

# **Evolutionary Ecology of the European Eel**

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*“Eels are derived from the so-called 'earth's guts' that grow spontaneously in mud and in humid ground; in fact, eels have at times been seen to emerge out of such earthworms, and on other occasions have been rendered visible when the earthworms were laid open by either scraping or cutting. Such earthworms are found both in the sea and in rivers, especially where there is decayed matter: in the sea in places where sea-weed abounds, and in rivers and marshes near to the edge; for it is near to the water's edge that sun-heat has its chief power and produces putrefaction. So much for the generation of the eel. “*

Aristotle, the History of Animals

The reputation of the European eel as an emblematic and mysterious species dates back to the Antiquity. Aristotle dedicated several parts of his treaty on “the History of Animals” to argue for spontaneous generations of the eel. He was wrong. The slimy European eels managed to avoid the intellectual grasp of one of the greatest thinkers of all time, but to his defense, one must say that they continued to puzzle Humanity a couple of millennia longer...and that they are probably still doing it so for some time.



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## Zusammenfassung

Zum effektiven Schutz lebender Ressourcen müssen, nach heutiger Auffassung, sowohl der ökologische als auch der evolutionäre Hintergrund von wirtschaftlich genutzten Arten berücksichtigt werden, um nachhaltige Managementpläne aufzustellen. Diese Perspektive stammt von der bisherigen Unfähigkeit traditioneller Methoden die Dynamik von marinen Fischbeständen einzuschätzen und zu bewahren: Weltweit kollabierende Fischbestände zeigen keinen Erholungstrend. Diese drastischen Szenarien gefährden nicht nur die Biodiversität der entsprechenden Ökosysteme und das Überleben einzelner Arten, sondern bedrohen auch das soziale und ökonomische Wohl von Gemeinschaften, die von diesen Ressourcen abhängig sind.

Ein kritisches Beispiel ist der Europäische Aal, welcher bereits seit Jahrhunderten in Europa gefangen und verwertet wird. In den 1980ern kam es zu einem Einbruch der Nachwuchszahlen, welche zu einer stark verminderten Populationsgröße führten, die sich bis heute nicht erholen konnte. Versuche den Europäischen Aal zu managen und seine Abundanz zu steigern, zum Beispiel in der Durchsetzung eines europaweiten Managementplanes in 2007, waren allerdings ineffektiv. Heute gilt der Europäische Aal laut IUCN als vom Aussterben bedrohte Tierart (critically endangered, CR). Der Populationseinbruch der 1980er zog das wissenschaftliche Interesse auf den Europäischen Aal und führte zur intensiven Erforschung seiner Ökologie und Evolution. Unter anderem waren mögliche ökologische Ursachen für den Populationseinbruch und die Erforschung der Evolution des Aals von großem Interesse, wobei eine scheinbare Abwesenheit einer strukturierten Population – Panmixie – festgestellt wurde. Obwohl die ökologischen und evolutionären Erkenntnisse über den Europäischen Aal große Anwendung im Schutz und der Erhaltung der Population finden, wurde die Verbindung der beiden Disziplinen nur sehr selten untersucht. Das Hauptziel meiner Doktorarbeit war daher die evolutionäre Ökologie des vom Aussterben bedrohten Europäischen Aals aufzuklären.

Zunächst haben wir einen multidisziplinären Ansatz gewählt, welcher Ozean Modellierungstechniken und Populationsgenetik verbindet, um die Rolle der Meeresströmungen auf die Evolution des Europäischen Aals zu untersuchen. Hiermit lieferten wir aussagekräftige Beweise dafür, dass die Ursache des Populationseinbruchs eine ozeanografische ist. Anhand der positiven Korrelation zwischen tatsächlichen und modellierten Nachwuchszahlen konnten wir eine windgetriebene Verbindung zwischen der Sargassosee (Laichgründe des Aals) und dem Golfstrom ausmachen. Außerdem konnten wir eine alternative Hypothese zur Panmixie formulieren: weibliche Philopatrie innerhalb bestimmter Bereiche der Sargassosee. Diese These wird gestützt durch die empirische und

modellierte genetische Differenzierung des Europäischen Aals zwischen Gebieten entlang der kontinentalen Verbreitungsgebiete.

Als nächstes untersuchten wir die Folgen des meeresströmungsgetriebenen Einbruches der Nachwuchszahlen, in Kombination mit der Einführung des Schwimmbblasen befallenden Parasiten, *Aguillicola crassus*, für die Populationsdynamik und das adaptive Potential des Europäischen Aals. Hierfür verglichen wir die genetische Diversität von neutralen Markern mit der von adaptiven Immun-Genen von zwei verschiedenen Generationen. Die Ergebnisse lassen vermuten, dass die Population einem Erholungstrend folgt, worauf die erhöhte genetische Diversität, vor allem der Immungene, der nachfolgenden Generation schließen lässt. Außerdem weist ein kürzlich entdeckter Flaschenhalseffekt in der genetischen Diversität von adaptiven Immungenen darauf hin, dass, trotz des Erholungstrends, das adaptive Potential des Aals immer noch stark beeinträchtigt sein könnte. Deshalb empfehlen wir den Europäischen Aal weiterhin als vom Aussterben bedrohte Tierart (critically endangered, CR) nach IUCN zu führen.

Zuletzt überprüften wir ob sich Anzeichen für weibliche Philopatrie als Fortpflanzungsstrategie finden lassen, wie im ersten Kapitel bereits angedeutet. Basierend auf den indirekten Messungen von Genfluss haben wir getestet, ob die, in der Aalpopulation gefundenen, matrilinearen Abstammungsgruppen evolutionäre Einschränkungen hervorrufen könnte welche Panmixie unterbinden. Unsere Ergebnisse lassen vermuten, dass dies wirklich der Fall sein könnte: zum einen war im Modell eine strukturierte Population statistisch wahrscheinlicher als reine Panmixie, zum anderen fanden wir Asymmetrien in Migrationsraten zwischen den Abstammungsgruppen. Diese Asymmetrien gehen auf die Tatsache zurück, dass eine Gruppe eine stark abweichende, in diesem Fall eine erhöhte, Migrationsrate aufweist. Unsere Vermutung ist, dass die Existenz von getrennten Laichgründen in der Sargassosee der Mechanismus sein könnte, welcher, nicht nur das Überleben, sondern auch das evolutionäre Potential des Europäischen Aals aufrechterhält.

## **Abstract**

The ongoing paradigm on the preservation of living resources argues that both the ecological and evolutionary background of exploited species should be taken into account as to devise effective and sustainable management practices. This perspective stems from the apparent ineffectiveness of traditional methods in preserving and predicting the dynamics of marine fish stocks: worldwide collapsing fish populations show no signs of recovery. These drastic scenarios severely compromise not only the ecosystems biodiversity and viability of the species, but also affect the social and economical welfare of communities that are dependent on those resources.

The European eel constitutes a critical example. For centuries, it served as prominent fishing item to a large number of communities all across Europe. However, the steep recruitment decline that occurred in the 1980s drove the eel population to the low numbers still observable nowadays. Management attempts to raise eel abundance were apparently inconsequent and culminated with a “critically endangered” conservation status attributed to the species and with the enforcement of a European-wide eel management plan in 2007. Still, the 1980s decline triggered an extensive scientific research on the European eel, which translated in significant improvements concerning the knowledge on its ecology and evolution. Amongst those one can name the identification of potential ecological drivers for the decline and the disclosure of the complex evolution of the species, where the apparent absence of a structured population – panmixia – became paradigmatic. Surprisingly, and despite those achievements having wide application in the conservation of the European eel population, the link between ecology and evolution only has seldom been investigated. The main objective of the present doctoral thesis was therefore to shed light on the evolutionary ecology of the endangered European eel.

First, we employed a multidisciplinary approach, which incorporated ocean modelling techniques and population genetics theory, to investigate the role of ocean currents in shaping the evolution of the European eel. Here we provided evidence for an oceanographic origin of the decline. A positive correlation between observed and modeled recruitment allowed the identification of a wind-driven oceanic pathway connecting the Sargasso Sea (the eel spawning ground) to the Gulf Stream. We were also able to put forward an alternative hypothesis to the paradigm of panmixia, namely, female philopatry within certain areas of the Sargasso Sea. The formulation of this hypothesis found support in empirical and modeled genetic differentiation amongst locations within the European eel’s continental range.

Second, we investigated the consequences of both the ocean-driven recruitment decline and of the introduction of the swim bladder parasite, *Aguilocola crassus*, in the post-decline dynamics and adaptive potential of the species. For that, we compared the genetic diversity of neutral markers and that of an adaptive immune gene between two distinct generations of European eels. Results indicated that the European eel population might be experiencing a recovery, as suggested by an increase of the genetic diversity between generations, particularly in the case of the immune gene. The detection of a recent bottleneck in the genetic diversity of the adaptive immune gene further suggested that despite the ongoing recovery, the adaptive potential of the species might still be severely affected. We suggest that the critical endangered status of the species should not be lifted.

Lastly, we explored whether signs of the possible female philopatric strategy suggested in Chapter I could be detected. Based on indirect measurements of gene flow, we tested if matrilineages identified in the eel population could impose evolutionary constraints to complete panmixia. Results suggested that indeed that could be the case: not only was a structured population model statistically favored over complete panmixia, but also asymmetries in the migration rates amongst the matrilineages were detected. By observing that those asymmetries were mainly due to the predominant matrilineage supplying migrants to the others, we suggested that the existence of segregated reproductive units at the Sargasso Sea might be the mechanism maintaining not only the viability but also the evolutionary potential of the species.

## **General Introduction**

### ***I – Evolution of the European eel***

#### *1. Overview of the phylogeny and demographic history of the species*

The European eel is one of the 16 species that compose the monophyletic clade of the freshwater eels, the genus *Anguilla* (Minegishi *et al.* 2005). The capacity to inhabit freshwater habitats have evolved once in the Anguillid family, a large group of exclusively ocean dwelling anguilliform fishes that comprises around 800 species (Inoue *et al.* 2010). The life history of the European eel (*Anguilla anguilla* Linnaeus 1758) encompasses both an oceanic and a freshwater phase, although diadromy (i.e. fish migrations from freshwater to saltwater and reverse) in this species is apparently facultative (Tsukamoto & Nakai 1998). The intrinsic relationship with oceanic environment reflects its deep ocean origin and reinforces the theory that the colonization of freshwater environments was an opportunistic use of a once empty niche (Inoue *et al.* 2010). Furthermore, the spawning grounds of all the members of the *Anguilla* genus are located in open ocean areas which further reflect the critical dependence on the oceanic phase for the completion of the life cycle.

Specifically for the European eel, the spawning grounds are known to be located in the Sargasso Sea area (Schmidt 1923) (Als *et al.* 2011; Kleckner & McCleave 1988; Tesch 2003). The Sargasso Sea is a region in the Northwest Atlantic Ocean that supports high primary production and higher levels of biodiversity than the surrounding oceanic environment (Venter *et al.* 2004). It is delimited by the Gulf Stream, a fast surface current of warm water that spans the northern limb of the North Atlantic gyre, the Azores current and the Caribbean current (Venter *et al.* 2004). It is also the spawning ground of a wide range of species, amongst them the American eel *Anguilla rostrata* (McCleave 1987). It has been proposed that these species diverged 3.4 to 5 million years ago (Jacobsen *et al.* 2014a; Minegishi *et al.* 2005) due to the closure of the isthmus of Panama (Jacobsen *et al.* 2014a). This major geological event is associated with an increased strength of the Gulf Stream, allowing eels to colonize the European coasts (Jacobsen *et al.* 2014a). Hypothetically, it created a mismatch in the spawning grounds between those foraging the American coasts – the

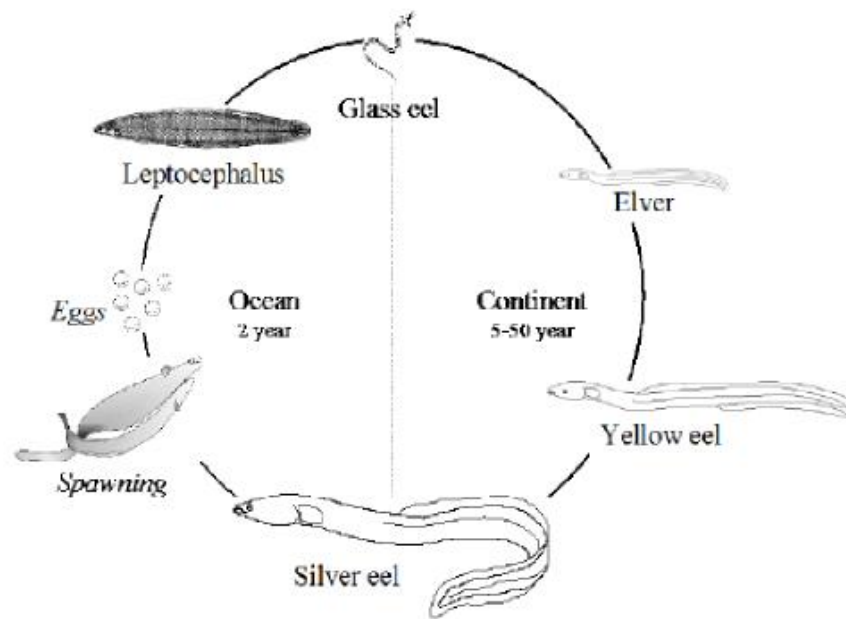
ancestral species – and those foraging the European coasts – the new species. It is nowadays known though, that the actual relationship between the two sisters' species extends beyond their sharing of the Sargasso Sea. Hybridization and introgression – gene flow between two species – exist between American and European eels (Albert et al. 2006; Gagnaire et al. 2009) as confirmed by transcriptome analyses (Gagnaire et al. 2012) and modelling of nuclear genetic frequencies (Wielgoss et al. 2014). Those studies revealed that hybridization between both species could create genetic patterns across a latitudinal range at continental coasts (Wielgoss et al. 2014) or originate incompatibilities between nuclear and mitochondrial genes that maintain different mitochondrial lineages in each species (Gagnaire *et al.* 2012).

Inferences on the demographic history of the European eel showed an intrinsic connection between eel population dynamics and large scale environmental factors, in particular, with late Pleistocene glaciations (Jacobsen *et al.* 2014a; Wirth & Bernatchez 2003). Cyclical (*circa* every 10 000 years) events of low temperatures affected the Northern Hemisphere through the extension of polar ice sheets towards southern latitudes (Hewitt 1996) probably reducing a great fraction of the European eel continental habitats. A reduction in the strength and position of the Gulf Stream related to those climatic changes was hypothesized to have led to unsuccessful post-hatching migrations, reducing the effective population size of the species to the levels observed nowadays (Wirth & Bernatchez 2003).

## *2. A brief description of the life cycle*

The life cycle of the European eel encompasses one of the largest cases of oriented migration reported in the animal kingdom (Figure 1). It starts in the Sargasso Sea where spawning and reproduction take place. Not much is known about the European eel reproductive biology. It has been reported though, that for the Japanese eel and for the giant mottled eel – two other species of the genus *Anguilla* – reproduction occurs once in a life time but with multiple spawning events (Tsukamoto *et al.* 2011). This possibility cannot be excluded for the European eel.





**Figure 1 – Life cycle of the European eel.** Source: Report of the joint EIFAAC/ICES working group on eels, 2011 (EIFAAC/ICES 2011).

After hatching the European eel larvae enter the Gulf Stream which promotes the connection from the Sargasso Sea to the foraging grounds in Europe and North Africa. Once in the Gulf Stream, larvae acquire the shape of a leaf-like organism – the *leptocephalus* – a key adaptation to thrive in the marine environment as it facilitates the transport by ocean currents (Helfman *et al.* 2009). Theoretical expectations suggest that feeding activity during this stage is crucial for leptocephali growth (Desaunay & Guerault 1997), metamorphosis into glass eels (Bureau Du Colombier *et al.* 2007) and subsequent survival up to the arrival at coastal habitats (Desaunay & Guerault 1997). However, the feeding ecology of the *leptocephalus* stage during the transatlantic migration remains largely unknown – an observation that can be extended to the great majority of the Anguillids species (Miller 2009). It has been suggested that Anguillids may feed on abandoned larvacean shells, since those items have been found in the gut content of non-Anguillid species that also possess leptocephalus larvae (Miller 2009). Laboratory experiments however failed to identify natural prey items that triggered any active feeding behavior in *Anguilla japonica* (Tanaka 2003). In addition, reports of Anguillids’ ability to absorb water and dissolved organic carbon and a particular morphological feature in the roof of the mouth that apparently forces water and particles down the

esophagus, rather suggests a passive feeding behavior (Miller 2009). This hypothetical feeding strategy may support speculated links between ocean productivity and recruitment levels (Friedland *et al.* 2007). Nevertheless, the leptocephalus is composed of substantial energy reserves stored throughout the body which, during the metamorphosis into glass eels, are utilized in the ossification and compression of the body (Miller 2009).

The metamorphosis into adulthood starts with the leptocephali arrival at the continental shelf (Figure 1). The trigger, or triggers, for this ontogenic shift are also largely unknown (Otake 2003; Tesch 2003), but environmental cues such as salinity, pressure (as in relation to bottom depth) and chemical components are thought to initiate the process (Miller 2009). Those cues may relate to the distance from a coastal or shore line environment, which, by triggering metamorphosis, permit glass eels to thrive in highly competitive and dynamic habitats such as estuaries or coastal areas. Key aspects of this metamorphic step encompass the ossification of the skull and vertebral column, development of olfactory organs, mild pigmentation and rearrangement of the digestive tract (Miller 2009; Tesch 2003). This process allows glass eels to actively swim, which facilitates selective tidal stream transport (McCleave & Kleckner 1982) and active feeding behavior upon reaching the foraging habitats (Tesch 2003). Those processes are known to be partially mediated by thyroid hormones secretion (Edeline *et al.* 2004).

The yellow eel stage, the stage which follows the glass eel stage, is characterized by the storing of nutritional reserves necessary for gonad maturation and spawning migration later in life. This life stage is thought to last between 3 and 15 years (Daverat & Tomas 2006). The broad temporal window is explained by 1) differences in growth and maturation patterns between males – shorter life span, faster maturation – and females – larger life span, slower maturation – and 2) habitat-specific conditions. The habitat conditions vary amongst rivers, estuaries, lagoons and marine environments as a function of their primary production (Dekker 2000a). Differences in primary production have also been evoked to explain migratory patterns of adult eels between marine and freshwater habitats (Daverat *et al.* 2006), despite the potential trade-offs linked to the osmorregulatory response (Kalujnaia *et al.* 2007). Nevertheless, the continental phase of European eels encompasses several life history strategies, such as diadromy or facultative catadromy,

reflecting the range of phenotypic plasticity of the European eel during the growth phase (Daverat *et al.* 2006).

The silvering process defines the next stage of an Anguillid life cycle. What activates this last ontogenic shift is unknown, but for instances, active swimming, lipid storage or stimulation through sexual hormones have been suggested as potential triggers (Durif *et al.* 2005). Silver eels can be morphologically distinguished from yellow eels due to the appearance of a dark lateral line that divides the body coloration in a white ventral region and a contrasting black dorsal region (Durif *et al.* 2006). From a physiological perspective, silver eels have more developed gonads (Durif *et al.* 2005), thicker skin, and enlarged eyes (Righton *et al.* 2012). Silver eels can further be divided into pre-migrants and migrants, a classification made to distinguish between those animals that are on the process of starting their spawning migration (migrants) from those apparently on the process of acquiring the physiological pre-requisites (pre-migrants) (Durif *et al.* 2005).

The spawning migration is the second large-scale migration of the Anguillid life cycle. It defines the nocturnal transition from foraging areas (rivers, lagoons, estuaries) to the open ocean waters. In the specific case of the European eel, it is known to occur from August until early Winter (Aarestrup *et al.* 2008). This first stage of migration is supposedly triggered by environmental conditions. More specifically, it has been observed that silver eels tend to move downstream during periods of new moon and seasons of high rainfall, as to avoid predation and facilitate swimming activity (Tsukamoto 2009). This period is followed by a resting phase at the transition zones between fresh and salt water (Aarestrup *et al.* 2008). Note, nothing is known with that regard about those eels that never enter the freshwater systems.

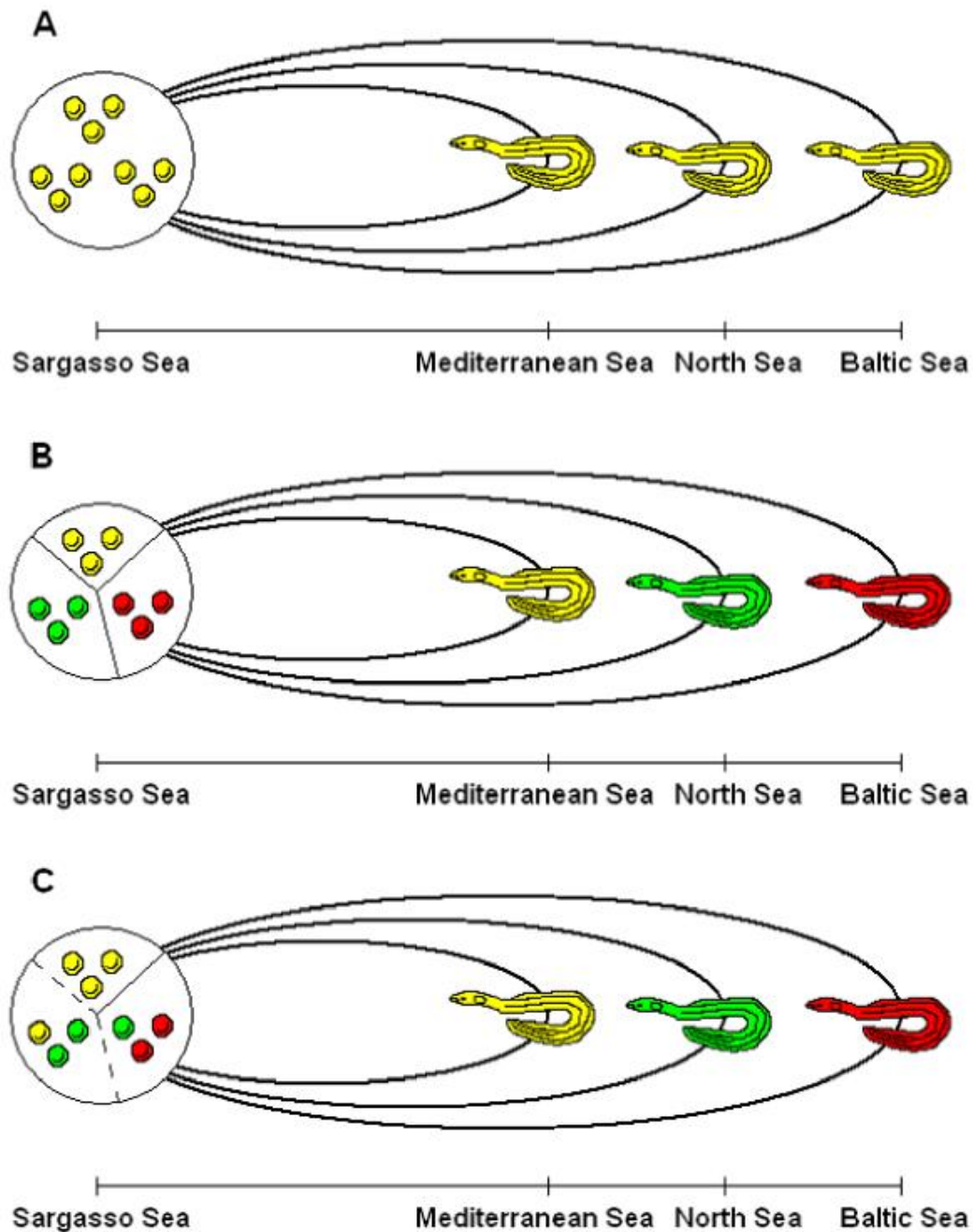
Details of the approximately 5000km long migration to the spawning grounds are only now being revealed (Aarestrup *et al.* 2009), and ongoing research focus on two main topics: orientation mechanisms and migratory routes. In relation to the first topic, evidence suggest that European eels – and Anguillids in general – are sensitive to the earth geomagnetism (Durif *et al.* 2013; Nishi *et al.* 2004). As for migratory routes, the inference of migratory routes greatly relies on tagging and satellite-based information of individual animals. This methodology has permitted the

characterization of the European eel migratory pathway during the first thousand of kilometers (Aarestrup *et al.* 2009), which can be resumed as a southward migration to presumably engage into the southern limb of the north Atlantic gyre. Although this pathway was thought to facilitate the swimming performance of adult eels, it has recently been shown that adult European eels are extremely efficient endurance swimmers (van Ginneken *et al.* 2005). This would allow them to perform the migration, complete the maturation process and eventually find partners in Sargasso sea relying on the energy reserves stored during continental phase (Righton *et al.* 2012).

