 14 pp.

Tsukamoto, K., and Kuroki, M., eds. 2014. Eels and Humans. Springer. Available at https://link.springer.com/book/10.1007/978-4-431-54529-3 (accessed 30 May 2023).

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

Tverin, M., Esparza-Salas, R., Strömberg, A., Tang, P., Kokkonen, I., Herrero, A., Kauhala, K., *et al.* 2019. Complementary methods assessing short and long-term prey of a marine top predator – Application to the grey seal-fishery conflict in the Baltic Sea. PloS one, 14: e0208694.

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.

Ubl, C., and Dorow, M. 2015. A novel enclosure approach to assessing yellow eel (*Anguilla anguilla*) density in non-tidal coastal waters. Fisheries Research, 161: 57–63.

Ubl, C., and Jennerich, H.-J. 2008. Aalmanagementplan – Flussgebietseinheit Warnow/Peene. 34 pp.

van den Thillart, G., van Ginneken, V., Korner, F., Heijmans, R., van der Linden, R., and Gluvers, A. 2004. Endurance swimming of European eel. Journal of Fish Biology, 65: 312–318 (accessed 3 August 2017).

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., and van den Thillart, G.E.E.J.M. 2000. Physiology: Eel fat stores are enough to reach the Sargasso. Nature, 403: 156–157.

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. Available at http://edepot.wur.nl/198496.

van Ginneken, Vincent J. T., and Maes, G. E. 2005. The European eel (*Anguilla anguilla*, Linnaeus), its Lifecycle, Evolution and Reproduction: A Literature Review. Reviews in Fish Biology and Fisheries, 15: 367–398.

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.

Vøllestad, L. A. 1992. Geographic variation in age and length at metamorphosis of maturing European eel: Environmental effects and phenotypic plasticity. Journal of animal ecology, 61: 41–48.

Walker, A. M., Godard, M. J., and Davison, P. 2014. The home range and behaviour of yellow-stage European eel *Anguilla anguilla* in an estuarine environment. Aquatic Conservation: Marine and Freshwater Ecosystems, 24: 155–165.

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.

Wee, S. L., Tye, C. T., and Bhatia, S. 2010. Process optimization studies for the dehydration of alcohol–water system by inorganic membrane based pervaporation separation using design of experiments (DOE). Separation and Purification Technology, 71: 192–199.

Westerberg, H. 1998. Oceanographic aspects of the recruitment of eels to the Baltic Sea. Bulletin Français de la Pêche et de la Pisciculture: 177–185.

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. Available at http://www.nrcresearchpress.com/doi/pdf/10.1139/cjfas-2016-0256 (accessed 3 July 2017).

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.

Wichmann, L. 2018. Aalbesatz als Managementoption – Evaluierung eines großskaligen Besatzversuchs: Jahresbericht 2017. 102 pp.

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

Winkler, H. M., Waterstraat, A., Hamann, N., Schaarschmidt, T., Lemcke, R., and Zettler, M. 2007. Verbreitungsatlas der Fische und Rundm¨auler, Großmuscheln und Großkrebse in Mecklenburg-Vorpommern: Natur und Text, Rangsdorf. (in German).

Wysujack, K., Dorow, M., and Ubl, C. 2014. The infection of the European eel with the parasitic nematode *Anguillicoloides crassus* in inland and coastal waters of northern Germany. Journal of Coastal Conservation, 18: 121–130.