



Magnetic and Celestial Orientation of Migrating European Glass Eels (*Anguilla anguilla*)

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MAGNETIC AND CELESTIAL ORIENTATION OF MIGRATING EUROPEAN
GLASS EELS (*ANGUILLA ANGUILLA*)

By

Alessandro Cresci

A DISSERTATION

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Doctor of Philosophy

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UNIVERSITY OF MIAMI

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MAGNETIC AND CELESTIAL ORIENTATION OF MIGRATING EUROPEAN
GLASS EELS (*ANGUILLA ANGUILLA*)

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Magnetic and Celestial Orientation of Migrating
European Glass Eels (*Anguilla anguilla*)

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The European eel (*Anguilla anguilla*) is a diadromous fish that spawns in the Sargasso Sea. As leptocephalus larvae, eels cross the Atlantic Ocean and reach the continental slope of Europe, where they metamorphose into post-larval glass eels. These reach the coast and facultatively enter freshwater. After 5-25 years or longer, as adult silver eels, they will migrate back from freshwater to the Sargasso Sea to spawn and die. At the glass eel stage, eels cross the continental shelf and recruit to estuaries, where they transition to a freshwater physiology. Extensive research has been conducted to understand the environmental cues, and behavioral responses, that guide glass eels during this migration. Previous work described the migratory behavior of glass eels with respect to chemical cues, tides, temperature and light intensity. However, little is known about whether glass eels rely on fixed frames of reference to find their way during migration, such as the geomagnetic field and celestial cues. This dissertation fills this gap by describing the orientation of glass eels using a combination of *in situ* observations and laboratory experiments. In this work, I report novel evidence that both magnetic and lunar-related orientation occurs in European glass eels. These new findings are integrated with the existing body of literature on the behavior of glass eels and a comprehensive and unifying hypothesis on their migration is proposed.

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Chapter 1. Introduction and General Methods

1.1 Introduction

1.1.1 The complex life history of the European eel

The European eel (*Anguilla anguilla*) is a diadromous fish that undertakes one of the most extraordinary migrations in the animal kingdom, and it constitutes a major fishery in Europe (Starkie, 2003). This species has been subject of fishing and farming practices for millennia (Dekker, 2018; Dekker, 2003a). However, the European eel is now critically endangered [International Union for Conservation of Nature (IUCN)] and its population has decreased dramatically since the 1960s (Drouineau *et al.*, 2018; ICES, 2018).

This species has been shrouded in mystery for millennia, stimulating curiosity and interest since at least the 4th century BC. At that time, the Greek philosopher Aristotle hypothesized in his “History of Animals” that eels originate through spontaneous generation from nonliving matter, specifically, from mud. This hypothesis was linked to the fact that the eel has an extremely complex life cycle (Fig. 1) such that, in ancient Greece, philosophers and men of science were not able to link the eels to their early-life stages. Two thousand years later (1886-1896) Yves Delage and Giovanni Grassi determined, for the first time, that the leaf-shaped creature called *Leptocephalus brevirostris* was actually the larval form of *Anguilla anguilla* (Bertin, 1965).

The struggle that scientists encountered in understanding the biology of the European eel was rooted in the multiple physiological and habitat changes that the species undergoes and that it nearly crosses the entire Atlantic Ocean twice, first as a larva and finally as an adult. The European eel spawns in the Sargasso Sea, and then migrates more than 5000 km towards the European coast (Schmidt, 1923; Miller *et al.*, 2015). After hatching in the

Sargasso Sea, eel leptocephali drift with the Gulf Stream (Tesch, 1977; Bonhommeau *et al.*, 2010) until they reach the continental slope of Europe and North Africa. There, the larvae metamorphose into the post-larval transparent glass eel (Tesch, 1980). Glass eels then start a complex journey, migrating from the continental slope, crossing the continental shelf, and eventually reaching coastal areas (Deelder, 1952; Tesch, 1977). After reaching the coast, glass eels recruit in estuarine environments, where some of them will start their upstream migration into fresh water (Tzeng *et al.*, 2000). Here, in brackish water, they transform into juveniles, known as elvers (Tesch, 1977). The body of the eel becomes wider, it develops skin pigment and the jaw enlarges, the composition of visual pigments changes, and they adapt their physiology to fresh water (Wood, Partridge and Grip, 1992; Ciccotti *et al.*, 1993). Thus, the eel develops all of the morphological and physiological features necessary for life in freshwater. However, some eels displays facultative catadromous behavior; remain in marine waters throughout their life while some switch between fresh and saltwater (Tsukamoto and Arai, 2001; Daverat *et al.*, 2006; Thibault *et al.*, 2007; Marohn, Jakob and Hanel, 2013).

The eels that enter freshwater will spend most of their lifetime there (5-25 years or more), to grow into the adult stage of yellow eel first, and then metamorphose into silver eels (Tesch, 1977; Durif *et al.*, 2009). Silver eels exhibit all of the morphological features of a mesopelagic, mid/deep water fish. The eyes increase in size, and the number of photoreceptors as well as the diameter of rods increases (more efficient in low-light conditions) (Pankhurst, 1982). Moreover, the body develops the countershading traits typical of pelagic fish. The silver eel is the adult migratory stage, which swims across the

Atlantic ocean to the spawning area in the Sargasso Sea (Schmidt, 1923; Righton *et al.*, 2016; Béguyer-Pon *et al.*, 2018), where they die after spawning (Fig. 1).

Deeper knowledge of the behavior of the European eel is required for a better understanding of their migratory strategies. Great advances were made in the 20th century in our knowledge of the behavior and the movement ecology of the European eel. Extensive sampling programs were conducted in the Atlantic Ocean to understand the distribution and vertical movement of eel leptocephali (Hanel *et al.*, 2014; Miller *et al.*, 2015), and direct observations of the migratory behavior of yellow and silver eels have been collected through the use of telemetry (Amilhat *et al.*, 2016; Righton *et al.*, 2016; Béguyer-Pon *et al.*, 2018). However, less is known about the migratory behavior of glass eels during their complex journey from the continental slope to estuaries.

Several laboratory studies have described the cues used by glass eels with special focus on chemical cues, such as odors (earthy odors, amino acids and bile salts to cite few) and salinity gradients. However, these cues might not be sufficient to explain their recruitment success at the estuaries. Chemical signals could be too weak in pelagic environments to guide the migration of glass eels from the continental slope to the coast, and they are also highly variable in coastal areas such as fjords.

1.1.2 Magnetic and celestial cues as candidate frame of reference

The earth's magnetic field represents a stationary frame of reference for navigation (Walker, Dennis and Kirschvink, 2002; Gould, 2010) and a growing body of literature reports its use in many animals, such as birds (Wiltschko and Wiltschko, 2001; Ritz *et al.*, 2004), reptiles (Lohmann and Lohmann, 1996; Lohmann, Putman and Lohmann, 2012),

fish (Takebe *et al.*, 2012; Durif *et al.*, 2013; Putman *et al.*, 2018), amphibians (Phillips, Jorge and Muheim, 2010), crustaceans (Boles and Lohmann, 2003; Ernst and Lohmann, 2016) and mammals (Tian *et al.*, no date; Begall *et al.*, 2013).

Celestial cues also represent a stable reference that is used by animals for orientation. For example, African dung beetles follow lunar-dependent polarized light to find their way in the Savannah (el Jundi *et al.*, 2015), salmon use a combination of celestial and magnetic cues to orient (Quinn, 1980; Quinn and Brannon, 1982) and European robins use celestial cues to re-calibrate their magnetic compass (Alert *et al.*, 2015).

Magnetic and celestial cues could play an important role in the migration of glass eels. However, whether glass eels use these cues to reach the coast and swim through the estuaries is unknown. This dissertation aims to investigate whether European glass eels follow magnetic and celestial cues during their migration towards the coasts of Europe.

1.2 The environment of glass eels in Norwegian fjords

This dissertation presents the results of research on glass eels collected in Norwegian fjords, where these recruit to freshwater habitats. Fjords are semi-enclosed basins with unique oceanographic features, such as deep coastal saltwater with freshwater inputs from glacier melting and river runoff, and a highly stratified water column.

Usually, fjords present an upper layer of brackish waters flowing from the head to the mouth of the fjord (Fig. 2). In wide fjords, the depth of this brackish layer depends on the ratio between the fluxes of outflowing brackish water and the incoming water from the river (Stommel and Farmer, 1953; Stigebrandt, 1981). This surface layer exchanges seawater through weak mixing processes with an intermediate layer flowing underneath,

towards the head of the fjord (Fig. 2) (Stigebrandt, 1981). Below this intermediate layer, there is a deep-water mass, enclosed between the sill (area where the sea bottom rises) at the entrance of the fjord and the coast (Fig. 2). Vertical mixing of the deep waters with the intermediate waters is very rare and occurs only in special cases in overmixed fjords (Stigebrandt, 1981). The salinity profile over the extension of the fjord is closely related to the salinity at the fjord mouth, the level of freshwater runoff and the geometrical dimensions of the fjord (Stommel and Farmer, 1953). Close to the head of the fjord, the salinity ranges from values as low as 10‰ (for the first meter at the surface) to 30‰ at the pycnocline, which can be 5-6 meters deep (Stigebrandt, 1981).

Due to their geometrical shapes and great depth, water circulation in fjords is unusual. For example, the tides, which are known to affect the behavior of glass eels, are affected by the geography of the fjords and can face energy losses (in tidal amplitude) inside the basin due to geometrical constrictions. In a fjord, tides can propagate as fast surface (barotropic) signals, which take only a few minutes to reach the head of large basins (Svendsen, 1995). The passage of barotropic tides over the narrow and shallow sill at the entrance of the basin generates vertical velocity, which causes an oscillation of the water layers. This oscillation creates internal tides that propagate in the fjord as internal waves with a period that coincides with the tidal phase (Svendsen, 1995). At the mouth of a fjord, if a sill is present, phenomena such as “tidal choking” can also occur, resulting in a reduced range of tidal elevation inside the fjord compared to the outside, and the generation of a fast/jet current at the mouth of the fjord (Stigebrandt, 1980). In the internal basin of a fjord, tidal currents can vary in intensity and direction with depth, decreasing in strength in deeper water layers due to the high stratification of the water column (Johnson *et al.*, 2011).

1.3 Animals and maintenance

Glass eels were collected manually using hand nets from the estuaries of several streams located around the Austevoll archipelago (Norway). The eels were generally found under rocks and sediment, during low tide. The collection site and the time of the year during which the eels were collected varied between experiments. The estuaries where most of the eels were caught were Vasseide (60.1122°N and 5.2298°E) and Stolmen (60.0082°N and 5.0788°E) (Fig. 3). The eels were collected between March and May, depending on the year and the experiment.

In the maintenance tanks, glass eels were re-acclimated to near full salinity seawater (32 ppt) after capture. They were kept in aerated aquaria in a temperature-controlled room at temperatures close to those experienced during the course of the deployments *in situ* and in the laboratory (7–10 °C). Live zooplankton (*Acartia* spp.) were provided as food, though later staging determined that the animals were at the pre-feeding stage. The animals were kept in 24h darkness or a 12h light and 12 dark cycle, depending on the experiments. Two-thirds of the volume of each aquarium was replaced every 48 hours to maintain water quality. Before deployments, glass eels were transferred from the large aquaria and placed in individual 500 mL white high-density polyethylene (HDPE) containers filled with fresh seawater at the same temperature as the aquaria. These cups were sealed and kept in a water bath or cooler to maintain temperature during transportation to the deployment sites (at sea or to the magnetic facility, see below).

1.4 General methods: *in situ*

1.4.1 Concept and equipment

An objective of this dissertation work was to assess the orientation behavior of glass eels at sea. For all the tests conducted *in situ*, a drifting in situ chamber (DISC, Paris *et al.*, 2008, 2013) was used. The DISC is a drifting transparent circular arena which was deployed in Norwegian coastal areas (Fig. 4). This drifting system is an acrylic structure equipped with a semi-open circular chamber, transparent to both small-scale turbulence and light. A drogue is connected to the lower side of the acrylic frame holding the chamber, allowing the device to drift with the current. A braided line attaches the upper extremity of the acrylic frame to a float, which adds positive buoyancy and allows operators to recover and re-deploy the DISC. The chamber in which glass eels swim is 40 cm wide (diameter), 15 cm deep, and is built entirely with transparent material. The bottom of the chamber is rigid and made of acrylic, while the walls are made of transparent fine mesh. The mesh was preferred to a rigid acrylic wall because it allows water and gas exchange, assuring that the fish in the arena could detect potential chemical cues and that the dissolved oxygen level would not decrease during deployments. The top of the arena was also made of transparent mesh.

Glass eels in the DISC were monitored using a video camera, while monitoring also external environmental cues. The device is equipped with a GOPRO camera, a HOBO light and temperature sensor, a GPS, three analog compasses and a custom Arduino® digital compass. The GOPRO camera records the behavior of glass eels from below the chamber (Fig. 4B). The digital compass is placed on the bottom plate of the frame of the DISC, oriented on the same axis of the camera. The analog compasses are attached to the acrylic

poles of the DISC frame and placed below the circular arena. This positioning avoids the compasses possibly serving as visual references for the animal in the chamber and allows the camera to include the compasses in its visual field. The Orientation With No Frame of Reference (OWNFOR) approach was applied to characterize the orientation of the glass eels in the apparatus while it was drifting (Paris *et al.*, 2008).

1.4.2 Field Trials

The purpose of each trial with the DISC was to observe the orientation of an individual glass eel. Each glass eel was placed in the circular arena while the DISC was held semi-submerged along the side of a small boat. This procedure requires 3 operators: 1 boat driver, 1 person holding the frame of the DISC and 1 person handling the glass eels. Once the glass eel is placed in the chamber, the lid is closed and secured using nylon zip ties or soft plastic tubes. The whole device is then gently released until it reaches the depth at which it will drift throughout the test. The depth at which the glass eel drifts depends on the distance between the arena and the float, which ranged between 4-5 m for all the experiments. This depth allows the animals to be exposed to visual cues while minimizing the impacts of surface waves on the chamber. Each animal is video recorded for 15 minutes in total, at the end of which the device is recovered and the glass eel is replaced with a new one. I considered the first 5 minutes as an acclimation period, observing the orientation behavior of glass eels during the last 10 minutes of each trial.

For most of the data collected for this dissertation, the DISC was deployed in the fjord of Langenuen (Northeast of Austevoll, 60°09'N, 5°28'E; Fig. 3; saltwater) where it drifted

with the current at a depth of 4-5 m, in water that was 70-100 m deep, 800-1000 m from the coast, at water temperatures ranging 6-10 °C.

1.4.3 Data analysis

The orientation of glass eels was determined through the analysis of the GOPRO images, tracking the position of the head of the eel in the circular arena over 10 minutes. The DISC was allowed to rotate, and the position of the eels was monitored with the digital or analog compasses. All of the images were processed using the DISCR tracking procedure, utilizing R and a graphical user interface provided by imageJ software (Irisson, Guigand and Paris, 2009; Irisson *et al.*, 2015). The code utilized is available at the web page Drifting In Situ Chamber User Software in R (<https://github.com/jiho/discr> written by Jean-Olivier Irisson at the Université Pierre et Marie Curie UPMC, released under the GNU General Public License v3.0. Date of access: 7/13/2016). Through the tracking procedure, the positional data and bearings (in units of magnetic degrees) of individual glass eels were collected with respect to the center of the chamber, at a temporal resolution of one frame per second. The images were geo-referenced with respect to the geomagnetic cardinal points, against the reference of the digital compass and the GPS.

Data analysis consisted of two steps. First, the mean orientation of each individual was computed from the bearings collected by the video tracking analysis. The mean of 600 data points, which represent the position of the fish each second (one position/sec over 10min period), was considered to be the orientation of each single individual. The data collected with the video tracking analysis provide the positional information of the glass eel using the top of the camera's field of vision as the frame of reference (the top of the

image corresponded to 0°). These tracks are considered the raw data. The digital compasses were used to derive information about the orientation of the eels with respect to the cardinal frame of reference. Each position of the eel was corrected with respect to the magnetic North. At the end of this procedure, the corrected tracks were used to compute the magnetic bearing of the glass eel, defined as the direction of motion of the fish (in °degrees) with respect to the magnetic North.

The ability of each individual to keep a specific magnetic bearing in the DISC was considered to be evidence of directionality. The significance of the directionality was assessed using the Rayleigh test of uniformity for circular data, and the level of convergence of the bearings towards one direction by the Rayleigh test r value (from 0 to 1) (Irisson, Guigand and Paris, 2009; Irisson *et al.*, 2015). Rayleigh's r values indicate the concentration of the positions of the fish in a specific section of the circular arena, or, in other words, the accuracy of the directionality. Statistical significance was set at $\alpha = 0.05$.

After assessing the orientation of each individual, the following step of the analysis focuses on evaluating whether the eels that had significant orientation at the individual level tend to orient towards a common direction. To accomplish this step of the analysis, the Rayleigh test of uniformity was applied to the values of all the mean individual bearings, testing whether the frequency distribution of the directions displayed by the individuals is significantly different from random ($\alpha = 0.05$).

1.5 General methods: magnetic laboratory

1.5.1 Concept and facility

The main goal of this dissertation is to investigate whether glass eels sense the Earth's magnetic field and use it for orientation. To pursue this goal, experiments were performed using a system that allows manipulation of the magnetic field in its 3 dimensions and to eliminate all other cues that might serve as a reference for orientation. The concept behind the experiments conducted in the magnetic lab was to isolate the magnetic field from all other environmental cues that are normally available at sea, such as chemical cues (odors and salinity gradients), temperature gradients, celestial bodies, wind and surface wave, currents and sounds. Because of the isolation of the magnetic field and its manipulation using electric currents, it is possible to infer whether the eels can use it as a frame of reference. A second goal of the experiments conducted in the lab was to understand the role of magnetic orientation in the broader context of the migration of glass eels. To accomplish that, the same experimental arena (DISC) used *in situ* was also used to observe the behavior of the glass eels in the laboratory, conducting tests of the same length and characteristics as those at sea. In this way, it was possible to compare the orientation behavior that the eels displayed when drifting at sea - with all environmental cues available - with the orientation behavior displayed in presence of the magnetic field only.

Laboratory experiments were conducted at the Institute of Marine Research's (IMR) magnetic research facility (60.12 N and 5.21 E: Hufthamar, Austevoll, Norway). The magnetic laboratory was designed to study the magnetic orientation of aquatic animals (Fig. 5A). It is equipped with a tri-axial electric coil system (Merritt, Purcell and Stroink, 1983; Kirschvink, 1992) with a design described in (Phillips, 1986). This system consists

of two sets of coils. One set of coils is used to compensate and cancel the Earth's horizontal magnetic field. The second set of coils is used to impose a magnetic field with the desired features. Electricity is supplied by a diesel generator located 220 m from the building, which is connected to an uninterruptible power supply (UPS, max 3 A) that stabilizes the electric current. The UPS is connected to an adjustable power supply, linked to a switchbox. The latter allows control of the coil system and to quickly switch between different preset current intensities, used to change the experimental conditions to which test animals are exposed.

At the center of the coils, there is a black circular tank (diameter = 1.40 m; height = 0.90 m, Fig. 5B) filled with seawater pumped from the sea 300 m away. The pump is located in a small fjord and pumps water from approximately 3 m beneath the sea surface. The pumped seawater is collected in a large storage tank located uphill with respect to the magnetic laboratory. The bottom of the circular tank is located at the middle of the electric coils, where there is the maximum homogeneity of the magnetic field. The support of the tank is made of wood and sits on a concrete block separated from the rest of the concrete bottom of the building, isolating the tank from any vibrations coming from activity, such as walking in the building. The room containing the tank and the electric coils is separated from the observer and the power supplies, which are located in an adjacent room.

The building is constructed of non-magnetic material (wood, aluminum, brass and non-ferrous stainless steel), and is far from any source of magnetic interference (163 m from the nearest electrical disturbance and 365 m from the closest building) (Fig. 5A-5B).

1.5.2 Laboratory Trials

The laboratory trials followed the same protocol as used *in situ*. In the laboratory, the DISC did not rotate as it does when it drifts at sea. As in the trials conducted *in situ*, the glass eels were tested individually and each eel was recorded for 15 minutes, with the first 5 minutes considered as an acclimation period. With the coil system, a magnetic field with the same total intensity as the ambient field (48.8 - 50 μT) was generated and the magnetic North was rotated. This was achieved by manipulating only the horizontal components (x, y) of the magnetic field, but not the vertical (z, unvaried). The inclination (73° , deviation $<1^\circ$) was not modified.

The trials were performed under four simulated magnetic field conditions. In each of these, the magnetic North in the laboratory was reoriented with the respect to the Earth's magnetic North (Phillips, 1986; Durif *et al.*, 2013). Thus, in each condition, the magnetic North in the tank was oriented to the Earth's magnetic East, South, West, or North (Fig. 6). Each glass eel experienced only one of the four magnetic conditions. The orientation of the magnetic North in the lab was changed using the switchbox before the animal was placed in the DISC.

The processing of the images was the same as that described for the experiments *in situ*. The orientation of each eel observed in the lab was analyzed with respect to the direction of the magnetic North in the laboratory during each specific test. Using this approach, any non-magnetic cue that could have influenced the orientation response of the animals was eliminated.

1.6 Statistical Analysis

The significance of the directionality at both the individual level (first order) and population level (second order) was assessed using Rayleigh's test of uniformity. Watson's Two-Sample test for homogeneity for circular data was used to compare between the overall orientations. Tests were performed using R, package 'circular'. In all tests, a p-value <0.05 was considered significant ($\alpha = 0.05$).

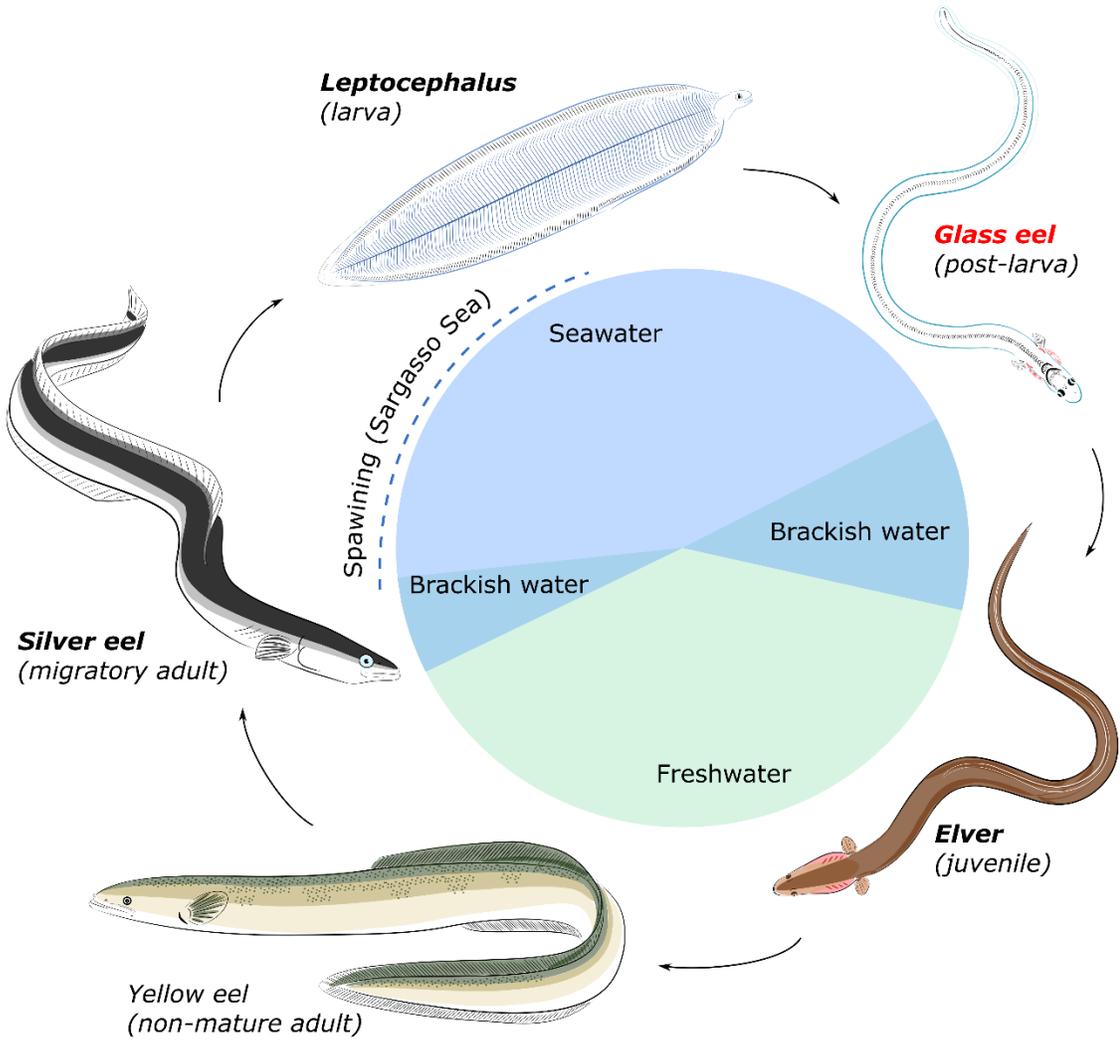


Figure 1 - Life cycle of the European eel (*A. anguilla*)

Eels hatch as leptocephalus larvae in the Sargasso Sea. As larvae, they drift across the Atlantic Ocean to the continental slope of Europe, where they metamorphose into post-

larval, transparent glass eels. The glass eels migrate across the continental shelf and eventually reach the brackish water of estuaries. After metamorphosing into pigmented juveniles, called elvers, they start the ascent into freshwater, where they grow into adult yellow eels. After some years, yellow eels become silver eels, which migrate for thousands of kilometers to the Sargasso Sea where they spawn and die. Artwork by A.Cresci.