### *3. On the foundation of the paradigm of panmixia*

The mode of reproduction of the European eel remains one of the most challenging topics in evolutionary biology. Indeed, since the discovery of the likely location of the spawning grounds in the Sargasso Sea – by Schmidt in the 1910s – assessing the population structure of the species has revealed to be a non-trivial exercise. For much it contributes the still unknown location of the reproductive unit within the Sargasso Sea area (Tesch 2003). The first attempts to understand the structure of the eel population can be traced back to the 1970s/1980s. By that time, two studies, one on genetic differences based on allozyme data (Pantelouris *et al.* 1970) and the other based on the counting of number of vertebrae (Boëtius & Harding 1985), suggested not only the existence of a structured eel population (Pantelouris *et al.* 1970) but also that a secondary spawning location could exist inside the Mediterranean (Boëtius & Harding 1985). Those theories were dismissed with a more comprehensive study performed in 1986 (Avisé *et al.* 1986), which, by sampling European eels from several continental locations argued for the existence of a single panmictic population i.e. panmixia, of European eels (Avisé *et al.* 1986). This theory set the foundations of the paradigm of panmixia in this species, and remained unchallenged for several years. By the late 1990s, theories suggesting the existence of segregated reproductive units within the Sargasso sea, and therefore contradicting panmixia – started to rise (Lintas *et al.* 1998). Empirical evidence for such came from the advances in the development of molecular markers that took place in beginning of the 21<sup>th</sup> century. From 2001 to 2005, studies on mtDNA and microsatellites suggested patterns of isolation by distance (Daemen *et al.* 2001; Maes & Volckaert 2002; Wirth & Bernatchez 2001) or isolation by time (Dannewitz *et al.* 2005) could occur amongst the eel continental population. The first is

characterized by a positive correlation between genetic differences and geographic distances, while the second is characterized by genetically distinct cohorts of individuals. These patterns are clear deviation from a panmictic mode of reproduction, as they were associated to genetically distinct cohorts further suggesting the existence of genetically distinct groups of progenitors separated by timing of spawning, or by the time their progeny arrives at European coasts (Figure 2). However, subsequent studies (2009 and 2011) with wider genomic tools (Palm *et al.* 2009) and geographic resolution (Als *et al.* 2011) once again rekindled the flame of the panmixia paradigm. The later study might be considered an hallmark in eel population genetics for two reasons. First, contrasting to all other studies so far presented, it was the only study for which several locations in the Sargasso Sea corresponding to the documented sites where leptocephali had been previously found were sampled (Als *et al.* 2011). The second was that no genetic differentiation was found but related individuals were sampled in close vicinity although excluded from analyses (Als *et al.* 2011). Lastly, single-generation local adaptation in mitochondrial genes was also shown to be a plausible explanation for the genetic differences amongst continental locations (Pujolar *et al.* 2014). Those results can explain, for example, the habitat specific growth and maturation of adult eels that results in unsynchronized spawning migrations across the distribution range. Unlike homing salmons (Dittman & Quinn 1996), eels do not target specific freshwater systems, which leads to a loss of the local adaptation signal from one generation to another (Pujolar *et al.* 2014).



**Figure 2 – Leading hypothesis to justify the evolution of population structure detected amongst European eels collected across coastal locations.** The colours represent different genetic backgrounds, while the circles represent migration pathways from and to the Sargasso Sea that characterize the life cycle of the European eel (Ragauskas & Butkauskas 2014). **A – panmixia** following (Palm *et al.* 2009): random mating in the Sargasso Sea is reflected in no genetic differentiation, amongst locations within the continental range. **B – Isolation by distance** as reported in (Wirth & Bernatchez 2001): geographic structure at spawning grounds stems from different migratory pathways which in turn are reflected in genetic differentiation amongst locations within the continental range. **C – Isolation by time** as reported in (Dannewitz *et al.* 2005): here, the structure at Sargasso is not so strict and allows for the mating of individuals from different spawning locations. Each location at Sargasso Sea is also associated with a migratory pathway, which, produces the waves of recruited glass eels genetically distinct from one another (Ragauskas & Butkauskas 2014).

## II – Contemporary dynamics of the eel population

### 1. The 1980s recruitment decline

For the last three decades, population biology of the European eel has caught even more attention of the scientific community. Much of that is due to the drastic decline in glass eel's recruitment observed since the 1980s (Moriarty 1990), Figure 3). The problematic of the collapse extends over social and economic aspects of European fishing industries (Dekker 2008). This is because European eel fisheries assure the sustainability of entire fishing communities, particularly in northern Europe and Biscay Bay (Dekker 2003b). There has been an apparent reduction in the landings of eels since the 1960's (Dekker 2008), but since eels are fished at all life stages, it is difficult to ascertain, for instances, the share of glass and adult eels in those reconstructed trends (Dekker 2000b). On another perspective, genetic signature of a recent population bottleneck, as it would be expected after a chronically low recruitment has never been detected (Pujolar *et al.* 2011).

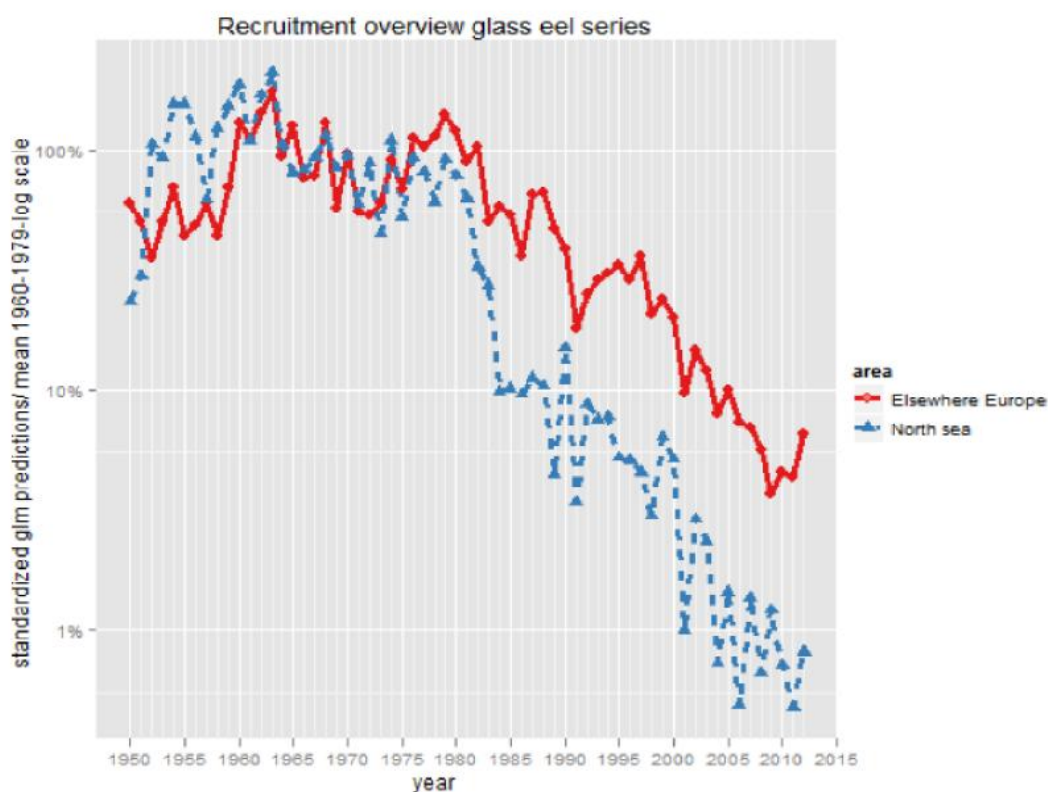


Figure 3 – European eel recruitment trends. Joint report of EIFAAC/ICES working group on eels (EIFAAC/ICES 2011).

Despite a heated debate, the origin of the steep decline and consequent low recruitment remain mainly unexplained to date (Astrom & Dekker 2007). Several non-mutually exclusive hypotheses have been proposed:

### *1.1. Lack of spawners – overfishing*

This hypothesis builds on the observation of low records of eel landings in the period prior to the 1980s decline (Dekker 2003a). The reduction in the number of mature, ready-to-spawn eels in the Sargasso Sea due to overfishing at the continental stage could have reduced the probability of each sex to find a mating partner. Consequently, the animals would die before mating (Dekker 2003a). In general, it is argued that the intense fishing activity period that followed the WWII (Pauly *et al.* 2002) might have greatly contributed to overall decline of the population.

### *1.2. Pollution*

Due to their relatively high position in the trophic chain at adult stage, eels tend to accumulate toxic components existing in the streams they inhabit and act as bio-accumulators (Geeraerts & Belpaire 2010). This concept defines the accumulation of toxic components in the lipid content of animal tissue that is transmitted vertically in the trophic chain through predation (Bryan *et al.* 1979). During the fastening spawning migration, toxins such as polycyclic aromatic hydrocarbons (PAHs), polychlorobiphenyls (PCBs) or heavy metals stored in the lipid reserves are possibly re-absorb and reduce the quality of spawners (Robinet & Feunteun 2002). Pollution in the continental range may also affect an individual's physiology, e.g. altering hormonal regulation, immune and nervous systems (Geeraerts & Belpaire 2010).

### *1.3. Parasitism*

The anthropogenic introduction of the swim bladder parasite *Anguillicola crassus* (Kuwahara, Niimi and Hagaki, 1974) a natural parasite of Japanese eels (*Anguilla japonica*) in European inland waters imposed an novel selective pressure on European eels (Kirk 2003). Originally from Taiwan (Wielgoss *et al.* 2008a), the parasite was first reported in 1982 in Germany and soon became pervasive across European freshwater streams (Taraschewski *et al.* 1987). Laboratory experiments showed that,



contrary to the Japanese eel (natural host of the *Anguillicola crassus*), the European eel is unable to mount an effective immune response to fight off the parasite (Knopf 2006). Eels whose swim bladder has sustained heavy damage due to *A. Crassus* infestation have a poor swimming performance compared to uninfected individuals (Palstra *et al.* 2007). In the continental phase though, parasite prevalence does not seem to impair fitness of infected eels (Lefebvre *et al.* 2013). However, it is clear that *A. Crassus* can act as a strong selective pressure during the fastening spawning migration (Palstra *et al.* 2007). This could occur either through the allocation of eel nutritional reserves to immune defence, or through the loss of flexibility of the swim bladder that would preclude the documented vertical migrations.

#### 1.4. Changes in the oceanic environment

Contrary to freshwater, which is a facultative environment for the European eel, the oceanic environment plays an essential role in the completion of its life cycle. Since the early 1990s, changes in the oceanographic conditions have been advocated for the decline and chronically low eel recruitment (Castonguay *et al.* 1994). Those changes speculatively relate to major climatic events such as the North Atlantic Oscillation (NAO), which mediate sea surface temperatures and ocean currents and constrain leptocephali migration and development (Knights 2003). Implications of such unfavorable conditions would be reflected in the recruitment trends. The NAO and the recruitment index of Den Oever (DOI) – the longest fisheries-independent time-series of eel recruitment – showed to be negatively correlated in a temporal lagged scale of 0 to 2 years (Kettle *et al.* 2008b). Declines in Sargasso Sea primary production have also been suggested to contribute for the low European eel recruitment trends (Friedland *et al.* 2007; Munk *et al.* 2010).

Concrete evidence on how the proposed factors provoked the decline remains disputable. For instance, the impact of pollution or of the introduced parasite in continental phase is well known, but the extent to which it affects future generations of eels is elusive. Similarly, proposed oceanographic influences neither provide a direct link nor explain the chronic low for almost 30 years. The sudden recruitment decline, but above all its chronic low that occurred in the

subsequent years, urged the fisheries stakeholders to devise strategies in order to mitigate the shortage of eels in European freshwater streams.

## *2. European eel management practices: linking decline and population structure*

With artificial reproduction out of reach (only recently artificial reproduction was successfully induced in Anguillids (Ijiri *et al.* 2011)), farming the full life cycle of the species under aquaculture conditions does not appear as a viable option. Instead, fisheries managers adopted a plan of farming only a part of the life cycle, namely, the transition from glass eels to yellow eels. By protecting and feeding eels through that transition, mortality rates amongst juvenile eels were greatly reduced. The replenishment of depleted streams was then made possible by collecting glass eels from locations where the recruitment decline was not so pronounced, such as Biscay bay, and trans-located them, as yellow eels, to the depleted freshwater systems (Feunteun 2002; Moriarty & Dekker 1997). The implementation of this practice was strongly backed up by up-to-date population genetic reports that pointed towards the existence of a single panmictic population of European eels (Aulsebrook *et al.* 1986). In practical terms, the paradigm of panmixia in the European eel ensured that managing the species as a single stock spanning all European fishing regions was possible and biologically safe. Nowadays, despite punctual reports of population structure challenging the paradigm of panmixia (Baltazar-Soares *et al.* 2014; Dannewitz *et al.* 2005; Maes & Volckaert 2002; Wirth & Bernatchez 2001), the European eel remains the only critically endangered species (IUCN) still exploited.

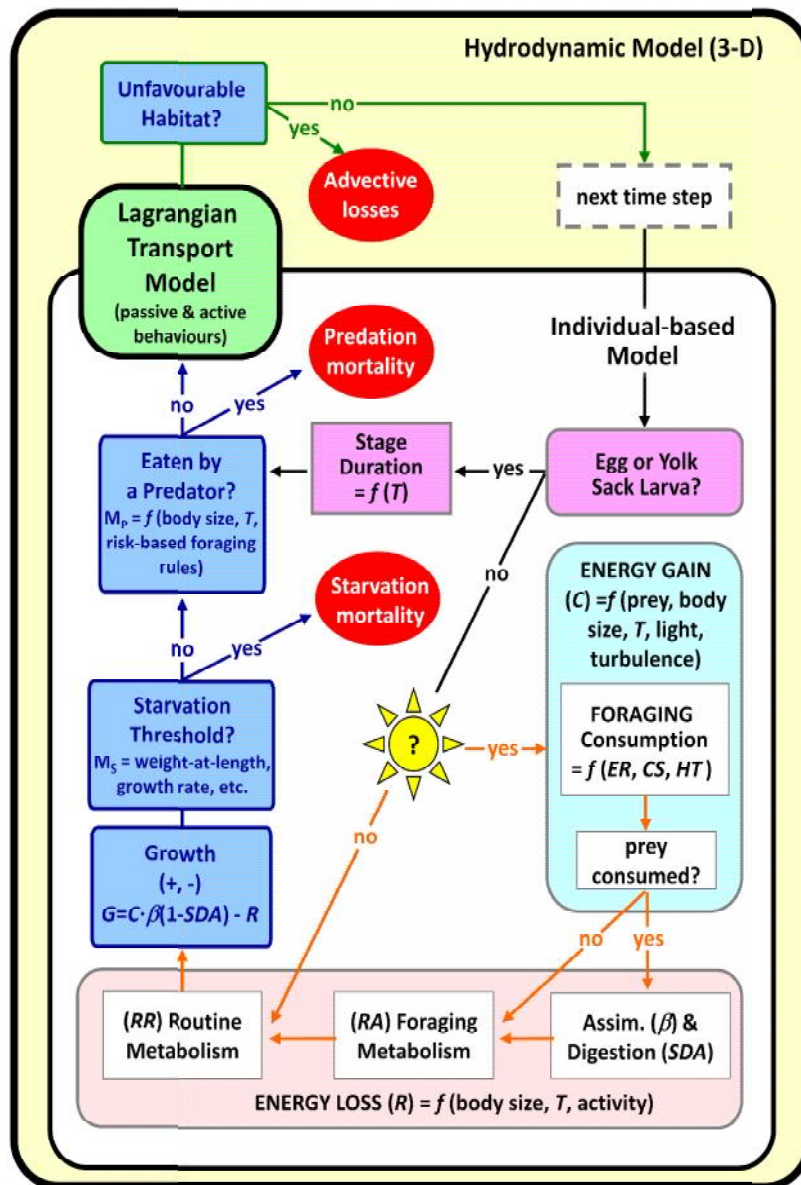
### ***III- Investigating the reasons behind the decline of eel population***

Effective management and conservation of the European eel critically depends on the clarification of the reasons behind the recruitment decline and its subsequent chronic low. For instances, if the reasons are derived from anthropogenic stresses, such as pollution or overfishing, measures can be taken in order to mitigate those pressures on eel population until recovery is reached. However, if the factors are extrinsic to anthropogenic actions, such as oceanography or the relationship with the introduced swim bladder parasite *A. crassus*, it becomes important to understand the extent to which they impact the eel demography and predict how changes in those pressures can further alter those dynamics. That is the reason why the focus of this thesis was the thorough investigation of how oceanography and incidence of the parasite pressure have shaped the contemporary demography and structure of the eel population.

#### ***1. Insights from biophysical modelling***

Due to the passive or near passive behavior of the early stages of the European eel, changes in oceanic patterns are part of the leading hypotheses for the decline and subsequent low of the species recruitment (Bonhommeau *et al.* 2008). In addition, it has been suggested that currents might influence population dynamics to the levels where signatures of population structure amongst coastal locations are detected (Kettle & Haines 2006). Overall, studies relying on ocean modelling that can be particularly informative because they allow a direct comparison between simulated dispersal and real recruitment patterns. To this end the incorporation of high resolution hydrodynamic models primarily developed by physical oceanographer is crucial. In a broader sense, oceanographers have developed several ways to access the dynamics of water mass systems: directly by i) measuring it from a static point (Eulerian), ii) measuring movement pathways of deployed drifters (Lagrangian), or indirectly, through iii) satellite-tracked buoys and iv) simulations of virtual drifters. The latest has become a particularly valuable tool when used in combination with particle tracking software (Fossette *et al.* 2012), since it allows to trace the movements of specific bodies of water. This was how Kettle and Haines (Kettle & Haines 2006) or Bonhommeau *et al.* (Bonhommeau *et al.* 2008) modeled European eel recruitment.

However, to explore how ocean dynamics affect the biological activities of marine fishes, it is also possible to parameterize physical models to meet biological criteria. Biophysical models emerge from *in silico* approaches that incorporate biotic and abiotic characteristics of any given ecological type. Conceptually, a biophysical model incorporates three components in addition to a hydrographic model: a particle tracking system to simulate the drift of virtual individual organism, an egg production model, to mimic the spawning activity, and a program that computes the distribution of the virtual organism as a function of time (Brickman *et al.* 2007). Of extreme importance for the predictive and exploratory capacity of the biophysical modelling was the implementation of Individual Based Models (IBMs) on hydrographic modelling. On top of tracking the virtual movement of individuals, IBMs allow to predict how biotic or abiotic characteristics of the surrounding environments can influence individual survival (Hinrichsen *et al.* 2011). IBMs are based on assumptions that each individual behaves towards maximizing its fitness. For example, predation is optimized to consume as many preys as available (Huston *et al.* 1988). Even though these techniques were known to ecologists since the 1980s, only later they have entered the fisheries biologist toolbox (Hinckley *et al.* 1996; Werner *et al.* 1993). Nowadays, studies coupling hydrodynamic models with IBMs are used to investigate connectivity between spawning and foraging areas, predation and starvation (Peck & Hufnagl 2012), and its influences in recruitment fluctuations of several fish species (Miller 2007) (Figure 4).



**Figure 4 – Schematic representation of an Individual-based-model embedded in a hydrographic model from Peck and Hufnagl (Peck & Hufnagl 2012).** The main components of these models are here represented as following: the large yellow box that envelops the schematic picture represents the hydrodynamic model component that confers realistic environmental conditions for interactions of the individual-based model, i.e. the second-in-size white box of the picture. The minor rectangles represent detailed conditional variables (yes/no, for instances) that define the overall probability of a successful individual development. In green, is the advection component that mimics the physical environment on which individuals move, which is directly connected to the hydrodynamic component.

It is clear that extending current modelling approaches on European eel towards the complexity of a spatially-explicit IBM is, so far, constrained by the knowledge gaps on key biological parameters of leptocephalus (Melià *et al.* 2013). For instances, optimal developmental temperatures, feeding ecology during transatlantic migration or growth patterns are largely unknown. However,

oceanographic models can be used to build null expectations regarding how the post-hatching transatlantic migration takes place, therefore exploring the importance of ocean currents on the evolution of European eel. This is particularly informative given the availability of data on European eel recruitment that extends past the 1980's decline event. No such study encompassing the extensive time-series of recruitment data (provided by FAO and ICES) exists up to date in the European eel. In addition and as previously mentioned, ocean models can also be used to explore, *in silico*, the extent to what transatlantic migration disturbs our perception of population structure, given that 1) the assessment of population structure in the European eel is often performed amongst continental locations and 2) there is no evidence of how reproduction actually takes place in the Sargasso Sea.

## *2. Insights from evolutionary theory*

Modern synthesis defines evolution as changes in allele frequencies across generations (Mayr & Provine 1998). Those changes are driven either by stochastic or deterministic events through the action of selection, migration, mutation or drift. Amongst these four evolutionary mechanisms, selection and drift are the main drivers of the loss of genetic diversity across generations within a population. While losses due to drift can be compared to the process of random sampling (Kimura & Ohta 1978), natural selection would favor determined alleles. However, selection does not act directly on the alleles. Instead, it acts on the phenotype, i.e. the expressed characteristics of the alleles or genotypes. Intrinsically connected to selection is the concept of fitness. Evolutionary theory defines it as the ability of individuals to thrive and reproduce in the environment that surrounds them (Orr 2009). Since that ability is granted by the phenotypic expression of a given genotype, the next generation of individuals will be mainly constituted by that genotype that will provide best thriving capacity in the given environment. The process of adaptation is therefore the result of the interplay of three key components: a selective pressure exerted by the environment, an individual response, or phenotypic trait, conferring increased fitness, and a genetic basis that confers heritability of the trait. The temporal scale of the adaptive process is mediated by the intensity of the selective pressure and is measured in generations (Schoener 2011). It is now though accepted that evolutionary and ecological effects act on overlapping time frame (Grant & Grant

2002; Lohbeck *et al.* 2012), and adaptive responses can occur as rapidly as from one generation to another (Eizaguirre *et al.* 2012b).