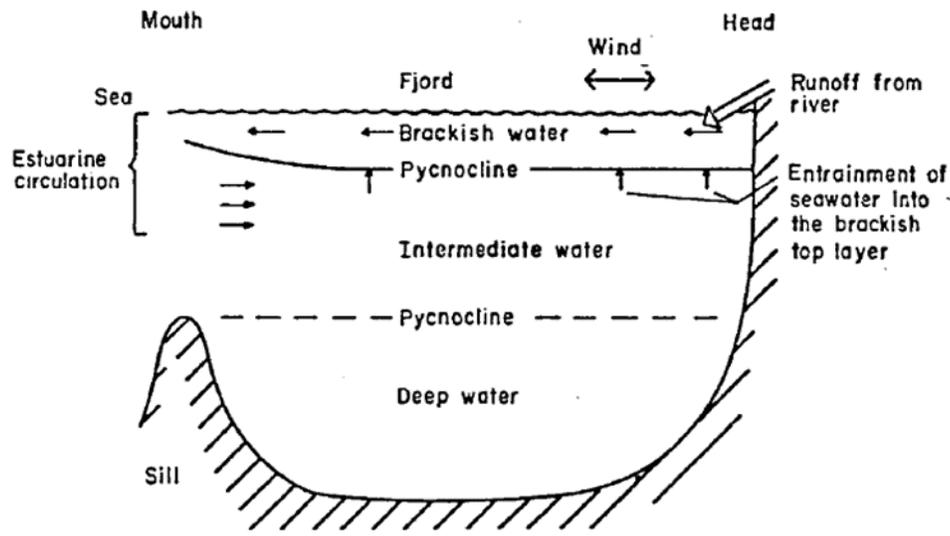


Figure 2 - Description of the water column in a typical fjord.

Vertical profile from the mouth (left) to the head (right) of a fjord (Stigebrandt, 1981)

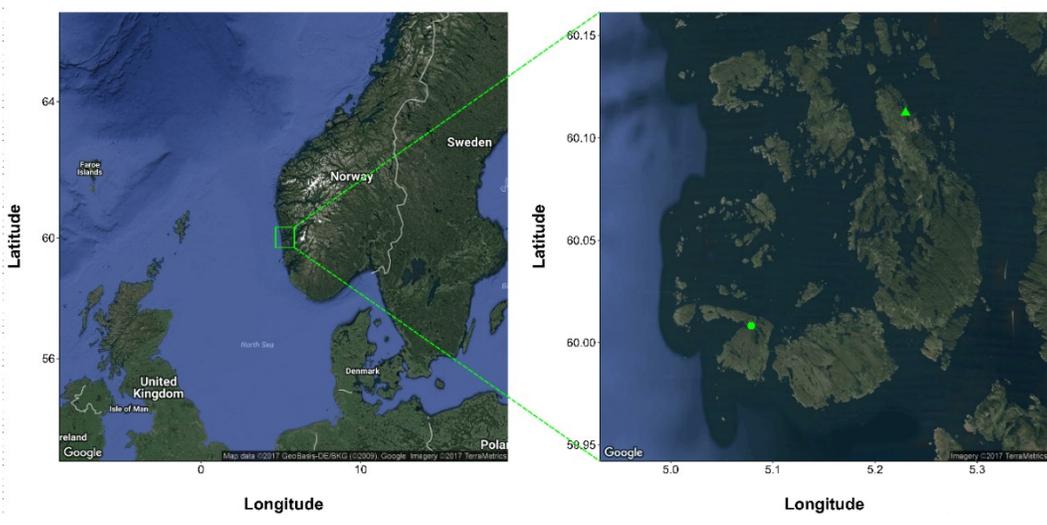


Figure 3 - Maps of the North Sea and the Austevoll archipelago.

The map on the right shows the area of Austevoll and the location of the two estuaries where most of the glass eels were collected for the experiments described in this dissertation: Vasseide (green triangle) and Stolmen (green circle).

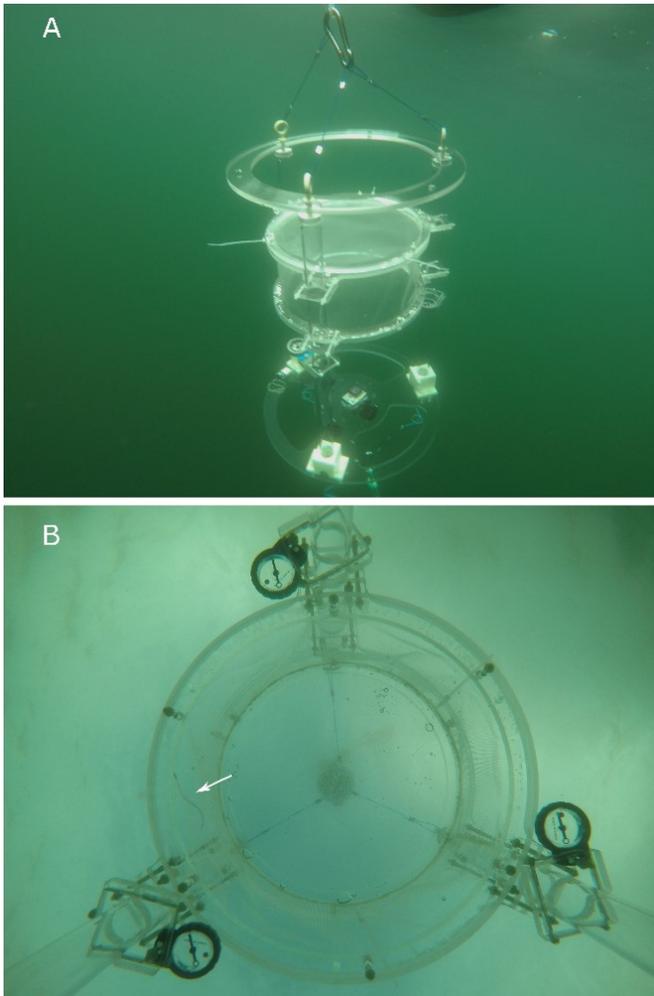


Figure 4 – Drifting in situ Chamber (DISC)

Panel A shows the DISC drifting in the fjords. Panel B shows the field of vision of the GOPRO camera placed underneath the chamber. The white arrow identifies the glass eel that is in the circular chamber.



Figure 5 - Magnetic laboratory.

A. Aerial view of the magnetic lab and the storage tank that collects the seawater pumped from the fjord. B. Inside the magnetic laboratory. The electric coil system surrounds a dark circular tank. The DISC is also visible in the picture, partially submerged in the circular tank.

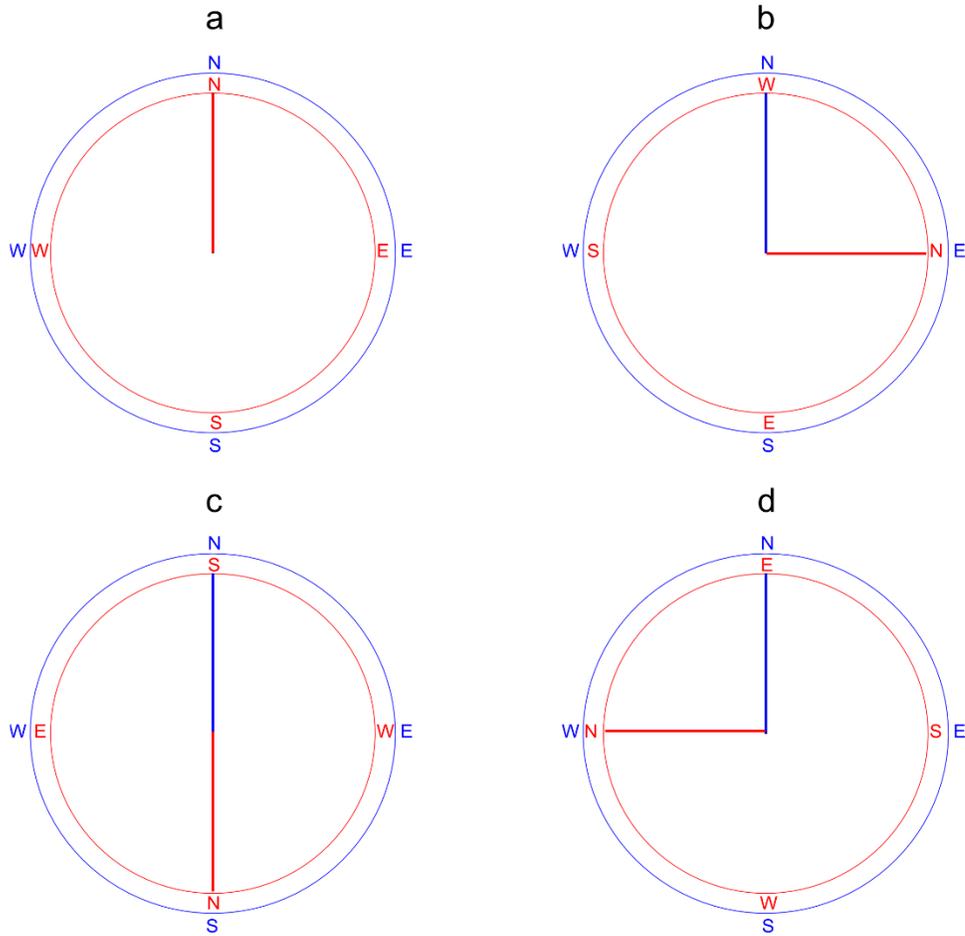


Figure 6 - Schematic diagram of the magnetic protocol.

Blue N, E, S, W represent the Earth's magnetic cardinal points. Red N, E, S, W correspond to the magnetic cardinal points in the testing tank. The red line highlights the orientation of the magnetic North and the blue line the orientation of the Earth's magnetic North.

Chapter 2. Glass eels (*Anguilla anguilla*) have a magnetic compass linked to the tidal cycle

2.1 Overview

The European eel (*Anguilla anguilla*) has one of the longest migrations in the animal kingdom. It crosses the Atlantic Ocean twice during its life history, migrating between the spawning area in the Sargasso Sea and Europe, where it is widely distributed. The leptocephalus larvae drift with the Gulf Stream and other currents for more than a year and metamorphose into glass eels when they arrive on the continental shelf and move toward coastal areas. The mechanisms underlying glass eel orientation toward the coast and into freshwater systems are poorly known. However, anguillid eels, including the glass eel life stage, have a geomagnetic sense, suggesting the possibility that they use Earth's magnetic field to orient toward the coast. To test this hypothesis, we used a unique combination of laboratory tests and in situ behavioral observations conducted in a drifting circular arena. Most (98%) of the glass eels tested in the sea exhibited a preferred orientation that was related to the tidal cycle. Seventy-one percent of the same eels showed the same orientation during ebb tide when tested in the laboratory under a manipulated simulated magnetic field in the absence of any other cue. These results demonstrate that glass eels use a magnetic compass for orientation and suggest that this magnetic orientation system is linked to a circatidal rhythm.

Supplementary material available online:

<https://advances.sciencemag.org/content/3/6/e1602007/tab-figures-data>

2.2 Background

The European eel (*Anguilla anguilla*) spawns at sea but spends most of its life in freshwater or coastal habitats throughout Europe and from Northern Africa to the North Cape in Norway. This critically endangered species [International Union for Conservation of Nature (IUCN)] is an important commercial resource, and its population has decreased markedly since the 1980s (International Union for Conservation of Nature (IUCN), 2015; Jacoby *et al.*, 2015; ICES, 2016). European Union member states are required to establish management plans to restore its population, and this has stimulated research on both conservation and management of eels (Righton and Walker, 2013). Stocking of postlarval glass eels and pigmented

juvenile eels (elvers) to locations where the population is most depleted is a widespread management practice. However, a deeper knowledge about the ecological consequences of eel releases is needed to improve the reproductive success of the transferred individuals (Dannewitz, 2003). Research about their orientation mechanisms is also important to assess how the translocation of restocked glass eels affects the way that they integrate spatial cues when entering coastal and inland habitats—cues that they may use when returning to the spawning areas later in life. The European eel migrates across the Atlantic Ocean twice during its life. Silver eels leave freshwater and coastal habitats from areas across their distribution range and migrate to the spawning area in the Sargasso Sea (Righton *et al.*, 2016). Eggs released at the spawning area hatch into leptocephalus larvae that drift with the currents. During this pelagic larval phase, leptocephali are transported toward the European continent (Johannes Schmidt, 1923; Tesch and Thorpe, 2003; Bonhommeau *et al.*, 2010; Miller *et al.*, 2015), and as they approach the continental shelf, they

metamorphose into the postlarval form, the glass eel (Tesch, 1980; Antunes and Tesch, 1997b). Glass eels migrate to coastal and freshwater habitats (Tesch and Thorpe, 2003; Daverat and Tomás, 2006), where they recruit as elvers. They then spend the growth phase of their life cycle as yellow eels (most often 5 to 20 years). Finally, toward the end of their life cycle, they transform into silver eels. Eel leptocephali might drift passively across the Atlantic with the currents. However, it is also possible that at some point during the later stages of the journey, they swim in an oriented manner (Bast and Strehlow, 1990; Tesch and Thorpe, 2003; Miller *et al.*, 2015). The leptocephali are transported from the Sargasso Sea to Europe via two main routes: one is northeastward, and one is a more direct route toward the east (Bonhommeau *et al.*, 2010). According to a length-frequency analysis of the large database of leptocephali sampled at sea, this pelagic larval phase may last from 17 to 28 months (Bonhommeau *et al.*, 2010). During the early larval pelagic phase, leptocephali migrate vertically in the upper 50 to 300 m (Castonguay and McCleave, 1987) and between 300 and 600 m as they approach the continental shelf (Tesch, 1980). Thus, considering the vertical migration of leptocephali, and the variability of the current velocity with depth, passive drifting alone seems insufficient to explain how the larvae complete their long migration. Furthermore, a coupled biophysical model predicted that setting particles to swim along a preferred bearing increased their survival and recruitment to the continental shelf compared to those modeled as passive drifters (Rypina *et al.*, 2014). Later in their life cycle, leptocephali metamorphose into the postlarval glass eel stage as they move onto the continental shelf in search of the outflows of freshwater streams. The mechanisms and environmental cues underlying the orientation of glass eels in pelagic and coastal areas are poorly known. Ichthyoplankton surveys and laboratory-based experiments

indicate that glass eels are attracted by terrestrial odor cues, low salinity, and colder temperature (Deelder, 1952; Creutzberg, 1961; Tzeng, 1985; Tosi, Spampanato, *et al.*, 1989; Tosi and Sola, 1993; Briand, Fatin and Legault, 2002). However, these cues provide directional information in the form of gradients that are highly variable because of weather conditions, glacier melt, and marine circulation patterns

(Farmer and Freeland, 1983). Thus, on their own, these cues are insufficient to explain the successful orientation of glass eels to freshwater outlets. Earth's magnetic field represents a stationary frame of reference for animal navigation (Walker, Dennis and Kirschvink, 2002; Gould, 2010), providing a directional cue that would allow any marine animal that could perceive it to orient in the absence of any other environmental cue (Tesch, 1974; Boles and Lohmann, 2003; Lohmann, Putman and Lohmann, 2008; Lohmann, 2010; Putman *et al.*, 2014, 2015). Animals of numerous taxa, ranging from insects to birds and mammals, can perceive Earth's magnetic field (Lohmann, 2010). In the marine environment, crustaceans such as spiny lobsters (*Panulirus argus*) are able to detect magnetic variations in the latitude and longitude and orient using magnetic maps (Boles and Lohmann, 2003). Similarly, Pacific salmon (*Oncorhynchus nerka*) and loggerhead sea turtles (*Caretta caretta*) use magnetic orientation for long oceanic migrations and homing to natal areas (Lohmann, Putman and Lohmann, 2008; Putman *et al.*, 2014, 2015). Laboratory-based studies demonstrated that eels are also able to detect magnetic fields at different life stages. As silver eels, they orient magnetically using a magnetic compass system (Tesch, 1974; Durif *et al.*, 2013). Additionally, an American silver eel tracked by a pop-up satellite tag followed a direct, quasi-straight route from the Canadian coast to the Sargasso Sea (Béguier-Pon *et al.*, 2015). This ocean transect is characterized by weak

horizontal thermohaline gradients that would not provide a consistent and reliable orientation cue, implying the possible involvement of magnetic orientation (Béguer-Pon *et al.*, 2015). Eels can also orient with magnetic fields at the yellow eel stage. Yellow eels of *Anguilla rostrata* orient to the northeast when placed in an octagonal chamber in the presence of Earth's magnetic field (Souza, Poluhowich and Guerra, 1988). Similarly, female yellow eels (*A. anguilla*) in a freshwater pond preferred shelters oriented along the south-southwest–north-northeast axis during the fall, suggesting a seasonally dependent magnetic orientation (Van Ginneken *et al.*, 2005). Additionally, European yellow eels (*A. anguilla*) can follow 90° directional shifts of the magnetic field (Tesch, Wendt and Karlsson, 1992). Eels may be able to detect magnetic fields very early in their life history. Glass eels of *Anguilla japonica* slow down their heart rate after conditioning with imposed weak magnetic fields (Nishi and Kawamura, 2005). Elvers of *A. rostrata* display orienting behavior related to the direction and intensity of the electric field, with higher turning frequency toward the anode (Zimmerman and McCleave, 1975; McCleave and Power, 1978). However, the link between turning directions of elvers and the magnetic field has not been demonstrated (Zimmerman and McCleave, 1975). Moreover, none of the experiments carried out on the orientation of early life stages of eels have been able to disentangle the effect of the magnetic field and a potential effect of the electrical current running through the experimental setup. Thus, clear behavioral evidence is necessary to understand whether glass eels use the geomagnetic field as a cue for orientation during their migration to freshwater. We tested the innate orientation ability of European glass eels, newly arrived at the Norwegian coast, using a unique combination of laboratory and in situ observations. We conducted the tests using a drifting in situ chamber (DISC; Fig. 7)

equipped with a circular arena in which glass eels were placed and their swimming and orientation were observed while the DISC drifted in situ in a fjord channel (Fig. 8). In addition, we submerged the arena in a circular tank in a magnetic laboratory on land, under manipulated simulated magnetic fields and with no other orientation cues (fig. S1). The data generated in these experiments were used to assess orientation of glass eels with respect to the magnetic field and the tidal cycle.

2.3 Results

The orientation behavior of glass eels was observed, one eel at a time; most exhibited a preferred compass direction. Furthermore, glass eels oriented with the magnetic field and changed their preferred direction with respect to the tidal phase (Fig. 9).

We tested each eel twice, equally distributing the number of tests between ebb and flood tides, over a period of 7 days (see tables S1 and S2). Thus, we tested each eel once during one tidal phase in situ and once during one tidal phase in the magnetic laboratory. The swimming speed of the glass eels did not change between the tidal phases or between in situ and laboratory testing (fig. S2).

2.3.1 *In situ*

For the *in situ* experiments, we deployed the DISC in the sea, where it was allowed to drift with the current. We tested 54 glass eels, placing each glass eel in the DISCS' circular transparent arena and recording their orientation behavior with a video camera. Through video-tracking analysis of the images, we computed the mean compass orientation of each glass eel (see Materials and Methods for more details). We found that during the tests, glass

eels tended to explore the environment, moving continuously around the edges of the arena. This behavior created noise in the accuracy of the directionality, resulting in some low Rayleigh's r values from the analysis of the orientation at the individual level (see tables S3 and S4). However, the distribution of the positions of the eels indicated that each animal displayed a specific section of the chamber (Rayleigh's $P < 0.05$); the mean position was interpreted as a preferred bearing. At the individual level, 98% oriented in a preferred compass direction (Rayleigh test, $P < 0.05$; Fig. 9A - B, and table S3). The eels tested during ebb tide showed a common orientation to the south ($n = 27$; Rayleigh's $P = 0.02$, $r = 0.38$; Fig. 9B), whereas the ones that were tested during flood tide did not exhibit a common orientation ($n = 26$; Rayleigh's $P = 0.43$, $r = 0.18$; Fig. 9A).

2.3.2 Magnetic laboratory

In the laboratory, 49 of the same glass eels that were tested in situ were observed in the DISC, which was submerged in a black tank inside a magnetic coil system (see the Supplementary Materials). The magnetic coil system made it possible to test each eel with a different configuration of the simulated magnetic field: The magnetic north in the laboratory was reoriented toward one of the four cardinal points of Earth's magnetic field in each test (fig. S3). The orientations of the glass eels were significant at the individual level (Fig. 9C - D), with 35 of the 49 eels displaying orientation (71%; Rayleigh test, $P < 0.05$; table S4).

During ebb tide, the eels tested in the laboratory showed the same common orientation as observed during the in situ experiments, orienting to the south ($n = 15$; Rayleigh's $P = 0.04$, $r = 0.46$; Fig. 9D). However, in addition, they also oriented significantly during flood tide,

but toward the opposite direction, to the north ($n = 20$; Rayleigh's $P = 0.02$, $r = 0.43$; Fig. 9C).

2.4 Discussion

The objectives of the experiments were to observe how glass eels orient in their environment and to test whether they can use the magnetic field as an orientation cue. To address these questions, we tested the orientation abilities of glass eels in a transparent circular arena, which was allowed to drift in a fjord channel. The same eels were then tested in the same arena under manipulated magnetic fields in the laboratory. To investigate the possible role of the tidal phase in the orienting behavior of glass eels, we conducted the tests during ebb and flood tides.

Glass eels exhibited a common orientation to the magnetic field whether they were in the sea or in the laboratory. Glass eels did not display any common orientation in situ during flood tide, but they oriented toward the south during ebb tide. In the laboratory, glass eels only had the magnetic field as an orientation cue, and they showed the same southward orientation during ebb tide. During flood tide, glass eels oriented toward the north. From these results, we conclude that glass eels can perceive the magnetic field and that they use it as a magnetic compass to orient their movements. In the laboratory, they displayed a tidally dependent orientation, and their directionality during ebb tide was consistent with that observed in situ during the same tidal phase, despite the fact that they could not sense the changes in the tide. This implies that their compass orientation is associated with an endogenous rhythm linked to the ambient tidal cycle.

In the laboratory, we manipulated the magnetic field rotating the magnetic vector on the horizontal plane, resulting in different directions of the magnetic north with respect to Earth's magnetic north. Under all configurations of the field, glass eels oriented using the rotated magnetic field as a frame of reference. This allowed us to control for any nonmagnetic bias (visual or olfactory), leaving only the magnetic field as an orientation cue.

Eels are widely distributed from continental Northern Norway to Northern Africa and far into the Mediterranean. At some point during their drift, glass eels must shift to a so-called "y axis orientation": The fish orient at right angles to the shoreline, which is designated as the x axis (Smith, 1985). In the sea, where there are no visual cues, this type of orientation is only possible with the use of a reference system such as the sun or the magnetic field. Because glass eels are active at night (Casamajor, Bru and Prouzet, 1999; Bardonnnet, Bolliet and Belon, 2005), using the latter has obvious advantages. As glass eels get closer to the shore, olfactory cues from freshwater plumes and inland water odors would also guide them (Tosi, Sola, *et al.*, 1989; Crivelli *et al.*, 2008).

Here, all the individuals were collected as they were entering a small stream (Fig. 8A - B). The south-north directionality that they exhibited during the experiments might be consistent with the hydrodynamic conditions at the site where the eels were collected, because the estuary of the stream is oriented toward the north. Although we cannot make definitive conclusions about the significance of their preferred orientation based on the present experiments, indirect evidence for this common orientation exists from Pacific sockeye salmon (*O. nerka*), whose migratory route toward the continent can be predicted using the drift of the geomagnetic field (Putman *et al.*, 2013). Other fish species can detect

the magnetic field and use it as a directional cue during migration. For example, adult yellowfin tuna (*Thunnus albacares*) can distinguish between geomagnetically relevant magnetic fields (Walker, 1984), and juvenile sockeye salmon (*O. nerka*) imprint the intensity of the magnetic field characterizing the area where they first encounter seawater (Putman *et al.*, 2013). As adults, salmon use this magnetic information during the long-distance phase of their migration to find their natal rivers (Putman *et al.*, 2013, 2014).

Glass eel orientation behavior was related to tidal phase. Like many other catadromous fishes, eels use selective tidal stream transport (STST) during the first phase of their upstream migration (Creutzberg, 1961; J. D. McCleave and Kleckner, 1982; Wippelhauser and McCleave, 1987; Laffaille, Caraguel and Legault, 2007). Glass eels rise in the water column during flood tide and drift passively toward the shore but swim at the sea bottom during ebb tide to migrate inshore and to avoid being carried back out to the open ocean (Creutzberg, 1958; Gascuel, 1986; Elie and Rochard, 1994; Bolliet *et al.*, 2007; Fukuda *et al.*, 2016). This behavior reduces the energetic cost of horizontal movement in coastal and estuarine areas (Tankersley and Forward Jr, 2001). Our *in situ* results are consistent with this scenario. Glass eels did not show any common orientation during flood tide. During this tidal phase, they would take advantage of the local current and drift toward the shore, and therefore would not need to orient. During ebb tide, on the other hand, they oriented in a specific direction, and this is consistent with the countercurrent behavior they would adopt to reach the shore or to avoid being pushed back out to the sea.

Glass eel orientation was consistently related to tidal phase in the laboratory, in the absence of any environmental cues. Orientation and swimming behavior linked to endogenous rhythms have been reported in various taxa. The vertical migration of the pelagic larvae of

the estuarine crab, *Rhithropanopeus harrisi*, is modulated by the lunar phase; the crabs rise during flood tide and descend during ebb tide when tested under laboratory conditions (Cronin and Forward Jr, 1979). Further examples come from the intertidal crustacean *Eurydice pulchra*, which exhibits swimming patterns regulated by an independent circatidal pacemaker (Zhang *et al.*, 2013). Furthermore, circalunar endogenous rhythms can influence or entrain the magnetic orientation of animals. The turning pattern of the flatworm *Dugesia dorocephala* is related to the lunar phase and is affected by manipulation of the surrounding magnetic field (Brown, 1962). This relationship between magnetic orientation and lunar phase has also been observed in marine animals. For example, when placed in a Y-maze, the opisthobranch *Tritonia diomedea* makes choices that are related to the geomagnetic field and are linked to the lunar phase (Lohmann and Willows, 1987). The tidally dependent orientation of glass eels observed in situ persisted under laboratory conditions, even in the absence of cues such as odor, salinity, or tidal ellipse and turbulence, suggesting that the mechanism underlying the observed tidally dependent orientation is likely an internal rhythm. Glass eels observed in artificial flumes displayed rhythmic swimming, the periodicity of which was synchronized to the tidal phase (Bolliet *et al.*, 2007). Moreover, glass eels of *A. rostrata* collected in tidal areas and tested in the laboratory showed a circatidal periodicity of 12.5 hours in their swimming behavior (Wippelhauser and McCleave, 1988). These findings support the hypothesis that the tidal orientation of glass eels depends on internal stimuli rather than environmental cues. During flood tide, eels were significantly oriented only in the laboratory. In the sea, eels could have integrated magnetic cues with additional information, such as celestial and chemical cues. Conversely, when tested in the magnetic laboratory, eels displayed an orienting

response based only on the magnetic compass system (that is, no other cues were available), and therefore, they might have used a different strategy to find freshwater outlets. Glass eels rely on salinity gradients as they arrive near fresh water (Deelder, 1958; Edeline and Elie, 2004), and reversing directions with the current could allow them to cover a larger perimeter in search of chemical cues.

The tidal phase–linked magnetic orientation of glass eels that we observed is consistent with the well-documented behavior of glass eels in terms of how they use STST or a tidal-related signal (for example, odor and salinity gradient) to migrate toward freshwater habitats. Therefore, management measures, such as stocking of glass eels in European regions where eels are most depleted, should consider the ability of the eels to orient magnetically with respect to their environment. This could improve the chances that the eels will correctly integrate spatial cues that may be useful later in their life cycle. If eels are able to record magnetic features during their shoreward migration as glass eels, they could potentially use this information for orientation later in life, for example, on their migration back to the spawning areas.

2.5 Conclusions

Glass eels have a magnetic compass, and their orientation abilities appear to be linked to the tidal phase. This is preliminary evidence that magnetic compass–guided movement behavior could be tuned by an endogenous rhythm in the early life stages of a fish. This compass-guided movement, regulated by an endogenous rhythm, may be present in many migratory species.

2.6 Methods

2.6.1 *In situ experiments*

In situ observations were conducted in a fjord channel situated between the island of Huftarøy (Norway) and the mainland. The 54 glass eels that were used in the deployments were collected just before they migrated up a small freshwater stream situated at 60.1122°N and 5.2298°E (Fig. 8). The collection site was an intertidal area with a rocky gravel bottom at the outflow. Eels were hiding under small rocks and were collected with small hand nets. The glass eels' behavior was observed *in situ* using the DISC (Fig. 7) (Paris *et al.*, 2008, 2013). This drifting system is equipped with a semiopen circular chamber, transparent to both small-scale turbulence and light. Glass eels in the DISC were filmed in their natural environment while monitoring external environmental cues. The device is equipped with a GoPro camera, a HOBO light and temperature sensor, a Global Positioning System (GPS) device, three analog compasses, and a custom Arduino digital compass. The Orientation with No Frame of Reference (OWNFOR) (Paris *et al.*, 2008) approach was applied to characterize the orientation of the glass eels in the apparatus while it was drifting.

We conducted 15-min orientation tests on the glass eels, considering the first 5 min as an acclimation period. Tests were performed over a period of 7 days, and distributed equally between ebb and flood tides (see tables S1 and S2). Tidal data were obtained from the tidal station BERGEN (ID 58; 60.3980°N, 5.3205°E; country, Norway; coastline, 040; station, 221). During each test, one individual was deployed in the DISC, and its movement was recorded by a GoPro camera, capturing video at 1 frame/s. The pictures were georeferenced with respect to the geomagnetic cardinal points, against the reference of the digital compass and the GPS device. The DISC was deployed in the fjord of Langenuen (northeast of

Austevoll; ~60.09°N, ~5.28°E; salt water), where it drifted with the current at an average speed of 0.09 m s⁻¹ at a depth of 3 m in water that was 100 to 200 m deep, 800 to 1000 m distant from the coast, at a water temperature of 6° to 7°C. The DISC was allowed to rotate, and the position of the eels was monitored with the compasses. The video was processed by the DISCR (drifting in situ chamber user software in R) tracking procedure (see the Supplementary Materials), using R and a graphical user interface provided by ImageJ software (Irisson, Guigand and Paris, 2009; Irisson *et al.*, 2015). We collected positional data and bearings (in units of magnetic degrees) of individual glass eels with respect to the center of the chamber at a rate of 1 frame/s.

Data analysis consisted of two steps. First, the mean orientation of each individual was computed from the bearings collected by the video-tracking analysis. The ability of each individual to keep a specific bearing in the DISC was considered to be evidence of directionality. We assessed the significance of the directionality using the Rayleigh test of uniformity ($P < 0.05$) and the level of convergence of the bearings toward one direction by the Rayleigh test r value (from 0 to 1) (Irisson, Guigand and Paris, 2009; Irisson *et al.*, 2015). Second, we assessed the significance of the directionality at the group level (that is, the overall directionality of all the individuals tested) to evaluate the common orientation of the glass eels. To accomplish this step of the analysis, we applied the Rayleigh test of uniformity to the values of all the mean individual bearings.

2.6.2 Experiments under simulated and manipulated magnetic fields

To further assess whether the orientation of glass eels was related to the geomagnetic field, we conducted experiments at the Institute of Marine Research's magnetic research facility

(60.1175°N, 5.2118°E; Hufthamar, Austevoll, Norway). We used 49 of the same eels that were tested *in situ*.

The magnetic laboratory is designed to study the magnetic orientation of aquatic animals. It is equipped with a triaxial electric coil system (see fig. S1A), with a design described by Merritt et al. (Merritt, Purcell and Stroink, 1983), connected to a power supply (maximum, 3 A). At the center of the coils, there is a black circular tank made of fiberglass (diameter, 1.40 m; height, 0.90 m; see fig. S1A) filled with seawater, which is pumped from the sea 300 m away. The building (see fig. S1B) is constructed of nonmagnetic material and is far from any source of magnetic interference (163 m from the nearest electrical disturbance and 365 m from the closest building). The experiments in the laboratory followed the same protocol as those *in situ*. The same DISC was submerged in the circular dark tank (see fig. S1C), and each eel was recorded for 15 min, with the first 5 min considered as an acclimation period. The laboratory is equipped with two nested electric coil systems. One was used to cancel out the horizontal component of the ambient field. With the second coil system, we were able to generate a magnetic field with the same total intensity as the ambient field (48.8 to 50 mT) and to reorient the magnetic north. The intensity and inclination inside the coil were set to match the ambient field (48.8 to 50 mT and 73°, with a deviation of <1°). Tests were carried out under four simulated magnetic field conditions where magnetic north was reoriented to the geographical east, south, west, and north (see fig. S3). Each glass eel experienced only one of the four magnetic conditions. Using this approach, we eliminated any nonmagnetic cue that could have influenced the orientation response of the animals. The number of tests conducted was equally distributed between

ebb and flood tides that occurred in the ocean during the trials (although eels could not detect the currents in the magnetic laboratory).

2.6.3 Statistical analysis

The significance of the directionality at both the individual level (first order) and population level (second order) was assessed using the Rayleigh test of uniformity. We used Watson's two-sample test for homogeneity for circular data for the comparison between the global orientations (Fig. 3). Tests were performed using R, packages "discr" and "circular." In all tests, a P value of <0.05 was considered significant.

2.7 Figures

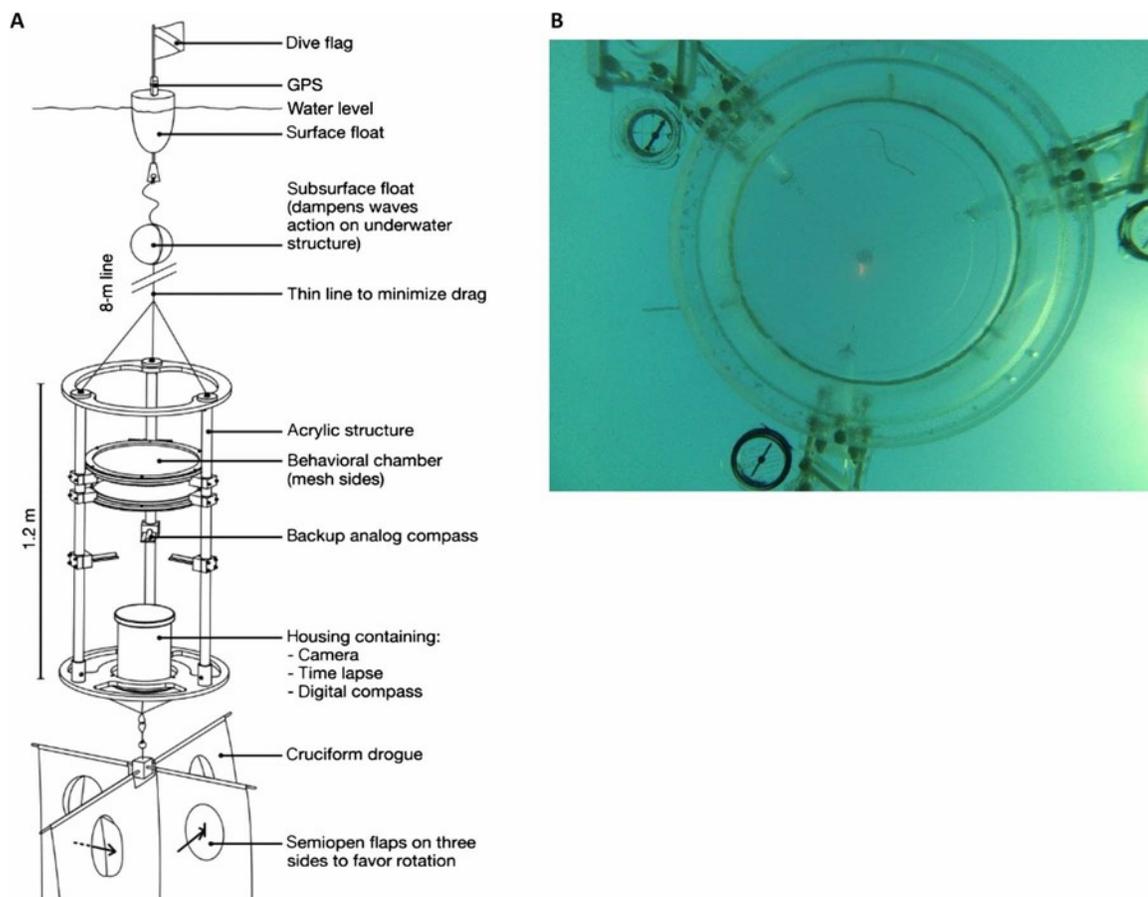


Figure 7 - Drifting in situ chamber.

(A) The main underwater unit is composed of a chamber (of a size selected to accommodate the swimming abilities of the glass eels being tested), the imaging system, and a set of sensors. The DISC configuration for the glass eels was a 41-cm-diameter chamber placed 35 cm above the camera. General protocol: During deployment, the DISC was submerged alongside a small boat, and one animal was inserted through the top the chamber. The DISC was then slowly released at a selected depth and location for a duration of 20 min (5-min acclimation and 10-min observation). (B) Example of an image from the upward-looking camera showing the position of the glass eel inside the chamber, the three analog compasses, the water surface, and the downwelling sunlight as seen through Snell's window.

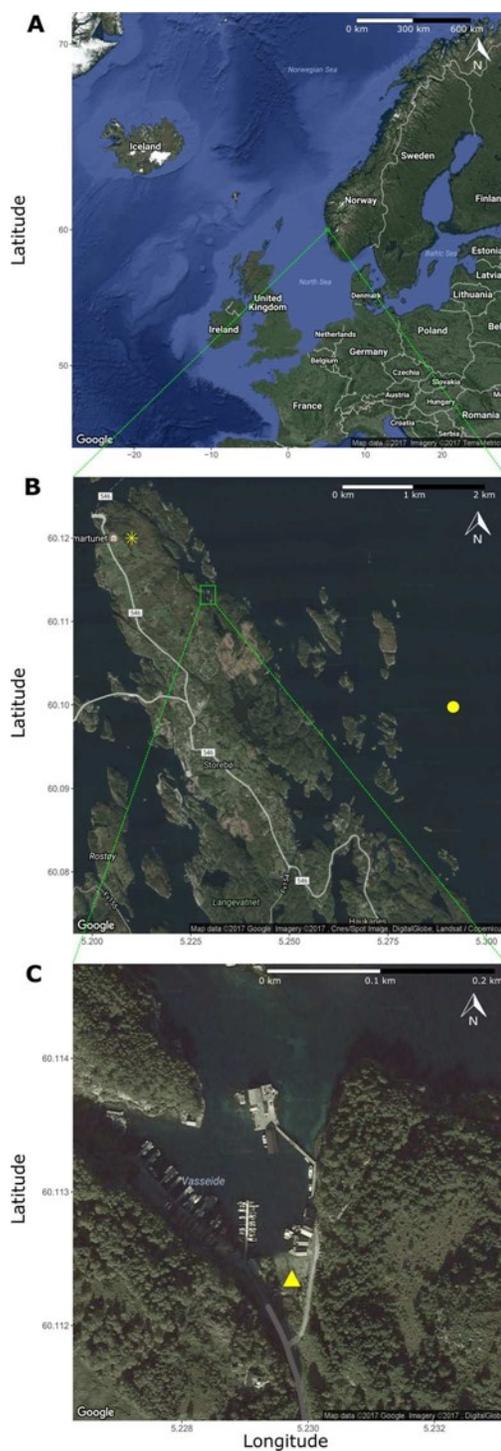


Figure 8 - Study location

(A) Northern Europe. (B) Austevoll location where experiments were conducted. The Austevoll archipelago is located between the North Sea (west) and the Bjørnafjorden fjord channel (east). The yellow circle (•) indicates the site of the *in situ* experiments, and the yellow asterisk (*) represents the location of the magnetic laboratory facility. (C) Zoomed satellite image of the stream estuary. The yellow triangle (▲) indicates the location of the

stream estuary where *A. anguilla* glass eels were collected. The brown zone between the triangle and the water is a rocky gravel intertidal area. The map was created using RStudio version 0.98.1103 (package ggmap, 2009–2014, RStudio Inc.) (www.rstudio.com).

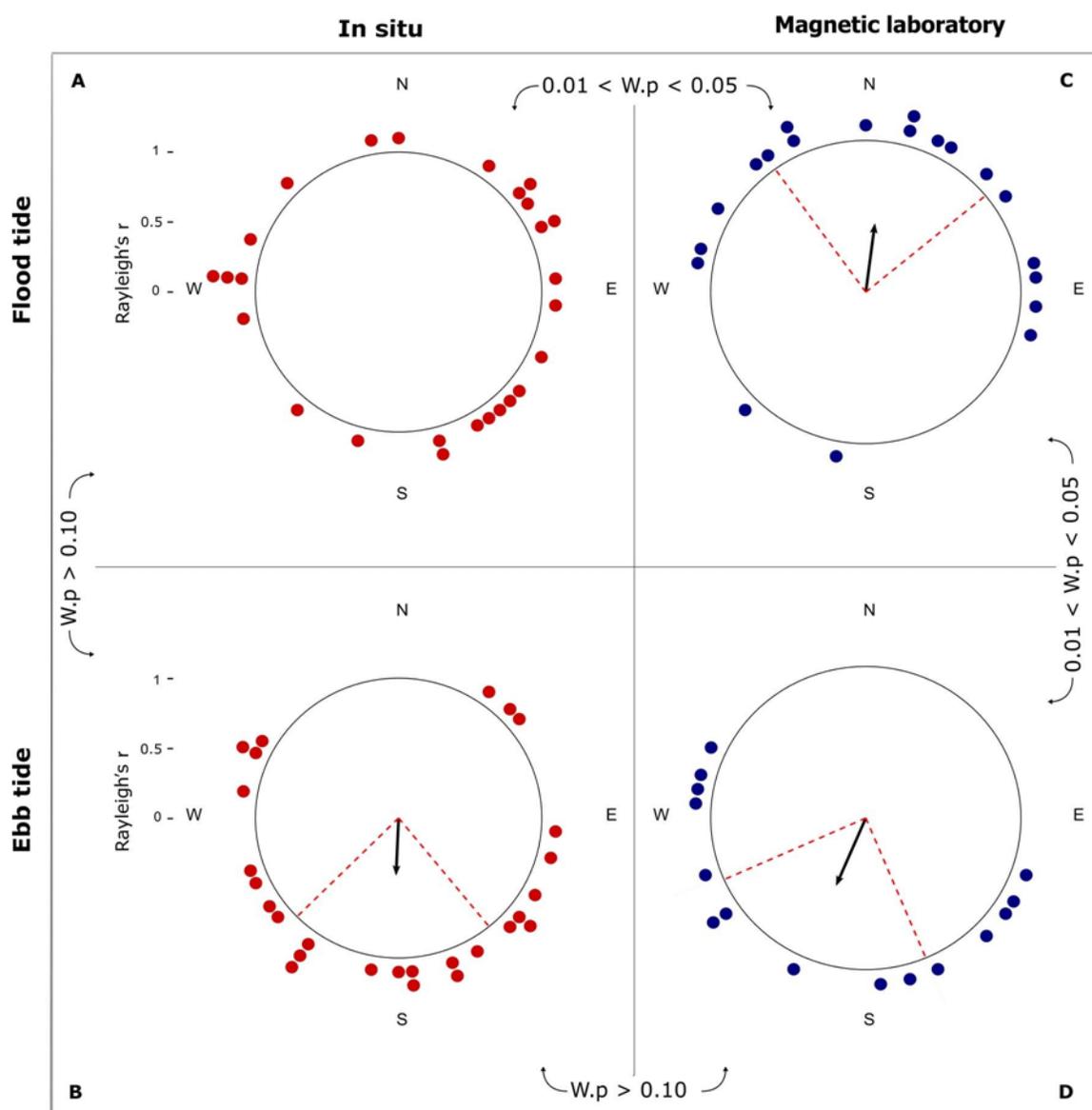


Figure 9 - Orientation of *A. anguilla* glass eels in situ and in the magnetic laboratory.

The mean values of the individual bearing angles are presented as red dots for the eels tested in situ and blue dots for the eels tested in the magnetic laboratory. N, E, S, and W represent the four main magnetic cardinal points. The length of the black arrows corresponds to the Rayleigh r value in each graph and their orientation to the mean group

direction. Red dashed lines indicate 95% confidence intervals. When the arrow and dashed lines are present, there is a significant directionality in orientation at the population level, according to the Rayleigh test of uniformity (5%). W_p are P values of the Watson's two-sample test of homogeneity. (A) Orientation of glass eels observed in situ during the flood tide ($n = 26$; Rayleigh's $P = 0.43$, $r = 0.18$). (B) Orientation of glass eels observed in situ during ebb tide ($n = 27$; Rayleigh's $P = 0.02$, $r = 0.38$). (C) Orientation of glass eels observed in the magnetic laboratory during flood tide ($n = 20$; Rayleigh's $P = 0.02$, $r = 0.43$). (D) Orientation of glass eels observed in the magnetic laboratory during ebb tide ($n = 15$; Rayleigh's $P = 0.04$, $r = 0.46$). The orientation behavior in (C) and (D) was displayed by the glass eels tested under rotated magnetic fields under laboratory conditions.

Chapter 3. The relationship between the moon cycle and the orientation of glass eels (*Anguilla anguilla*) at sea

3.1 Overview

Links between the lunar cycle and the life cycle (migration patterns, locomotor activity, pulses in recruitment) of the European eel (*Anguilla anguilla*) are well-documented. In this study, we hypothesized that the orientation of glass eels at sea is related to the lunar cycle. The European eel hatches in the Sargasso Sea and migrates across the Atlantic Ocean towards Europe. Upon reaching the continental shelf, the larvae metamorphose into glass eels, and migrate up the estuaries, where some individuals colonize freshwater habitats. How glass eels navigate pelagic waters is still an open question. We tested the orientation of 203 glass eels in a transparent circular arena that was drifting in situ during daytime, in the coastal Norwegian North Sea, during different lunar phases. The glass eels swimming at sea oriented towards the azimuth of the moon at new moon, when the moon rose above the horizon and was invisible, but not during the other moon phases. These results suggest that glass eels could use the moon position for orientation at sea and that the detection mechanism involved is not visual. We hypothesize a possible detection mechanism based on global-scale lunar disturbances in electrical fields and discuss the implications of lunar-related orientation for recruitment of glass eels to estuaries. This behavior could help glass eels to reach the European coasts during their marine migration.

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3.2 Background

The European eel (*Anguilla anguilla*) undertakes a long distance migration of >5000 km twice during its life, from the spawning areas in the Sargasso Sea to the European coast and back (Johannes Schmidt, 1923). After hatching in the Sargasso Sea, eel leptocephalus larvae drift with the gulf stream (Tesch, 1977; Bonhommeau *et al.*, 2010) until they reach the continental slope of Europe. There, they metamorphose into the transparent post-larval glass eel (Tesch, 1980), which then migrate across pelagic waters towards coastal areas (Deelder, 1952; Tesch, 1977). Glass eels are attracted to estuaries and most of them will migrate upstream into freshwater habitats. There, eels will spend most of their lifetime (5-30 years) as yellow eels before becoming silver eels (Tesch, 1977; Durif *et al.*, 2009). Silver eels then navigate back to the Sargasso Sea where they spawn and presumably die (J Schmidt, 1923; Righton *et al.*, 2016).

The mechanisms that glass eels use to orient towards the coast are not well understood. Eels have an extremely sensitive olfactory system (Atta, 2013) and glass eels are possibly attracted by chemical cues, such as freshwater plumes containing inland odors (Tosi, Spampanato, *et al.*, 1989; Tosi and Sola, 1993), when they swim near the coast. Additionally, glass eels sense the Earth's magnetic field and use it as a reference compass mechanism to orient (Cresci, Paris, *et al.*, 2017), as do many other species (Gould, 2010). Specifically, glass eels showed a magnetic swimming direction on a North-South axis, which was possibly related to the tidal flows of the stream estuary where they were collected (estuary flowing towards the North). However, while tidal flow can affect the swimming behaviour of glass eels in estuarine and shallow coastal areas (J. McCleave and Kleckner, 1982), the nature of the directional stimulus that glass eels use to swim in a

specific compass direction in open pelagic waters is unknown. Orientation at sea could be based on magnetic cues alone, which would provide glass eels with a perceptual mechanism to maintain an innate compass direction. However, in the case of glass eels, orientation at sea does not follow a direct trajectory, and changes in orientation behaviour occur in association with the tidal cycle (Cresci, Paris, *et al.*, 2017). Thus, the orientation mechanism involved at this stage of their migration might be more complex and multifaceted, and the magnetic sense could also serve as a stable frame of reference for the interpretation of other directional cues (e.g. salinity gradients, odor plumes and water currents).

The movement ecology and behaviour of many species of anguillids, from the larval to the adult stage, is related to the phase of the moon. For example, the hatching of leptocephali of Japanese eel (*A. japonica*) is synchronized with the new moon cycle (Tsukamoto *et al.*, 2003). Leptocephalus larvae of Atlantic eels (*A. Anguilla* and *A. rostrata*) change their depth distribution in the open ocean according to the moon cycle, swimming deeper during full moon (Kracht, 1982). The arrival of glass eels at the coast and estuaries is related to the moon cycle (De Casamajor, Bru and Prouzet, 2001). Pacific glass eels (*A. Japonica* and *A. marmorata*) show the highest recruitment to coastal areas at new moon, and *A. bicolor pacifica* recruits mostly during full moon (Yamamoto, Mochioka and Nakazono, 2001). The arrival of glass eels of *A. australis* and *A. dieffenbachii* to the New Zealand coast is linked to the moon cycle, with the highest number of eels observed during new moon and full moon (Jellyman and Lambert, 2003). This pattern was observed at several locations in New Zealand (Jellyman, 1979). There is also evidence that the behaviour of adults is linked to the moon cycle (Adam and Elie, 1995; Bruijs and Durif, 2009). Adult yellow eels tagged

in the Mediterranean Sea swim slower and closer to the bottom during full moon (Tesch, 1989). Interestingly, freshwater adult silver eels migrate downstream towards the sea mostly around new and full moon, independent of the degree of lunar illumination (Boetius, 1967; Bruijs and Durif, 2009; Verhelst *et al.*, 2018). We hypothesized that the moon could also provide glass eels with a compass direction at sea that could guide them in reaching the coast.