Hence, the application of evolutionary theory to European eel research represents another opportunity (in addition to ocean modelling) to investigate the recruitment decline and infer the potential effects of its low level on the evolutionary potential of the species. This is because reductions in population size, or bottlenecks, are known to lead to decreases in genetic diversity. Small population with low genetic diversities are more exposed to the effects of genetic drift, which might results in inbreeding depression, fixation of deleterious mutations or constrain adaptation to changing environmental conditions (Reed & Frankham 2003). That is why genetic diversity, alongside with species diversity and ecosystems diversity, is acknowledge as one of the three universal indicators of biodiversity (Frankham 1995).

Curiously, and despite more than 30 years of continuously low recruitment, genetic screens of the eel population revealed no recent loss of genetic diversity (Pujolar *et al.* 2011). This was already indirectly suggested by a previous study (Pujolar *et al.* 2009a) that searched for heterozygosity-fitness correlations and showed no heterozygote advantage, i.e. positive correlation between fitness indicator and genetic diversity. This is because heterozygote-fitness correlations are predicted to arise in populations whose genetic diversity has been reduced, particularly if the genetic markers used for such correlations are neutrally evolving (Szulkin *et al.* 2010)

The use of neutrally evolving markers however, precludes the inference of the role of a selective pressure at the onset of the population decline. It has been proposed though, that the ideal framework to assess the viability of a natural population would aggregate information regarding the genetic diversity of both neutral and adaptive genes (Hendry *et al.* 2011; Radwan *et al.* 2010). While the former provide general information on factors that might affect the evolutionary potential, such as level of inbreeding, effective population sizes or migration rates, the latter are often used to directly assess the adaptive potential of species by either targeting regions of the genome known to directly respond to a selective pressure or through identification of genomic regions that may indicate local adaptation (Allendorf *et al.* 2010).

Given the introduction of the swim bladder parasite, *A. crassus* in European inland waters, the use of adaptive genes related to parasite resistance seems to be critical to infer the viability of the European eel population. Despite being often regarded as one of the causes for the recruitment decline, its impacts on the host population have only been explored from an ecological perspective. As previously mentioned, it is commonly accepted that European eels are not able to mount a proper immune response against this parasite (Knopf 2006) and its prevalence damages eels' swim bladders consequently impairing the swimming performance of infected animals (Palstra *et al.* 2007). Apparently infection does not have a negative impact on the fitness of individuals during the continental life phase (Lefebvre *et al.* 2013), and no susceptibility to infection was found to be explained by genome-wide levels of heterozygosity (Pujolar *et al.* 2009a). Those observations are not surprising given that 1) the European eel is the only final host of *A. crassus* in European freshwaters and parasite virulence should not be so extreme to kill the host during the continental such as it reproduces and spreads (Kirk 2003) and 2) no vital function is known for the swim bladder during continental phase (Tesch 2003). It results that the parasite prevalence would only manifest lethal to an infected European eel when the swim bladder acquires biological relevance for the fish for the previously mentioned reasons.

As the use of neutral loci has failed to detect parasite mediated pressure (Pujolar *et al.* 2009a) genes of the Major Histocompatibility Complex (MHC) – components of the vertebrate adaptive immune system with a known role in parasite resistance (Janeway *et al.* 2005) – are ideal candidates to infer whether *A. crassus* have played a decisive role in the European eel decline or not

### 3. Major Histocompatibility Complex (MHC)

#### 3.1. Function and structure of the MHC gene family

The MHC is a highly polymorphic gene family (Apanius *et al.* 1997; Klein *et al.* 2007) that controls the adaptive response of the vertebrate immune system (Janeway *et al.* 2005). MHC genes encode for cell-surface glycoproteins that bind pathogen- or parasite-derived peptides in specific regions of their structure, namely, the peptide binding region (PBR) (Klein *et al.* 2007). Those foreign peptides



are either the products of enzymatic reactions performed by host cells in their cytoplasm or are bound in the extracellular space. The former represents reaction to intracellular pathogens, such as cancer or virus-derived proteins and the later to extracellular pathogens, such as nematode or cestode parasites (Janeway *et al.* 2005). Those distinct functional mechanisms justify the division of MHC in two classes, the MHC class I and the MHC class II respectively. The genomic organization of those classes diverge between Teleosts, like the European eel, where each class is found in separate chromosomes, and all other jawed vertebrates, where the MHC region is a single gene-dense cluster with both class I and II tightly linked (Wegner 2008).

Independently of the MHC classes, the ability to bind diverse antigens is a function of the genetic composition of the PBR: the higher the genetic diversity of PBR, the wider the range of parasite-derived antigens individual can mount an adaptive response against (Eizaguirre & Lenz 2010a). Specifically for the MHC class II, studies have focused on the exon 2 of the  $\beta$  chain of the protein (Wegner 2008). This genomic region encodes the PBR and is therefore responsible for the extreme polymorphism of the gene (Eizaguirre & Lenz 2010a; Sommer 2005; Spurgin & Richardson 2012). The high polymorphism of particular regions of the MHC genes often translates into high nucleotide diversities within populations, and high number of alleles within individuals. These observed patterns of diversity find no match in any other regions of the vertebrate genome encoding for functional proteins (Klein *et al.* 2007). This fact has puzzled researchers ever since its discovery and multiple hypothesis have been put forth to justify its creation and maintenance.

### *3.2. Trans-species polymorphism*

Due to its ability to respond to pathogen threats, the characterization of MHC represents a hallmark in the evolutionary history of immune systems. Phylogenetic analyses revealed an old, common ancestry of MHC genes that extends back to the first jawed vertebrates, further detecting old divergent clades that surpass species boundaries. This phenomenon is called trans-species polymorphism (Klein *et al.* 2007) and has been observed e.g. in turtles (Stiebens *et al.* 2013a), sticklebacks (Lenz *et al.* 2013), frogs (Bos & Waldman 2006) and humans (Reche & Reinherz 2003) amongst many others (see (Klein *et al.* 2007) for a review). Trans-species polymorphism presumably

reflects, at a macro-evolutionary time scale, the ecological relevance of MHC polymorphism which is currently acknowledged to be maintained through balancing selection acting on the peptide-binding regions (Klein *et al.* 2007).

### *3.3. Parasite-mediated selection*

Given the ubiquity of pathogens (Poulin 2011; Windsor 1998) and the known role of MHC genes in immunity the mechanisms of balancing selection proposed to drive polymorphism at MHC have been understood under the general theory of parasite-mediated-selection (PMS) (Bernatchez & Landry 2003; Eizaguirre & Lenz 2010a; Spurgin & Richardson 2012).

PMS is proposed to mediate MHC diversity through three main mechanisms: heterozygote advantage, frequency-dependent selection and fluctuating selection (Spurgin & Richardson 2012). The heterozygote advantage hypothesis states that heterozygous individuals at MHC loci can respond to a large diversity of parasite-derived antigens therefore be able to resist a broader range of pathogens than homozygous individuals (Hughes & Nei 1988). The rare allele advantage hypothesis proposes that high frequency MHC alleles in a population are counter adapted by the parasite, leading to an increase in frequency of the rare alleles (Eizaguirre *et al.* 2012a; Takahata & Nei 1990). Lastly, the hypothesis of fluctuating selection states that spatial and temporal variation in parasite communities are responsible for the MHC genetic diversity of host populations, as each one would be locally adapted to the respective parasite community (Hill 1991). These hypotheses are not mutually exclusive, and may also relate to the strength of the selective pressure posed to the parasite and time since host-parasite relationship occurred (Spurgin & Richardson 2012). Importantly, and as a mechanism of natural selection, PMS is responsible for losses of genetic diversity. Therefore, to understand the MHC diversity one also needs to evoke the mechanisms able to generate, on equally fast temporal scales, novel genetic diversity.

### *3.4. Generating genetic novelty*

The maintenance of standing genetic variation at MHC, i.e. polymorphism existing in a population, is a function of selection intensity, mutation rate and effective population size (Sommer 2005). Still,

selective sweeps promoted by long periods of intense parasite pressure can deplete MHC genetic diversity in a population through selection for specific resistant alleles (Sommer 2005). Similarly and through long periods of reduced population sizes, reductions in genome wide genetic diversity due to genetic drift may affect specific regions such as the MHC (Spurgin *et al.* 2011). After such scenarios, how does MHC recovers genetic diversity? The pertinence of this question is underlined in the ongoing hypothesis of host-parasite co-evolution (Liow *et al.* 2011; Van Valen 1974), which predicts an evolutionary arm races between hosts and parasites in order to counter adapt each other. Indeed, it is critical for the viability of a population to rapidly recover MHC variability in order to fight off the emergence of evolving parasite threats.

Excluding migration, long standing debates on the processes that drive the regeneration of MHC diversity within a population have led evolutionary biologists to consider mechanisms additional to single point mutations as the drivers of the regeneration of MHC diversity (Spurgin *et al.* 2011; Wegner 2008). At molecular level, one can name gene conversion and recombination, which, in brief, are non-reciprocal transfers of segments of DNA between two homologous chromosomes during meiosis (Betran *et al.* 1997). Those processes differ mainly in the amount of genetic information exchanged during each event. Gene conversion is often used to define the exchange of small continuous segments of DNA (Betran *et al.* 1997), while recombination is a recurrent term when larger sections of chromosomal regions are exchanged. Apparently and when referring to MHC, recombination is often associated with inter allelic exchange, or exon shuffling (Ohta 1991), while gene conversion refers to minor, intra allelic exchanges (Yeager & Hughes 1999). These mechanisms maybe particularly important in bottlenecked or founder populations, since novel genetic variation can be created upon minimal levels of divergence between the homologous sequences, as suggested by field studies of birds (Spurgin *et al.* 2011), ungulates (Schaschl *et al.* 2006) and fish (Reusch & Langefors 2005). Lastly, MHC can be found in high copy number in many genomes (Sommer 2005; Star *et al.* 2011), which might potentiates the action of the above mentioned mechanisms.

### 3.5. Applications to the eel population

Surprisingly, and despite the putative role of the parasite *A. crassus* in the decline of the European eel's recruitment, the genetic diversity of eel MHC has never been assessed. In addition to inferring the role of the parasite in the onset of recruitment collapse, the urge for such clarification is justified by the critical demographic period of chronically low recruitment, which *per se* could have decreased the genetic diversity of the adaptive gene and therefore compromised the adaptive potential of the species.

## ***Thesis outline***

The main objective of this thesis was to investigate the evolutionary ecology of the European eel, in the light of the recent and well documented recruitment and population decline that occurred in the 1980s. By exploring potential drivers, I (and colleagues) attempted to identify both ecological and evolutionary constraints to the viability of the European eel species and shed light on several unknown aspects of the eel biology. In chapter I, we inferred the role of ocean currents on the evolution and contemporary demography of the species. It includes an integrated approach that combines population genetic theory and ocean modelling. In chapter II, we evaluated the genetic status of the eel population by comparing two distinct generations of eels and analyzing both neutral and adaptive genetic markers. The adaptive marker of choice, the Major Histocompatibility Complex, also allowed testing for the hypothesis that argues for a decisive role of the swim bladder parasite *A. crassus* in the recruitment decline of the European eel. Finally, in chapter III, we built on the results of the previous chapters, namely, the identification of matrilineal lineages possibly linked to female philopatric behaviors (chapter I) and the putative recovery of the eel population (chapter II). In this chapter, we utilized three consecutive cohorts of glass eels and performed measurements of male mediated gene flow, i.e. male migration, amongst the hypothetical female demes and test how their putative existence can be important for the species viability.



# Recruitment Collapse and Population Structure of the European Eel Shaped by Local Ocean Current Dynamics

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## Summary

Worldwide, exploited marine fish stocks are under threat of collapse [1]. Although the drivers behind such collapses are diverse, it is becoming evident that failure to consider evolutionary processes in fisheries management can have drastic consequences on a species' long-term viability [2]. The European eel (*Anguilla anguilla*; Linnaeus, 1758) is no exception: not only does the steep decline in recruitment observed in the 1980s [3, 4] remain largely unexplained, the punctual detection of genetic structure also raises questions regarding the existence of a single panmictic population [5–7]. With its extended Transatlantic dispersal, pinpointing the role of ocean dynamics is crucial to understand both the population structure and the widespread decline of this species. Hence, we combined dispersal simulations using a half century of high-resolution ocean model data with population genetics tools. We show that regional atmospherically driven ocean current variations in the Sargasso Sea were the major driver of the onset of the sharp decline in eel recruitment in the beginning of the 1980s. The simulations combined with genotyping of natural coastal eel populations furthermore suggest that unexpected evidence of coastal genetic differentiation is consistent with cryptic female philopatric behavior within the Sargasso Sea. Such results demonstrate the key constraint of the variable oceanic environment on the European eel population.

## Results and Discussion

### Oceanographic Modeling

We studied the effect of mesoscale currents and their variation on the European eel (*Anguilla anguilla*) over more than half a century using a novel high-resolution ocean model [8, 9], atmospherically driven with improved reanalysis products [10]. In silico, we released  $8 \times 10^6$  virtual eels (v-eels) in an

area, depth, and time range reflecting the putative spawning area of the species [11, 12], allowing them to disperse [13] following realistic ocean conditions. This experiment was repeated annually for the period between 1960 and 2005. We subsequently defined v-eels as “successful” if they reached the continental shelf (25°W meridian) within a 2-year period within the simulation [14]. With this approach, we confirmed the existence of an ocean bifurcation pathway [15] that emerges only at sufficient spatial model resolution [16] and also a strong year-to-year variability in numbers at the European coastlines [17] (Figure 1). The north branch of the ocean bifurcation reflects the presence of European eel at high latitudes; the southern branch suggests the presence of eel larvae around the Canary Islands and Madeira, a prediction supported by field data [18]. The confirmation of such results provides an important demonstration of the resolution power of our novel model.

Owing to the extended period over which our model iterated variation in oceanic conditions, we were able to investigate the relative role of interannual to decadal oceanic variability on the eel recruitment: particularly, when comparing recruitment prediction from v-eels with actual observed recruitment available in International Council for the Exploration of the Seas (ICES) reports, the ocean model was strong in predicting both annual fluctuations and the collapse of observed recruitment ( $F_{VR \times \text{time}} = 35.08$ ;  $p < 0.001$ ; Figure 2). Interestingly, the significant interaction in our statistical linear model between v-eels and the period (before/after) of the major recruitment collapse shows that the correlation between oceanic fluctuations and eel recruitment was lost. Such significant interaction suggests that the lack of recovery in the European eel recruitment after the notorious decline was associated with other exogenous pressures such as parasites, pollutants, and/or lack of spawners [19–22]. Nonetheless, our study gives conclusive evidence for an oceanographic onset of the recruitment decline of the European eels.

Our analyses also revealed that years showing high dispersal rates were characterized by predominantly westward currents in the variable flow regime east of the Bahamas [23], providing a “shortcut” of the much longer route to the Gulf Stream through the Caribbean Sea. In those years, a large fraction of the v-eels can reach the Gulf Stream in a matter of weeks (Figure 1). In years with lower dispersal rates, the shortcut was absent, so that v-eels could only follow the extended migration route through the Caribbean Sea. We identified that the existence of the shortcut is dependent on the regional wind characteristics shaping the details of the western part of the subtropical gyre (see Figure S1 available online). Note that the general spreading pattern is not significantly affected by depth of v-eel release (e.g., 300 m) or longer dispersal periods (e.g., 3 years) (data not shown).

The spatial and temporal variability of the currents observed in the Sargasso Sea revealed that the spawning ground of the European eel was highly dynamic and that such variation strongly affected eel recruitment (Figure S1). What, then, are the consequences of such heterogeneous environments on genetic structure in coastal Western European eel populations? This question is important because conflicting reports

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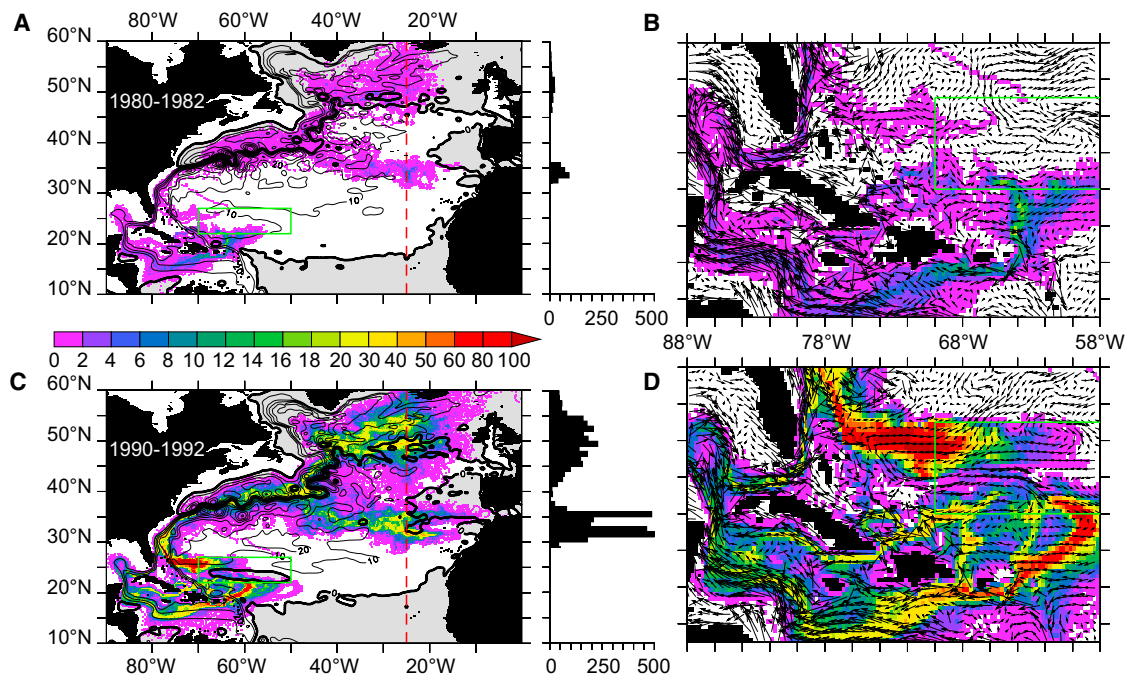


Figure 1. Simulated v-Eel Dispersal Rates

(A and C) Examples of low (A; 1980–1982) and high (C; 1990–1992) dispersal rates (in  $10^{-2}$  eels/m<sup>2</sup>) from the released area (50°W–70°W; 22°N–27°N) in the Sargasso Sea (green box) toward 25°W within 2 years. Oceanic circulation is contoured by the horizontal stream function (1-year average). Histograms show the number of v-eels arriving at 25°W, binned at 1° resolution and summed over the first 100 m of the water column.

(B and D) Close-up of dispersal rates and ocean currents, averaged over the first 3 months after release for low and high years.

regarding the existence of a panmictic breeding system in eels have raised questions regarding the existence of a single, randomly mating population. Such conclusions have also important implications for conservation and fisheries management, as numerous early-life-stage eels are translocated among watersheds in order to support fisheries, possibly affecting sensory cues required to return to the Sargasso Sea [18, 24].

#### In Silico Population Genetics

We first examined this question in silico by (1) generating two genetically distinct spawning scenarios—panmixia versus female philopatry (Figure S2)—within the high-resolution ocean circulation models and (2) comparing genetic signatures of artificially created populations at the 25°W meridian. Overall analysis showed that under the scenario of panmixia, no spatial or temporal genetic structure was detectable on European coasts (analysis of molecular variance [AMOVA]; 99% of overall variation within all populations;  $p < 0.001$ ). Conversely, the scenario of female philopatry constrained the distribution of both spatial (among sites within years = 1.2% of overall variability,  $p < 0.01$ ) and temporal (among release events = 8.6% of overall variation,  $p < 0.001$ ; within continental sites, among release events = 9.8% of overall variation,  $p < 0.001$ ) genetic variability in European populations (Tables S1A and S1B).

In spite of a homogenizing effect of the ocean, the overall degree of in silico estimated  $F_{ST}$  differentiation was higher under the female philopatry scenario than under panmixia (panmixia  $F_{ST} = 0.01 \pm 0.006$  [SEM]; female philopatry  $F_{ST} = 0.03 \pm 0.01$ ;  $t = 2.14$ ,  $df = 12.65$ ,  $p = 0.05$ ). Interestingly, spatial pairwise comparisons among continental v-eel populations (Table S2) revealed that observable genetic structure can result from both the panmixia and female philopatry scenarios, especially

in years of low recruitment. Those structures were not linked to any obvious form of isolation by distance (all Mantel tests  $p > 0.001$ ; Table S3). Pairwise comparisons (Table S2) of modeled genetic structure across different temporal periods also revealed significantly higher genetic differentiation under female philopatry than under panmixia (Student's test;  $t = 5.49$ ,  $df = 7.26$ ,  $p < 0.0001$ ), suggesting that a nonpanmictic mode of evolution may result in an isolation by time [6]. Considered together, our results unify previous conflicting reports regarding the evidence for a panmictic mode of reproduction in European eels, as even under this mode of evolution, under low recruitment conditions, departure from signature of random mating can exist on European coasts [6, 7, 25]. Our model outputs provided support for the hypothesis that the genetic signature of spatially structured (or even panmictic) distribution within the Sargasso Sea should be reflected as observable genetic structure in eels recruiting to European coastal populations.

Because the relative number of successfully arriving v-eels produced by each event was negatively correlated with the mean  $F_{ST}$  values associated with the female philopatry scenario (Spearman rank correlation:  $\rho = -0.69$ ,  $p = 0.04$ ), we predict that any observed genetic structure will be stronger under conditions of low recruitment. No such relationship was observed under the scenario of panmixia (Figure S2).