To investigate the influence of the moon on the orientation of European glass eels, we observed the orientation behaviour of 203 glass eels in situ during daytime, while drifting in a transparent circular behaviour arena, the Drifting In Situ Chamber (DISC, Fig. 10) in the coastal Norwegian North Sea (Fig. 11) during the four main moon phases: full moon, third quarter, new moon and first quarter. Additionally, we compared glass eel orientation to the position of the moon relative to the horizon. The data collected were utilized to assess the relationship between the orientation direction of the glass eels and the direction of the moon (moon azimuth). Furthermore, the influence of multiple lunar-related factors such as moon phase, moon position relative to the horizon (above/below), and tide (ebb/flood) on the orientation and swimming speed of glass eels was explored.

3.3 Results

The experiments were designed to investigate whether the moon influences the orientation of glass eels throughout the lunar cycle. Glass eels (150) were tested individually while drifting in the DISC in the North Sea. The dataset also includes the orientation data of 53 glass eels tested under the same conditions published in Cresci *et al.*, 2017. Altogether, the dataset includes 203 individual glass eels observed at sea (Table 3.1). DISC deployments

were conducted during the 4 main moon phases: full moon, third quarter, new moon and first quarter (Table 3.1).

For each glass eel, we calculated the mean orientation direction (mean bearing) and its significance based on video recordings (details of the data collection and statistical analysis are presented in Figure S1). Of the 203 glass eels observed, 175 (86%) showed non-random orientation and a significant mean bearing (Rayleigh's $P < 0.05$). This proportion of eels showing orientation was highest during new moon and first quarter (96% and 93%, respectively), was lower during full moon (80% of the eels oriented) and was lowest during the third quarter (62%). The associated R values (Rayleigh test, ranging from 0 to 1) were 0.30 ± 0.16 and 0.30 ± 0.13 (mean \pm SD) during new moon and first quarter, 0.26 ± 0.14 during full moon and 0.14 ± 0.06 during third quarter.

We compared mean individual orientations of the 175 significantly orienting glass eels between the different tidal and lunar configurations (Table 3.2). Concerning the orientation directions, we considered three variables: the orientation direction of every glass eel (α_{north}), the azimuth of the moon, and the angle between the orientation of the eel and the azimuth of the moon (α_{moon}) (Fig. 12). Only glass eels tested during the new moon and first quarter showed a common orientation, which was southward (Fig. 13C and 13D). This common orientation also corresponded to a significant orientation to the moon azimuth. During flood/new moon (Fig. 13C), tests were performed while the moon azimuths were in opposite directions (East-West) (Fig 13C. column ii). Under this configuration, the behavior of glass eels did not change, and they still oriented towards the azimuth of the moon (Fig. 13C, column iii). However, there was no common orientation towards magnetic south (Fig. 13C, column i).

The orientation towards the new moon azimuth was independent of the tidal phase. When all of the data collected during the new moon were combined, there was a highly significant orientation towards the moon ($N = 80$, Ray. mean bearing = 11.2° , $r = 0.38$, $P = 0.000006$; Fig. 14A iii) and a significant orientation to the magnetic South ($N = 80$, mean bearing = 180° , $r = 0.22$, $P = 0.02$; Fig. 14A i).

During first quarter (Fig. 13D), when the moon starts becoming brighter, a common orientation towards the Southeast was observed (Fig. 13D, column i), along with a significant for orientation with respect to the moon azimuth (Fig. 13D, column iii), although only when the moon was above the horizon (occurring only during flood tide) (Fig. 13D, column ii). Both common orientations (Fig. 13D, column i and iii) towards magnetic south and the moon azimuth disappeared when the moon fell below the horizon (during the ebb tide) (Fig. 13D, column ii). No significant patterns in orientation direction were observed during full moon, when the moon was always below the horizon (Fig. 13A), and third quarter (Fig. 13B).

Since the tests were conducted during the day, we also analyzed the orientation data according to the sun azimuth and the light intensity during the tests (SI, section 2, Fig. S5-S8). Neither had a significant relationship to the orientation of the glass eels (See section 2 of the SI).

The swimming speed of glass eels was significantly faster when the moon was above the horizon (median = 3.0 cm/sec) compared to when the moon fell below the horizon (median = 2.6 cm/sec) (Wilcoxon test $p = 0.0002$) (Fig. S3).

Table 3.1 - Experimental conditions during the Drifting In Situ Chamber (DISC) deployments to test the effect of the moon phase on the orientation of glass eels (*Anguilla anguilla*). N: the number of glass eels.

Moon phase	N	Period	Moon illumination (%)
<i>Full moon</i>	57	11-16 April 2017	80-99%
<i>Third quarter</i>	32	19-20 April 2017	42-52%
<i>New moon</i>	53 30	17-21 April 2015 27 April 2017	0-10%
<i>First quarter</i>	31	3-4 May 2017	57-67%

Table 3.2 - Summary statistics of the orientation of glass eels (*Anguilla anguilla*) according to magnetic north, tidal and moon phase and the moon's position at the horizon. N = number of eels. Mean bearings represent the topographical bearing of the eel relative to magnetic North or to the moon azimuth (0° = toward the moon, 180° = away from the moon). These are only presented when significant (Rayleigh test, r is the angular dispersion, p = Rayleigh's p value). The moon position, either below or above the horizon is provided for each test configuration. Fig. = reference figure where those data are displayed.

Moon phase	Tidal phase	Moon at horizon	N	Magnetic north			Moon azimuth			Fig.
				Mean bearing (°)	r	P	Mean bearing (°)	r	P	
				α_{north}			$\alpha_{azimuth}$			
Full	<i>Flood</i>	Below	20	-	0.09	0.86	-	0.05	0.96	4A
	<i>Ebb</i>	Below	26	-	0.17	0.48	-	0.29	0.10	4A
Third q.	<i>Flood</i>	Below	11	-	0.09	0.92	-	0.06	0.97	4B
	<i>Ebb</i>	Above	9	-	0.48	0.12	-	0.49	0.11	4B
New	<i>Flood</i>	Above	35	-	0.17	0.37	3°	0.31	0.04	4C
	<i>Ebb</i>	Above	45	195°	0.42	0.0002	16°	0.43	0.0001	4C
First q.	<i>Flood</i>	Above	13	147°	0.64	0.003	75°	0.68	0.0014	4D
	<i>Ebb</i>	Below	16	-	0.32	0.18	-	0.35		4D

3.4 Discussion

Glass eels oriented towards the azimuth of the moon at sea only during specific phases of the lunar cycle. Specifically, a significant orientation was observed when the moon was above the horizon at new moon. With these conditions the visibility of the moon is minimal (at this time, the moon is invisible). However, we did not observe patterns in the orientation behaviour during full moon and third quarter. Compared to the new moon phase, the features of the lunar cycle reverse during full moon. At new moon, the moon is above the horizon mostly during the day (and below the horizon during the night), while at full moon the moon is above the horizon during the night. Such features of the lunar cycle might explain the difference in behaviour observed in this study.

Several biological processes are linked to the phases of the moon. Diel vertical migration of zooplankton is entrained to the lunar cycle during the long Arctic winter (Last *et al.*, 2016). Mass spawning of corals occurs during certain full moon and last-quarter nights of the year (Harrison *et al.*, 1984). Similar correlations were observed in shrimps: growth rate and molting of the penaeid shrimp *Penaeus vannamei* coincides with lunar phase (Griffith and Wigglesworth, 1993). The lunar cycle is also correlated with the spawning of many fish. For example, Nassau grouper (*Epinephelus striatus*), Atlantic killifish (*Fundulus heteroclitus*), salmonids and other reef fishes all spawn during particular lunar phases (Taylor *et al.*, 1979; Taylor, 1984; Colin, 1992; Donahue *et al.*, 2015).

The orientation of glass eels in our study was related to the moon's azimuth and its position relative to the horizon, suggesting that the moon might serve as a possible additional cue during their migration towards land. This moon-related orientation could play a role during

the migration of glass eels across the continental shelf, in addition to chemical and tidal cues and/or when those cues are missing or unreliable. Glass eels are known to use odors (Tosi and Sola, 1993; Sola and Tongiorgi, 1996, 1998), salinity gradients (Edeline, Dufour and Elie, 2005) and selective tidal stream transport (STST) (Creutzberg, 1961; J. D. McCleave and Kleckner, 1982; Gascuel, 1986; Tankersley and Forward Jr, 2001; Beaulaton and Castelnaud, 2005) in coastal and brackish water. However, these cues could be absent or less reliable in pelagic areas or far from large freshwater inputs coming from land. Moon-related orientation might work together with the magnetic compass during the pelagic step of the migration over the continental shelf. One possibility is that the moon might serve as a directional stimulus and the magnetic compass as a frame of reference. Another possibility is that the two systems might alternate when the moon is undetectable, and eels might use the compass to maintain a course learned during previous new moon phases. These hypotheses require further research.

Glass eels oriented towards the moon azimuth only at new moon (when the moon was always above the horizon), and partially during the first quarter but with lower precision (Fig. 13). However, during the first quarter, this relationship disappeared (when the moon was below the horizon). Glass eels also swam at significantly higher speed when the moon was above the horizon. These results suggest that glass eels only detect the position of the moon when it is above the horizon at new moon. However, the presence of the moon above the horizon is important but it is not the only factor that plays a role. When the moon is above the horizon, the moon phase also matters. The orientation behaviour of the glass eels changed according to the 3 moon phases during which the moon was above the horizon: third quarter, new moon and first quarter. During third quarter, despite the moon being

above the horizon during half of the tests, the glass eels did not follow the direction of the moon. Similarly, during first quarter, glass eels had a common orientation direction when the moon was above the horizon, but they did not orient towards the moon azimuth with the same precision as observed during new moon.

3.4.1 Hypothetical mechanism for the detection of the moon azimuth

The behaviour of the glass eels showed lunar-related patterns in orientation only when the moon was invisible to them (at new moon). Therefore, the mechanism involved in the detection of the moon azimuth during the day cannot be visual. The moon's gravitational pull (1) and the change in electric flux caused by the moon (2) represent two other possible mechanisms explaining glass eel orientation towards the moon azimuth.

The gravitational pull of the moon has significant effects on large masses, such as water in the oceans. It has a much smaller effect on small masses, such as planktonic organisms. The gravitational pull of the moon on a person weighing 80 Kg is 0.0027 N, which is 105 lower than the gravity of the Earth (785 N) (Bevington, 2015). Thus, although theoretically possible, observable biological effects of the moon phase on the glass eel behaviour are probably not due to the lunar gravitational pull.

The moon generates electrical disturbances at the level of the Earth's surface, which depend on the relative positions of the moon, the Earth and the sun (Bevington, 2015) (Fig. 15). During full moon (Fig. 15B), the moon faces the dark side of the Earth (moon above the horizon only at night). During this phase, the Earth is between the moon and the sun. Consequently, the motion on its own orbit causes the moon to cross the Earth's magnetotail (Fig. 15B) and, in doing so, disturbs it. The Earth's magnetotail contains an earthward-

directed electric field (Zelenyi, Artemyev and Petrukovich, 2010), and when the moon passes through it, there is an ion exchange between the moon's surface and the Earth's magnetotail (Kimura and Nakagawa, 2008). This phenomenon causes variations in the electric field at the level of the Earth's surface. Measurements of electric fields during the lunar cycle revealed that, during full moon nights, there is a positive voltage increase from ≤ 1 V/m to 1-2 V/m, with peaks up to 16 V/m (Bevington, 2015). A similar electric disturbance also occurs at new moon, but it is caused by a different astronomical process (Kimura and Nakagawa, 2008; Bevington, 2015). At new moon, the moon is between the Earth and the sun (Fig. 15A). The solar radiation (solar wind) impacts the surface of the moon and creates a lunar wake that propagates downstream towards the Earth (Fig. 15A). The obstruction of the solar wind causes an accumulation of negative electric charges on the side of the moon facing the Earth (anti-solar side) (Kimura and Nakagawa, 2008). Thus, the moon becomes an "electric dipole" propagating a negatively charged electrical field downstream (Kimura and Nakagawa, 2008) to the Earth's surface during daytime. These electrical phenomena potentially influence the behaviour of many electrosensitive animals both on land and in the aquatic environment (Bhattacharjee *et al.*, 2000; Mercier *et al.*, 2011; Bevington, 2015; Morley and Robert, 2018).

Clear examples of biological effects of weak electric fields comparable to those caused by the moon come from spiders, which use atmospheric electricity to take off from the ground and fly hundreds of kilometers, a phenomenon known as "ballooning" (Morley and Robert, 2018). These electric fields could also affect marine animals, as seawater is a conductive medium (Bevington, 2015). This could also explain why biological phenomena such as spawning of multiple deep-sea species are correlated with the lunar cycle (Mercier *et al.*,

2011), at depths to which the moon's illumination penetrates at very low intensities during dark nights, and only in clear water (Kaartvedt, Langbehn and Aksnes, 2019). Interestingly, the glass eels observed in this study showed a preferred orientation direction towards the azimuth of the moon during daytime at new moon, which is when the electrical field of the new moon impacts the Earth's surface.

Eels are among the taxa of marine organisms that are electrosensitive. Previous work investigating the effect of weak electric fields (<1 V/m) on the orientation of juvenile American eels (*A. rostrata*) revealed that, when swimming in a square arena under artificial electric fields, eels turned towards the anode (negative charges), and that reversing the polarity of the electric field inverted their turning direction (Zimmerman and McCleave, 1975). The authors also suggested that eels could use electric fields present in the ocean for orientation. Similar experiments were conducted on the European eel (using adult eels), although such high sensitivity was not observed (Enger, Kristensen and Sand, 1976). The results from Zimmerman and McCleave are consistent with the hypothesis that glass eel orientation towards the moon azimuth could possibly involve the perception of lunar cycle-related electric fields.

The lack, or partial lack, of moon-related orientation during third quarter and first quarter (Figure 13B - D) is also consistent with the electric field hypothesis because during these lunar phases there are no electric disturbances of the moon to the Earth's surface (Bevington, 2015). In future work we intend to investigate whether glass eels orient towards the moon during full moon nights using infrared cameras, and conduct experiments on glass eels' sensitivity to electric fields comparable to those caused by the moon.

3.4.2 How orienting towards the moon could help glass eels during the pelagic landward migration

Following the azimuth of the moon at new moon could be play a role in the recruitment of the European eel across Europe.

The European eel constitutes a single panmictic population (Palm *et al.*, 2009; Als *et al.*, 2011) that inhabits marine and freshwater habitats from Northern Norway to Morocco (Tesch, 1977). The entire population spawns in the same area in the Sargasso Sea (Johannes Schmidt, 1923; Miller *et al.*, 2015). This means that the European eel must possess a generic orientation mechanism to reach the continent while also supporting latitudinal spread across Europe. At new moon in Austevoll, Norway (where the tests were conducted), during the period when glass eels arrive at the coast (March-May), moonrise is approximately eastward (Fig. 14C). The moon then moves on its path above the horizon all the way southward and sets below the horizon approximately westward (Fig. 14C). This means that swimming towards the moon azimuth when the moon is above the horizon at new moon would lead to an average swimming direction towards the South (Fig. 14C). This average southward orientation direction at new moon is consistent with our observations: glass eels significantly oriented towards the moon azimuth and this corresponded to a southward direction (Fig. 14A). At a larger scale, the direction of the moonrise and the moonset at new moon during the period of arrival of glass eels does not change much with latitude. In the Canary Islands (southernmost point of the distribution of the European eel) (Schmidt, 1931; Lorenzo Perera, Jiménez Medina and Zamora Maldonado, 1999), the moon still rises eastward and sets westward (Fig. 14C). Thus, the average orientation direction of glass eels would always be South during this moon phase,

independent of latitude (Fig. 14B - C). Additionally, during full moon at night, the moon has the same path compared to that during new moon; it rises East, it moves southwards, and it sets in the West. Thus, if glass eels have similar moon-driven orientation during full moon nights (when the electrical disturbance of the moon is the strongest, Fig. 14B), they would swim southward for approximately 8 days a month (~4 days of new moon, ~4 days of full moon), when the moon is above the horizon. This is consistent with the timing of recruitment of many species of glass eel, which peaks both at new and full moon (De Casamajor, Bru and Prouzet, 2001; Yamamoto, Mochioka and Nakazono, 2001; Jellyman and Lambert, 2003).

Following from the above, it is possible to develop several working hypotheses about recruitment of glass eels to the European coasts (Fig. 14B). Glass eels metamorphosing on the continental slope at the northern edge of the North Sea (Fig. 14A, location a) need to actively swim towards the south to exit the Norwegian Atlantic Slope Current (NwASC) (which otherwise would transport them to the Arctic) and enter the North Sea. The NwASC flows between the Faroe Island and Scotland and travels northward following the North Sea and the Norwegian continental slope, at an average speed above 30 cm/sec (Orvik and Niiler, 2002). From our in situ data, we observed that glass eels were swimming at 3.02 ± 0.68 cm/sec (mean \pm SD). Laboratory measurements on the critical speed (U_{crit}) of glass eels indicate higher values of 11-13 cm/sec (Wuenschel and Able, 2008). Swimming to the South, towards the moon azimuth, would play an important role especially at the boundary areas of the NwASC, where exiting the current or drifting with it could make the difference between entering the North Sea and successfully recruiting to the coast, or drifting to the Arctic.

In the central part of the species' distribution (Fig. 14B, location b), orienting towards the South could facilitate migration over the continental Celtic Shelf and the Porcupine basin, and towards the Bay of Biscay (Fig. 14A location b). This bay is a critical recruitment area for the European glass eel and it is where the largest glass eel fishery in Europe takes place (87 % of all European glass eel fished) (Beaulaton and Briand, 2007).

Further South, glass eels drifting offshore off the coast of Portugal would encounter the Portugal Coastal Current (PCC) (Martins, Hamann and Fiúza, 2002). Here, a wide (1000 km) and slow (10-30 cm/sec) current flows steadily, year-round, towards the South (Wooster, Bakun and McLain, 1976; Batteen *et al.*, 2000). Under these oceanographic conditions, swimming towards the moon could shorten the duration of the journey of the glass eels arriving from more northern areas and migrating to the Canary Islands. However, it is important to consider that over the latitudinal range of the European glass eel distribution, the altitude (zenith) of the moon significantly increases at lower latitudes, making the moon a less horizontal directional cue at lower latitudes. Thus, moving more South than the Canary Islands, the moon would reach altitudes over 80° above the horizon, and much of its directionality would be lost. We will test these scenarios and hypotheses in future work using numerical models.

3.5 Methods

3.5.1 Animals and maintenance

Glass eels were collected using hand nets at several estuarine streams located around the Austevoll archipelago (Norway) (Fig. 11), before they migrated into freshwater. The eels

were found under rocks and sediment, at low tide, and the majority of them were collected from the stream estuaries of Stolmen (60.0082 N and 5.0788 E, Fig. 11E) and Vasseide (60.1122 N and 5.2298 E, Fig. 11F). The eels were collected during the recruitment period at the estuarine streams, between March and April of 2015 and 2017. None of the animals used in this study were pigmented (developmental stage: V-VI (Tesch, 1977)), and they did not have food in the gut.

Glass eels were kept in 20 L maintenance tanks, where they were re-acclimated to near full salinity seawater (32 ppt) after capture. They were kept in aerated aquaria in a temperature-controlled room set to ambient conditions similar to those that the glass eels encountered when they arrived at the coast and would experience during the *in situ* deployments of the DISC (ranging between 6 and 10 °C). Animals were not fed (they were at the pre-feeding stage) and were kept in 14h light and 10h dark cycle (following the daylength at the study location during the observation period). Two-thirds of the volume of each aquarium was replaced with filtered seawater every 48 hours to maintain water quality. The seawater was provided by the filtering system at the Institute of Marine Research's Austevoll Research Station, which collects seawater from the Langenuen fjord at a depth of 160 m. Before being used for the deployments at sea, glass eels were taken from the large aquaria and placed in individual 500 mL white plastic containers filled with seawater at the same temperature as the aquaria. These cups were kept with the lid on but non-sealed and in a cooler to maintain temperature during transportation to the deployment sites (Fig. 11).

3.5.2 *In situ* observations

For all of the tests conducted *in situ*, we used the Drifting In Situ Chambers (DISCs) (Paris *et al.*, 2008, 2013) (Fig. 10), a drifting transparent circular arena that was deployed in

Norwegian coastal areas near where the eels were collected (Fig. 11). The DISC has an acrylic structure including a circular chamber, transparent to both small-scale water movement and light. A drogue connected to the bottom of the acrylic frame holding the chamber allows the DISC to drift with the current. A fine braided line attaches the top of the acrylic frame to a surface float, which allows easy recovery and re-deployment of the DISC. The chamber in which individual glass eels swam one at a time was 40 cm wide (diameter) and 15 cm deep. The chamber was semi open, as the bottom was rigid and made of acrylic, while the walls and the top were made of transparent fine mesh. The mesh is preferred to a rigid acrylic wall because it allows water and dissolved gas exchange, assuring that the fish in the arena could detect potential chemical cues and that the dissolved oxygen level would not decrease during deployments.

The behaviour of glass eels in the DISC was observed using a GOPRO HERO 4 camera. The device is also equipped with a HOBO Pendant® Temperature/Light 64K Data Logger - UA-002-64, a GPS locosys gw-60, three analog compasses and a Star-Oddi DST Magnetic digital compass. The GOPRO camera records the behaviour of glass eels viewed from underneath the chamber, looking towards the water surface (Fig. 10B). The digital compass is placed on the bottom plate of the frame of the DISC, oriented on the same axis as the camera. The analog compasses are attached to the acrylic poles of the DISC frame and placed below the circular arena (Fig. 10B). This positioning eliminates the possibility that the compasses are a visual reference for the eel.

The Orientation With No Frame of Reference (OWNFOR) (Paris *et al.*, 2008) approach was applied to characterize the orientation of the glass eels in the apparatus while it was drifting.

3.5.3 Deployments *in situ*

The experiments were designed to investigate whether glass eel orientation was related to the lunar cycle. Thus, DISC deployments were conducted during the 4 main moon phases: full moon, third quarter, new moon and first quarter. Data on moon position, moon illumination and moon phase were obtained from the Norwegian calculator www.timeanddate.com/moon (Copyright © Time and Date AS 1995–2018). Data about the tide were obtained from the Norwegian Mapping Authority (www.kartverket.no). All of the deployments were conducted during the daytime.

The purpose of each trial with the DISC was to observe the orientation of glass eels, one at a time. Each glass eel was placed in the circular arena while the DISC was held semi-submerged along the side of a small boat. Once the glass eel was placed in the chamber, the lid was closed and secured using soft plastic tubes. The DISC was then gently released until it reached the depth at which it would drift throughout the test. The depth at which the glass eel drifted ranged between 4-5 m for all of the experiments, which is consistent with the depth range at which glass eels migrate at sea (Creutzberg, 1961). Each animal was video recorded for 15 minutes, at the end of which the DISC was recovered and the glass eel was replaced with a new one. The first 5 minutes were considered as an acclimation period; the orientation behaviour of glass eels was observed during the last 10 minutes of each trial (Paris *et al.*, 2013; Cresci, Paris, *et al.*, 2017).

The DISC was deployed in the fjords of Langenuen (Northeast of Austevoll, 60.09 N, 5.28 E; saltwater) and in Stolmen (Southwest of Austevoll, 60.00 N, 5.04 E) (Fig. 11), where it

drifted in water that was 70-100 m deep, 300-500 m from the coast, at water temperatures ranging between 6-10 °C.

3.5.4 Data analysis

The orientation of glass eels was determined through the analysis of the GOPRO images, tracking the position of the head of the eel in the circular arena every second for 10 minutes (a schematic diagram of all the steps of the circular analysis are in figure S1). The DISC was allowed to rotate, and the position of the eels with respect to the Earth's magnetic North was monitored using the digital compasses. The video frames were processed using the DISCR tracking procedure, utilizing R and a graphical user interface provided by imageJ software (Irisson, Guigand and Paris, 2009; Irisson *et al.*, 2015). Using this tracking procedure, we collected the positional data (in units of magnetic degrees) of the glass eel with respect to the center of the chamber, which were considered as bearings. The images were geo-referenced with respect to the geomagnetic cardinal points, against the reference of the digital compass. The code utilized is available at the web page Drifting In Situ Chamber User Software in R (<https://github.com/jiho/discr> written by Jean-Olivier Irisson (Université Pierre et Marie Curie UPMC), released under the GNU General Public License v3.0.

Data analysis consisted of two steps. First, the mean orientation of each individual was computed from the bearings collected by the video tracking analysis. The mean of 600 data points, which represent the bearing of the fish in the drifting chamber at each second (one position/sec over 10 min period), as the orientation of each individual (Paris *et al.*, 2008; Irisson, Guigand and Paris, 2009; Paris *et al.*, 2013; Faillettaz *et al.*, 2015). Each

bearing of the eel was corrected with respect to the magnetic North using the digital compass. At the end of this procedure, we used the corrected tracks to compute the magnetic bearing of the glass eel, defined as the direction of motion of the fish (in degrees) with respect to the magnetic North.

The ability of each individual to keep a specific magnetic bearing while swimming in the DISC was considered evidence of directionality (Paris *et al.*, 2008; Irisson, Guigand and Paris, 2009). The significance of the directionality was assessed using the Rayleigh test of uniformity for circular data, and the level of convergence of the bearings towards one direction using the Rayleigh test r value (from 0 to 1) (Curry, 1956; Irisson, Guigand and Paris, 2009; Irisson *et al.*, 2015). The Rayleigh's r values indicate the concentration of the positions of the fish in a specific section of the circular arena, or, in other words, the accuracy of the directionality of the swimming behaviour observed. An outcome was considered statistically significant when $p < 0.05$ ($\alpha = 0.05$) (Irisson, Guigand and Paris, 2009).

After assessing the orientation of each individual, the next step of the analysis focused on evaluating whether the eels tested in the DISC have a common, collective trend in orientation (i.e. whether they go towards a common direction). To accomplish this step of the analysis, we applied the Rayleigh test of uniformity to the values of all the mean individual bearings, testing whether the frequency distribution of the directions displayed by the individuals was significantly different from random (95% confidence interval, $\alpha = 0.05$) (Irisson, Guigand and Paris, 2009).

The swimming speed of the eels was calculated from the video tracks, dividing the distance that the animal swam at every frame in the chamber by time observed (1 s). The average

of all the speed values calculated every second throughout one deployment was considered as the average speed of one glass eel.

3.6 Figures

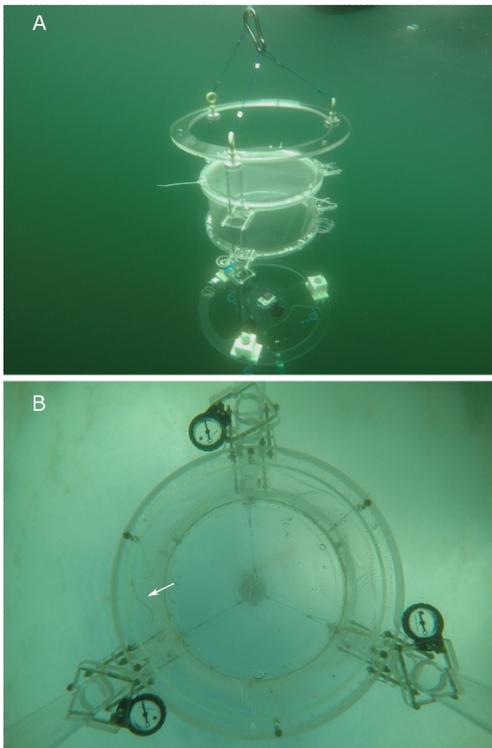


Figure 10 - DISC used to observe the glass eels (*Anguilla anguilla*) *in situ*.

A: view of the DISC drifting in the North Sea. The acrylic frame, the circular behavioural arena and the GOPRO camera placed underneath are visible in the picture. **B:** view from the GOPRO camera underneath the arena. The white arrow points towards a glass eel swimming in the arena. Below the arena, attached to the poles, 3 analog magnetic compasses which were used, together with a digital compass, to monitor the orientation of the glass eels with respect to the magnetic North.

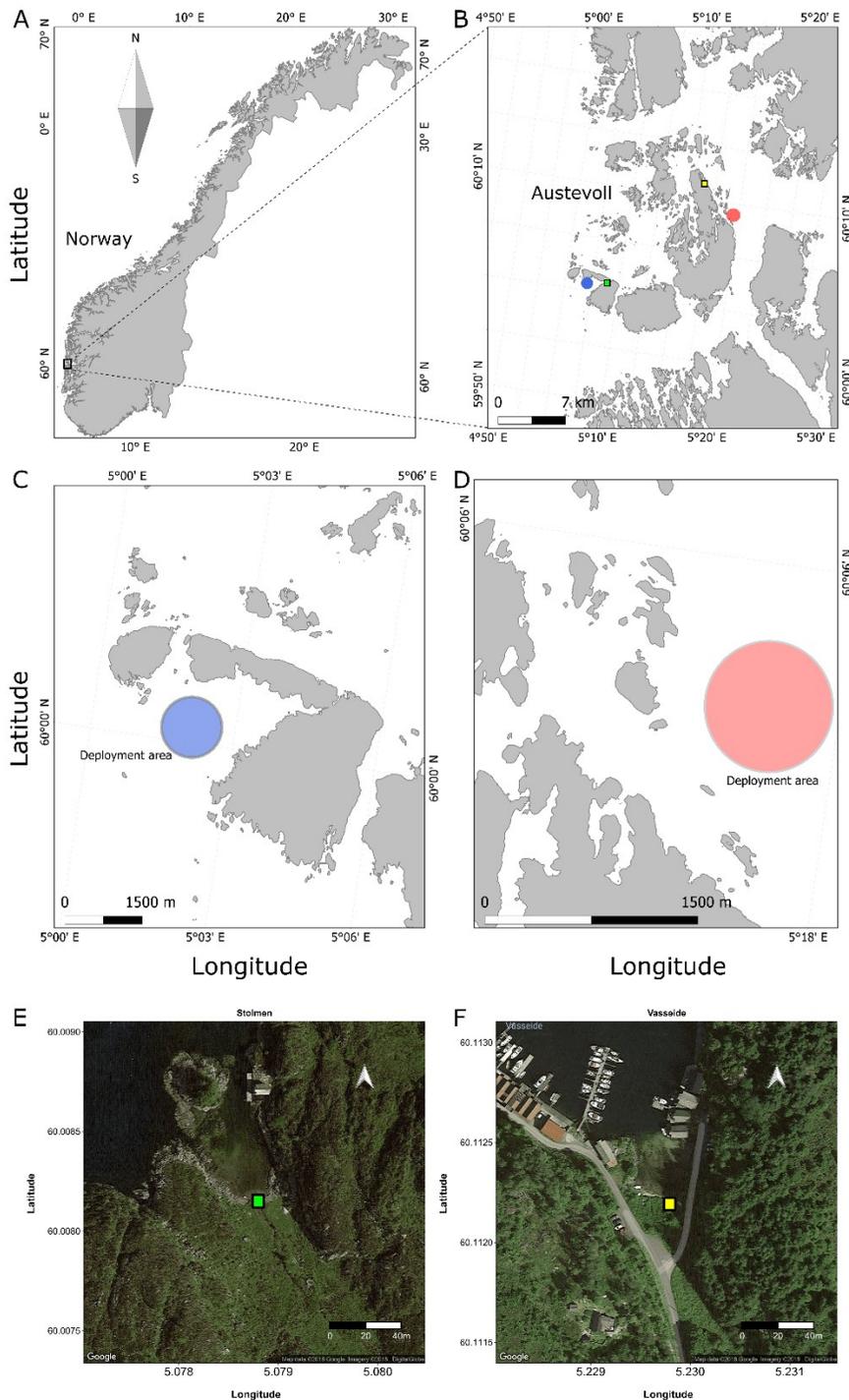


Figure 11 - Figure 2. Map of the locations where glass eels (*Anguilla anguilla*) were observed drifting in the DISC in situ.

A. map of Norway. B. map of the archipelago of Austevoll. C. and D. maps of the deployment areas of Stolmen (blue) and Langenuen fjord (red). E. and F. estuaries where glass eels were collected: Stolmen estuary (green box) and Vasseide estuary (yellow box).

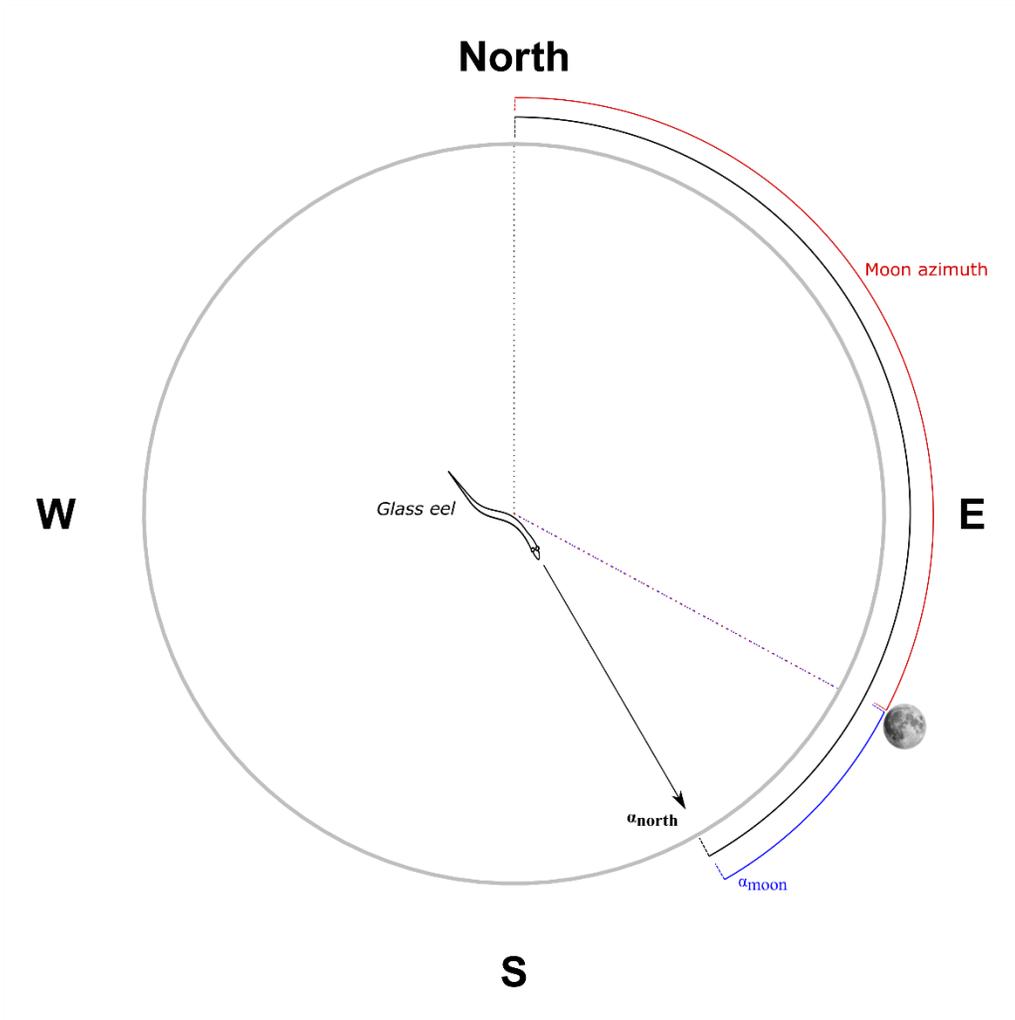


Figure 12 - Diagram of the circular variables considered in this study.

At the center of the circle, a glass eel (*Anguilla anguilla*) swimming at sea on the horizontal plane, with respect to the Earth's magnetic North, East, South and West. The black arrow shows the orientation direction of the eel (α_{north}), which is the angle between the mean bearing of the eel and the magnetic North (black arch). The **moon azimuth** is the red arch, which is the angle between the magnetic North and the orthogonal projection of the moon onto the horizon. The blue arch is the angle between the orientation direction of the eel and the moon azimuth (α_{moon}) is the angle between the orientation of the eel and the moon azimuth.

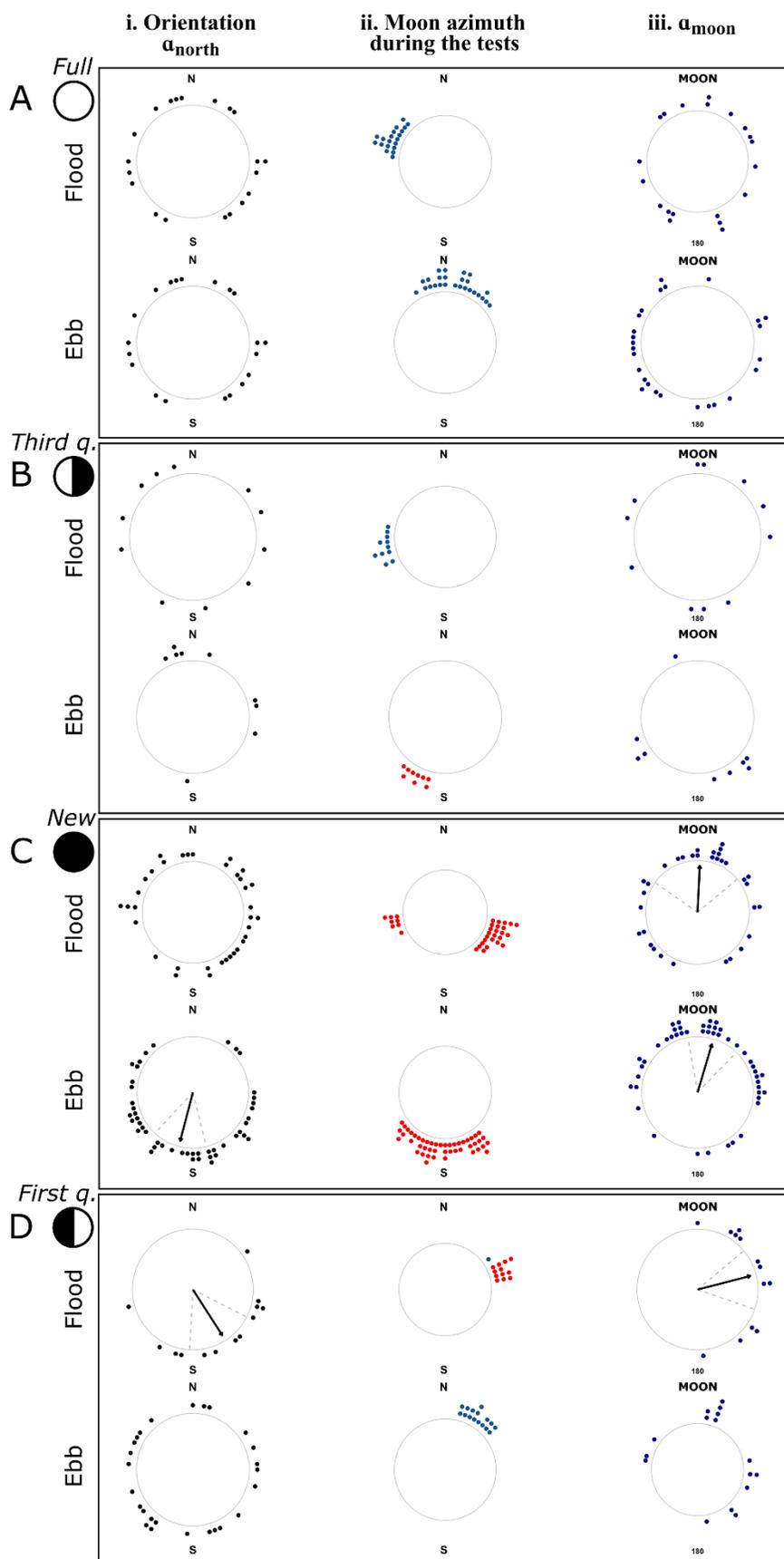


Figure 13 - Orientation of glass eels (*Anguilla anguilla*) at sea and moon-related orientation.

Orientation of the glass eels, the azimuth of the moon, and orientation with respect to the azimuth of the moon (as described in Fig. 3), during each of the 4 main moon phases (**A**: full moon, **B**: third quarter, **C**: new moon, **D**: first quarter) and during each tidal phase (flood/ebb). The frame of reference of each plot is indicated: **N** = magnetic North, **MOON** = direction of the moon at the horizon (azimuth). In the first and third column, significant preferences of orientation direction are shown by a black arrow starting from the center of the circle and pointing towards the mean orientation direction. Dashed grey lines are the 95% confidence intervals around the mean. The circular plots are empty when there was no significant preference of orientation direction. The first column **i. Orientation (α_{north})** shows the orientation of glass eels with respect to the magnetic North (0°) and South. Each black data point represents the mean bearing of a single glass eel. Only the glass eels that had a significant individual orientation are presented. The second column, **ii. Moon azimuth during the tests**, shows the direction of the moon azimuth during each DISC deployment. The points are blue if the moon was below the horizon and red if the moon was above the horizon. The third column, **iii. (α_{moon})**, shows how far the glass eels were orienting from the moon azimuth (**MOON**, top of the plot= 0°). The angular difference between the orientation of each eel and the azimuth of the moon is shown as a navy-blue data point. Significant collective orientation towards the direction of the moon is shown as a black arrow pointing towards the top of the plot (at new moon and first quarter).

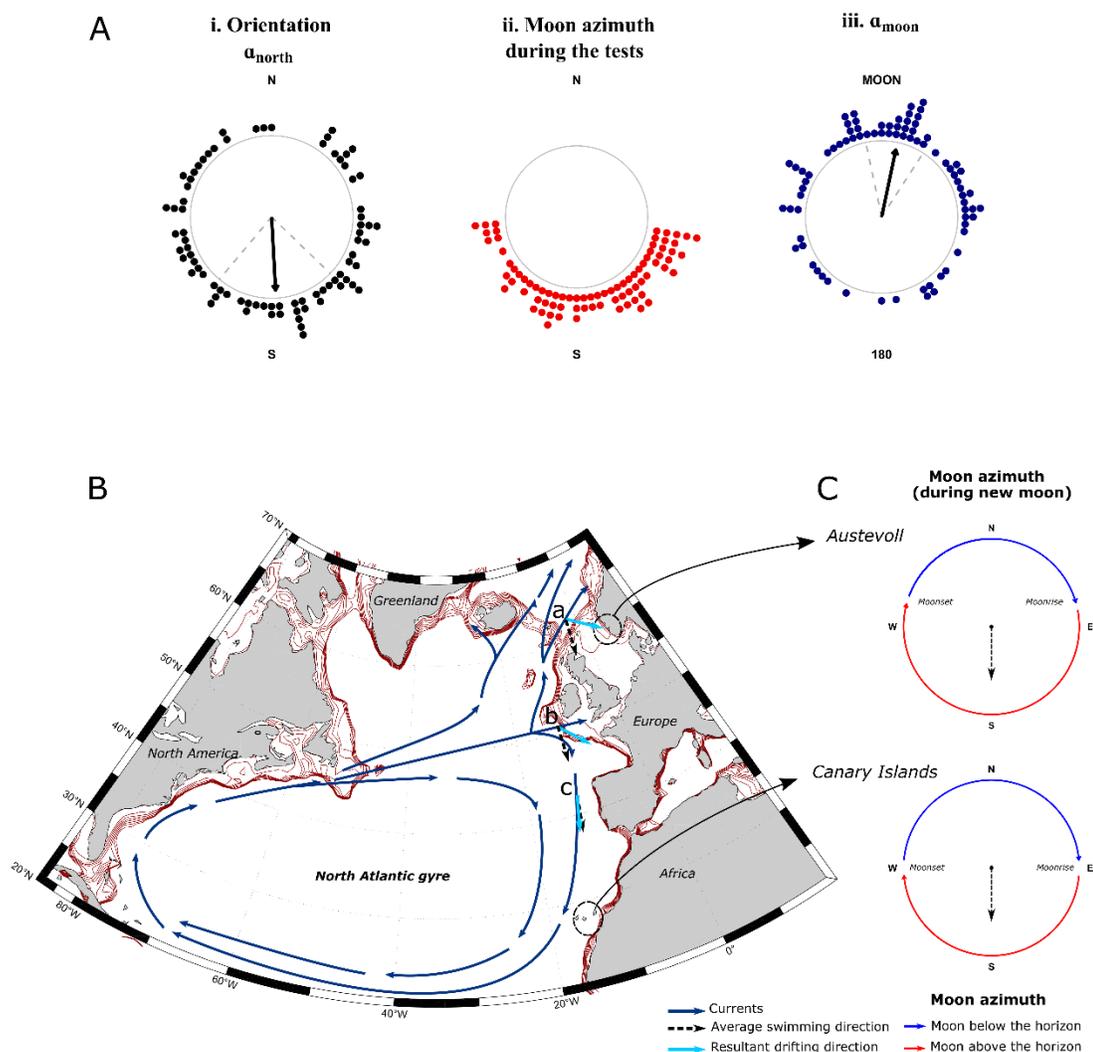


Figure 14 - Schematic illustration of the potential advantages of the moon-driven orientation during the migration of European glass eels (*Anguilla anguilla*).

A: Pooled data of collective orientation (α_{north}) (**A.i**), moon azimuth during the tests (**A.ii**) and orientation with respect to the moon azimuth (α_{moon}) (**A.iii**) at new moon. The circular plots have the same features as described in Figure 5. **B:** map of the North Atlantic Ocean with a simplified schematic representation of the great North Atlantic gyre and the main surface currents in proximity of the European continental shelf (navy-blue arrows). The continental shelf is visible from bathymetric red lines (0-700 m, with 100 m interval between the lines). **C:** simplified plots of the moon path at the horizon at Austevoll, Norway (where the experiments were conducted and close to the Northern limit of the area of distribution of glass eels), and at the Canary Islands, West of Africa (Southernmost point where glass eels are found) during the new moon periods of March-May 2017. The plots show approximate moonrise and moonset with respect to the magnetic North (with around

10° of possible variation). Red arrows show the moon path at the horizon when the moon is above the line of the horizon, and the blue arrows when the moon is below the line of the horizon. Dashed black arrows in both **B** and **C** show the average swimming direction that the glass eels would have swimming towards the direction of the moon when the moon is above the horizon, at new moon (South, as showed by the *in situ* orientation data displayed in **A**). In **B**, we report 3 possible case scenarios of the drifting direction (sky-blue arrows) resulting from the passive drifting caused by the currents and the moon-driven orientation (South oriented). **B.a** shows that swimming towards the moon could potentially help glass eels to exit the Norwegian Current, enter the North Sea and arrive at the coast. **B.b** shows that the moon-oriented swimming could help the glass eels coming from the Gulf Stream to arrive at the Bay of Biscay, the area with the highest recruitment of glass eels. **B.c** shows that the moon-oriented swimming could help glass eels to reach also the Canary Island, at the Southernmost point of their distribution.

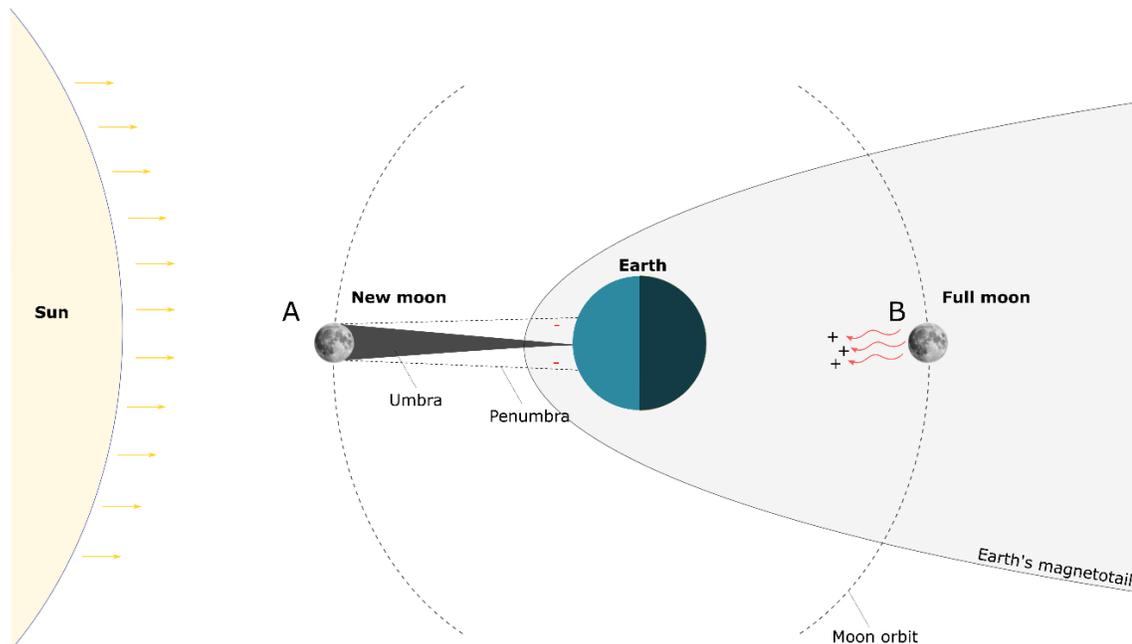


Figure 15 - Diagram of the electrical disturbance of the moon to the Earth's surface (modified from: Bevington, 2015).

A: at new moon, the moon faces the bright side of the Earth (moon above the horizon during daytime) and the solar wind impacts the moon. This creates an umbra and a penumbra, which are shadows of the moon body carrying negative electric charges to the Earth's surface. **B:** at full moon, the moon faces the dark side of the Earth (moon above the horizon at nighttime). Following its orbit, the moon impacts the Earth's magnetotail and creates an electric disturbance of positive charges propagating towards the Earth's surface.

Chapter 4. Glass eels (*Anguilla anguilla*) imprint the magnetic direction of tidal currents at their juvenile estuaries

4.1 Overview

The European eel (*Anguilla anguilla*) hatches in the Sargasso Sea and migrates to European and North African freshwater. As glass eels, they reach estuaries where they become pigmented. Glass eels use a tidal phase-dependent magnetic compass for orientation, but whether their magnetic direction is innate or imprinted during migration is unknown. We tested the hypothesis that glass eels imprint their tidal-dependent magnetic compass direction at the estuaries where they recruit. We collected 222 glass eels from estuaries flowing in different cardinal directions in Austevoll, Norway. We observed the orientation of the glass eels in a magnetic laboratory where the magnetic North was rotated. Glass eels oriented towards the magnetic direction of the prevailing tidal current occurring at their recruitment estuary. Glass eels use their magnetic compass to memorize the magnetic direction of tidal flows. This mechanism could help them to maintain their position in an estuary and to migrate upstream.