#### Molecular Analyses of Natural Populations

After our in silico examination of the role of different modes of reproduction in v-eel populations, we tested for signatures of genetic structure in natura based on the hypotheses emitted from the ocean current models. Hence, we sampled yellow-phase eels from contemporary populations from 13 different



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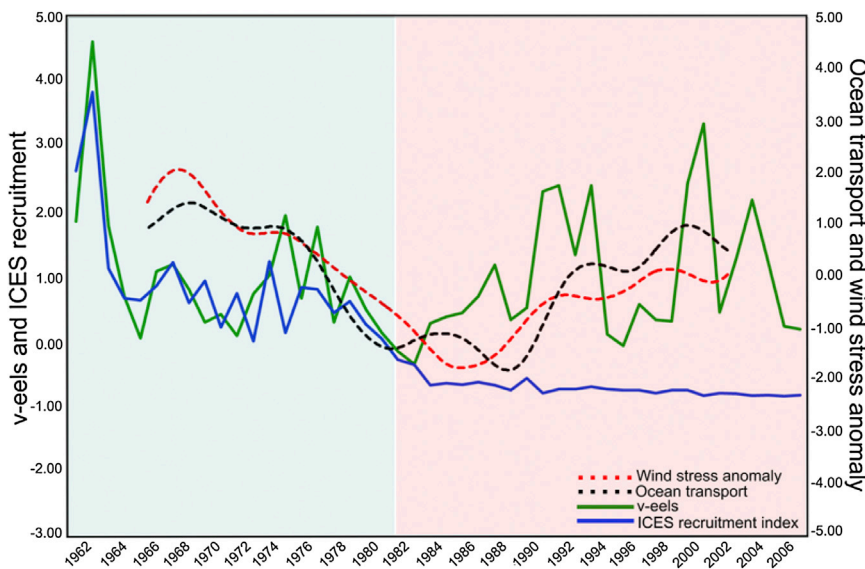


Figure 2. Natural Recruitment, Simulated Virtual Recruitment, and Wind Forcing between 1962 and 2007

Left y axis: ICES-NS natural recruitment index (blue curve) and virtual recruitment (v-eels, green curve). Right y axis: ocean transport (dashed black line) at 70°W, integrated between 24°N and 27°N. The wind stress anomaly (dashed red line) was integrated between 24°N and 28°N and between 60°W and 50°W (values were multiplied by  $-1$  for representation purposes). For transport and wind stress, a 121-month Hanning filter was applied to focus on decadal timescales.

sites located along the natural marine-freshwater salinity gradient inhabited by this species [26]. This strategy was devised to screen variation across both large and small geographic scales. We assessed polymorphism of the *ND5* region of the mitochondrial genome (which should best reflect female-mediated structure such as philopatry) as well as 17 nuclear loci (which should provide a more contemporary picture of mating systems). Information on natural populations can be found in Table S5. Consistent with some of the previous findings using nuclear markers [5, 6], we found weak but significant genetic structure among some sampling locations (Table S6). More striking however, was the strong and significant genetic structure detected using the maternally inherited mtDNA (Figure 3A; Table S6), which was significantly higher than that shown using nuclear markers (mtDNA  $F_{ST} = 0.11 \pm 0.002$  [SEM]; microsatellites  $F_{ST} = 0.02 \pm 0.0001$ ;  $t = 7.96$ ,  $df = 9$ ,  $p < 0.001$ ). Although this pattern may arise from slower allelic fixation of microsatellites and a 4-fold higher effective population size of nuclear DNA (nDNA) compared to mtDNA [27], lower levels of nuclear differentiation are generally thought to arise from female structured populations and male-mediated gene flow through opportunistic mating [28]. Deeper investigations on mtDNA gene phylogeny showed multiple lines of evidence supporting the existence of subpopulations at the source location (Figures 3B and S3; Tables S5, S6, and S7).

Importantly, the overall order of magnitude of  $F_{ST}$  detected via mtDNA sequencing reflected the higher  $F_{ST}$  levels predicted from our in silico scenario simulating female philopatry. This correlation and the maternal inheritance of mtDNA [29] suggest the discovery of a previously unreported mode of reproductive behavior in the European eel, where females are philopatric to and within locations in the Sargasso Sea, whereas males maintain gene flow by returning earlier than females to the spawning ground where they may mate opportunistically [18, 30]. Although the mechanisms underlying the homing behavior in this species are not well understood [31] and may be linked to the Earth's magnetic field [24], life history strategies of this kind are common both in aquatic and terrestrial organisms [28, 32].

In summary, a process of atmospherically driven dispersal by ocean currents connects the putative spawning grounds of the European eel and the Gulf Stream, greatly enhancing

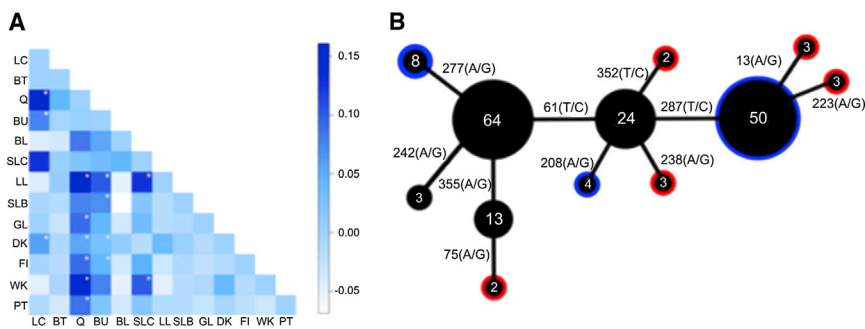
the arrival of juveniles at the European coast. When atmospheric-oceanographic conditions shift and this mechanism is absent, eel recruitment is low, explaining the onset of the large-scale collapse in recruitment that occurred during the 1980s. Following

the crash, the capacity of the eel population to recover not only was limited by a reduced supply of potential recruits but was further diminished by the effects of a multitude of anthropogenic impacts, combining to limit the probability of recovery of this ecologically and economically important species. To compensate for the shortage of eels in European freshwater systems, management measures such as stocking of eels across large geographical scales have been put in place. The assumption of a panmictic breeding system was thought to limit any consequences of such movement of individuals, but our work suggests that this may have unexpected impacts and furthermore may affect the recovery of this species. Finally, our work highlights the potential power of combining oceanographic modeling with modern population genetics, and the fusion of the two approaches will likely represent a valuable tool to understand the fundamental basis of species' evolutionary biology and ultimately optimize conservation programs.

**Experimental Procedures**

**Oceanographic Modeling**

We investigated the effects of oceanographic variability along the known dispersal pathway connecting the European eel's spawning grounds (Sargasso Sea) and the European coast by utilizing a global ocean circulation model with a very high resolution ( $1/20^\circ$ ,  $\sim 4$  to 5 km grid size) in the North Atlantic between 32°N and 85°N (VIKING20), accomplished by a two-way nesting approach [8] into the ORCA025 model [33] based on the NEMO code [9]. Owing to its very high resolution, which was identified as an important prerequisite for a realistic simulation of eel dispersal [16], advanced numerics [34], and a synoptic atmospheric forcing of the period 1948–2007 [10], our model allows the investigation of spatiotemporal variability of oceanic circulation influences with much improved verisimilitude. A detailed description of the VIKING20 ocean model is provided in the Supplemental Experimental Procedures and Figure S4. In short, using a Lagrangian tracking technique [13], we released  $8 \times 10^6$  virtual eels (v-eels) in an area and depth range reflecting the putative spawning area of the European eel [11, 12], following results and discussion for vertical distribution in [14]. Release was performed during the month of May (from the 1<sup>st</sup> until the 31<sup>st</sup>) [35]. We then calculated the dispersion of the v-eels with the transient three-dimensional flow field of the base model. The procedure was repeated for every year during the 1960–2005 period. Particles reaching the eastern North Atlantic (25°W) within 2 years of advection were defined as successful migrants [14] and entered subsequent recruitment and genetic analyses.



**Figure 3. Observed Genetic Differentiation and Phylogeny in the mtDNA Gene**

(A) mtDNA pairwise comparison matrix among 13 eel populations here represented as a heatmap (Arlequin v3.5 R graphical interface). White asterisks identify pairwise locations shown to differ significantly in haplotype composition. Significant  $F_{ST}$  values ranged between 0.058 (Q/DK) and 0.16 (Q/LL).

(B) Phylogeny and haplotype relationships are shown here in the format of a simplified haplotype network (no unique haplotypes). Numbers inside each haplotype represent that haplotype's frequency; branching numbers represent polymorphism defining the haplotypes. Red and blue markers reflect the neighbor-joining tree; blue = 50 < bootstrap < 60; red = 60 < bootstrap.

### Virtual and Natural Recruitment

The hypothesis that ocean currents drive European eel recruitment and decline was tested by the statistical comparison of natural [36] and virtual recruitment. The recruitment data set used in this study corresponded to generalized linear model of recruitment for the North Sea, hereafter referred as “ICES,” as it incorporates the longest recruitment index for the European eel, Den Oever [36]. Both types of recruitment were standardized to their z scores for direct comparisons. For statistical purposes, we defined “decline” as the time point where natural recruitment z scores became consistently negative; the factor “time” was introduced to delimit the periods “before” and “after” the population collapse. The relationship between natural and virtual recruitment before and after the decline was inferred by linear models run in R [37]. Ocean transport and wind forcing were also standardized to their z scores.

### In Silico Population Genetics

We integrated the eel genetic component to the oceanic model by splitting the released particles into ten different mtDNA haplotypes. These haplotypes were distributed either randomly or along ten subareas within the Sargasso Sea. Here, we aimed to simulate the consequences on eel distribution at continental sites of a panmictic spawning ground versus a contrasting scenario of complete genetic structure which would correspond to the population signature of female philopatry within the spawning ground. Subsequently, successfully arriving (i.e., within the 2-year period) v-eels were split, on the European coast, into an equal amount of ten populations—each population spanning 4° latitude (Figure S2). To discriminate any effects of temporally and spatially isolated samplings on genetic structure under both spawning scenarios, we performed two AMOVAs: (1) among release events and (2) among artificial populations at continental sites. The capacity of the release events to generate genetic structure at the coast was also examined by calculating Wright's index ( $F_{ST}$ ) pairwise comparisons among artificial populations. Isolation by distance was calculated among artificial populations. To this end, geographical distances were converted according to the relation 1° latitude = 110 km. Finally, to investigate the possible link between recruitment and population structure at continental sites under the proposed spawning scenarios, we correlated each release event's averaged  $F_{ST}$  with the proportion (Table S1) of successfully arriving particles.

### Molecular Analyses and Populations Genetics

The presence of genetic structure among European eel coastal locations was evaluated by sampling yellow eels spanning 13 locations (Table S2A) across both small (within Ireland) and large (additional four continental sites) geographical scales. A total of 240 individuals were examined for a section of the *ND5* (355 bp) mitochondrial gene as well as 17 nuclear loci. Population structure was accessed through calculation of  $F_{ST}$  values between populations. We also added eight American eel (*A. rostrata*) sequences to test for neutral evolution of the mitochondrial marker. Detailed descriptions of molecular protocols, analyses, and software used are given in the Supplemental Experimental Procedures.

### Supplemental Information

Supplemental Information includes four figures, seven tables, and Supplemental Experimental Procedures and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2013.11.031>.

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## ***Chapter II (submitted manuscript)***

### **Evaluation of adaptive potential of the European eel population suggests a recovery of its genetic status.**

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#### **Abstract**

The integration of evolutionary theory in conservation programs has greatly improved our ability to protect endangered species. A growing body of literature suggests that dissociating evolutionary processes from management activities may have detrimental consequences on the viability of endangered species. The European eel (*Anguilla anguilla*) has undergone a drastic population decline in the 1980s and since experiences a low recruitment. By 2011, the recruitment was down to 1% of the 1960-1979 reference level. Through the screening neutrally evolving markers, mtDNA and microsatellites, as well as polymorphism at the genes of the Major Histocompatibility Complex (MHC), we here assessed the impact of population decline not only in the overall genetic diversity but also to the adaptive potential of the species. Tracking the evolution of MHC genes is particularly relevant as the European eel also faces a large scale parasitic invasion by the recently introduced swim bladder nematode, *Anguillicola crassus*. Here, we present evidence that both the recruitment collapse and the invasion by the parasite have left signatures of a past genetic bottleneck event supported by an ongoing population expansion/recovery at neutral markers. Importantly, we found that the MHC not only provides the best evidence for a genetic signature of both the recruitment decline and the parasite invasion but above all it brings conclusive evidence for a recovery positively affecting estimates of genetic diversity - crucial for the adaptive potential of the species.

## Introduction

Preserving natural biodiversity while allowing species to maintain their adaptive potential is the challenge of modern conservation biology (Frankham *et al.* 2002) ; (Brodersen & Seehausen 2014, *in press* ). Anthropogenic activities impact global ecosystems and reduce population sizes of species, whether by shrinking or fragmenting available habitats, overexploitation, or disruption of population dynamics (Allendorf *et al.* 2008; England *et al.* 2010; Thomas *et al.* 2004). The smaller the population is, the more it is vulnerable to environmental, demographic and genetic factors (Keith *et al.* 2008). Even slight shifts in these factors can greatly constrain the population viability and ultimately lead to extinction (Frankham 2005). Genetic factors are particularly important as they may not manifest immediately after population bottlenecks but their effects prevail in the population even if the population size recovers to viable levels (Spielman *et al.* 2004). It is then clear that populations have complex dynamic functioning and therefore evolutionary genetics provide an ideal framework for conservation biologists to monitor population changes and viability (Hendry *et al.* 2011).

Major contributions of evolutionary principles to the study of wild populations have focused on estimating fluctuations of effective population sizes, genetic diversities or effects of genetic drift (Frankham 1995), (Waples & Do 2010). When populations experience a reduction in the number of individuals, drift acts as the main evolutionary force (Hedrick 2004), resulting in loss of genetic diversity (Hartl & Clark 1997). Low genetic diversity in turn, increases the risks of inbreeding depression or fixation of deleterious mutations (Lynch *et al.* 1995). The effect of genetic drift may also further reduce the adaptive potential of species (Castro-Prieto *et al.* 2011); under extremely reduced effective populations sizes, variation at adaptive markers (i.e. genomic regions under selection) becomes susceptible to drift, preventing adaptation to sudden environmental changes (Hedrick 2004; Ouborg *et al.* 2010; Willi *et al.* 2006). Despite the preponderant role of genetic drift in small populations, the genetic assessment of a population has traditionally been inferred by analyzing neutrally evolving genetic loci (see (McMahon *et al.* 2014)).

Populations are also subjected to natural selection. In the event of natural or anthropogenic factors causing population decline, conservation measures solely based neutrally evolving markers may be insufficient in establishing appropriate management programs (Hendry *et al.* 2011). Independently inferring genetic diversity using neutral or adaptive loci may also skew the estimates of genetic parameters of a population. Drift blurs the genetic signature of adaptation while natural selection can reduce genetic diversity at neutral markers. Therefore, management or conservation strategies devised to protect exploited or wild endangered populations should expand their toolbox beyond

neutrally evolving markers to assess adaptive potential of a species using adaptive genetic markers (Eizaguirre and Baltazar-Soares 2014, *in press*).

The genes of the Major Histocompatibility Complex (MHC) have repeatedly been shown to be suitable candidates to evaluate the adaptive potential of endangered populations (Sommer 2005; Stiebens *et al.* 2013b). This highly polymorphic multigene family (Apanius *et al.* 1997; Klein *et al.* 2007) plays a decisive role in controlling the vertebrate adaptive immune system by presenting self- and pathogen-derived peptides to T-cells (Janeway *et al.* 2005). Pathogen-mediated selection is acknowledged to be the primary factor maintaining MHC polymorphism in a population (Eizaguirre & Lenz 2010b; Eizaguirre *et al.* 2012b; Spurgin & Richardson 2012). For management and conservation purposes, the connection between the presence of pathogens and the shift in MHC allele frequencies (Eizaguirre *et al.* 2012b) may be particularly informative as an indirect way to detect the naturally or anthropogenically-derived emergence of new diseases (Sommer 2005).

The European eel (*Anguilla anguilla*) is a highly migratory, semelparous species with spawning grounds located in the Sargasso Sea and foraging grounds spread across European and North African coastal and inland waters (Tesch 2003). The post hatching migration phase is facilitated by local currents in the Sargasso sea that connects the spawning grounds with the Gulf Stream (Baltazar-Soares *et al.* 2014) and consequently with the European foraging grounds (Bonhommeau *et al.* 2008; Kettle *et al.* 2008b; Munk *et al.* 2010). Changes of local currents in the Sargasso Sea may be at the onset of the drastic decline in recruitment observed in the beginning of the 1980s (Baltazar-Soares *et al.* 2014; Moriarty 1990). Despite a chronically low recruitment for ~30 years, no genetic signature of a population bottleneck has been reported using neutral markers (Pujolar *et al.* 2011). Reasons for the low recruitment have been attributed to the lack of spawners (Dekker 2003a), pollutants (Robinet & Feunteun 2002), productivity changes in the Sargasso Sea (Friedland *et al.* 2007), and incidence of the invasive swim bladder parasite, the nematode *Anguillicola crassus* (Kirk 2003). Originally from Taiwan (Wielgoss *et al.* 2008a), this parasite was first reported in 1982 in Germany and soon became pervasive across European freshwater streams (Taraschewski *et al.* 1987). European eels are highly susceptible to this parasite (Knopf 2006) and severe infections can impair swimming performance (Palstra *et al.* 2007). Even though the parasite has only a weak impact on these fish during the continental phase of their life cycle (Lefebvre *et al.* 2013), completing the spawning migration back to the Sargasso Sea may be impaired with a damaged swim bladder that resulting from *A. crassus* infection .

Our study focuses on assessing the population genetic status of the European eel in the light of the documented low recruitment and of the introduction of the swim bladder nematode parasite. Inferences are made based on a region of the mitochondrial gene (*ND5*) and 22 nuclear microsatellite loci for evaluating the present genetic diversity and patterns of demographic events. In addition, we sequenced the highly polymorphic exon 2 of the MHC-class II B gene to assess adaptive diversity. Altogether we aim to link the recruitment collapse, the invasion by the parasite and the evolution of genetic diversity in this species. Using this multi-loci approach, we provide conservation managers with a realistic framework to monitor genetic diversity and infer population-wide adaptive potential.

## Material and methods

### *Study scheme*

A total of 683 eels were sampled which included 202 silver eels captured across 13 European inland water locations (Tab. 1) and 481 glass eels (Tab. 1) from four cohorts captured just upon arrival at European coasts. Number of individuals per sampling point, year of capture, developmental stage, and GPS locations can be found in Supp. Tab. 1. Amongst the 683 individuals, 327 were genotyped at the exon 2 of the MHC class II B gene. These individuals were chosen because they belong to populations with sufficient sample sizes for robust analyses.

Since a component of the weak but significant population structure punctually reported in the European eel system is associated with reproductive isolation by time (Dannewitz *et al.* 2005), we grouped our samples into two major age cohorts independently of their location of capture: “*silver eels*” and “*glass eels*”. Considering the complex life cycle and generation time reported in this species - 12 to 15 years (Tesch 2003) – we assumed this partition to represent at least one discrete generation. This grouping not only minimized the effects of any possible single-generation spatial structure (Pujolar *et al.* 2014), but also allowed to test for the evolution (i.e. allele frequency shifts) of immune genes between distinct generations.

### *Neutrally evolving mitochondrial marker*

#### *Genetic estimates of diversity, differentiation and demography - at the population level & cohort level*

For all samples the mitochondrial NADH dehydrogenase 5 (*ND5*) was sequenced following (Baltazar-Soares *et al.* 2014). Haplotype diversity (*Hd*) and nucleotide diversity ( $\pi$ ) were calculated for each sampling location in DnaSP v5 (Librado & Rozas 2009). Genetic structure was estimated using Arlequin v3.5 with 10000 permutations, (Excoffier & Lischer 2009). Moment-based demographic parameters that test for changes in effective population size were calculated for each sampling location in DnaSP v5 under the assumption of mutation-drift equilibrium. Tajima’s *D* (Tajima 1989) and raggedness’s *r* (Rogers & Harpending 1992) were also calculated in DnaSP v5. Confidence intervals were estimated through coalescence simulations using 1000 permutations. We evaluated the nucleotide mismatch pairwise distributions (Rogers & Harpending 1992) within each location. These distributions were compared to expected distributions under a constant and sudden population expansion (Librado & Rozas 2009). The same methods were used for comparing “*glass eels*” and “*silver eels*” cohorts.



### ***Neutrally evolving nuclear marker***

#### *Genetic estimates of diversity, differentiation and demography - at the population level*

Twenty-two microsatellite loci were used (Als *et al.* 2011; Pujolar *et al.* 2009b; Wielgoss *et al.* 2008b) and complete amplification protocols can be found in the Supp. text 1. Nei's unbiased heterozygosity ( $H_e$ ), observed heterozygosity ( $H_o$ ) and  $F_{IS}$  were calculated for each sampling location in GENETIX (1000 bootstrap, (Belkir K 1999). Rarefied allelic richness ( $A_r$ ) was calculated for each sampling location in HP-RARE v1.0 (Kalinowski 2005a). Genetic structure amongst sampling locations was inferred through pairwise comparisons in Arlequin v3.5 and Bayesian clustering in STRUCTURE v2.3.3 (Pritchard *et al.* 2000). STRUCTURE was run assuming a maximum number of possible groups of  $K = 26$ , i.e. representing the sum of all spatial and temporal partitions of our samples.

Genetic signatures of a bottleneck were tested for each location using the tests available in BOTTLENECK (Cornuet & Luikart 1996). A two-phase mutation model was assumed with 10% of the loci allowed to evolve through stepwise mutation (Kimura & Ohta 1978). Allele frequency distributions were also calculated for each location (Cornuet & Luikart 1996).

#### *Genetic estimates of diversity, differentiation and demography - at the cohort level*

In order to compare genetic diversity and demographic histories between the two cohorts and to avoid sampling bias from disproportionate number of samples in the two groups ("*glass eels*"  $n=481$  and "*silver eels*"  $n=202$ ), we performed 10 rounds of resampling of the data without replacement using PopTools (Hood 2010), hereafter referred to as "replicates". Replicates were performed based on 50 individuals. This standardization is crucial to validate future comparisons, as it has been long acknowledged that sample size affects the detection of the genetic signatures of recent bottlenecks (Luikart *et al.* 1998) and the estimation of effective population size (Waples & Do 2010).

Deviations from Hardy-Weinberg equilibrium (HWE) were calculated for each replicate in Arlequin v3.5 (10000 permutations). Nei's unbiased heterozygosity ( $H_e$ ), observed heterozygosity ( $H_o$ ), allelic richness ( $A_r$ ) and  $F_{IS}$  were calculated and compared between groups of replicates with two-sided t-tests in FSTAT (1000 permutations) (Goudet 1995). The distribution of genetic variance between "*glass eels*" and "*silver eels*" was assessed with an analysis of molecular variance (AMOVA, Arlequin v3.5) amongst groups of replicates.

Demographic history was inferred using two approaches. First, we evaluated the possible genetic signature of the recent population decline using BOTTLENECK (1000 iterations) as previously described.

Second, we estimated the effective population size ( $N_e$ ) of each replicate of “*silver eels*” and “*glass eels*” using the linkage-disequilibrium method implemented in NeEstimator V2.01 (Do *et al.* 2014). We utilized  $P_{crit} = 0.05$ , since lower  $P_{crit}$  can overestimate  $N_e$  (Waples & Do 2008). All estimates were obtained with the composite Burrows method (Weir 1990). The unweighted harmonic mean was calculated for each group according to the following equation:  $\widehat{N}_e = \frac{j}{\sum_{i=1}^j (1/N_{e(i)})}$  where  $j$  is the number of replicates,  $i$  is a given replicate and  $N_{e(i)}$  is the  $N_e$  estimate of the  $i$ th replicate (Waples & Do 2010).

### **Adaptive marker: diversity and demography of the MHC**

We amplified the exon 2 of the MHC class II gene that encodes for the peptide-binding groove of the molecule following a characterization protocol developed by Bracamonte *et al.* (*in preparation*), whose description of amplification and genotyping procedure can be found in the Supp. text 1. Individual MHC allele numbers, nucleotide diversity, and individual average nucleotide p-distance (Eizaguirre *et al.* 2012a) were calculated at each sampling location and for each group, i.e. “*silver eels*” and “*glass eels*”, in DnaSP v5 and using custom Perl scripts. MHC allele pools were compared amongst sampling locations and between “*silver eels*” and “*glass eels*” with analyses of similarity (ANOSIM) using Primer v6 (Clarke 1993) following Eizaguirre *et al.* (2011), Eizaguirre *et al.* (2012a) (1000 permutations). Correlation between MHC divergence and neutral structure was calculated using a Mantel test between pairwise  $F_{ST}$  matrices (mtDNA and microsatellites) and pairwise Bray-Curtis similarity matrices (MHC).