Supplementary material available online:

<https://www.nature.com/articles/s42003-019-0619-8#Sec11>

4.2 Background

The European eel (*Anguilla anguilla*) is a migratory species that crosses the Atlantic Ocean twice during its life (Fig. 16). After hatching in the Sargasso Sea (Johannes Schmidt, 1923), eel leptocephali larvae move with the gulf stream more than 5000 km until they reach the continental slope of Europe (Tesch, 1977; Miller *et al.*, 2015). There, they metamorphose into the post-larval transparent glass eel (Fig. 16) (Tesch, 1980). At this stage, glass eels migrate across the continental shelf to the coast (Deelder, 1952; Tesch, 1977). After reaching the coast, glass eels enter estuaries, where some of them continue their migration upstream into freshwater (Tzeng *et al.*, 2000). The eels that enter freshwater spend most of their lifetime (5–25 years) there, growing first into the adult yellow eel stage, and then into silver eels (Fig. 16) (Tesch, 1977). Silver eels then navigate back to the Sargasso Sea where they spawn and die (Fig. 16) (Johannes Schmidt, 1923; Righton *et al.*, 2016; Bégué-Pon *et al.*, 2018).

The European eel is a commercially important species that is critically endangered [International Union for Conservation of Nature (IUCN)]: eel populations have declined precipitously since the 1980s (Dekker, 2003a; Jacoby *et al.*, 2015; ICES, 2018). Much research on the conservation and management of eels has been undertaken (Righton and Walker, 2013; Castonguay and Durif, 2016), driven to some extent by the requirement for member states of the European Union to develop management plans for the recovery of eel populations. The arrival of the early life stages to estuaries is an important phase of their migration, and the number of recruiting glass eels has been consistently declining over the past decades (Dekker *et al.*, 2003; ICES, 2018). Thus, a better understanding of the

dynamics of this step of the migration, including deeper knowledge of the orientation mechanisms involved, is needed.

Glass eels use multiple spatial and sensory cues for orientation (De Casamajor, Bru and Prouzet, 1999; Edeline, Dufour and Elie, 2005; Cresci, Paris, *et al.*, 2017), which could hypothetically be imprinted and used years later during their return journey to the spawning areas. It is possible that eels imprint spatial information from the environment at several steps during their migration, such as at the time of hatching or during the journey to the European coast. Imprinting is associated with a broad variety of processes in animals, including food and habitat preference, host selection in parasitic animals, and homing (Immelmann, 1975; Dodson, 1988; Brothers and Lohmann, 2015). Imprinting is a fast-learning process that typically occurs during a specific life history phase (Lorenz, 1935; Immelmann, 1975; Dodson, 1988). Imprinting of spatial cues, such as olfactory cues in salmon (Dittman and Quinn, 1996), occurs in several aquatic animals. Animals can also imprint information from the magnetic field of the Earth. Aquatic species such as Pacific salmon (*Oncorhynchus nerka*) (Putman *et al.*, 2013) and sea turtles (*Caretta caretta*) (Brothers and Lohmann, 2015) imprint specific features of the Earth's magnetic field and use this to orient during migration. The European eel is a long distance migrator that also uses magnetic fields to orient during migration (Tesch, 1974; Durif *et al.*, 2013). However, whether imprinting of magnetic cues occurs, either at the larval or glass eel stage (or both), remains unknown.

Glass eels recruit at tidal estuaries along the European coast, where they are exposed to the alternation of ebbing and flooding tidal currents. In aquatic environments, fish display an unconditioned response to water currents (rheotaxis), which can be positive (the fish swims

into the current) or negative (the fish swims in the same direction as the current) (Arnold, 1974; Chapman *et al.*, 2011). Rheotaxis is a major component of fish orientation in tidal estuaries in which currents can be fast and visibility low (Arnold, 1974). In such situations, fish use rheotaxis for both upstream migration and upstream-oriented station holding behavior to minimize energy expenditure in flowing water (Baker and Montgomery, 1999). Glass eels display both of these rheotactic behaviors when they recruit to estuaries, showing rhythmic patterns of positive or negative rheotaxis synchronized to the tidal phase (Bolliet *et al.*, 2007). This tidal-dependent orientation is important during migration, as glass eels exploit tidal flows to enter brackish and freshwater using “selective tidal stream transport” (STST) (J. D. McCleave and Kleckner, 1982; Wippelhauser and McCleave, 1987; Beaulaton and Castelnaud, 2005; Bolliet and Labonne, 2008). This constitutes a deeply rooted behavior in many species of fish and invertebrates inhabiting tidal estuaries (Gibson, 2003).

In order to orient with respect to water flow, fish rely on multiple sensory cues, including visual, vestibular and tactile (Bak-Coleman *et al.*, 2013; Montgomery, Bleckmann and Coombs, 2013). Rheotaxis in fish is also influenced by changes in the magnetic field at intensities as low as the Earth’s (Cresci, De Rosa, *et al.*, 2017; Cresci *et al.*, 2018), suggesting that magnetic fields could be one of the reference cues that fish use to orient in the presence of flowing water. Glass eels exhibit rheotaxis in the presence of tidal currents and sense magnetic cues: our previous work showed that glass eels use the magnetic field for orientation using a magnetic compass mechanism, switching magnetic direction according to an endogenous tidal rhythm in the absence of a current (Cresci, Paris, *et al.*, 2017). Specifically, eels oriented to the magnetic south with the ebb tide and to the north

with the flood tide (Cresci, Paris, *et al.*, 2017). While these results show that glass eels use a magnetic field-based compass mechanism, the stimuli that cause glass eels to swim towards specific magnetic directions at this stage of the migration are unknown.

We tested the hypothesis that the magnetic orientation direction of glass eels (i.e. the orientation direction relative to the direction of the magnetic field) is imprinted from the tidal currents at the estuaries where they recruit. To test this hypothesis, 222 glass eels were collected at stream estuaries flowing towards different cardinal directions (north, southeast, south and northwest) (Fig. 17). The magnetic orientation of glass eels was observed in a magnetic laboratory facility, using an electric coil-system (Fig. S1) that modified the direction of the magnetic field (Fig. S2), while depriving the glass eels of any other external cues that they could use for orientation.

4.3 Results

The magnetic orientation behavior of glass eels was observed following the methods described in our previous work (see methods section).(Cresci, Paris, *et al.*, 2017) In brief, a circular transparent chamber (Drifting In Situ Chamber, ‘DISC’; Fig. S1B) (Paris *et al.*, 2008) was submerged in a circular black tank in a magnetic laboratory (‘MagLab’; <https://fishlarvae.org/facilities/magnetoreception-test-facility/>) located in Austevoll, Norway (60.1175 N, 5.2118 E; Fig. S1). The MagLab is equipped with a triaxial electric coil system that allowed us to manipulate the magnetic field to which glass eels were exposed in the experimental tank.

Glass eels were collected at four stream estuaries located around the Austevoll archipelago (Fig. 17), before they migrated into freshwater. The tidal estuaries were selected according

to their geographical features, as we searched for estuaries flowing towards different cardinal directions. Glass eels were collected at Vasseide (60.1122 N and 5.2298 E, flowing to the north), Torvesund (60.0294 N, 5.3016 E, flowing to the southeast), Vinnesvåg (60.0088 N, 5.2583 E, flowing to the south), and Stolmen (60.0082 N and 5.0788 E, flowing to the northwest) (Fig. 17). Upstream of these tidal areas (for example, in Torvesund, where the stream changes direction), there was no action of the tides and only pigmented elvers were present. The dataset used in this study is composed of the orientation data of 222 glass eels. These include the re-analysis of the orientation data from our previous work of 49 glass eels tested in the MagLab in 2015 (eels coming from Vasseide), (Cresci, Paris, *et al.*, 2017) and 173 glass eels newly collected from Vinnesvåg, Torvesund and Stolmen (S, SE and NW oriented estuaries; Table 4.1). Glass eels were individually exposed to different configurations of the magnetic field: the magnetic north in the laboratory was rotated towards one of the four cardinal points of the Earth's magnetic field, and each eel was exposed to only one of the four magnetic conditions used in this study (Fig. S2). The orientation of each glass eel was then assessed with respect to the rotated magnetic north in the laboratory (Fig. S2).

Each eel was tested in the magnetic lab between the peaks of high and low tide, during one of the rising/lowering tides (ebb/flood) occurring along the Austevoll archipelago. The magnetic orientation direction of each eel was then analyzed with respect to the magnetic direction of the tidal current occurring contemporaneously at the eel's recruitment estuary. Thus, in our reference system, we considered the magnetic direction of the tidal flow with respect to the Earth's magnetic field) as 0° . Finally, we computed the angular difference

between the magnetic orientation direction of each eel and the magnetic direction of the tidal flow.

The overall proportion of glass eels showing a preferred magnetic orientation direction was 70% (155 out of 222). This proportion however changed depending on the estuary, but it was always above 50% (Table 4.2). Glass eels significantly oriented to the magnetic direction of the prevailing tidal flow that was occurring at their recruitment estuaries during the tests. Their average magnetic orientation direction was 359° ($N = 155$, Rayleigh's $p = 0.0018$, $r = 0.2$; Fig. 18), and it matched the magnetic direction of the incoming tidal flow (0° , Fig. 18).

Table 4.1 - The estuaries where the glass eels (*A. anguilla*) were collected are listed. Orientation of the estuary is the cardinal direction towards which each of the estuaries flows. n: number of glass eels tested in the magnetic laboratory listed by stream of provenience. The dates of the tests are also indicated.

Estuary	Orientation of the estuary	n	Dates of the tests
<i>Vasseide</i>	N	49	16-22 April 2015
<i>Vinnesvåg</i>	S	36	9-16 May 2016
<i>Torvesund</i>	SE	24	13-16 May 2016
<i>Stolmen</i>	NW	113	28 April – 18 June 2017

Table 4.2 - Proportion of glass eels (*A. anguilla*) showing a significant magnetic orientation direction. n: number of glass eels tested in the magnetic laboratory listed by stream of provenance. The Table shows the number of glass eels displaying significant orientation and the proportion of eels that oriented.

Estuary	n	Orienting glass eels	Proportion of orienting eels
<i>Vasseide</i>	49	35	71%
<i>Vinnesvåg</i>	36	20	56%
<i>Torvesund</i>	24	15	62%
<i>Stolmen</i>	113	85	75%
TOTAL	222	155	70 %

4.4 Discussion

Glass eels use their magnetic compass to memorize the magnetic direction of the currents at the estuaries where they recruit. This is evidence that these fish are capable of forming and retaining a “magnetic memory” of the direction of water currents, and to use it to orient in moving water during migration.

Satellite-tracks of loggerhead sea turtles (*Caretta caretta*) and fur seals (*Callorhinus ursinus*) indicate that they detect the downstream direction of currents in open waters, implying the use of magnetic cues (Ream, Sterling and Loughlin, 2005; Mencacci *et al.*, 2010). It was also hypothesized that elasmobranchs, that navigate by detecting electromagnetic fields (Carey, Scharold and Kalmijn, 1990), could sense the direction of oceanic currents through the electricity produced by the friction of water moving over the sea bottom (Kalmijn, 1982). When visual reference points were present, the interaction between the magnetic sense and orientation to water flows was observed in shoaling

zebrafish (*Danio rerio*), which change their orientation in flowing water according to the direction of the magnetic field (Cresci, De Rosa, *et al.*, 2017).

The results described in this study add evidence that eels detect and form a memory of the magnetic direction of currents, supporting the possibility that these fish – and possibly others - have the sensory capacity to integrate magnetic and rheotactic information and use them for orientation. Analogous results involving learning of magnetic cues were reported at a later stage in the life cycle of the eel: silver eels displaced between different locations learned the compass direction of their displacement (Durif *et al.*, 2013). In that study, orientation was dependent on temperature, demonstrating their ability to modulate their response to the magnetic field according to other environmental signals.

The “magnetic memory” of the flows at the recruitment estuaries described in this study might represent a specific case of imprinting. In its original definition (Lorenz, 1935), imprinting describes a learning process that occurs in a restricted, sensitive time of the life of an animal, and that this memory is stable and retained over time. Considering the glass eels, it is possible that they imprint the rhythm of the tide and the magnetic direction of flows during a specific, possibly “sensitive” period, when they first enter the estuary, which constitutes a life history transition from seawater to freshwater physiology. Previous studies on salmon smolts show that imprinting can occur in such transition periods associated with surges in plasma thyroxine (Dittman, Quinn and Nevitt, 1996). Furthermore, otolith analyses revealed that the time that glass eels spend at the estuaries could be very long as some never enter freshwater (Tzeng *et al.*, 2000). Thus, this memory could be retained and used for a significant portion of the life of a glass eel, before the elver stage. However, it is also possible that these results may represent a phenomenon of

continuous ongoing-learning, and that this “magnetic memory” of flows could remain flexible over time. For example, eels might adjust their magnetic heading to varying hydrodynamic conditions at the estuary, or further upstream, where the magnetic direction of the current could change. These are interesting scenarios that we intend to address in future work. Furthermore, the estuaries selected in this study are quite rectilinear systems, but it is possible that highly curvilinear estuaries are harder for glass eels to navigate and future work should investigate whether this affects recruitment to such sites.

In movement ecology, the concept of imprinting has been mostly associated with the return of animals to natal areas (homing). Among aquatic species, the most studied case is that of salmon. As smolts, salmon record the specific combination of odors from their natal stream, which they will use as adults to guide their spawning migration upstream (Dittman and Quinn, 1996; Ueda, 2012). In addition to olfactory cues, there is evidence that salmon imprint magnetic information when they make first contact with seawater, which they use later in life to find the coastal area where their natal estuary is located (Putman *et al.*, 2013). Similarly, sea turtles imprint the geomagnetic field associated with their natal beach, which they use years later to return and nest (Brothers and Lohmann, 2015). In the case of the eel, the magnetic imprinting observed in glass eels has a different importance for their migration. Glass eels learned the magnetic direction of currents at their recruitment estuary. This estuary will be the same as that which they encounter years later during the descent from the stream to start their oceanic spawning migration as adult silver eels. Whether eels keep a “magnetic memory” of their estuary through adulthood, and whether this constitutes a reference to start the marine phase of the spawning migration in the right direction, is unknown. For example, it is possible that adult eels recognize the direction of the

alternating tidal flows experienced as glass eels when they descend the estuary, and that this constitutes a trigger to undertake the last physiological and behavioral changes at the start of their long marine migration. However, this hypothesis needs to be tested in future work.

The use of the magnetic sense to imprint the direction of currents at estuaries could also help glass eels at this earlier phase of their migration. During their estuarine residency, glass eels undergo physiological and morphological changes, developing pigmentation, developing jaws and teeth, and adapting to freshwater (Tesch, 1977). Thus, glass eels need to maximize the energetic efficiency of their swimming behavior at the estuary, both to keep their position and to migrate upstream (Bureau Du Colombier *et al.*, 2007). In environments such as streams, rivers or estuaries, fish orient with respect to the current by using visual and tactile cues (such as the bottom) (Montgomery, Baker and Carton, 1997; Bak-Coleman *et al.*, 2013). Our results show that glass eels also use their magnetic sense as a reference to orient against the direction of the flow. This could help them in several ways, such as keeping the right compass course when visual reference points are lost or obscured (turbid water in rivers or muddy streams), or when there is no physical contact with the substrate. This would be of great importance especially for glass eels recruiting to large, long tidal estuaries such as the Gironde in France, where the effect of the tidal flows extends for kilometers. Moreover, in such large estuaries the water is turbid, and temperature and salinity are subjected to high variability. Thus, the use of the magnetic compass system described in this study would have obvious advantages in such environments, providing a fixed reference for orientation.

Although glass eels oriented along the magnetic direction of tidal currents at their recruitment estuaries, there was unaccounted for interindividual variability (Rayleigh's $r = 0.2$). Variation in orientation response is dependent on the internal state (or behavioral traits) of the animals, which significantly contribute to their decision to move (Nathan *et al.*, 2008). The internal state (often classified as “proactive” and “reactive”, or “migratory” and “non-migratory” phenotypes) plays an important role in the magnetic sense of fish, up to the point that it can make the difference between responding or not responding to magnetic stimuli (Cresci *et al.*, 2018). European eels display significant differences among individuals concerning their tendency to migrate. Experiments using flume tanks showed that glass eels (*A. anguilla*), elvers and yellow eels display different tendencies to migrate and can be separated into migrants that actively move upstream or downstream and non-migrant that do not show a particular tendency to migrate (Bureau Du Colombier *et al.*, 2007; Imbert *et al.*, 2008). It is possible that those glass eels not showing any preferred magnetic orientation direction have a non-migrant phenotype. Future work should repeat these experiments on magnetic imprinting after dividing the glass eels according to their motivation to migrate and dividing migrating glass eels into upstream vs. downstream migrants. This could be accomplished using an experimental setup similar to that used by Bureau Du Colombier and coworkers, with a flume tank connected to upstream and downstream traps (Bureau Du Colombier *et al.*, 2007).

Glass eels learn the features of the environments that they encounter during migration. Future work should investigate whether migrating silver eels that are translocated at the glass eel stage have more difficulty in finding their way towards the sea compared to non-translocated ones. If they do, then the results of this study would be relevant to management

plans that include the restocking of glass eels in freshwater where the population is most depleted.

4.5 Methods

4.5.1 Animals and maintenance

The glass eels were collected in Norway, before their upstream freshwater migration between March and June of 2015, 2016 and 2017. They were caught using hand nets searching under rocks and sediment at low tide. None of the animals used in this study were pigmented (developmental stage: V-VI (Tesch, 1977)) and they did not have food in the gut. Glass eels were kept in 20 L maintenance tanks, where they were re-acclimated to near full salinity seawater (32 ppt) after capture. They were kept in aerated aquaria in a temperature-controlled room set to ambient conditions similar to those of the local Langenuen fjord (ranging between 6 and 10 °C). Animals were not fed (they were at the pre-feeding stage) and were kept in 14 h light and 10 h dark cycle (following the daylength at the study location during the observation period). Two-thirds of the volume of each aquarium was replaced with filtered seawater every 48 hours to maintain water quality. The seawater was provided by the filtration system at the Institute of Marine Research's Austevoll Research Station, which collects seawater from the Langenuen fjord at a depth of 160 m. Before the tests, glass eels were taken from the large aquaria and placed in individual 500 mL white plastic containers filled with seawater at the same temperature as the aquaria and transported to the magnetic laboratory (MagLab) in a cooler to keep the temperature stable.

4.5.2 Experiments and data analysis

The experiments in the laboratory followed the same protocol as described in Cresci et al. 2017 (Cresci, Paris, *et al.*, 2017). The MagLab is designed to study the magnetic orientation of aquatic animals. It is equipped with a triaxial electric coil system (Fig. S1C), with a design described by Merritt et al. (Merritt, Purcell and Stroink, 1983), connected to a power supply (max. 3 A). At the center of the coils, there is a black circular tank made of fiberglass (diameter, 1.40 m; height, 0.90 m; see Fig. S1C) filled with seawater, which is pumped from the sea 300 m away. The building (see fig. S1A) is constructed of nonmagnetic material and is far from any source of magnetic interference (163 m from the nearest electrical disturbance and 365 m from the closest building; Fig. S1A).

For the present study, we used the DISC ('Drifting In Situ Chamber', Fig. S1B) (Paris *et al.*, 2008) as a behavioral chamber, submerged in the circular dark tank (see fig. S1C). The chamber of the DISC in which individual glass eels swam one at a time was 40 cm wide (diameter) and 15 cm deep. The chamber was semi open, as the bottom was rigid and made of acrylic, while the walls were made of transparent fine mesh. The mesh is preferred to a rigid acrylic wall because it allows water and dissolved gas exchange. The top of the chamber was covered with opaque white plastic, which diffused light uniformly in the chamber. Light intensity in the tank was low (0 lum/ft² from HOBO light sensor on the bottom plate of the DISC frame).

The behavior of glass eels in the DISC was observed using a GOPRO HERO 4 camera placed on the bottom plate of the DISC acrylic frame, underneath the chamber (Fig. S1B, Fig. S1D). The DISC was equipped with an analog compass attached to the acrylic poles

of the DISC frame and placed below the circular arena. This positioning eliminates the possibility that the compass would be a visual reference for the eel.

The laboratory is equipped with two nested electric coil systems. One was used to cancel out the horizontal component of the ambient field. With the second coil system, we were able to generate a magnetic field with the same total intensity as the ambient field (48.8 to 50 μT) and to reorient the magnetic north. The intensity and inclination inside the coil were set to match the ambient field (48.8 to 50 μT and 73° , with a deviation of $<1^\circ$).

Each eel was observed for 15 min, with the first 5 min considered as an acclimation period (Paris *et al.*, 2013; Cresci, Paris, *et al.*, 2017), under one of the four simulated magnetic field conditions, with the magnetic north reoriented to the Earth's east, south, west, or north (see Fig. S2 and Table S1). Each glass eel experienced only one of these 4 magnetic conditions (Table S1). Using this approach, we eliminated any nonmagnetic bias that could have influenced the orientation response of the animals. Moreover, the MagLab is designed to cancel out all the possible external cues that animals could use for orientation except for the magnetic field, as the animals are not exposed to water flows, odor plumes, sunlight or any celestial cues. All tests were conducted during daytime. Data about the tide were obtained from the Norwegian Mapping Authority (www.kartverket.no). The direction of flow in the estuaries was assessed in situ using an analog compass.

The orientation of glass eels was determined through the analysis of the GOPRO images, tracking the position of the head of the eel in the circular arena every second for 10 minutes. Magnetic north had a different orientation in the laboratory during each test, and the position of the eels with respect to the magnetic north in the laboratory was monitored using the analog compasses. The video frames were processed using the DISCR tracking

procedure, utilizing R and a graphical user interface provided by imageJ software (Irisson, Guigand and Paris, 2009; Irisson *et al.*, 2015). Using this tracking procedure, we collected the positional data (in units of magnetic degrees) of the glass eel with respect to the center of the chamber, which were considered as bearings. The code utilized is available at the web page Drifting In Situ Chamber User Software in R (<https://github.com/jiho/disc>) written by Jean-Olivier Irisson (Université Pierre et Marie Curie UPMC), released under the GNU General Public License v3.0.

Data analysis consisted of two steps. First, the mean orientation of each individual was computed from the bearings collected by the video tracking analysis. The mean of 600 data points, which represent the bearings of the eel in the chamber at each second (one position/sec over 10 min period), was considered to be the orientation of each individual (Irisson, Guigand and Paris, 2009; Paris *et al.*, 2013). The orientation of each eel was then corrected with respect to the rotated magnetic North induced with the coils in the laboratory.

The ability of each individual to keep a specific magnetic bearing while swimming in the DISC was considered evidence of directionality (Paris *et al.*, 2008; Irisson, Guigand and Paris, 2009). The significance of the directionality was assessed using the Rayleigh's test of uniformity for circular data (Curry, 1956; Irisson, Guigand and Paris, 2009; Irisson *et al.*, 2015). An outcome was considered statistically significant when $p < 0.05$ ($\alpha = 0.05$) (Irisson, Guigand and Paris, 2009).

After having assessed the orientation of each individual, the following step of the analysis evaluated whether the eels tested in the DISC show a significant pattern in their orientation, or whether they go towards a common direction. To accomplish this step of the analysis,

we applied the Rayleigh test of uniformity to the values of all the mean individual bearings, testing whether the frequency distribution of the directions displayed by the individuals was significantly different from random (95% confidence interval, $\alpha = 0.05$) (Irison, Guigand and Paris, 2009).

4.6 Figures

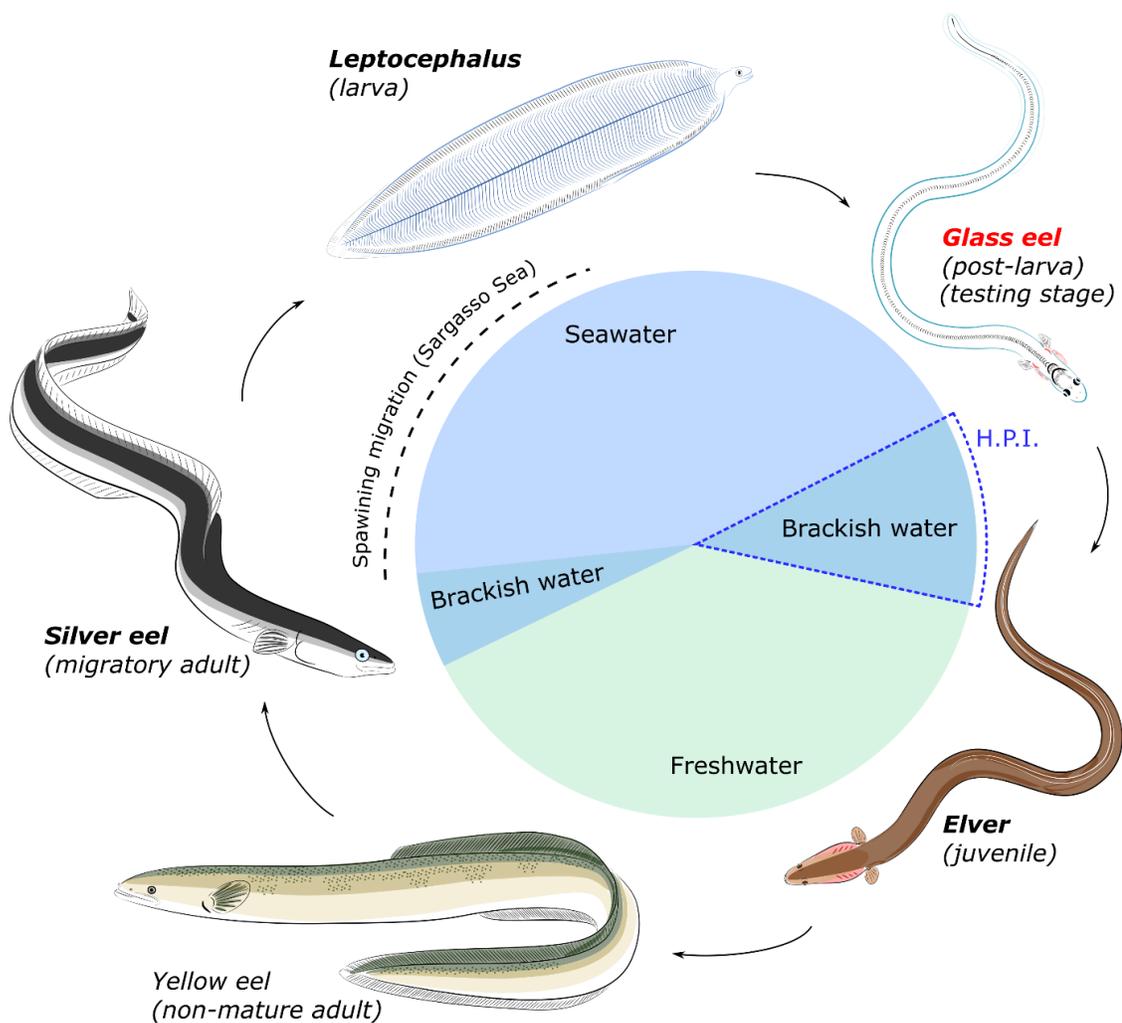


Figure 16 - Life history of the European eel (*A. anguilla*) and hypothetical period of imprinting.

Eels hatch as leptocephalus larvae in the Sargasso Sea. As larvae, they drift across the Atlantic Ocean to the continental slope of Europe, where they metamorphose into post-larval, transparent glass eels. The glass eels migrate across the continental shelf and eventually reach the brackish water of estuaries. After metamorphosing into pigmented juveniles, called elvers, they start the ascent into freshwater, where they will grow into adult yellow eels. After some years, yellow eels undergo another metamorphosis into silver eels, which migrate for thousands of kilometers to the Sargasso Sea where they spawn and die. Eels used in this study were at the stage of glass eel (red font), and the Hypothetical Period of Imprinting (H.P.I.) is highlighted by a dashed blue polygon.

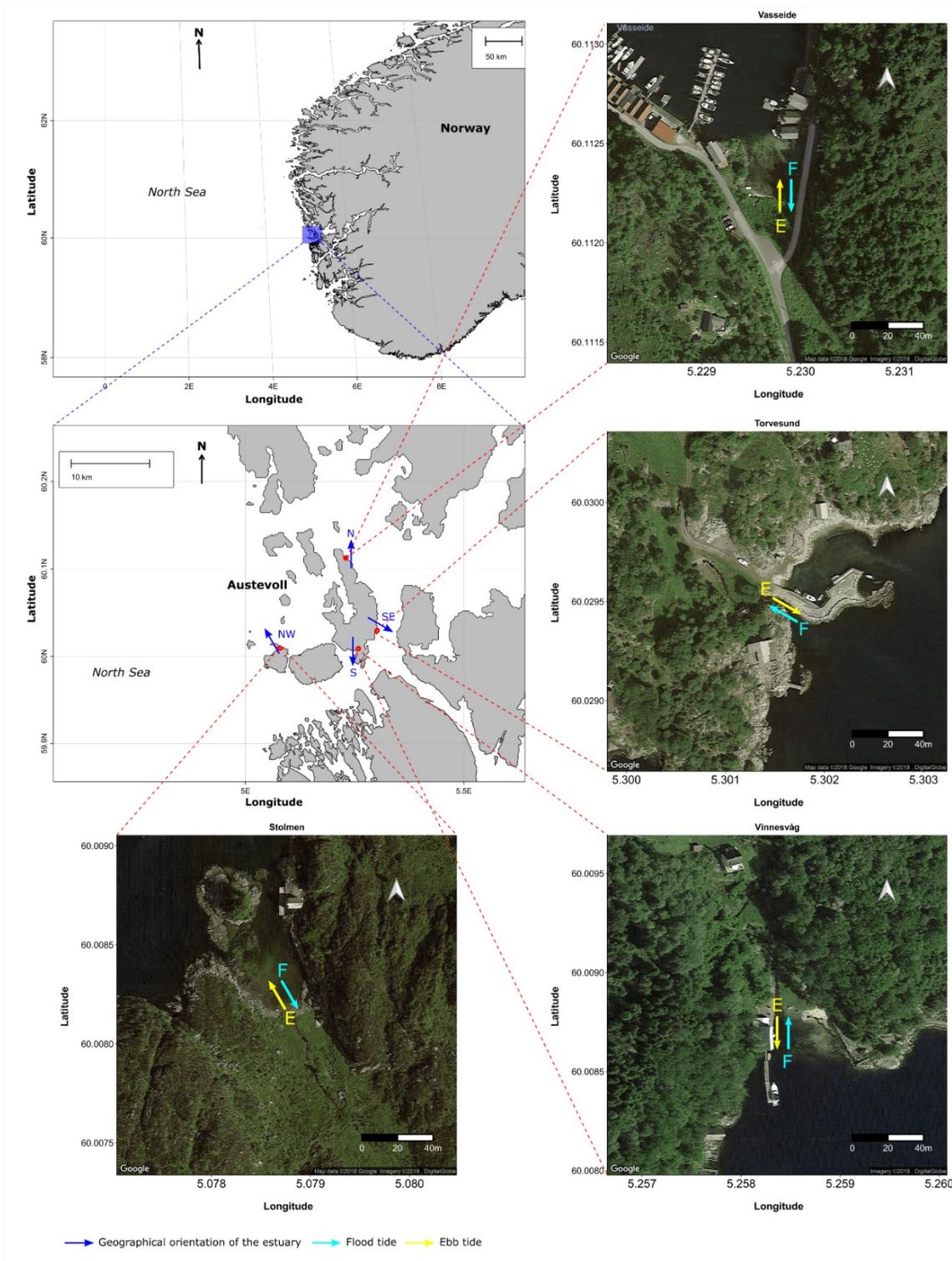


Figure 17 - Estuaries where glass eels (*A. anguilla*) were collected and the direction of tidal currents.

Maps show Norway (upper left) and the Austevoll archipelago. Red points show the location of the estuaries. Blue arrows and blue cardinal points show the magnetic direction towards which the estuaries flow; arrows start from the freshwater side and point towards the seawater side. The satellite images show the aerial view of each one of the four estuaries. Sky-blue and yellow arrows show the magnetic direction of the tidal currents at each of the estuaries (E = ebb current, F = flood current).

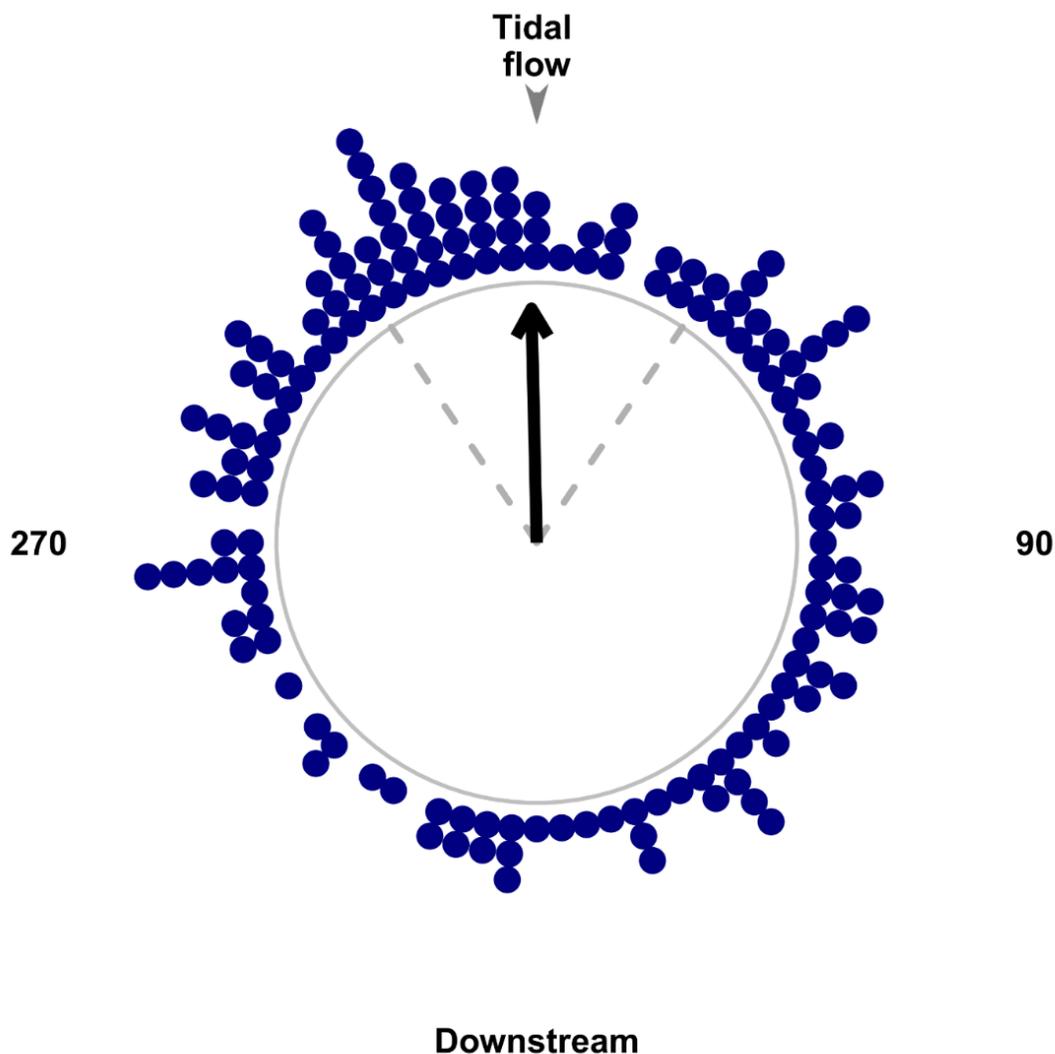


Figure 18 - Magnetic orientation of glass eels (*A. anguilla*) with respect to the magnetic direction of the tidal flows.

In the circular plot, the outer gray circle represents the x axis. The angle between the magnetic orientation of each glass eel that significantly oriented (Rayleigh's $p < 0.05$ on individual tracks) and the direction of the tidal flow (top of the plot) is shown as a navy-blue data point ($N = 155$). The bottom of the plot represents the downstream direction of the flow. Significant (Rayleigh's $P < 0.05$) collective orientation towards the upstream magnetic direction of the tidal flow is shown as a black arrow pointing towards the top of the plot. The direction of the arrow point towards the mean orientation direction of the glass eels. Dashed grey lines show the 95% confidence interval around the mean direction of the eels. For clarity, data are displayed binned by 5° .

Chapter 5. Conclusions: a comprehensive hypothesis on the migration of glass eels (*A. anguilla*): from the continental slope to estuaries – a review

5.1 Overview

The European eel (*Anguilla anguilla*) is a diadromous fish that spawns in the Sargasso Sea. As larvae, eels cross the Atlantic Ocean and reach the continental slope of Europe, where they metamorphose into post-larval glass eels. These will reach the continent, and some of them will enter freshwater, some will stay marine and some will move between fresh water and seawater. After 5-25 years or more, as silver eels, they will migrate back from freshwater to the Sargasso Sea to spawn and die. The glass eel stage is a critical step during which the eels cross the continental shelf and recruit to estuaries, where they will facultatively transition to freshwater. Extensive research has been conducted to understand the behavioral mechanisms that glass eels use and the underlying environmental cues that guide them during migration. Glass eels follow odors and salinity gradients, they avoid light and they change orientation and depth according to the tides. Recent work revealed that European glass eels also use the earth's magnetic field and lunar cues to orient. However, while we have knowledge of many aspects of their orientation behavior, a unifying theory describing how glass eels migrate from the continental slope to freshwater is lacking. The goal of this review is to develop a comprehensive hypothesis on the migration of European glass eels, integrating previous knowledge on their orientation behavior with recent findings on magnetic and celestial orientation. This review follows the journey of a hypothetical glass eel, describing the nature and the role of orientation cues involved at each step. I propose that, although glass eels have the sensory capacity to use multiple cues at any given time, their migration is based on a hierarchical succession

of orientation mechanisms dictated by the physical properties of the environments that they occupy: 1) lunar and magnetic cues in pelagic water, 2) chemical and magnetic cues in coastal areas and 3) odors, salinity, water current and magnetic cues at the estuaries.

5.2 Background and goal of the review

The European eel has been an important species for fishing and farming practices for millennia (Dekker, 2003b, 2018; Starkie, 2003). However, this species has decreased dramatically since the 1960s (Drouineau *et al.*, 2018; ICES, 2018) and it is listed as critically endangered [International Union for Conservation of Nature (IUCN)]. Thus, a deeper knowledge of the behavior of the European eel is required for a better understanding of the migratory strategies of this species, which is important for the management of the eel stocks. Great advances were made in the 20th century on our knowledge of the behavior and the movement ecology of the European eel. Extensive sampling programs have been conducted in the Atlantic Ocean to understand the horizontal and vertical distribution of eel leptocephali (Miller *et al.*, 2015), and direct observations of the migratory behavior of yellow and silver eels have been collected through the use of telemetry (Amilhat *et al.*, 2016; Righton *et al.*, 2016; Béguer-Pon *et al.*, 2018). However, less is known about the migratory behavior of post-larval glass eels during their complex journey from the continental slope to estuaries.

Several laboratory studies have described the cues used by glass eels with special focus on chemical cues, such as odors (e.g. green odors, amino acids and bile salts) and salinity gradients. Numerous observations from the field come from sampling in brackish and/or

fresh water, which together with studies on otolith microstructure, provide valuable insights on such aspects as: timing of arrival at the coast, duration of the migration, and timing of metamorphosis. Recent work investigated the orientation abilities of glass eels with respect to the Earth's magnetic field (Cresci, Paris, *et al.*, 2017) (Cresci et al. 2019 – in press) and lunar cues (Cresci et al. 2019 in review), at sea and under laboratory conditions, suggesting that glass eels may use both magnetic and lunar orientation mechanisms during migration.

However, while many single pieces of the complex puzzle that underlies the orientation and migratory behavior of glass eels have been reported, a holistic hypothesis describing how European glass eels migrate from the continental slope to estuaries is lacking.

In this review, I integrate the existing body of literature on the behavior of glass eels with recent findings on magnetic and lunar-driven orientation and propose a comprehensive theory. I hypothesize that the migration of glass eels is a multistep, hierarchical succession of orientation mechanisms involving multiple external cues operating at different spatiotemporal scales. I propose that, although glass eels have the sensory ability to use multiple environmental cues at a given time, they use different subsets of those cues and orientation mechanisms at any given time, according to the physical properties of the environment at each specific step of the migration, from the continental slope to estuaries. This will be presented by following the journey of a hypothetical glass eel in space and time during its migration. Thus, I first review aspects about the metamorphosis from leptocephali into glass eels that are relevant to understand how the journey of glass eels begins. Next, I suggest how glass eels might use lunar signals, odors, salinity gradients, water currents and the Earth's magnetic field (EMF) to navigate from pelagic shelf waters

to shallow estuaries. Finally, I describe the orientation dynamics associated with residency in estuaries, where glass eels become pigmented into elvers.

5.3 Metamorphosis from leptocephalus to glass eel on arrival at the continental slope

Eel leptocephali cross the Atlantic Ocean from the spawning areas in the Sargasso Sea to the European continental slope, where they undertake a metamorphosis into glass eels (Fig. 19A, Step 1). Researchers started studying this metamorphosis over a century ago (Schmidt, 1906), and several aspects such as the timing of the metamorphosis, the age and the spatial distribution (vertical and horizontal) of metamorphosing larvae, were partially described. However, which environmental conditions trigger this event, and which behavior and orientation strategies the metamorphosing larvae use to move towards the coast is unknown. The metamorphosis of leptocephali larvae into glass eels takes 18 to 52 days (Arai, Otake and Tsukamoto, 2000). During this period, they swim at greater depth during the day and shallower depths during the night (Castonguay and McCleave, 1987). When they reach the proximity of the European continental slope, they descend to depths of 300-600 m during the day, while ascending to 35-100 m during the night (Tesch, 1980). This diel vertical migration appears to be conserved through metamorphosis, as glass eels show similar patterns of vertical distribution in coastal water, influenced by both light and tides (Creutzberg, 1961; Bardonnnet, Bolliet and Belon, 2005).

Sampling cruises conducted with midwater trawls provided important information on the areas where the metamorphosis occurs. Between 1971 and 1977, data from cruises off the Iberian coast using an Isaac Kidd Midwater Trawl indicated that the metamorphosis occurs offshore of the continental slope (Tesch, 1980). Specifically, leptocephali were collected

outside the margins of the shelf but only one glass eel was found over the shelf (Tesch, 1980). Sampling conducted in 1991 with the same trawls off the Iberian coast, performed at depths of 50 m, 75 m and 100 m, confirmed that leptocephali can be found offshore of the slope (more abundant at 100 m) (Antunes and Tesch, 1997b). However, while leptocephali undertaking metamorphosis almost never occur over the continental shelf (Antunes and Tesch, 1997a; Miller *et al.*, 2015), glass eels can be found beyond the continental shelf margin in pelagic waters, from 50 to more than 1000 m deep and up to 300-600 km offshore of the slope (Antunes and Tesch, 1997a, 1997b). Thus, while the end point of the distribution area of metamorphosing leptocephali has been identified as the continental shelf, the areas and the environmental triggers associated with the beginning of the metamorphosis from leptocephalus to glass eel are not well known.

With metamorphosis, as the buoyancy of glass eels and the kinetics of their swimming (anguilliform swimming) differ from those of the larvae, changes in swimming strategy and behavior might also occur. As leptocephalus larvae, eels are adapted to live in the upper layers of the mesopelagic zone (down to 600 m). Experiments on early life stages of Japanese eel (*A. japonica*), a closely related species with the same morphological features of *A. anguilla*, revealed that from the earliest larval stages, leptocephali constantly increase their buoyancy as their body length increases, becoming positively buoyant at approximately 30 mm in total length (Tsukamoto *et al.*, 2009). However, as metamorphosis begins, the buoyancy of the body starts decreasing, crossing a phase of neutral buoyancy to finally become negatively buoyant as glass eels (Tsukamoto *et al.*, 2009). When larvae undertake metamorphosis into glass eels, the body structure changes dramatically: the body surface shrinks, the cartilage turns into bone, and the cross-section of the body becomes

cylindrical (Tesch, 1977). All of these changes increase the density of the body, and glass eels remain negatively buoyant until they reach freshwater (Hickman, 1981). Furthermore, although they possess a swim bladder, it will not become fully functional (gas-filled) until glass eels reach freshwater (Hickman, 1981). All of these modifications suggest that the buoyancy control of glass eels relies only on their swimming abilities, which at this stage play a key role in their movement ecology.

At metamorphosis, the behavior of this fish switches from a larval phase, which feeds and mainly drifts with ocean currents, to a non-feeding, actively migrating stage, which will cross the shelf and recruit to the coast (Tesch, 1977; Miller, 2009). This event represents the beginning of the journey of glass eels, which navigate through a great variety of environments relying on complex, multisensory orientation mechanisms.

5.4 Over the continental shelf: lunar and magnetic cues in pelagic water

Once metamorphosis has occurred, glass eels cross the continental shelf from the continental slope towards coastal water. This is the least understood phase of the migration of glass eels as most glass eel sampling has been conducted at stations located in estuaries or farther into freshwater, where they become pigmented (Harrison *et al.*, 2014). However, there is some information on the vertical distribution of glass eels swimming in open water, especially around the continental slope. As already mentioned, glass eels can be found at depths ranging between 50 and 1000 m when offshore of the shelf (Antunes and Tesch, 1997a, 1997b). More in proximity to the slope, one glass eel was collected SW of the Isle of Ouessant in 1974 at a depth of 140 m (Tesch, 1980). While moving over the shelf, glass eels ascend the water column and swim at shallower depths. Glass eels sampled at the

entrance of the Baltic Sea, in the Skagerrak and Kattegat areas, were found at depths down to 50 m (Hagstrom and Wickström, 1990). More inshore, in the shallow coastal area of the Dutch Wadden Sea, glass eels were found from the surface down to 8 m, displaying patterns of vertical movement which followed the tidal and light cycles (Creutzberg, 1961). However, while there is some indication of how glass eels move vertically at sea, there is almost no information on whether glass eels perform horizontal orientation during the pelagic phase. This is because glass eels are too small and fragile for today's tagging-telemetry technology and observing their behavior at sea remains challenging. The only data on horizontal orientation of glass eels at sea available to date come from studies conducted in the Norwegian area of the North Sea (Cresci *et al.*, 2017; Cresci *et al.*, 2019). These studies indicate that glass eels use orientation mechanisms based on the earth's magnetism and possibly the lunar cycle, which are both cues that are available in open water. Specifically, glass eels have an internal magnetic compass which they use for orientation and as a frame of reference (Cresci *et al.*, 2017). This means that glass eels can sense the direction of the earth's magnetic north, east, south and west.

Behavioral tests on the orientation of glass eels at sea conducted throughout the lunar cycle indicate that their orientation direction is linked to the lunar phase (Cresci, Durif, Paris, Thompson, *et al.*, 2019). This adds to a large body of literature reporting the connection between the moon phase and the activity pattern of the eel, at all life stages. The lunar cycle affects leptocephalus larvae, which hatch in the open ocean and change their depth according to the moon phase (Kracht, 1982; Tsukamoto *et al.*, 2003). The arrival of glass eels at the coast is lunar-dependent, with peaks of abundance at new and full moons (De Casamajor, Bru and Prouzet, 2001; Yamamoto, Mochioka and Nakazono, 2001; Jellyman

and Lambert, 2003), and at the stage of yellow and silver eels (adult stage) the migratory behavior of the eels changes according to the moon phase, both at sea and in freshwater (Tesch, 1989; Bruijs and Durif, 2009; Verhelst *et al.*, 2018).

The observations of glass eels *in situ* revealed that they orient towards the moon azimuth and in an average southward direction at new moon, when the moon is invisible and above the line of the horizon (Cresci, Durif, Paris, Thompson, *et al.*, 2019). The moon azimuth could provide glass eels a magnetic direction towards which they swim under these conditions of the lunar cycle (Cresci, Durif, Paris, Thompson, *et al.*, 2019) (Fig. 19A, Step 2). Glass eels might also remember this direction using the magnetic compass as their frame of reference when the lunar phase switches and the moon is no longer detectable. As the lunar-related orientation was observed at new moon, these findings show that it is not based on vision, rather it could depend on global-scale disturbances in electrical fields (Cresci, Durif, Paris, Thompson, *et al.*, 2019). However, this hypothesis needs to be tested by observing the orientation of glass eels in the laboratory under an artificial electric field with the same characteristics as those caused by the motion of the moon.

The path of the moon above the horizon, at new moon, in the Northern hemisphere begins in the east (moonrise), continues southward, and ends west (moonset). Therefore, following the moon under these specific conditions results in glass eels orienting (on average) towards the south. The advantages of this orientation mechanism are still unknown, but considering the large and mesoscale circulation around Europe, from the North Atlantic drift (flowing northeastward) to the end of the Azores Current (flowing east-northeastward), orientation towards the south could potentially help glass eels recruit to

coastal areas, independent of latitude. This hypothesis will be tested in the future using biophysical dispersal models.

5.5 Navigating coastal water: odors and salinity gradients

Eels possess one of the most sensitive olfactory systems among fishes and olfaction plays a central role in their life (Huertas, Canário and Hubbard, 2008). At the larval stage (leptocephalus) eels already possess 12-15 folds in the olfactory epithelium (Tesch, 1977), which is the same number possessed by adult salmonids, well known for their acute olfaction (Dittman and Quinn, 1996; Ueda, 2012). Additionally, comparing olfaction in relation to vision in adults, the ratio of the surface area of the olfactory epithelium (OE) compared to the surface of the retina in the eye ranges between 14 and 140% in freshwater fish, but in eels the surface of the OE is more than 600% of that of the retina (Tesch, 1977). At the glass eel stage, the OE is also very developed, presenting both ciliary and microvillar receptors at the center of the olfactory lamellae, and cell aggregates that are likely to be precursors of ciliated, supporting cells and receptors in the periphery (Sola, Giulianini and Ferrero, 1993). Eels can detect highly diluted olfactory cues and exhibit different thresholds for detection depending on the chemical. The sensitivity to olfactory cues varies through the life cycle and depends on sex, life stage, environment (freshwater, seawater) and maturation.

After crossing the pelagic area over the continental shelf, glass eels reach shallower, coastal water, where they encounter water masses with a broad range of characteristics. The European coast along which glass eels recruit is very diverse in terms of bottom topography, physical/chemical and biological features (Babin *et al.*, 2003). Furthermore,

freshwater discharge also varies greatly along the European coast depending on the location. As an example, basins like the Baltic Sea and areas surrounding main European rivers are influenced by large freshwater discharges (Hordoir *et al.*, 2013). These rivers transport odors and create salinity gradients, which can serve as orientation cues for both adult fish (Dittman and Quinn, 1996) and late-stage fish larvae (Paris *et al.*, 2013; Foretich *et al.*, 2017).

As a glass eel, *A. anguilla* could potentially rely on odors to navigate coastal waters up to the estuaries (Fig. 19A, Step 3), and extensive research has been conducted to investigate the behavior of glass eels of multiple species in relation to odors (Table 5). Glass eels of the European eel respond to a broad variety of chemical cues, such as salinity gradients (Sola and Tongiorgi, 1996). The attraction of glass eels to freshwater varies among individuals and is associated with higher locomotory activity and reduced growth rate (Edeline, Dufour and Elie, 2005). Moreover, glass eels coming from the sea and adapted to saltwater seem to be less attracted to freshwater compared to individuals that are already adapted to freshwater (Tosi *et al.*, 1988).