Minimum number of recombination events ( $Rm$ ) and estimates of recombination rate ( $R$ ) were calculated after Hudson and Kaplan (1985) in DnaSP v5, as well as the relative ( $R/\theta$ ) contribution of recombination ( $R$ ) and point mutations ( $\theta$ ) in the generation of genetic diversity (Reusch & Langefors 2005). Gene conversion was investigated using  $\psi$  that measures the probability of a site to be informative for a conversion event ( $\psi > 0$ , (Betran *et al.* 1997)) between “*glass eels*” and “*silver eels*”, using a sliding window method (window length = 2, step size = 1) implemented in DnaSP v5.

Overall positive selection was estimated with a Z-test implemented in MEGA v5 (Tamura *et al.* 2011). We tested for signs of codon-specific positive selection using maximum likelihood site models using CODEML implemented in PAML v4.4 (Yang 2007) and the mixed effects model of evolution mixed effects model of evolution (MEME) (Murrell *et al.* 2012) implemented in the Datamonkey web server (Delport *et al.* 2010; Pond & Frost 2005). Detailed description of each method can be found in Supp. text 2.

Sites under positive selection were concatenated (Positively Selected Sites, PSS) to infer demography assuming selection as the main evolutionary mechanism responsible for the change in population demography. We also concatenated the remaining sites (nPSS) and performed the exact same analyses as for the PSS – allowing a direct comparison. Nucleotide mismatch pairwise distributions were calculated for both PSS and nPSS of both “*glass eels*” and “*silver eels*” under the assumption of a constant population size and sudden expansion using DnaSP v5. Demographic reconstructions were performed through coalescent-based Bayesian skyline plots (BSP) (Drummond *et al.* 2005) in BEAST v1.8 (Drummond & Rambaut 2007). Because of several characteristics of the MHC including a deviation from a neutral mode of evolution, recombination or gene conversion events (Spurgin *et al.* 2011) and trans-species polymorphism (Lenz *et al.* 2013), we did not attempt to associate the substitution rate to a clock-calibrated evolution. As such, we fixed a molecular clock and assumed three different mutation rates: 0.2, 1 (the default) and 5 substitutions per time unit respectively. The substitution model was chosen in jModeltest (Tamura-Nei: Tn93) (Darriba *et al.* 2012; Guindon & Gascuel 2003) and also inserted as parameter in BEAST’s runs. Markov chain run was set to a length of  $1 \times 10^8$ . Demography was reconstructed for PSS and nPSS of both “*silver eels*” and “*glass eels*”. Piecewise constant skyline model allowing for five skyline groups were used in three independent MCMC runs. We then compared the marginal probability distributions of several parameters amongst the runs. Lastly, we constructed lineage-through-time plots. These plots reflect accumulation of lineages through time translated for a given dated phylogeny (Nee *et al.* 1992).

## Results

### ***Neutrally evolving mitochondrial DNA***

#### *Molecular indexes, population structure and demography amongst sampling locations*

355 bp of the mtDNA *ND5* in 683 European eels revealed 102 haplotypes including 73 singletons. Forty-eight randomly picked singletons were verified by independent re-sequencing to eliminate possible risks of sequencing errors. Amongst sampling locations, haplotype diversity ranged between 0.575 (BU) and 0.934 (GL). Nucleotide diversity ranged between 0.003 (G\_SPA) and 0.008 (GL), with an average of 0.005 (+/- 0.001) amongst sampling locations (Supp. Tab. 1). Pairwise  $F_{ST}$  comparisons computed from haplotype frequencies amongst the 26 geographically confined groups revealed no significant tests after correction for multiple tests (Narum 2006), Supp. Tab. 2).

Almost all sampled locations showed negative Tajima's  $D$  values suggestive of population expansion or of putative population subdivision (Tajima 1989). The three exceptions are GER, which belong to a closed system where individual input is solely mediated by stocking (Prigge *et al.* 2013), as well as G\_TITA and G\_WENG, both of which have low sample sizes. Mismatch distribution analyses performed at the population level showed the typical pattern of a sudden population expansion where the peak differs from zero (Rogers & Harpending 1992) (Fig. 1).

#### *Molecular indexes, population structure and demography between generations*

Haplotype diversity and genetic diversity between generations (i.e. "silver eels" and "glass eels") were very similar:  $Hd_{\text{silver eels}} = 0.821$ ,  $Hd_{\text{glass eels}} = 0.842$ ;  $\pi_{\text{silver eels}} = 0.0048$ ,  $\pi_{\text{glass eels}} = 0.0049$ . No evidence for genetic structure based on haplotype frequency distributions was detected between these groups,  $F_{ST} = -0.0003$ ,  $p = 0.138$ . Both groups also had negative and significant Tajima's:  $D_{\text{silver eels}} = -2.053$ ,  $D_{\text{glass eels}} = -2.357$ , both  $p < 0.05$ .

Mismatch distribution analyses revealed that both "silver eels" and "glass eels" display the distribution of expanding populations, suggesting that the overall pattern is not driven by a single generation and has a true biological origin (Fig. 2).

## **Neutrally evolving nuclear markers**

### *Molecular indexes, population structure and demography amongst locations*

Across populations,  $H_e$  ranged between 0.6869 (G\_NIRL) and 0.7581 (PT),  $H_o$  between 0.5568 (G\_TITA) and 0.6658 (DK) and the average number of alleles per locus varied between 3.5455 (G\_VFRA) and 15.7727 (G\_AD2012).  $F_{IS}$  varied between 0.0380 (G\_NIRL) and 0.2537 (Q) (Supp. Tab. 1). Even though  $F_{ST}$  estimates are very low, pairwise comparisons revealed 7 statistically significant pairwise comparisons after correction for multiple tests (Supp. Tab. 2). STRUCTURE analyses did not show any signs of population clustering as expected under the weak observed differentiation.

None of the sampled locations showed either heterozygote excess or a mode shift in allele frequencies, characteristic genetic signatures left by a bottleneck in a population (Fig. 1).

### *Molecular indexes, structure and demography between generations*

As for the “silver eels” and “glass eels” standardized-replicates, 21 loci were used in the subsequent analyses because locus (AjTr-45) consistently deviated from Hardy-Weinberg equilibrium in all “silver eels” replicates. No significant differences for  $H_e$  ( $H_{e\text{ silver eels}} = 0.733$ ,  $H_{e\text{ glass eels}} = 0.731$ ,  $p = 0.54$ ),  $Ar$  ( $Ar_{\text{silver eels}} = 11.736$ ,  $Ar_{\text{glass eels}} = 11.826$ ,  $p = 0.46$ ) and  $F_{IS}$  ( $F_{IS\text{ silver eels}} = 0.152$ ,  $F_{IS\text{ glass eels}} = 0.162$ ,  $p = 0.07$ ) were found between the two generations.  $H_o$ , however, was significantly higher in the “silver eel” group ( $H_{o\text{ silver eels}} = 0.621$ ,  $H_{o\text{ glass eels}} = 0.612$ ,  $p < 0.01$ ). The AMOVA between “silver eel” and “glass eel” groups of replicates revealed a pattern of isolation by time ( $F_{CT} = 0.002$ ,  $p < 0.001$ ), supporting our a-priori assumption that those groups represent clear age structured cohorts.

None of the replicates showed evidence of heterozygote excess or deficiency. In addition, averaged allele frequencies of neither “silver eels” nor “glass eels” deviated from an expected L-shape distribution. However, we found that the averaged allele frequencies distribution observed in the “silver eels” group showed the signature of a 5-generations-old bottleneck identified from computer simulations by (Luikart *et al.* 1998). This is particularly evident in the distribution of the two most common allele classes, 0.8-0.9 and 0.9-1.0 (Supp. Fig. 1). This signature was not visible anymore in the “glass eel” group (Supp. Fig. 1).

Estimates of the effective population size,  $N_e$ , ranged between 0 - 625.2 for “silver eels” and 0 - 2708.9 for “glass eels”. The harmonic mean of effective population size estimates amongst replicates,  $\hat{N}_e$ , resulted in  $480.9 < \hat{N}_{e\text{ silver eels}} < 2941.7$  and  $1380.2 < \hat{N}_{e\text{ glass eels}} < 3506.0$  (Supp. Tab. 3), suggestive of a population expansion.

## **Adaptive marker: the MHC**

### *Molecular indexes and population structure*

We sequenced a 247 bp fragment of the exon 2 of the MHC class II region (91% of the total size of the exon) in 327 individuals using 454 sequencing technology. We detected 229 different amino acid coding variants. Among those, 226 (98%) were found to be unique but present in both independent replicated reactions (Supp. Info. 1). Amongst locations, nucleotide diversity ranged between 0.10185 (LL) and 0.13797 (BT). The mean number of alleles per individual ranged between 2 (Q, SE = 0.298) and 4 (G\_BU, SE = 0.392) (Supp. Tab. 4) and revealed to significantly differ amongst sampled locations (ANOVA:  $F = 1.674$ , d.f.= 17,  $p = 0.046$ ). However, post hoc pairwise comparisons showed no significant differences between pairs of populations after correction for multiple testing (all  $p > 0.05$ ). The mean nucleotide divergence (p-distance) ranged between 0.078 (BL) and 0.141 (FI) (Supp. Tab. 4) and was significantly different between sampled locations (ANOVA:  $F = 1.860$ , d.f.= 17,  $p = 0.021$ ). Post hoc pairwise comparisons revealed two significant comparisons after correction for multiple testing (GER vs FI,  $t = -3.551$ ,  $p = 0.045$ , GER vs G\_AD2011,  $t = -3.961$ ,  $p = 0.010$ ), suggesting a reduced MHC divergence in the German population.

The ANOSIM showed no significant differences amongst populations in the MHC allele pools ( $R=0.001$ ,  $p = 0.98$ ). No correlation was found amongst populations between Bray-Curtis similarity matrices on MHC and pairwise  $F_{ST}$  of both mtDNA ( $R^2 < 0.0001$ ,  $p = 0.58$ ) and microsatellites ( $R^2 < 0.0001$ ,  $p = 0.62$ )

Between generations, no difference in MHC allele pools were observed ( $R=-0.011$ ,  $p=0.87$ ). Interestingly, “*glass eels*” had a significantly higher individual mean number of alleles (“*glass eels*” = 3.423, SE = 0.166; “*silver eels*” = 2.856, SE = 0.101;  $F = 8.819$ , d.f. = 1,  $p = 0.003$ ) and a significantly higher individual mean nucleotide p-distance (“*glass eels*” = 0.117, SE = 0.006; “*silver eels*” = 0.101, SE = 0.004;  $F = 4.577$ , d.f. = 1,  $p = 0.032$ ) (Tab. 2). Both the nucleotide diversity ( $\pi$ ) and the number of minimum recombination events ( $Rm$ ) detected between “*silver eels*” and “*glass eels*” were similar ( $\pi_{\text{glass eels}} = 0.118$ ,  $\pi_{\text{silver eels}} = 0.123$ ;  $Rm_{\text{silver eels}} = 11$ ;  $Rm_{\text{glass eels}} = 10$ ) while the  $R/\theta$  ratio was slightly higher in “*silver eels*” ( $R/\theta_{\text{silver eels}} = 2.174$ ;  $R/\theta_{\text{glass eels}} = 2.089$ ).

### *Testing for gene conversion*

Gene conversion segments with an average nucleotide length of 4bp were detected within the “*glass eels*” group but not in the “*silver eel*” group. The average  $\psi$  of the whole segment was found to be 0.0002 (Fig. 3). This value is high enough to ascertain the occurrence of conversion events, but not robust enough to determine the exact length of the observed tracts (Betran *et al.* 1997).

Furthermore, at  $\psi < 0.05$ , the probability of an informative site to be involved in recombination more than once is expected to be negligible.

#### *Testing for positive selection*

Model-based tests using CODEML revealed 11 sites under positive selection while MEME identified 27 sites that have experienced episodic events of positive selection (Supp. Tab. 5). The discrepancies between the two methods reflect the different assumptions underlining the fixed effect models implemented in CODEML and the mixed effect models of MEME. Positively selected sites detected by both methods matched 10 out of 19 antigen binding sites identified in humans by X-ray crystallography (Reche & Reinherz 2003) (Fig. 3, Supp. Info. 1). Due to the functional role of the MHC, all amino acid sites that have experienced at least episodic events of selection were kept for further analyses (Fig. 3)

#### *Demographic analyses*

We aimed to test whether both positively selected sites (PSS) and non-positively selected sites (nPSS) shared identical demographic history. All mismatch distributions indicated a clear deviation from a constant population size, fitting a scenario where a major demographic event occurred (Fig. 4). The frequency distribution of pairwise differences showed different peaks for both PSS and nPSS (PSS  $\text{pairwise differences} = 20$ ; nPSS  $\text{pairwise differences} = 10$ ). Those peaks likely arise from the maintenance of old lineages known to exist in genes exhibiting trans-species polymorphism as is expected of the MHC (Klein *et al.* 2007). It was also possible to observe peaks in PSS and nPSS in the frequency of pairwise differences equaling 1. These peaks represent recent genetic diversity. No differences in patterns were detected between “*silver eels*” and “*glass eels*” (Fig. 4).

Bayesian demographic reconstructions revealed a steep decline very close to present time. This pattern is characteristic of a genetic bottleneck and is shared by all reconstructions independently of the substitution rates (Figs. 4). Noticeably, the decline is less abrupt in nPSS suggestive of a stronger influence of natural selection but also a contribution of neutral processes in the decline (Fig. 4). Furthermore, and when considering slower substitution rates, a wider high density probability interval is visible near  $t=0$  for the “*glass eels*”. This further implies a scenario of genetic diversity recovery in sites where the selective pressure is not acting directly. This scenario is also supported by the lineages through time plots that reveal a very recent burst of lineage diversification (Fig. 5).

The three independent MCMC runs clearly overlap the distributions of the posterior, likelihood and skyline estimates (Supp. Fig. 2), assuring that the demographic profiles observed were not a product of the Bayesian stochasticity, but rather a real and reproducible pattern.



## **Discussion**

Here, we present an extensive evaluation of the genetic diversity and demographic history of the European eel (*Anguilla anguilla*) using multiple genetic markers and a large collection of individuals. This work was motivated by the uncertainty over the cause of the steep decline in European eel recruitment observed in the 1980s and the spread of the swim bladder parasite, *Anguillicola crassus*. We expand on previous work (Pujolar *et al.* 2011; Wirth & Bernatchez 2003) by considering not only neutral markers (mtDNA or microsatellites) but also evaluating the adaptive potential of the species sequencing the immunogenes of the MHC. Despite the MHC being a major component of the immune system and an excellent marker of genetic diversity for endangered population, no formal evaluation of its variation and evolution in relation to demography exists for the European eel. The overarching goal was to provide a framework for conservation managers to evaluate diversity and demographical history based on variable genetic markers.

### *Genetic diversity and demography from a neutral, geographical perspective*

Firstly, the mtDNA screening provides an initial perspective on how genetic drift acts in the eel population. Due to the sampling scheme, we could test its effects amongst foraging locations. First, we evaluated genetic diversity, i.e. haplotype and nucleotide diversity. Under neutrality, those indexes are predicted to be a function of population size (Frankham *et al.* 2002). Therefore, in the drastically declined European eel population, we expected to find an overall low genetic diversity. Furthermore, and due to reports of panmixia (Als *et al.* 2011; Pujolar *et al.* 2014), we also expected coherent patterns amongst sampled locations with respect to the demographic history of this species. Instead, we found a wide range of nucleotide diversity (0.003-0.008), haplotype diversity (0.575-0.934) and Tajima's *D* estimates amongst locations. The high variation in genetic indexes amongst sampled geographical areas implies that processes act differently across the European eel continental distribution. In our opinion, two scenarios may explain these patterns. On the one hand, those estimates can result from the post-hatching transatlantic migration, since simulations showed that theoretical segregated spawning grounds would leave variable genetic signatures across continental locations under low recruitment (Baltazar-Soares *et al.* 2014). On the other hand, single-generation local selection acting on certain regions of the European eel genome could also affect diversity indexes (Pujolar *et al.* 2014). Noteworthy, some populations have been stocked, as is the case for the German population where no natural recruitment is possible due to barriers to migrations (Prigge *et al.* 2013). Such translocations thus have the potential of mixing population specific signatures therefore observations should be interpreted with caution.

Secondly, due to their bi-parental mode of inheritance, microsatellites offer the possibility to evaluate the dynamics of the eel population controlling for the effects of transatlantic post-hatching migration and possible maternally structured spawning grounds (Baltazar-Soares et al. 2014). In addition, since higher genetic diversities are more sensitive to effects of genetic drift, microsatellites are ideal to detect subtle shifts in the population dynamics (England et al. 2010). Here as well, we hypothesized that the chronically low recruitment in European eel observed since the 1980s left a genetic signature of a bottleneck. Considering the different sampling locations, we found no signature of a bottleneck in any sampling site. Not only were the estimates of allelic richness ( $A_r = 2,710-2,960$ ) and heterozygosity estimates ( $H_e = 0,687-0,758$ ) very similar, but neither mode shifts nor heterozygote excesses were observed (Fig. 1 and Supp. Fig. 1). These results are in line with a study from Pujolar *et al* (2011), which was conducted in 12 sampling locations (3 locations sampled across a temporal range) and that employed 22 EST-linked microsatellites (Pujolar *et al.* 2011). The apparent homogeneity of the allelic indexes amongst locations that we detected matches the expectations of a panmictic population (Als *et al.* 2011) or of maternally structured-spawning grounds with males maintaining gene flow (Baltazar-Soares *et al.* 2014). However, the low but significant  $F_{ST}$  observed between some of the locations probably reflects a pattern of isolation-by-time known to be major structuring factor at neutral nuclear loci in this species hence partly refuting the idea of a panmictic mode of evolution (Dannewitz *et al.* 2005).

#### *Genetic diversity and demography from a neutral, temporal perspective*

Because of some possible, though unlikely, sampling biases linked to locations, we also focused our study on temporal variation of genetic diversity. Here as well, our mitochondrial DNA results showed i) no evidence for a genetic bottleneck, ii) no differences in nucleotide and haplotype diversities between “*silver eels*” and “*glass eels*”, and iii) no signature of bottleneck in the frequency distribution of pairwise mismatches. Despite our expectations of bottleneck, the later analysis rather supports the perspective of a sudden population expansion. Negative Tajima’s  $D$  estimates reinforce this perspective and could reflect either the existence of segregated spawning grounds or single-generation local selection.

Investigating the distribution of the genetic variance observed at microsatellites, we found a significant  $F_{CT}$  ( $F_{CT} = 0.002$ ,  $p < 0.001$ ) between “*silver eels*” and “*glass eels*” replicates confirming our a-priori assumption of each group representing a distinct generation. This result on the one hand strengthens our interpretation of mtDNA isolation by time, but also allows excluding possible confounding factors associated to overlapping generations from the demographic estimates (Cornuet & Luikart 1996; Waples & Do 2010).

As we detected no evidence of heterozygote excess in any of the replicates, nor any differences between allelic richness of “*silver eels*” and “*glass eels*”, we provide conclusive evidence that the collapse of the recruitment did not translate into an observable genetic bottleneck occurring in a very recent past as also previously suggested (Pujolar *et al.* 2011). Contrary to our expectations, in-depth demographic analyses revealed that the eel populations might actually be experiencing a recovery or a rapid population growth. Several lines of evidence support this interpretation. Firstly, we estimated ~20% higher effective population size in “*glass eels*” replicates (harmonic mean  $N_e=3506.0$ ) compared to “*silver eels*” ( $N_e = 2941.7$ ). These contemporary estimates are near the lower confidence intervals of historic effective population sizes previously reported ( $5000 < N_e < 10000$ ; (Wirth & Bernatchez 2003);  $3000 < N_e < 12000$ ; (Pujolar *et al.* 2011)). Secondly, we observed fewer alleles in the most frequent class of allele frequencies in “*silver eels*”. The apparent reduction of the most frequent allele class may reflect a smooth transitory stage between a past bottleneck still detected in “*silver eels*” and a growing population scenario observed in “*glass eels*”. Even though the lines of evidence are robust, limits in detecting smooth recovery from past demographic events should be interpreted with caution (Luikart *et al.* 1998). Nonetheless, the high number of markers and individuals used in this study could be sufficient to detect contemporary signatures of a bottleneck if it had existed. Here we could speculate that the hypothetical 5-generation old bottleneck (~10-15 years per generation) detected only in “*silver eels*” may relate to a major drop in European eel recruitment that occurred in the beginning of the 1960s (EIFAAC/ICES 2011). Although the recruitment followed the natural trend shaped by ocean dynamics until the definite collapse in the 1980s (Baltazar-Soares *et al.* 2014), it is possible that the 1960s drop had a major impact on the overall genetic diversity of the species. By the crash in the 1980s, the population would have already been depleted from its original genetic diversity that would translate to no effect of genetic drift, at least in neutrally evolving markers. This is a scenario that surely deserves further studies.

Overall, our observations suggest the genetic signature of a recovery of the European eel. However, contemporary estimates of neutral diversity and effective population size are far from historical levels –emphasizing the need for continuous conservation efforts.

### ***Impact of natural selection on the demography of the European eel***

In this study, we extended the evaluation of genetic diversity and demography to the evolutionary analysis of the adaptive genes major Histocompatibility complex. The choice of this marker was motivated by the recent invasion of the European freshwater systems by the nematode parasite, *A. crassus*, for which the MHC was found to respond to in the paratenic host, the three-spined stickleback (Eizaguirre *et al.* 2012b). Using the exon 2 of the MHC class II B gene, we evaluated 1) genetic diversity, which might have been affected by the recruitment collapse and introduction of *A. crassus*, and 2) allele frequency shifts between generations, which would be a signature consistent with parasite mediated selection.

#### *Lower diversity indexes in “silver eels” point towards a selective sweep*

Using next-generation sequencing, we identified a total of 229 MHC alleles amongst 327 individuals. This indicates that the diversity within this species is far from low and directly compares to observations made in wild populations of other fishes that are not endangered, such as the half-smooth tongue sole (88 MHC class II alleles amongst 160 individuals (Du *et al.* 2011) or the three-spined stickleback (36 MHC class II alleles amongst 197 individuals (Eizaguirre *et al.* 2011). Because next generation sequencing is thought to generally overestimate the number of MHC alleles detected (Babik *et al.* 2009; Lighten *et al.* 2014; Sommer *et al.* 2013), we took multiple precautions to avoid artifacts, and found high reproducibility supporting the true existence of these MHC variants.

Generally, our results point towards a pattern of single generation selection imposed by the incidence of *A. crassus*, followed by an ongoing recovery of the genetic diversity of the gene. First, “*silver eels*” exhibited lower mean number of alleles and lower mean nucleotide distance than “*glass eels*”. This supports the perspective that the “*silver eel*” generation was under a selective pressure that reduced its pool of MHC alleles to fewer and more similar alleles. We can speculate that alleles that were able to best recognize parasite-derived antigens were positively selected in the population. This single generation selection event imposed by the parasite is consistent with a scenario of single-generation signatures of local adaptation reported in this species at a large geographical scale (Pujolar *et al.* 2014). Second, we identified two short gene conversion events in “*glass eels*”, representing new events generating genetic novelty within the MHC that may not be independent of demography and population sizes (Klein *et al.* 2007; Martinsohn *et al.* 1999; Spurgin *et al.* 2011). Altogether, the higher genetic diversity we are repeatedly detecting across genetic markers between “*glass eels*” and “*silver eels*” could represent a natural recovery of the population as a consequence of the evolution of resistance. Our study hints for an in-depth correlation between individual immune gene diversity and parasitism. Nonetheless, if the parasite invasion had triggered an adaptive

response, we hypothesized that it would be detected in the demography of the functional genetic diversity of the MHC.