Glass eels are also attracted to inland odors, which could be associated with the flora and micro fauna responsible for decomposition of detritus in fresh water (Sorensen, 1986). The attraction to odors is dependent on the salinity of the water. Inland odors such as geosmin (trans-1,10-dimethyl-trans-9-decalol) attract glass eels (Tosi and Sola, 1993; Sola, 1995), and could play a role in the migration to estuaries. Interestingly, geosmin seems to be an attractant in freshwater but a repellent in seawater (Tosi and Sola, 1993). However, maze choice experiments on the closely related New Zealand species *A. australis* (shortfin eel) and *A. dieffenbachii* (longfin eel) show that neither of these two species responded to

geosmin, indicating that attraction to inland odors might depend on the habitat and the species (McCleave and Jellyman, 2002).

One of the main hypotheses for the migration of catadromous and anadromous fish into fresh water is the “pheromone hypothesis”, which proposes that fishes from different life stages release particular odors into the water (pheromones) that will function as attractants for conspecifics which are migrating towards freshwater. Glass eels are attracted to odors coming from conspecifics. Two maze-choice experiments showed that glass eels of *A. rostrata* preferred water coming from “washes” of conspecifics (Schmucker *et al.*, 2016). Moreover, glass eels are more attracted to more concentrated odors from conspecifics, showing a logarithmic increase of response depending on the concentration of the stimulus (Schmucker *et al.*, 2016). More interestingly, the attraction to conspecifics is stage-dependent as it disappears in pigmented elvers, when they might shift to a solitary life style (Schmucker *et al.*, 2016). This was confirmed by another study conducted on glass eels and elvers using a two choice-maze assay, which demonstrated that glass eels are attracted to odors coming both from other glass eels and older elvers (Galbraith *et al.*, 2017). However, elvers do not show attraction for other elvers or glass eels (Galbraith *et al.*, 2017). These results suggest that glass eels could rely on odors from conspecifics for their migration in coastal areas, and that when they pigment into elvers this behavior changes and new cues possibly become dominant. However, this does not explain how the glass eels that arrive first find the estuary.

In the context of the pheromone hypothesis, amino acids are potential candidate compounds that might act as pheromones. Electro-olfactogram records from glass eels show that, among the amino acids, L-asparagine and L-glutamine are the strongest stimuli

and glass eels show thresholds of detection up to $10^{-10} \text{ mol}^{-1} \text{ L}^{-1}$ (Crnjar *et al.*, 1992). However, this attraction to amino acids weakens as eels become elvers (Crnjar *et al.*, 1992). Attraction to amino acids also depends on their concentration and the salinity of the water. For example, D-alanine acts as an attractant in freshwater at concentrations of 10^{-9} M but acts as a repellent at higher concentration (10^{-7} M) or if detected in seawater (Table 1) (Sola and Tongiorgi, 1998). Similarly, D-asparagine attracts glass eels in fresh water and has the opposite effect in seawater (Table 5) (Sola and Tongiorgi, 1998). Table 5 summarizes the current knowledge on attraction/repulsion that various chemical compounds have on glass eels, together with threshold detection concentrations.

The behavior of glass eels with respect to olfactory cues is complex. Glass eels are attracted by multiple chemical cues and these behavioral responses are dependent on multiple internal and external factors. For this reason, a comprehensive hypothesis on the odor or the combination of salinity gradients and odors that attract glass eels to estuaries has yet to be formulated. However, the morphological prominence and extreme sensitivity of the olfactory system, together with empirical evidence of attraction to odors, suggest that olfaction likely plays a central role in navigating coastal waters and finding estuaries. Once the glass eels reach the estuaries, salinity and odors are likely to continue contributing to their orientation. However, at this stage of the migration, glass eels encounter fast moving, turbulent tidal waters and the migratory strategy switches to mechanisms such as selective tidal stream transport (STST) and magnetic “imprinting” of the currents. This facilitates their retention as well as upstream migration in this new and challenging environment.

Table 3 – List of chemical compounds that cause attraction/repulsion of glass eels. The compound, the odor category to which they belong, and their role in eliciting behavior in glass eels are presented. The salinity (fresh water = **FW**, saltwater = **SW**) at which a specific compound elicits a specific behavior is also presented. Minimum concentration thresholds at which attraction or repulsion is observed are indicated. **CS** = conspecifics; **aa** = amino acid; *= the role changes at different concentrations.

Chemical cue	Odor category	Role	Water	Detection threshold	Reference
Geosmin	Earthy odor	Attractant Repellent	FW SW	10^{-13} mg/L 10^{-13} mg/L	(Tosi and Sola, 1993)
MMP 2-methyl-3-methoxypyrazine	Green odor	Attractant Repellent Attractant Attractant	FW SW Brackish (30‰) Brackish (15‰)	10^{-13} mg/L 10^{-13} mg/L 10^{-13} mg/L 10^{-9} mg/L	(Sola, 1995; Sola and Tongiorgi, 1996)
ETMCE 2-isobutyl-3-1-ethyl-2,2,6-trimethylcyclohexanol	Green odor	Attractant Repellent Attractant Attractant	FW SW Brackish (30‰) Brackish (10‰)	10^{-13} mg/L 10^{-13} mg/L 10^{-13} mg/L 10^{-9} mg/L	
MT 4-methylthiazole	Green odor	Attractant Repellent	FW SW	10^{-12} mg/L 10^{-11} mg/L	
L-MF L-2-methylfenchol	Earthy odor	Attractant Repellent	FW SW	10^{-12} mg/L 10^{-12} mg/L	
D-MF D-2-methylfenchol	Earthy odor	Attractant	FW	10^{-9} mg/L	
IBMP 2-isobutyl-3-methoxypyrazine	Green odor	Attractant Repellent	FW SW	10^{-11} mg/L 10^{-9} mg/L	
TMCE 1,2,2,6-tetramethylcyclohexanol	Earthy odor	Attractant Repellent	FW SW	10^{-11} mg/L 10^{-11} mg/L	
IPMCET 4-isopropyl-7-methylcyclohexathiazole	Green odor	Attractant	FW	10^{-10} mg/L	
Fresh water (0‰)	Salinity difference	Attractant	Eels kept in FW		(Tosi <i>et al.</i> , 1988)
D-glutamine	aa (CS)	Attractant Attractant	FW SW	10^{-7} M 10^{-7} M	(Sola and Tongiorgi, 1998)

D-glutamic acid	aa (CS)	Attractant	FW	10^{-7} M	
		Attractant	SW	10^{-8} M	
D-asparagine	aa (CS)	Attractant	FW	10^{-7} M	
		Repellent	SW	10^{-7} M	
D-alanine*	aa (CS)	Attractant	FW	10^{-9} M	
		Repellent	FW	10^{-7} M	
		Repellent	SW	10^{-8} M	
β -alanine	aa (CS)	Attractant	FW	10^{-9} M	
		Attractant	SW	10^{-9} M	
L-asparagine	aa (CS)	Stimulant OE	-	10^{-9} M	(Crnjar <i>et al.</i> , 1992)
L-glutamine	aa (CS)	Stimulant OE	-	10^{-9} M	
Conspecific odor (<i>A. rostrata</i>)	Conspecific wash	Attractant	FW	0.2 g of glass eels L ⁻¹ h ⁻¹	(Schmucker <i>et al.</i> , 2016)
Glycocholate	Bile salts (CS)	Attractant	FW	10^{-11} M	(Sola and Tosi, 1993)
		Attractant	SW	10^{-10} M	
Taurodeoxycholate	Bile salts (CS)	Attractant	FW	10^{-11} M	
		Attractant	SW	10^{-10} M	
Taurocholate	Bile salts (CS)	Attractant*	FW	10^{-11} M	
Cholate	Bile salts (CS)	Attractant*	FW	10^{-11} M	
		Attractant*	SW	10^{-11} M	
Deoxycholate	Bile salts (CS)	Attractant*	FW	10^{-14} M	
		Attractant*	SW	10^{-10} M	
Glycochenodeoxycholate	Bile salts (CS)	Attractant*	FW	10^{-12} M	
		Attractant*	SW	10^{-11} M	
Taurochenodeoxycholate	Bile salts (CS)	Attractant*	FW	10^{-14} M	
		Attractant*	SW	10^{-12} M	
Taurine	Taurine (CS)	Attractant	FW	10^{-12} M	
		Attractant*	SW	10^{-9} M	

5.6 Arrival at the estuaries: pigmentation, Selective Tidal Stream Transport (STST) and magnetic imprinting.

After the pelagic and coastal phases of their journey, glass eels eventually reach estuaries along the European and North African coast (Fig. 19A, Step 4). This is a critical step of their migration, as glass eels that reach estuaries will transition into juveniles, and some will start swimming upstream, into freshwater. The period that glass eels spend in estuaries before moving into freshwater varies; it can last from a few weeks to years (until silvering) in the case of brackish water residents (Tzeng, Severin and Wickström, 1997; Jessop *et al.*, 2008). The tendency of glass eels to migrate upstream is correlated with both their body condition (Edeline *et al.*, 2006) and hormonal activity (Edeline *et al.*, 2005). Furthermore, eels display facultative catadromy, as some never go into freshwater and some move multiple times between brackish and freshwater (Tsukamoto and Arai, 2001; Daverat *et al.*, 2006; Thibault *et al.*, 2007; Jessop *et al.*, 2008; Marohn, Jakob and Hanel, 2013). This further complicates the understanding of glass eels' behavior in estuaries and fresh waters. Estuaries present conditions and physical properties that differ significantly from any other environment that eels encounter beforehand, both as larvae and as glass eels. Estuaries are characterized by freshwater input, dynamic changes in salinity, low visibility and abrupt variation in hydrodynamic conditions due to changes in freshwater outflow and tides. Extensive research has been conducted to understand the behavior of glass eels in tidal estuaries and what environmental factors regulate this migratory step (Harrison *et al.*, 2014). It has been known for more than 50 years that the migratory behavior of glass eels in tidal areas follows the alternation of ebbing and flooding tides, using a mechanism known as Selective Tidal Stream Transport (STST) (Creutzberg, 1961; J. D. McCleave and

Kleckner, 1982; Gascuel, 1986; Wippelhauser and McCleave, 1987, 1988; Tankersley and Forward Jr, 2001; Beaulaton and Castelnaud, 2005). This is of particular importance considering that glass eels have relatively low critical swimming speeds (U_{crit}) that ranges between 10-12 cm/s, which they can sustain for only a short time (Langdon and Collins, 2000; Wuenschel and Able, 2008). Thus, glass eels rely on tidal flows to maximize the energetic efficiency of their migration upstream, moving upward in the water column during the flood tide and swimming down to the bottom during the ebb tide (Deelder, 1958; Jellyman, 1979; J. D. McCleave and Kleckner, 1982; Dou and Tsukamoto, 2003). This is further supported by the observation that catches of glass eels in the upper layers of water column occur mainly during the flood tide (Creutzberg, 1961; J. McCleave and Kleckner, 1982). Furthermore, empirical observations confirmed that glass eels tend to bury themselves in the sandy bottom during ebb tide (Trancart *et al.*, 2012). Although the STST is accepted as one of the main mechanisms involved in the upstream migration of glass eels, there is evidence that the behavior of glass eels in tidal areas is more complex.

Sampling data of Australasian glass eels in the Waikato River in New Zealand suggest that glass eels have an active phase of the migration also during the ebb tide, when they shoal and aggregate next to the river shores (Jellyman and Lambert, 2003). This behavior was integrated with the more classic concept of STST (involving mainly vertical movement), proposing that glass eels keep performing active swimming also during slack/early ebb tide, but along the margins of the river/stream (Harrison *et al.*, 2014). Sampling of *A. anguilla* glass eels in a large French estuary indicates that (at least in that area) only 15-19% of the glass eels migrated using STST as their sole form of transport, suggesting that active swimming might also play a role (Beaulaton and Castelnaud, 2005). This was

concluded by looking at peaks of abundance of glass eels both at the entrance of the estuary and further upstream, indicating a residency time of 20 days, and a migration speed of 3-4 km/day, which could not be explained by STST only (Beaulaton and Castelnaud, 2005). The migratory behavior of glass eels in tidal estuaries is also associated with other factors, such as location (Creutzberg, 1961; J. D. McCleave and Kleckner, 1982; Jellyman and Lambert, 2003), temperature (De Casamajor, Bru and Prouzet, 2001; Laffaille, Caraguel and Legault, 2007), light (Jellyman, 1979; De Casamajor, Bru and Prouzet, 1999; Bureau Du Colombier *et al.*, 2007) and lunar cycle (De Casamajor, Bru and Prouzet, 2001; Jellyman and Lambert, 2003). Thus, the mechanisms by which glass eels migrate through estuaries and the specific cues that they use to find their way at this stage is complex.

Water currents (which also transport odors) caused by freshwater inputs and tides constitute one of the main directional cues for glass eels. Fish orient in moving water by an unconditioned response termed rheotaxis, which can be positive (the fish swims into the current) or negative (the fish swims with the current) (Arnold, 1974; Chapman *et al.*, 2011). Fish also use rheotaxis in tidal estuaries, as the currents can be fast and in many cases the visibility is low (Arnold, 1974). In such conditions, glass eels and other species display rheotaxis for two main tasks: upstream or downstream migration and upstream-oriented station-holding behavior, which minimizes energy use in flowing water (Baker and Montgomery, 1999; Bolliet and Labonne, 2008) (Fig. 19B1 - B2).

In laboratory conditions, glass eels use rheotaxis to synchronize their swimming and orientation with the alternation of simulated tidal flows, showing rhythmic patterns of positive or negative rheotaxis mostly synchronized to the tidal period (Bolliet *et al.*, 2007). Furthermore, experiments showed that glass eels are able to memorize the timing of the

tidal flows (but with less precision) after switching from alternating currents to constant conditions (Bolliet *et al.*, 2007). An analogous ability of glass eels to develop an endogenous rhythm entrained to the tidal phase was observed in American eels (*A. rostrata*), which showed swimming patterns synchronized with the tide when tested in the laboratory (Wippelhauser and McCleave, 1988). This memory of the tide also plays a role in orientation with respect to the earth's magnetic field, as glass eels switch compass direction according to the tide when observed in a magnetic laboratory in the absence of flow (Cresci *et al.*, 2017).

Thus, currents and tides are important directional orientation cues in the estuary, determining the main directions towards which glass eels swim. Recent work indicates that this tidal-dependent rheotactic orientation also involves the eel's magnetic sense, seemingly through a process of magnetic "imprinting" (Cresci *et al.*, 2019). Specifically, glass eels collected from estuaries that were oriented along different cardinal directions, with associated ebbing and flooding tidal currents flowing in different directions, were observed in a magnetic laboratory. In the absence of currents, glass eels oriented against the magnetic direction of the prevailing tidal current occurring at their recruitment estuary (Cresci *et al.*, 2019). These results suggest that glass eels' orientation against the tidal flow involves both rheotaxis and sensing magnetic fields (Fig. 19B1 - B2) as glass eels are able to form and retain a "magnetic memory" of the direction of the tidal currents.

For glass eels, such magnetic imprinting could increase both the efficiency of station-holding behavior and upstream migration using STST (Fig. 19B1 - B2). Specifically, the imprinting suggests that glass eels are aware of the compass direction of the current through their magnetic sense (Fig. 19B1 - B2). This would allow them to orient with greater

efficiency in moving water, especially when physical contact with the bottom and visual cues are lost in the estuaries, where the water is turbid most of the time. This behavior, together with rheotaxis, could also help glass eels to keep their position at the estuaries (station-holding behavior) while undertaking the morphological and physiological changes to become elvers.

5.7 A comprehensive hypothesis and directions for future research

During their journey, glass eels encounter a multiplicity of environments, with dramatic differences in physical, chemical and biological features. Glass eels migrate from the pelagic water of the continental slope, passing through the continental shelf and coastal areas to finally reach brackish water estuaries. Based on the combination of previous literature describing the complexity of the behavior of glass eels, and recent findings on magnetic and lunar-related orientation, I propose that the migration of glass eels is a multi-step process, and that each step is driven by different orientation cues depending on the dominating physical and hydrodynamic conditions at that particular step (Fig. 19). Thus, there is not one main behavioral strategy underlying the migration of glass eels, but rather glass eels have the ability and plasticity to switch orientation mechanisms depending on which cues are available at each specific step. This ability allows glass eels to migrate through a broad variety of environments using a multi-step process involving a hierarchical succession of cues. Specifically, moving from the continental slope to the coast: 1- lunar and magnetic cues, 2- chemical (odors and salinity) and magnetic cues, 3) rheotaxis (water currents), chemical cues and magnetic imprinting.

The literature on the migratory behavior of glass eels highlights that many aspects of this complex process are not fully understood, and that follow up research is required. Starting from the pelagic phase of the migration, more research is needed to better describe the relationship between the orientation and the lunar cycle. Specifically, *in situ* observations of lunar-related behavior have so far only been conducted during daytime. Glass eels orient towards the moon during new moon, when the moon is always above the horizon during the day, but below the horizon during the night. The opposite is true during full moon – the moon is below the line of the horizon during the day but it's above it at night. Future work should investigate the orientation behavior *in situ* at night, with special focus on full moon nights. Furthermore, full moon nights constitute one of the phases of the lunar cycle during which, the electric disturbances (caused by the motion of the moon on its orbit) occur. If electric fields are involved in the lunar-related orientation, full moon nights could play an important role for oriented, directional swimming at sea.

Future work should thus investigate the possible mechanisms involved in the lunar-related orientation. This could be done by reproducing in the laboratory electric fields with the same features of those associated with the moon's orbit and testing the orientation of glass eels exposed to them. The role of lunar-related orientation in the recruitment of the glass eels to estuaries should be investigated using biophysical models of dispersal integrated with the empirical observations on lunar-related orientation behavior.

Future work should also investigate the possible relationship and the relative contribution of olfactory and magnetic cues during the migration in coastal waters. This could be achieved under laboratory conditions by manipulating the direction of odor plumes in an

artificially rotated magnetic field. With such a setup, it would be possible to assess how the direction of odor plumes affects the magnetic orientation of glass eels.

One aspect of the migration of glass eels that needs more research is the interindividual variability in orientation behavior. Previous work on olfactory, rheotactic and magnetic cues highlighted that glass eels display strong variability in orientation responses, which might be dependent (among other factors) on different internal states, or so-called migratory urge. Future work should repeat experiments such as those involving two-choice maze trials (olfactory cues), or those on magnetic imprinting, but after dividing the glass eels in “migratory” and “non-migratory” (proactive/reactive) individuals. This would highlight the role of the internal state of glass eels in their orientation behavior.

5.8 Figures

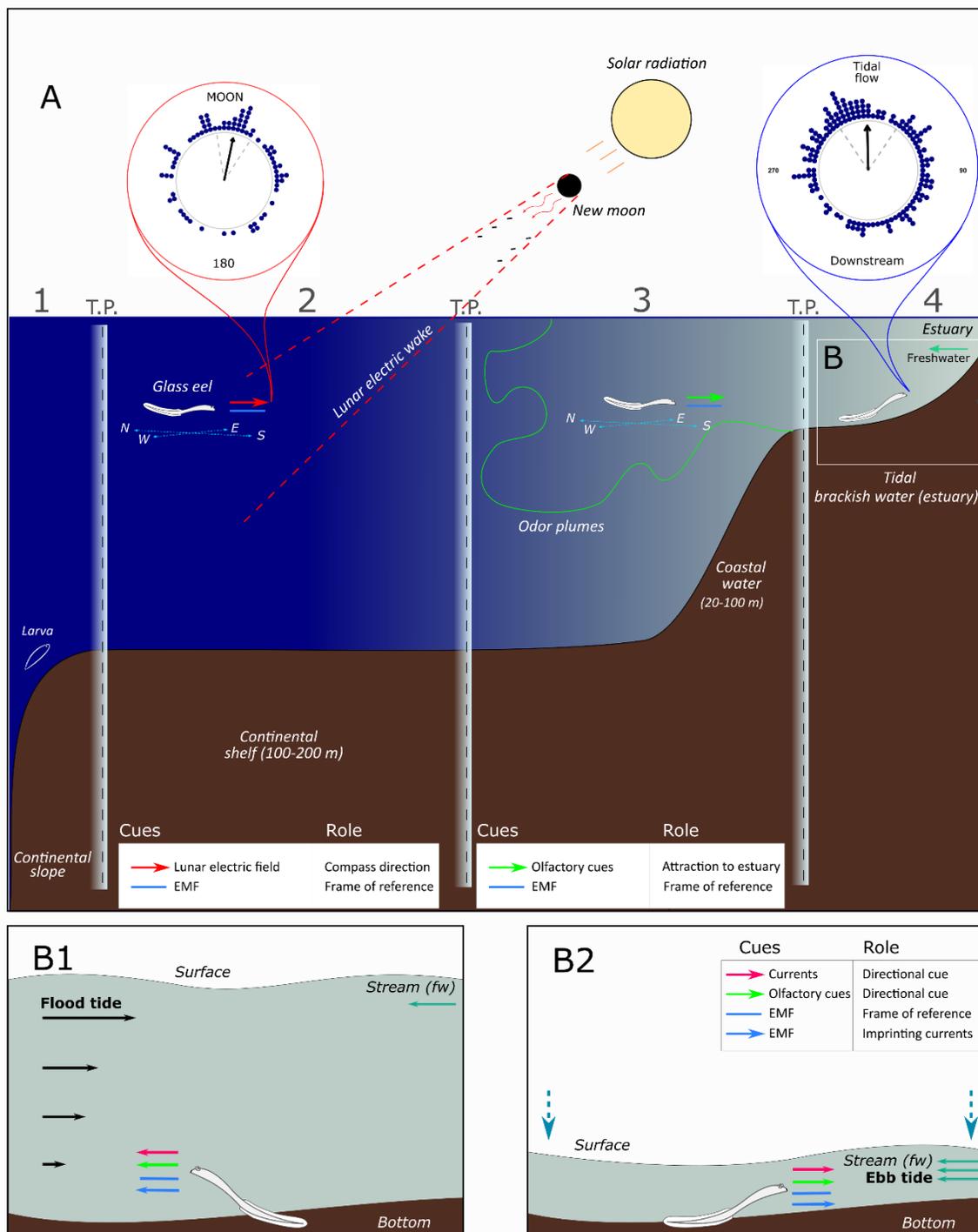


Figure 19 – Diagram of the orientation behavior and mechanisms of glass eels during migration.

This schematic figure shows all of the steps in the migration of glass eels from the continental slope up to estuaries. The diagram is theoretical and does not apply to a specific

geographical area. This figure does not take into account vertical movement related to STST (Selective Tidal Stream Transport), as it focuses on horizontal orientation. **A:** numbers indicate the steps of the migration, which are separated by vertical dashed lines. **T.P.:** Transition Phase between steps. All of the cues involved at each step are designated by arrows (if the cue serves as a directional cue) and lines (if the cue acts as frame of reference). All of the cues are color coded. Dashed blue arrows with the cardinal points (N, E, S, W) represent glass eels' magnetic compass, an orientation mechanism that glass eels possess at all steps. **Step 1:** leptocephali reach the continental slope and metamorphose into post-larval glass eels. **Step 2:** glass eels swim across the continental shelf in pelagic water. Glass eels orient towards the azimuth of the moon at new moon. The plot of the orientation response of glass eels *in situ* with respect to the moon azimuth described in Cresci et al., 2019 is also shown (red circle), where the blue data points show the angle between the orientation of the glass eels and the azimuth of the moon (0° , top of the plot), the black arrow shows the average direction of the glass eels and the gray dashed lines the 95% confidence intervals. At this step, the moon azimuth might serve as a directional cue (red arrow) coupled with the magnetic compass (Earth's Magnetic Field, EMF), which could act as a frame of reference (blue line). Lunar-related orientation was hypothesized to depend on the moon's electric wake (red dashed lines). **Step 3:** closer to the coast, glass eels might predominantly follow odor plumes and salinity gradients (chemical cues, green arrow). **Step 4:** when glass eels reach the estuaries (**B**), they use multiple orientation cues. Here, the alternation of flood and ebb tides causes changes in water depth, switch of current direction and alternation of different combinations of odors and salinity gradients. Rheotaxis (orientation to water current, fuchsia arrow) is one of the main components of the orientation of the eels at this step. During flood tide (**B1**), the water gets deeper and saline currents flow from the seaward side of the estuary into the estuary (black arrows). However, the current from the stream with fresher water (fw, light green arrow) still flows in the opposite direction, potentially closer to the surface. If glass eels perform station holding behavior, they would orient them against the prevailing current (flooding current). They accomplish this using rheotaxis, detecting odors and/or salinity gradients transported by the flooding current, and imprinting the magnetic direction of the flow using the magnetic compass as a frame of reference. This behavior at the estuary is also exhibited when the tide switches to ebb tide (**B2**), during which the water becomes shallower and the current faster and dominated by fresher water from the stream. The plot of the orientation response associated with learning of the magnetic direction of tidal flows (magnetic imprinting) that occurs at this step is shown in the blue circle (**Step 4, B**). The plot shows the magnetic orientation of glass eels with respect to the direction of the flow (magnetic upstream = 0° , magnetic downstream = 180°). Glass eels form a memory of the magnetic direction from which the tidal currents flow and swim towards that direction (the magnetic upstream).

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