#### *Contemporary loss of MHC diversity suggests a recent event of selection*

The nematode *A. crassus* was presumably introduced in the European freshwater systems by the beginning of the 1980's (Taraschewski *et al.* 1987). Its introduction provides an excellent biological calibration to evaluate its impact on the evolution of diversity of the MHC. More specifically, we expected signature of selection by the parasite to be reflected in positively selected sites of the MHC variants. In total, we detected 27 sites to be under or that have experienced positive selection along their evolutionary history. This provides the opportunity to link selection and demographical changes in the species.

Bayesian skyline plots - graphical analyses linking effective population size and genetic diversity across a time scale - showed a steep decline of the effective population size as time approaches present. This pattern is visible independently of the substitution rates and is reproducible with independent runs, suggestive of a real pattern and not of an artifact. Two main factors can explain such a profile. On the one hand, it could be attributed to the terminal branching typical of phylogenies of genes evolving under balancing selection (Richman 2000), amongst which the MHC is a classical example (Klein *et al.* 2007). On the other hand, it could be attributed to a scenario of parasite-mediated selection exerted by the spread of *A. crassus* across the European freshwater systems. As *A. crassus* was virtually unknown to the host species before its introduction in the ecosystem, the frequency of the alleles, or group of functionally similar alleles, that confer resistance against it would either be low or even absent in the population (Eizaguirre *et al.* 2012b). The selection for those rare variants could have triggered the major loss of diversity we observed in the demographic plots and confirmed by the lower diversity indexes of the "silver eels".

Noticeably, the steep decline is visible for both positively and non-positively selected sites, suggesting the interplay between demography and selection. Hence, we uphold the suggestion that the MHC shows signs of decreased diversity linked to both recruitment collapse and the invasion of the parasite. This is coherent with 1) the idea that local currents in the vicinity of the Sargasso Sea, the spawning ground of the species, are at the onset of the recruitment collapse (Baltazar-Soares *et al.* 2014) but also with 2) the spread of *A. crassus* that has affected the species and its lack of immediate recovery (Taraschewski *et al.* 1987).

Interestingly, the allelic lineage diversity of the MHC showed constant increase with a particular burst as we approach present times, as indicated by lineages-through-time-plots. Such diversification is consistent with our observation of genetic diversity generated by gene conversion and recombination within the MHC region but also, and particularly, is a typical signature of recovery after a genetic bottleneck in genes under balancing selection (Richman 2000). This scenario is supported by the mismatch pairwise distribution graphs, that in addition to the old allelic lineages expected to exist within the MHC (Klein *et al.* 2007), clearly shows the emergence of new, more recent lineages.

#### *Concluding remarks*

In summary, both the recruitment collapse and the spread of the invasive *A. crassus* nematode experienced by the European eel species have left indirect signatures of a past genetic bottleneck event mainly identified by what seems to be an ongoing population expansion/recovery at neutral markers. The best evidence for a genetic signature of both major events the eel had to face is provided by the evaluation of the adaptive genes of the Major Histocompatibility Complex. It revealed not only stronger signatures of past bottleneck but above all clear signs of recovery and larger estimates of diversity, beneficial for the adaptive potential of the species. Besides the specific contribution to eel fisheries management, our study highlights the increased resolution that analyses of adaptive genes add to management and/or conservation of wild populations. In particular, given the extreme standing variation of MHC genes, evaluating demographic history of polymorphic genes may provide practitioners with a more “real-time” tool to monitor the population status.

#### **ACKNOWLEDGMENTS**

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## Tables

Population	n	nHap	S	Hd	$\pi$	Tajima-D	He	Ho	Avg Nr alleles/locus	Ar	FIS
<i>silver eels</i>	202	34	33	0,821 (0,2230-0,8423)	0,00481	-2,05326 (-1,6500-1,9464)	0,7438 (0,2528)	0,6233 (0,2376)	16,364	15,97	0,1649 (0,1450 - 0,1802)
<i>glass eels</i>	481	85	65	0,842 (0,1941-0,843)	0,00489	-2,35741 (-1,5842-1,94939)	0,7427 (0,2598)	0,6215 (0,2304)	18,546	16,26	0,1610 (0,1488 - 0,1710)

**Table 1 - Molecular indices of "silver eels" and "glass eels"**. n = number of samples used; nHap = number of haplotypes; S = segregation sites; Hd = Haplotype diversity;  $\pi$  = nucleotide diversity; He = expected heterozygosity; Ho observed heterozygosity; Ar = Rarefied allelic richness; Fis = Inbreeding coefficient; Values in brackets represent confidence intervals, with the exception of He and Ho which represents standard deviations\* = p<0,05;

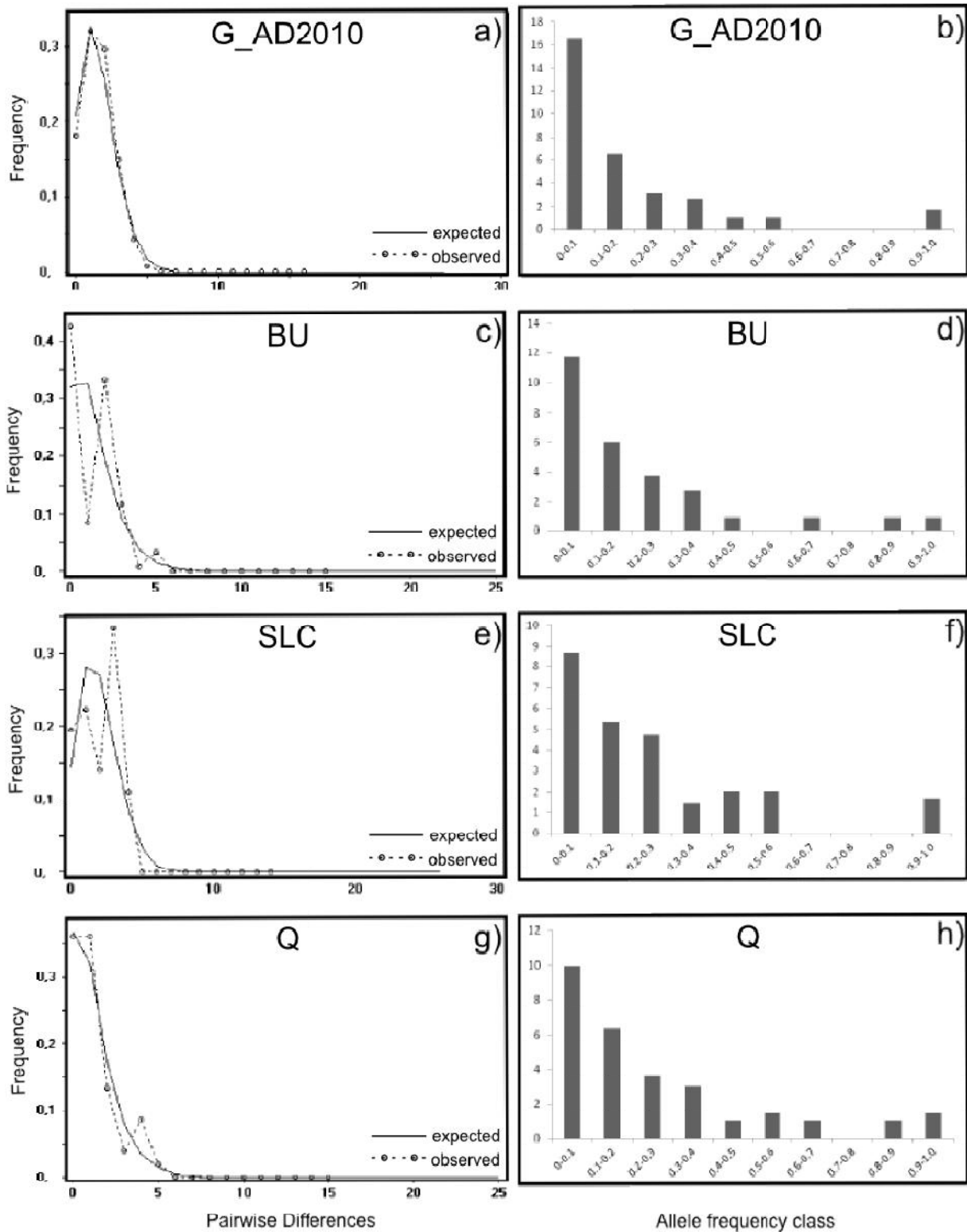
\*\*=p<0.001

Life Stage	nAlleles	nIndividuals	nHap	S	h	$\pi$	nr alleles/ind	se	dist_nt	se	R	$\theta$	Rm	R/ $\theta$
<i>glass eels</i>	332	97	115	100	0,9811	0,1179	3.423	0,166	0,117	0,006	48,0000	22,9830	10	2,0885
<i>silver eels</i>	654	230	184	115	0,9820	0,1232	2.856	0,101	0,101	0,004	53,0000	24,3850	11	2,1735

**Table 2 - Molecular indices of MHC for "silver eels" and "glass eels"**.. nHap = number of haplotypes; S = segregation sites; Hd = Haplotype diversity;  $\pi$  = nucleotide diversity; k = average number of differences; nr alleles/ind = average number alleles per individual with respective standard error (se); dist\_nt = average nucleotide distance per individual with respective standard error (se); R = recombination rate;  $\theta$  = mutation rate; Rm = minimum number of recombination events detected; R/ $\theta$  = ratio of recombination and mutation

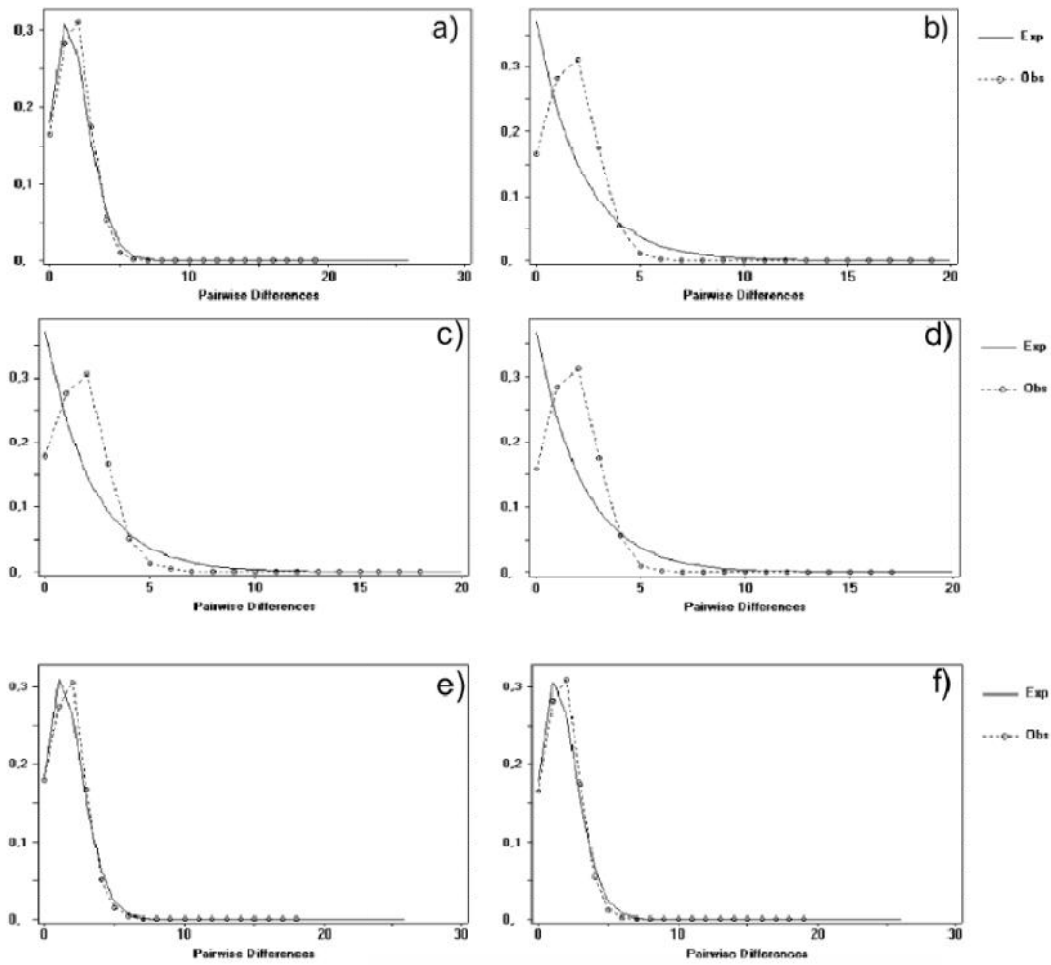


**Figures**



**Fig. 1 – Demographic analyses per sample locations.** Nucleotide mismatch pairwise distribution (**left column: a), c), e) and g)** and allele frequency distribution (**right column: b), d), f) and h)**) of locations whose samples exhibit different demographic patterns described below. In the mismatch graphs, full lines represent expected distribution under sudden population expansion, and dotted lines the observed distribution. The x-axis denotes the number of pairwise mismatches

and y-axis the frequency. It is possible to observe the signature of an expanding population in **a**), stable population or recovery from bottleneck **c**) and **e**), and stable population in **g**). In the allele frequency distribution plots, the bars correspond to allele frequencies, x-axis corresponds to allele frequency classes and y-plots to number of alleles. The sample locations are the same as the ones depicted in the mismatch graphs. All of them depict a normal L-shape distribution typical of a non-bottlenecked population.

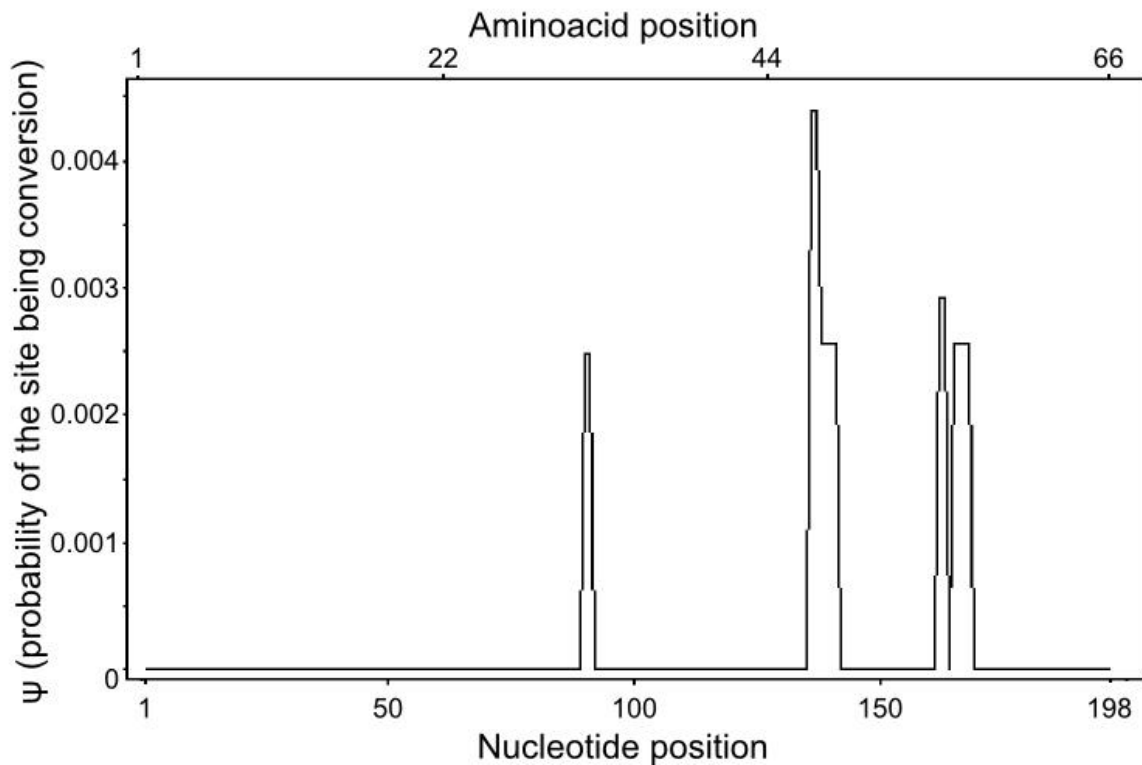


**Fig. 2 – Demographic analyses on the eel population.** In **a)** and **b)** the nucleotide mismatch pairwise distribution amongst all samples assuming **population expansion (a)**, and **constant or stable population size (b)**. From **c)** to **f)**, the nucleotide mismatch pairwise distribution amongst “*silver eels*” and “*glass eels*”. Both “*silver eels*” – **(c)** and **(d)** - and “*glass eels*” – **(e)** and **(f)** - showed similar patterns of deviation from a constant population towards expansion sudden population expansion. The x-axis shows pairwise differences and the y-axis the respective frequency distribution

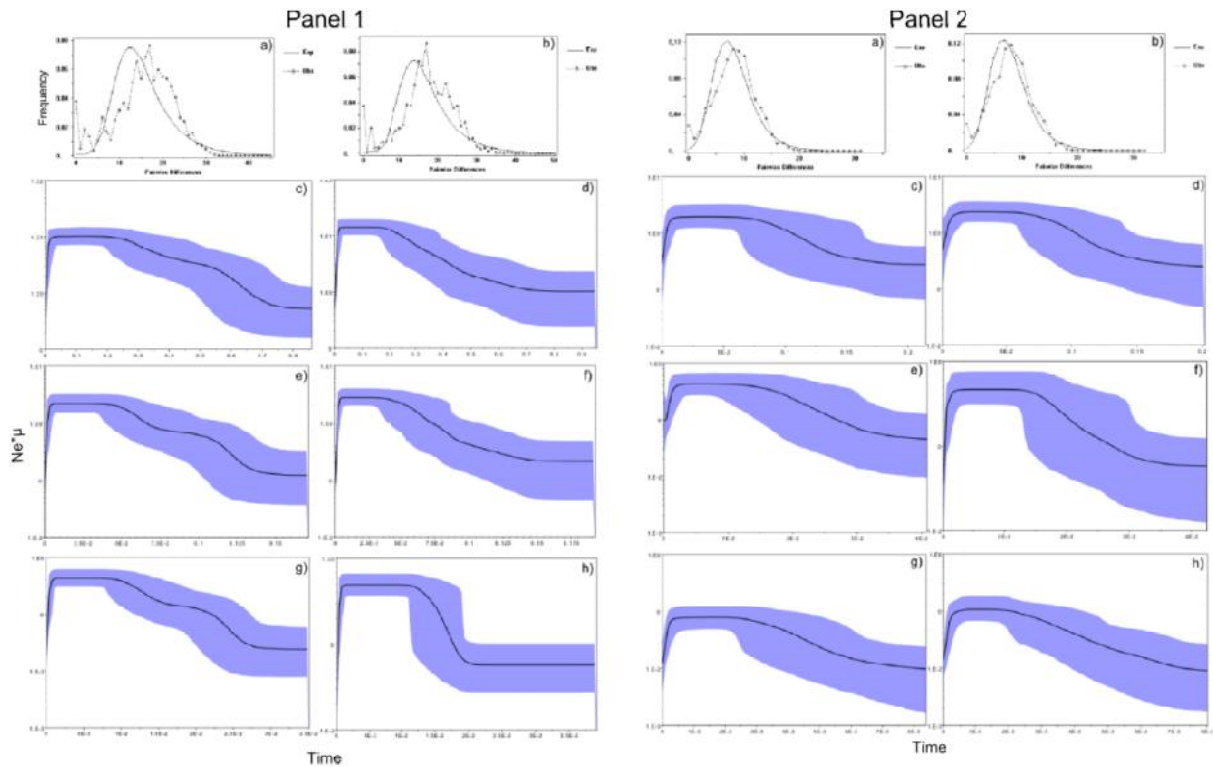
a)

	1	22	44	66
Anan-DXB0	QDLEFIDRYI FNKLEYARYN	STLNKFIGYT	EHGVKNADRW	N-RDGEAERQ HANLDSYCRH NAEL--SFN
Anan-DXB1	.....LL.....	.....AL..Q...L	.....S..G.	.T...G...P .....R
Anan-DXB2	.....L..V.....G.....	.....Y.....	.....E..F	.....D...T...G...P .....R
Anan-DXB3	...Y.S.....I.....	.....L..H.EI.	.....S..QT	.TY..G.....P .....R
Anan-DXB4	.....F.....	.....F.....	.....G.....	.....G.....P .....R
Anan-DXB5	.....D.....	.....D.....	.....T...G.....	.....G.....P .....R
Anan-DXB6	.....S..V.....L.....	.....E..A	..QE..P...	.....G..KP .....R
Anan-DXB7	.....F.....EI.	.....K..S..QT	.....G.....	.....G.....P .....R
Anan-DXB8	.....N..Q.....FL.....	.....L.....L	.....E..P...	.....G..KP .....R
Anan-DXB9	...Y.G.....V.....	.....D.....E..L	.....A..P	.....S.....
Anan-DXB10	.....FL.....	.....L.....EIF	..Q..STD.	.....I..EG.....
#DRB1*010101	ERVRLLE.C. Y.QE.SV.FD	.DVGEYRAV.	.L.RPD.EY.	.SQKDLL.QR R.AV.T... .YGVGE..T
	+++ + ++ +	+	++ -	- * - * - - + - - + + + + +

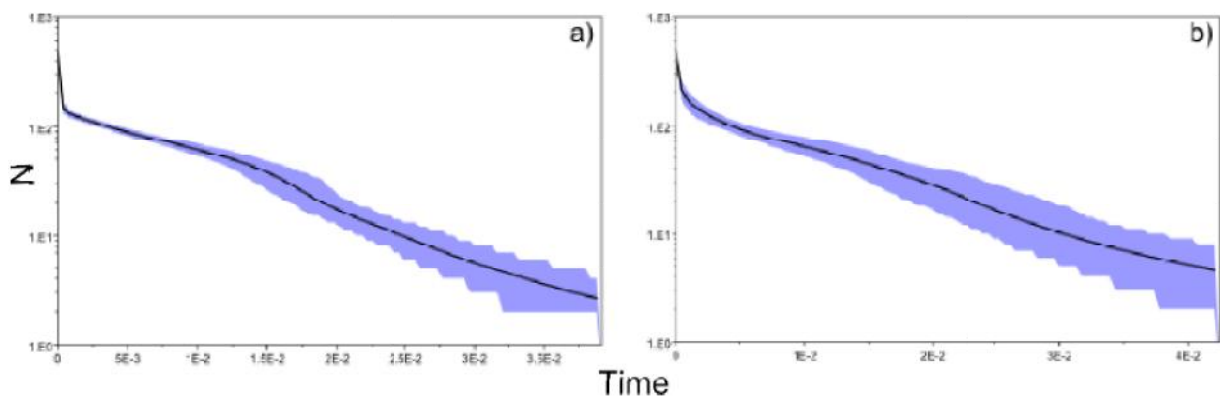
b)



**Fig. 3 – Landmarks in the allelic sequence of exon 2 of MHC class II  $\beta$  of the European eel:** a) Aminoacid alignment with human HLA-DRB1. For simplification purposes, only some of the alleles are shown. + denotes human antigen binding sites, - T cell receptor contact sites and \* sites that putatively interact with both. Sites estimated to be experiencing or have experienced positive selection are highlighted in green (identified by CODEML only), red (identified by MEME only) and blue (identified by both methods). b) Sliding window graph of  $\psi$ . Measures of the probability ( $\psi$ ) of a site being informative of conversion event in relation to the position in the alignment (in base pairs). Here it is possible to observe the two gene conversion tracts detected amongst “glass eels”, i.e. the regions 137-141 and 166-168.



**Fig. 4 – Demographic history of positively selected sites (PSS) and non positively selected sites (nPSS).** Panel 1 refers to estimates obtained with PSS only: mismatch distributions of a) “glass eels” and b) “silver eels”. Below, Bayesian skyline plots of “glass eels”, considering 0.2 substitutions/ unit of time, c), 1 substitution/unit of time, e), and 5 substitutions/unit of time, g). On the right, Bayesian skyline plots of “silver eels”, considering 0.2 substitutions/ unit of time, d), 1 substitution/unit of time, f), and 5 substitutions/unit of time, h). X-axis represents “time”. The lack of a clock-like evolution did not allow the definition of a time unit. Y-axis is an estimate of the product of  $N_e$  \* mutation rate ( $\mu$ ) per unit of time. The black line represents the mean  $N_e$  and the blue shading the 95% HPD (high probability density) interval. Panel 2 refers to estimates obtained with nPSS only. Mismatch distributions of “glass eels” a) and “silver eels” b). Below, Bayesian skyline plots of “glass eels”, considering 0.2 substitutions/ unit of time, c), 1 substitution/unit of time, e), and 5 substitutions/unit of time, g). On the right, Bayesian skyline plots of “silver eels”, considering 0.0002 substitutions/ unit of time, d), 1 substitution/unit of time, f), and 5 substitutions/unit of time, h).



**Fig. 5 – Lineages-through-time-plots (LTT):** Lineage diversification for a) PSS and b) nPSS, with both graphs showing a recent burst of lineage diversification. These graphs were built with “silver eels” using the substitution rate of 5. Like in Bayesian skyline plots, no unit of time is defined. The y-axis represents the number of lineages through time.



## **Chapter III (submitted manuscript)**

### **Asymmetric gene flow amongst matrilineages maintains the evolutionary potential of the endangered European eel**

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#### **Abstract**

Using evolutionary theory to predict the dynamics of populations is one of the aims of the emerging field of evolutionary conservation. In endangered species, whose geographic range extends over continuous areas, the predictive capacity of evolutionary-based measures greatly depends on the accurate identification of reproductive units. The endangered European eel (*Anguilla anguilla*) is a highly migratory fish species whose recruitment has undergone a steady low since the steep decline in the beginning of the 1980s. Despite punctual observations of genetic structure, the population is viewed as a single panmictic reproductive unit. Using a combination of mitochondrial and nuclear loci, we indirectly evaluated the hypothesis that female philopatry within the Sargasso Sea constrains the contemporary evolution of the species. For that, 403 glass eels from three distinct cohorts were measured, weighed and screened for genetic variation. Over the consecutive years of sampling, we detected an increased in both body condition and allelic richness – suggestive of a population recovery. We also identified three major matrilineages hypothetically representing female philopatric demes. Interestingly, not only we found lower levels of gene flow amongst matrilineages than expected under complete panmixia but we also found that there is a strong asymmetric gene flow amongst those matrilineages. Altogether our results suggest the existence of population recovery and constraints to panmixia linked to matrilineages. We uphold the suggestion that this structure maintains the adaptive potential of the species and explains that, despite the drastic population collapse, no genomic signature of bottleneck have ever been recorded.

## ***Introduction***

The field of evolutionary conservation aims at identifying the processes and mechanisms contributing to the maintenance of the adaptive potential of species and uses this knowledge to improve management (Eizaguirre and Baltazar-Soares 2014, *in press*). Particularly the use of genetic markers has paved the way for population genetics to become a discipline in which the boundaries and evolution of natural populations can be assessed (Hedrick & Hurt 2012). This is because *population genetics* allows interpreting the distribution and maintenance of genetic variation within intra-specific groups of individuals that, considering proximity, have higher chances to mate with each other (Waples & Gaggiotti 2006). This definition of population, based on reproductive interactions, may facilitate the critical but challenging task of identifying boundaries of natural populations, particularly in those species whose breeding groups are not spatially explicit (Sugg *et al.* 1996); (Manel *et al.* 2007). Identifying such boundaries is important since reduced gene flow amongst breeding groups of conspecific individuals is known to facilitate the evolution of local adaptation (Eizaguirre *et al.* 2012a), but at the same time can hinder the spread of beneficial mutations (Manel *et al.* 2003), and, in extreme cases, can even lead to inbreeding depression (Frankham 2005).

Barriers to gene flow amongst natural populations can either be the product of geographical or evolutionary constraints. For instance, habitat fragmentation resulting from the advances of ice sheets followed by secondary contact of populations after glacial retreat during Quaternary explains the contemporary distribution of many terrestrial and marine species/populations in the northern hemisphere (Hewitt 2000). Selection (Schluter 2009), mate choice (Kirkpatrick 2000), habitat choice (Via 1999) or sex-biased dispersal (Pusey 1987) on the other hand, may establish cryptic genetic structures independently of physical barriers. Amongst those, consequences of sex-biased dispersal, which states that one sex is philopatric while the other shows no natal site fidelity (Prugnolle & De Meeûs 2002) remain elusive (Stiebens *et al.* 2013b). It has been suggested that the created structure associated to philopatry could maintain the genetic diversity and evolutionary potential of species/populations (Stiebens *et al.* 2013b) through inbreeding avoidance due to 1) the existence of genetic differences between the dispersive and the philopatric sex leading to high heterozygosity amongst progeny (Prout 1981) or 2) the movement of the dispersing sex in order to avoid kin mating (Perrin & Mazalov 2000; Pusey 1987). In addition, recent evidence showed that despite female philopatry amongst endangered loggerhead turtles, the occurrence of male-biased dispersal is critical to maintain the adaptive potential of the species by mediating the transfer of immune genes amongst nesting locations (Stiebens *et al.* 2013b). The identification of such cryptic behaviors is



therefore crucial for endangered populations - especially amongst those which suffered recent population decline where the adaptive potential may already be eroded.

The European eel (*Anguilla anguilla*) is such a species. It is a highly migratory fish whose life cycle uses the entire North Atlantic basin (Tesch 2003). The connection between Sargasso Sea, where mating takes place, and foraging grounds, in European and North African coasts, is greatly facilitated by the ocean currents of the North Atlantic gyre (Bonhommeau *et al.* 2008; Kettle & Haines 2006). More specifically, local currents connecting the Sargasso Sea to the Gulf Stream have a preponderant role in mediating recruitment success (Baltazar-Soares *et al.* 2014). It has even been suggested that wind-driven anomalies in those connecting currents established the onset of the collapse in European eel recruitment in the 1980s (Baltazar-Soares *et al.* 2014). The recruitment levels have since then remained extremely low, affecting the abundance of adult eels in their continental range (Dekker 2008). Recent evidence though, suggests that the population might be recovering: large estimates of genetic diversity and an upward trend in the effective population size have indeed been reported (Baltazar-Soares *et al. submitted*).

Even though the European eel population is viewed as a single reproductive unit (Pujolar *et al.* 2014), punctual observations of genetic structure among coastal locations (Baltazar-Soares *et al.* 2014; Dannewitz *et al.* 2005; Wirth & Bernatchez 2001) suggest that the Sargasso Sea may not support a single and homogenous spawning ground (Baltazar-Soares *et al.* 2014). Particularly, the pattern of isolation by time identified by Dannewitz *et al.* (2005) across glass eel cohorts reflected temporal genetic discontinuities amongst reproductive events at Sargasso Sea. A scenario that was further extended by the use of hypothesis-driven ocean models testing predictions of female philopatry within the Sargasso Sea as a possible source of genetic structure (Baltazar-Soares *et al.* 2014). The endangered status of the European eel calls for clarifying the putative existence of the actual genetic structure because such cryptic organization could be associated to local adaptation and contribute to maintaining the adaptive potential of the species (Stiebens *et al.* 2013b). In the European eel, female philopatry could theoretically relate to 1) higher chances of transatlantic migration success and therefore increased recruitment (Baltazar-Soares *et al.* 2014), 2) insurance of fertilization by returning to the same place instead of seeking partners in the large region that is the Sargasso Sea (Sheldon 1994).

Sex-biased dispersal is commonly identified using indirect measures of gene flow (such as  $F_{ST}$ ) obtained from bi-parentally and maternally inherited genetic markers as reported in turtles (Bowen *et al.* 2004), waterfowl ducks (Peters *et al.* 2012) or great white sharks (Pardini *et al.* 2001). However,

it has been suggested that it might not be conclusive when the breeding structure of the species is not well defined (Prugnolle & De Meeûs 2002). This particularly applies to the European eel: expeditions to the Sargasso have, so far, been unable to detect a single event of reproduction, which, together with failure to sample mature individuals at those locations, constrain direct observations and hence conclusive argumentation towards or against female philopatry in this species. A solution to access the possible structure in the spawning ground of European eel is to inverse the perspective and focus on the possible outcomes of philopatry if it existed. In our specific case, it would consist in grouping individuals into matrilineages and compare the observed patterns with predicted genetic signatures left by panmixia. Particularly site-specific mitochondrial haplotypes have been associated to colony-specific evolutionary lineages in female philopatry in bats (Kerth *et al.* 2000; Rossiter *et al.* 2005) or linked to different nursery areas (Keeney *et al.* 2005). This means that by grouping individuals caught on European coasts for their matrilineage we can evaluate whether gene flow is bi-directional and equal amongst matrilineage groups. Meeting those assumptions would be suggestive of a panmictic mode of reproduction. Failing to reveal such patterns would suggest that philopatry constrains panmixia and hence contribute to the species' evolution.

To test whether matrilineages result from the evolution of philopatry, we first screened 3 consecutive cohorts of glass eels - representing over 400 individuals – for mitochondrial DNA variation and grouped the found haplotypes into different lineages supposedly representing female philopatric units. Second, using 22 microsatellite markers and fitness proxies, we explored the possible evolutionary constraints that might maintain those matrilineages in the population. To this end, we measured the rates and directionality of gene flow amongst female philopatric units and performed heterozygosity fitness correlations.

## **Material and methods**

### *Indicator of individual fitness: “condition index after arrival”*

This study includes a total of 403 glass eels from three distinct cohorts captured in the mouth of the river Adour in France. Individuals were captured within the 4<sup>th</sup> week of December of 2010 (n=157), 2011 (n=127) and 2012 (n=121). Specimens were dried with absorbing tissue, weighed (total weight +/- 1 mg), measured with an electronic caliper (total length, +/-0.01 mm) and clipped for DNA analyses. In addition, we added eight sequences of American eels (*Anguilla rostrata*), the sister species of the European eel, amplified in a previous study (Baltazar-Soares *et al.* 2014).

We calculated the relative condition index ( $Kn$ , (Le Cren 1951), which is a robust method to analyze individual condition in relation to average of all sampled population (Froese 2006)..  $Kn$  was calculated following:  $Kn = W/aL^b$ , where,  $W$  and  $L$  are weight and length respectively,  $a$  is the intercept of the  $\log(L)$ - $\log(W)$  regression, and  $b$  is the slope of this regression. To investigate whether we could fit a single regression line common to all the cohorts (and therefore consider a species-specific growth), we first perform an analyses of covariance with  $\log(W)$  as dependent variable and  $\log(L)$  and independent variable with “cohort” as fixed effect. Since all our fish were captured at the glass eel stage at the mouth of the Adour river, we hypothesized that  $Kn$  provides indications on the condition that each individual have reached after their transatlantic migration and is therefore referred to as “condition index at arrival”

### **Genetic diversities, structure and matrilineages**

All samples were sequenced for the mitochondrial NADH dehydrogenase 5 (*ND5*) gene following (Baltazar-Soares *et al.* 2014). Haplotype diversity ( $Hd$ ) and nucleotide diversity ( $\pi$ ) were calculated for each cohort in DnaSP v5 (Librado & Rozas 2009). To infer genetic structure, a test of pairwise comparisons of haplotype frequencies amongst cohorts was performed using Arlequin v3.5 (10000 permutations (Excoffier & Lischer 2009).

In line with a recent study proposing the existence of female philopatry within the Sargasso Sea (Baltazar-Soares *et al.* 2014), we attempted to identify major mtDNA lineages (matrilineages), and used them as proxy of the philopatric spawning groups, as it has been observed amongst animals that follow this strategy. For that purpose, we created a mtDNA haplotype list in DnaSP v5 and drew a network using NETWORK v4.6.1.2 (Bandelt *et al.* 1999). Eight sequences of American eels (*Anguilla rostrata*), the sister species of the European eel were used as an out-group. We calculated a median

joining network with the subsequent parameters: frequency criteria inactive, epsilon of 35, and the transversions weighted 8 times more than transitions, as suggested by analyses of transition/transversions bias performed on Mega v5 (Tamura *et al.* 2011). Lastly, the network was subjected to a maximum parsimony optimal post-processing (Polzin & Daneshmand 2003). Connection ambiguities, common in complex and large data sets such as ours (Bandelt *et al.* 1999), were solved by parsimonious choice of the most frequent connections observed amongst all shortest trees produced by the optimal post-processing step. Individuals were then grouped into three lineages (A, B and C) with respect to their network location and connection to the 3 most frequent haplotypes. That grouping was also performed within cohorts, which resulted in glass eels to be distributed amongst 9 groups (3 main haplogroups x 3 cohorts). Hereafter we will refer to those groups as “demes”.

### ***Matrilineages historical demography***

The hypothesis of female philopatry assumes that the strategy has evolved as a response to the variable hydrodynamic environment in the Sargasso Sea and hence relies on matrilineages to have variable population dynamics. In order to verify this assumption, we performed independent demographic reconstructions for each lineage using the software package BEAST v1.8 (Drummond & Rambaut 2007). Based on the long term low recruitment that affected the eel population since the 1980s, we expected to detect a collapse of all matrilineages close to present time. Furthermore, if independent units exist, we predict variable rates of declines.

To parameterize the reconstruction of independent Bayesian skyline plots (Drummond *et al.* 2005) for evaluating the demographic history of the different matrilineages, we estimated the mutation rates and created a Yule’s birth-death tree (Gernhard 2008) with three datasets: one based on *A. anguilla* only, another on *A. rostrata* only and one based on all samples. We defined a normal distribution (mean = 5.8 mya, standard deviation =  $\pm 0.5$  mya) setting the initial tree root height at  $5.8 \times 10^6$  years, according to the time since the most recent common ancestor (TMRCA) of *A. anguilla* and *A. rostrata* reported by (Minegishi *et al.* 2005). Markov chain was set to  $1 \times 10^8$ . This procedure allowed us to infer the specific mutation rate of the gene.

## ***Genetic footprints of male mediated gene flow amongst philopatric demes: insights from nuclear DNA***

### *Genetic diversities, structure and contemporary demography*

All samples were screened for variation at 22 microsatellites. The amplification was performed in four PCR multiplexes of four to six microsatellite loci developed for the European eel (Als *et al.* 2011; Pujolar *et al.* 2009b; Wielgoss *et al.* 2008b); Baltazar-Soares *et al in prep*). Reactions were performed in a total volume of 10  $\mu$ l and followed the QIAGEN<sup>®</sup> Multiplex PCR kit's recommendations. Genotyping was performed on an ABI<sup>®</sup> 3100 Genetic Analyzer. Alleles were called in GENEMARKER<sup>®</sup> v. 1.91 (Softgenetics LLC, State College, PA). We calculated the heterozygosity ( $H_e$ ), inbreeding coefficient ( $F_{IS}$ ), and rarefied allelic richness ( $A_r$ ) in GENETIX (1000 bootstrap, (Belkir K 1999) and HP-RARE v1.0 (Kalinowski 2005b), respectively, for each deme within a cohort.

Firstly, to evaluate and compare the magnitude of genetic differentiation between nuclear and mitochondrial markers, we calculated the  $F_{ST}$  amongst cohorts in Arlequin v3.5 (10000 permutations). Then, we used STRUCTURE v2.3.3. (Pritchard *et al.* 2000) to infer possible structure without *a-priori* bias linked to sampling. STRUCTURE was run assuming a maximum number of clusters ( $k$ ) of 9, i.e. the total number of demes in the dataset. Secondly, to investigate the distribution of molecular variance amongst the possible 9 demes, we performed an AMOVA with "cohort" as higher hierarchical group in Arlequin v3.5 (10000 permutations). Lastly, we assessed the recent demographic history of each deme speculating that genetic diversity, migration rates and heterozygosity fitness correlations can be explained by extreme demographic processes (Beerli & Felsenstein 1999; Frankham 1995; Reed & Frankham 2003) and could have unevenly affected each matrilineage. Scenarios of bottleneck were inferred by heterozygosity excess and allele frequency shift analyzes implemented in BOTTLENECK (Cornuet & Luikart 1996).

### *Individual-based genetic indexes*

At the individual level, we calculated the internal relatedness ( $IR$ ) and the homozygosity by loci ( $HL$ ) indexes in R version 2.13.2 (Fox 2005) using the *Rhh* package (Alho *et al.* 2010).  $IR$  compares parental half genotypes (two alleles are compared at each locus) within an individual.  $IR$  ranges from -1 (outbred) to 1 (inbred), where 0 is the score of individuals born from the random pairing of unrelated parents (Amos *et al.* 2001).  $HL$  is a homozygosity index that on top of what has been described for  $IR$ , considers the contribution of each locus, rather than each allele, while estimating allele frequencies (Aparicio *et al.* 2006). Such difference between both indexes might be particularly informative in the

presence of migration amongst reproductive units (Aparicio *et al.* 2006). For instance, since *IR* weighs the contribution of alleles based on their frequency, homozygous individuals carrying rare alleles (brought in the population through migration) are attributed higher *IR* index than those homozygous individuals carrying more common ones (Aparicio *et al.* 2006). Those differences stand out when comparing the correlation strengths of *IR* and *HL* with population-based inbreeding coefficients, such as the  $F_{IS}$  (Wright 1922). Under asymmetric migration, this would translate in a lower correlation coefficient ( $r$ ) between mean *IR* and  $F_{IS}$  in comparison with correlation coefficients obtained between mean *HL* and  $F_{IS}$  of receiving demes, if rare or low frequency alleles would be transported through migration. Hereafter we will refer to the correlation coefficients  $r(\text{mean } IR, F_{IS})$  and  $r(\text{mean } HL, F_{IS})$  as  $R_{IR}$  and  $R_{HL}$  respectively. Lastly, *IR* and *HL* were calculated independently for each deme, ensuring that those metrics were weighed by the allelic frequencies of the deme alone and not of the whole data set.

#### *Measuring the gene flow amongst female philopatric demes*

One of the primary goals of this study was to investigate the gene flow amongst putative female philopatric demes represented by different matrilineages. By grouping the samples in matrilineages, the gene flow would necessarily be the product of male migration. Here, using Bayesian inference methods (Beerli 2006; Beerli & Felsenstein 2001) implemented in the software Migrate-n v.3.6.4 (Bertorelle *et al.* 2009), we 1) compared the possibility that the three matrilineages could represent separate reproductive units and 2) calculated the effective number of migrants amongst those units. For this, we created two population models. In the first one (model I), the three matrilineages were considered to be part of a single panmictic population, while in model II each matrilineage was regarded a segregated philopatric deme with the possibility for symmetric migrations (i.e. bi-directional gene flow) to exist. Lastly, to identify the most likely model, we compared the marginal likelihoods of each model (Mlog) (Beerli & Palczewski 2010). Specifications on Migrate-n v.3.6.4 protocol are available in the supplementary material (Supp. Info. 1).

#### *Heterozygote-fitness correlations*

Relationships between individual condition index ( $K_n$ ) and individual genetic indexes (*HL* and *IR*) were analyzed using linear models, with “matrilineage” nested into “cohort”. To avoid confounding effects of *HL* and *IR* on the linear model, we calculated the residuals of their correlation and used those residuals as independent variable in the linear model. All statistical analyzes were performed in R 2.13.2 (Fox 2005).

## **Results**

### ***Variation in length (L), weight (W) and condition index (Kn) amongst cohorts***

Although sampled within the same week every year, mean fish length upon arrival at the European coasts significantly varied and ranged from  $66.59 \pm 3.49$  mm in the 2010 cohort to  $71.60 \pm 3.55$  mm in the 2012 cohort (ANOVA *cohorts*,  $F = 85.83$ ; d.f. = 2,458;  $p < 0.001$ ). Mean fish weight also significantly varied and ranged from  $0.23 \pm 0.04$  g in the 2010 cohort to  $0.33 \pm 0.05$  g in the 2012 cohort (ANOVA *cohorts*,  $F = 184.50$ ; df = 2,458;  $p < 0.001$ ). Analyses of co-variance supported the use of a single regression line to calculate the condition index (*Kn*) of all individuals (Tab S1 and S2). The comparisons of condition index amongst cohorts revealed also statistical significant differences in *Kn* (ANOVA *cohorts*,  $F = 48.22$ ; df = 2;  $p < 0.001$ ) (Fig. 1). Post-hoc pairwise t-tests revealed all comparisons to be statistically significantly different ( $t_{2010-2011} = 4.065$ ;  $t_{2010-2012} = 9.817$ ;  $t_{2011-2012} = 5.320$ , all  $p < 0.001$ ).

### ***Evolution and demographic history of the matrilineages***

Amongst cohorts, the haplotype diversity ranged between 0.818 (2010) and 0.861 (2011) and nucleotide diversity between 0.004 (2010) and 0.005 (2012) (Tab. 1), which is not low for an endangered species. No evidence of population structure based on mtDNA was found amongst cohorts, suggesting stable structure over time (higher  $F_{ST\ 2010vs2011} = 0.0002$ ,  $p = 0.36$ ).

The haplotype network revealed the existence of three major haplotypes and 68 satellite haplotypes (Fig. 2). The parsimonious resolution of connection ambiguities allowed us to delimitate three matrilineages (but see Fig S1 for all possible networks). Each consists of the main haplotype and the satellite haplotypes that directly relate to it. We named those matrilineages “A”, “B” and “C” (Fig. 2). Matrilineage A was consistently the most represented, accounting for ~ 50% of the total number of individuals in each cohort.

Interestingly, differences in the demographic patterns could be observed amongst those major lineages: matrilineage A shows a steeper growth phase surrounded by two plateaus of constant size. The onset of the growth phase occurred ~ 1.5 million years ago (mya) and ended ~ 0.5 mya. This pattern contrasts with the single phase of constant growth that can be observed in the demographic plots of matrilineages B and C that drags throughout the historical timeline investigated (Fig. 3).

### ***Assessment of the genetic diversity of each matrilineage***

For the group-based indexes i.e. allelic richness (*Ar*), Nei’s unbiased heterozygosity (*He*) and  $F_{IS}$ , we found that only the allelic richness *Ar* significantly differed amongst cohorts (ANOVA<sub>*Ar*</sub>:  $F = 7.01$ , d.f. =

2,  $p=0.03$ ) with the 2012 cohort showing the highest level (mean  $Ar_{2010} = 9.780$ ,  $SD=0.130$ ; mean  $Ar_{2011} = 10.073$ ,  $SD=0.161$ ; mean  $Ar_{2012} = 10.117$ ,  $SD=0.005$ ). Neither  $He$  nor  $F_{IS}$  significantly varied amongst cohorts (ANOVA $_{He}$ :  $F = 2.864$ ,  $d.f.= 2$ ,  $p=0.134$ ; ANOVA $_{FIS}$ :  $F = 0.107$ ,  $d.f.=2$ ,  $p=0.9$ ). No deme-based indexes significantly differed amongst matrilineages either (ANOVA $_{Ar}$ :  $F = 0.45$ ,  $d.f.=2$ ,  $p=0.66$ ; ANOVA $_{He}$ :  $F = 1.56$ ,  $d.f.= 2$ ,  $p=0.29$ ; ANOVA $_{FIS}$ :  $F = 0.40$ ,  $d.f.=2$ ,  $p=0.68$ ) (Table. 2).

In relation to the individual-based diversity indexes ( $HL$ ,  $IR$ ), we found no differences amongst cohorts, amongst matrilineages, or amongst demes (ANOVA $_{HL}$ :  $F=0.710$ ,  $df_1, df_2=8,377$ ;  $p=0.685$ ; ANOVA $_{IR}$ :  $F=0.585$ ,  $df_1, df_2=8,377$ ;  $p=0.792$ , Table 2).

Investigating possible genetic structure without a priori information on demes or cohort did not reveal any sign of grouping while ranging  $K$  from 1 to 9 in STRUCTURE. Amongst cohorts, even though very low,  $F_{ST}$  comparisons revealed to be statistically significant after correction for false-discovery rate (Narum 2006);  $F_{ST\ 2010/2011} = 0.002$ ,  $p=0.02$ ). Similarly, one pairwise  $F_{ST}$  comparison amongst demes revealed to be statistically significant after correction for multiple testing, ( $F_{ST\ 2010A/2011B} = 0.005$ ,  $p=0.01$ ). The AMOVA showed no statistical support for any sort of grouping, attributing 99% of genetic variance to within lineages. Altogether, these analyses confirm a pattern of isolation by time, as reported previously for the eel population. Lastly, no heterozygosity excess or allele frequency shifts, signs of genetic bottleneck, were detected in any of the 9 demes (Supp. Info. 2).

### ***Comparing models: assessing the likelihood of structure and the direction of gene flow***

Comparison of the average marginal log likelihoods (Mlog) of model I (panmixia) and model II (3 demes with symmetric migration possible) showed that model II is accepted over model I (model I $_{MLog} = -5400.113$ ; model II $_{MLog} = -3740.630$ , Bayes factor (model II $_{MLog} -$  model I $_{MLog}$ ) = 1659.483). We then calculated the effective number of migrants ( $N_e m$ ) predicted by model II, following  $N_e m = M_{j>i} * \theta_i$ . Results revealed the existence of asymmetric migration amongst the hypothetical philopatric demes. In particular, deme A always acted as source for the others demes. This was clearly evident in the cohort of 2010, where emigration ranged between 30 and 60  $N_e m$ , while immigration was reduced to 1  $N_e m$ . In addition, in the cohort of 2012, emigration from deme A to deme C was also one the highest observed in this study, 83  $N_e m$ , which again contrasted with immigration of 1  $N_e m$  (Tab. 3 and Fig. 4). Modes and confidence intervals of  $\theta$  and  $N_e m$  can be found in Table S3 and S4.



### ***Impacts of gene flow on the genetic diversity of matrilineages***

The likelihood of gene flow amongst the hypothetical female philopatric demes to transport rare alleles was investigated by comparing the coefficients of  $R_{IR}$  (correlation coefficient between mean  $IR$  and  $F_{IS}$ ) and  $R_{HL}$  (correlation coefficient between mean  $HL$  and  $F_{IS}$ ) considering the 9 possible demes. Under the observed asymmetries in gene flow amongst demes, one would expect  $R_{HL} > R_{IR}$  if immigration would bring new or low frequency alleles to the receiver demes. Here, we detected a coefficient  $R_{IR}$  of 0.93 ( $p < 0.001$ ) and a coefficient  $R_{HL}$  of 0.88 ( $p = 0.002$ ). The incorporation of  $HL$  and  $IR$  as independent explanatory variables to  $F_{IS}$  in a linear model confirmed that  $IR$  explains a higher proportion of  $F_{IS}$  than  $HL$  ( $t_{IR} = 2.380$ ,  $p = 0.06$ ;  $t_{HL} = -0.543$ ,  $p = 0.61$ ;  $R^2 = 0.84$ ,  $p = 0.002$ ). Therefore, it is possible to conclude that the gene flow amongst demes does not transport rare or low frequency alleles further suggesting that mating within demes is the main source of new genetic diversity.

### ***Heterozygosity-fitness correlations***

To explore potential drivers of the variations in condition index ( $Kn$ ) observed amongst cohorts or amongst demes, which would imply matrilineage-specific fitness, we fitted a linear model where we included potential effects of mitochondrial lineage (mtDNA) and individual diversity indexes such as  $HL$  and  $IR$ . We found that  $Kn$  varied amongst cohorts ( $F_{\text{cohort}} = 48,654$ ,  $p < 0.001$ , Tab. 4) and marginally negatively correlated with  $HL$  ( $F = 3.714$ ,  $p = 0.055$ ). This suggests that the fitness trait here measured, “condition index upon arrival”, are not directly linked to the evolutionary constraints maintaining the matrilineages in the eel population but may relate to genetic factors.

## Discussion

The main objectives of this study were 1) to test whether indirect genetic evidence exist to support the perspective of female philopatric units which would constrain the evolution of panmixia in the eel population and 2) to assess consequences of those matrilineages on the genetic diversity and dynamics of gene flow. Because sampling mature adults in the Sargasso Sea is challenging, our idea was to group glass eel individuals from consecutive cohorts according to their matrilineage (mtDNA) and then use nuclear markers to infer the likelihood of those matrilineages to represent segregated breeding units. Several lines of evidence suggest that to be the case. Firstly, by comparing two possible population models, one representing complete panmixia and the other representing a population structured by matrilineages, we found stronger support for the data to be best explained by segregated units. Secondly, inferences of gene flow amongst matrilineages revealed that migration is asymmetric which is inconsistent with a panmictic mode of reproduction. Furthermore, results suggest that rare alleles are maintained within each deme rather than being transported through gene flow. Altogether, our study brings indirect genetic support for the hypothesis of female structured spawning grounds in the European eel and suggests that such behavior might contribute to the maintenance of the evolutionary potential of this endangered species, and also playing a key role in the ongoing recovery of the population.

### *Definition, historical demography and contemporary structure of mitochondrial lineages*

By constructing a haplotype network, we were able to identify three major matrilineages. This grouping does not relate to a spatially explicit geographic origin in the Sargasso Sea, but is a representation of the typical site-specific haplotype frequencies, signature of philopatry, that can be observed amongst colonies of bats (Kerth *et al.* 2000) or islands of female turtles (Stiebens *et al.* 2013b), for instances. Upon their definition, we investigated the demographic history of each matrilineage. From a contemporary perspective, we observed that all lineages exhibited a subtle and recent growth where the genetic signature of the 1980s collapse is clearly absent. This observation is consistent with the lack of signature of bottleneck (Pujolar *et al.* 2011) and even the recent discovery of a molecular signature of an ongoing recovery at adaptive genes (Baltazar-Soares *et al.*, *in prep*). Furthermore, we observed that all matrilineages are likely experiencing an historical growth phase though with contrasting shapes: the most common lineage displays a steeper and more pronounced expansion compared to the two others. According to the Bayesian skyline plots, that expansion has started apparently  $\sim 1.25$ mya, and lasted for  $\sim 1$  mya, matching a high velocity phase of the Gulf Stream western boundary current (Kaneps 1979). This result mainly reinforces the key role of the

Gulf Stream in the population dynamics of the European eel (Baltazar-Soares *et al.* 2014; Bonhommeau *et al.* 2008; Kettle *et al.* 2008b). Furthermore, it suggests that the observed higher frequency of the most common lineage might be a consequence of its rapid historical expansion. The demographic profiles observed for the two other matrilineages further suggest that they have experienced a sustained, steady growth, possibly underlying the recovery of the eel population. Curiously, we were not able to detect the demographic profiles presented in Jacobsen (Jacobsen *et al.* 2014a), i.e. the decline and recovery *circa* 200 000 years ago. Since our analyses were performed in a single mitochondrial gene (*ND5*) and given the variable evolutionary histories amongst mitochondrial genes (Simon *et al.* 1994), it is possible our signal to be specific to *ND5*. However, the high sample size of this study (total of 403 individuals) ensures that the pattern we observed is not an artifact but has a biological origin.

#### *Measures of gene flow: insights into the structure and dynamics of the European eel population*

The definition of matrilineages allowed testing for the statistical robustness of segregated units for matrilineages vs. a complete panmictic mode of reproduction. We used Bayesian statistics coupled with coalescent theory, a framework that is becoming increasingly acknowledged as an ideal inferential approach to test connectivity amongst putative populations (Beerli & Palczewski 2010; Lee *et al.* 2013). The comparison of marginal log likelihoods of a full panmictic population *versus* population structured by the three demes showed that the latest was accepted as a potential true model (Bayes factor: model II<sub>Mlog</sub> - model I<sub>Mlog</sub> = 1659.483). Although this methodology does not provide conclusive results regarding the true structure of a population (Beerli & Palczewski 2010), the high acceptance rate of the harmonic mean estimator in identifying panmixia (70%) from a true panmictic population (Beerli & Palczewski 2010) suggests that complete panmixia is unlikely to be the underlying structure observed in our samples. Evolutionary constraints to complete panmixia observed amongst individuals collected immediately after their post-hatching transatlantic migration hints for selection acting on early life stages. This hypothesis might not be totally surprising, since the gene we here used, the *ND5*, was shown to be under selection in this species (Jacobsen *et al.* 2014a). Therefore, given the role of *ND5* in the metabolic pathway, the maintenance of matrilineages in the population could be associated with different energetic costs of the post-hatching transatlantic migration that have evolved as a function of i) spawning location and ii) oceanographic conditions. Altogether, it reinforces the hypothesis that evolutionary constraints maintain these matrilineages in the eel population, contrasting with random sorting processes that are thought to mediate the fixation of lineages in completely panmictic populations (Avice *et al.* 1984).

In addition, we detected a strong asymmetry in the migration rates amongst some philopatric demes. Specifically, over the sampled period, the most common matrilineage always acted as a source for the other demes. Asymmetries in migration rates amongst philopatric demes have been attributed to sex-biased dispersal in highly migratory species such as sperm whales (Lyrholm *et al.* 1999), salmonids (Fraser *et al.* 2004) or turtles (Stiebens *et al.* 2013b) and reflect the contrast between opportunistic mating of one of the sexes and faithfulness to specific spawning conditions of the other. Given that we group samples by matrilineage, the asymmetries here reported directly reflect male mediated gene flow.

Two critical insights are gained from these observations. The first is that asymmetries in gene flow can generate low but significant  $F_{ST}$  amongst philopatric demes ( $0.001 < F_{ST} < 0.006$ ) as observed in our study ( $F_{ST_{AD2010A/AD2011B}} = 0.005$ ,  $p = 0.01$ ) similar to those supporting the reports of isolation by time (Dannewitz *et al.* 2005). Male mediated gene flow amongst female philopatric sites might be a possible explanation for those observations, therefore providing indirect support for the hypothesis of female philopatry. The second insight gained from the asymmetric gene flow is linked to the suspected ongoing recovery of the eel population. Here, the reported high frequency of individuals belonging to matrilineage A in the eel population could have resulted from a recent high reproductive success of this deme, which, by providing males to other philopatric demes, might have had been crucial for the ongoing recovery.

#### *Heterozygosity-fitness correlations: linking demography and evolution?*

Lastly, in order to infer whether different matrilineages are associated with variable fitness traits, we performed heterozygosity-fitness correlations using body condition post-transatlantic migration as fitness trait of interest. Assuming that female philopatry evolved to maximize successful transport of offspring under given oceanic conditions, lineage-specific fitness is a reasonable expectation. Variable recruitment could be related to variation in condition index amongst the different matrilineages, linking recruitment and lineage-specific fitness. However, we observed that “cohort” is the major effect determining condition index variation. This means that either the condition index upon arrival is not directly related to matrilineage fitness, or that variation in the condition index is linked to ocean-mediated recruitment success (Baltazar-Soares *et al.* 2014). For example, if ocean currents promote a faster transatlantic migration of the 2012 cohort (in comparison with 2010) those individuals would have consumed less internal nutritional reserves and therefore obtained higher condition index at arrival.

## **Concluding remarks**

Because direct sampling in the Sargasso Sea is a challenge not yet overcome, to understand the evolution of the European eel and design appropriate conservation programs new theories have to be proposed and tested. Here, we built on previous modelling work which suggested that a female structured spawning ground would explain the punctual reports of genetic structure in a population otherwise considered panmictic. Artificially creating those female philopatric groups and testing for the population signature against that of panmixia supported the hypothesis that multiple (in time or space) spawning grounds are likely to exist. Such philopatric demes can explain why despite a drastic recruitment collapse down to <10% of historical record, no genetic signature of bottleneck were observed. Structured spawning grounds with male mediated gene flow permit the species to maintain its adaptive potential which turns out crucial for the viability of the species at low recruitment rates.

## **Acknowledgments**

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## Tables

<i>cohort</i>	<i>n</i>	<i>nHap</i>	<i>S</i>	<i>Hd</i>	$\pi$	<i>He</i>	<i>Ar</i>	<i>F<sub>IS</sub></i>	<i>Kn</i>
2010	155	31	30	0.818	0.004	0.734	14.520	0.159	0.944 (0.091)
2011	127	34	31	0.861	0.005	0.747	15.210	0.165	0.993 (0.114)
2012	121	34	35	0.851	0.005	0.748	15.400	0.166	1.061 (0.089)

**Tab. 1 – Genetic diversity and condition index of each cohort.** *n* = number of individual analyzed, **nHap** = number of haplotypes, **S** = segregation sites,  $\pi$  = nucleotide diversity, *He* = heterozygosity, *Ar* = rarefied allelic richness, *F<sub>IS</sub>* = inbreeding coefficient, *Kn*= condition index. Values in brackets represent standard deviation.

<i>Matrilineages</i>	<i>n</i>	<i>He</i>	<i>Ar</i>	<i>F<sub>IS</sub></i>	<i>HL</i>	<i>IR</i>	<i>Kn</i>
<b>2010</b>							
A	82	0.734	9.780	0.159	0.304 (0.101)	0.164 (0.123)	0.934 (0.086)
B	35	0.731	9.650	0.184	0.322 (0.091)	0.177 (0.117)	0.943 (0.100)
C	33	0.737	9.910	0.134	0.284 (0.097)	0.140 (0.119)	0.970 (0.089)
<b>2011</b>							
A	58	0.737	9.900	0.162	0.305 (0.108)	0.163 (0.132)	0.988 (0.108)
B	35	0.751	10.100	0.151	0.288 (0.104)	0.153 (0.121)	1.005 (0.122)
C	32	0.760	10.220	0.193	0.305 (0.089)	0.168 (0.118)	0.989 (0.119)
<b>2012</b>							
A	62	0.744	10.120	0.172	0.311 (0.097)	0.170 (0.110)	1.061 (0.090)
B	32	0.742	10.120	0.130	0.279 (0.124)	0.131 (0.150)	1.055 (0.090)
C	27	0.760	10.110	0.194	0.314 (0.094)	0.180 (0.117)	1.068 (0.090)

**Tab. 2 – Genetic diversity and condition index of each matrilineage, within cohort.** *n* = number of individuals analyzed, *He* = heterozygosity, *Ar* = rarefied allelic richness, *F<sub>IS</sub>* = inbreeding coefficient, *HL* = homozygosity per loci, *IR* = internal relatedness, *Kn* = condition index. Values in brackets represent standard deviation.



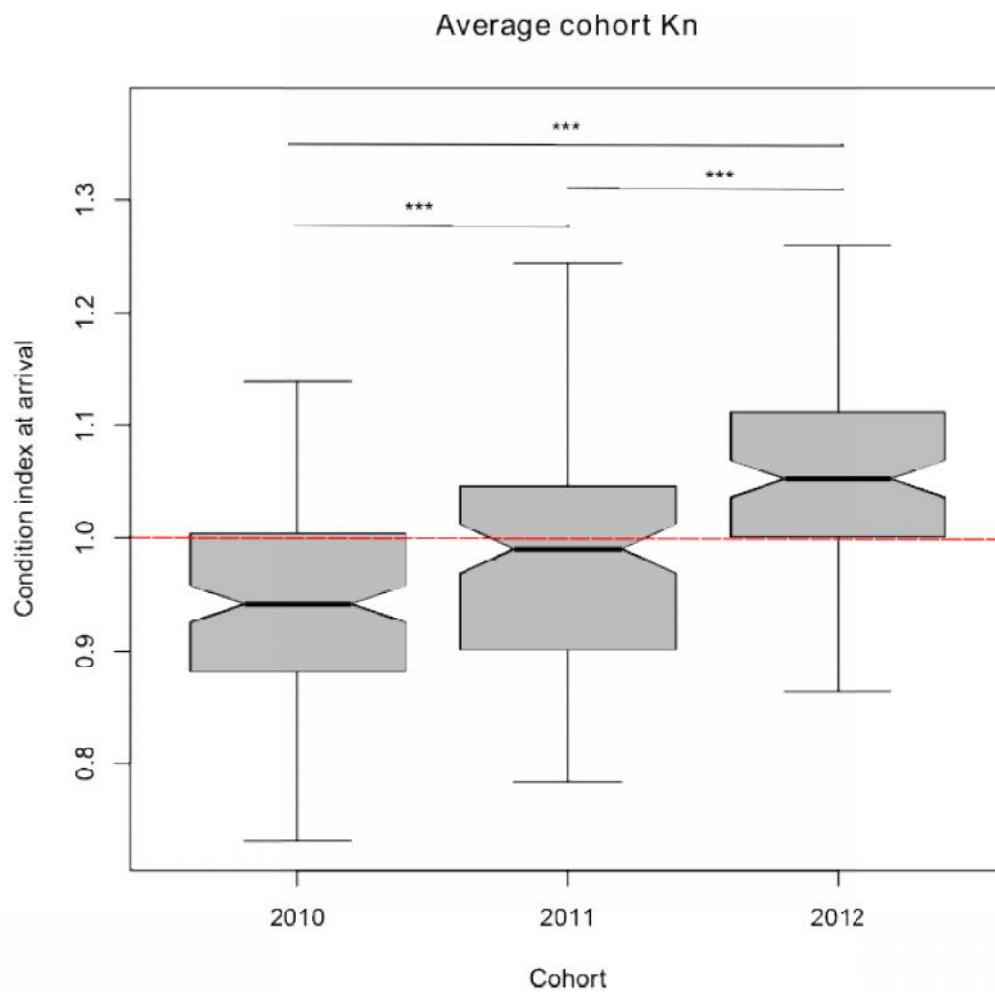
	2010	2011	2012
$\Theta_A$	0.067	2.600	0.067
$\Theta_B$	2.067	2.067	0.067
$\Theta_C$	2.467	4.600	4.200
$N_e m$ B→A	1.311	56.334	1.178
$N_e m$ C→A	1.267	49.400	1.311
$N_e m$ A→B	40.645	44.779	1.178
$N_e m$ C→B	32.379	35.133	0.778
$N_e m$ A→C	46.867	87.400	82.601
$N_e m$ B→C	38.645	78.200	49.001

**Tab. 3 – Mutation scaled effective population sizes ( $\Theta$ ) and effective number of migrants ( $N_e m$ ) of model II.**

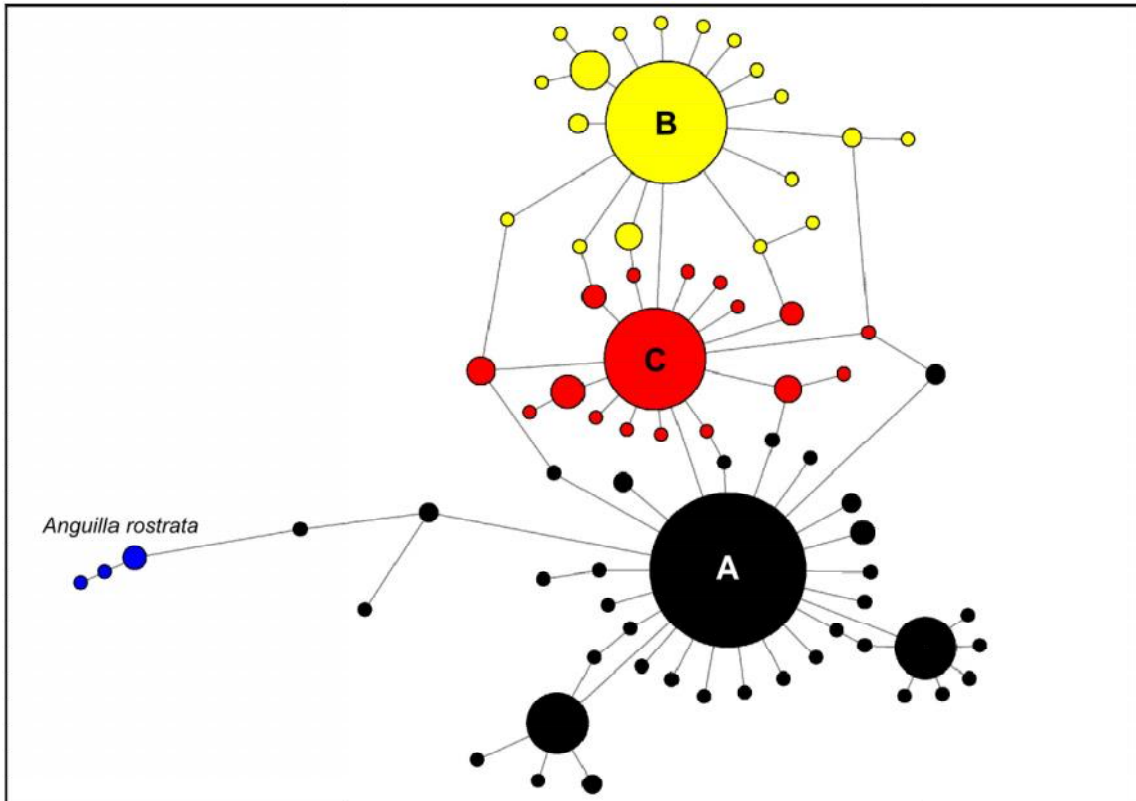
$Kn^{\sim}$	<i>Df</i>	<i>F</i>	<i>P</i>
<i>Rds</i>	1	3.714	0.055
IR	1	0.235	0.628
cohort	2	<b>48.654</b>	<b>&lt;0.001*</b>
<i>Rds</i> :IR	1	0.048	0.828
cohort:mtDNA	6	0.653	0.688
<i>Rds</i> :cohort	2	1.104	0.333
IR:cohort	2	1.237	0.292
<i>Rds</i> :cohort:mtDNA	6	1.891	0.082
IR:cohort:mtDNA	6	1.114	0.354
<i>Rds</i> :IR:cohort	2	0.585	0.558
<i>Rds</i> :IR:cohort:mtDNA	6	0.990	0.432

**Tab. 4 – Effects of genetic variables on condition index.** The effect of each variable was tested through an ANOVA, following the formula  $Kn^{\sim}Rds*IR(\text{cohort}/\text{mtDNA})$ . *Rds* = residuals of the correlation of *HL* and *IR*, *mtDNA* = matrilineage; \* represents a significant effect.

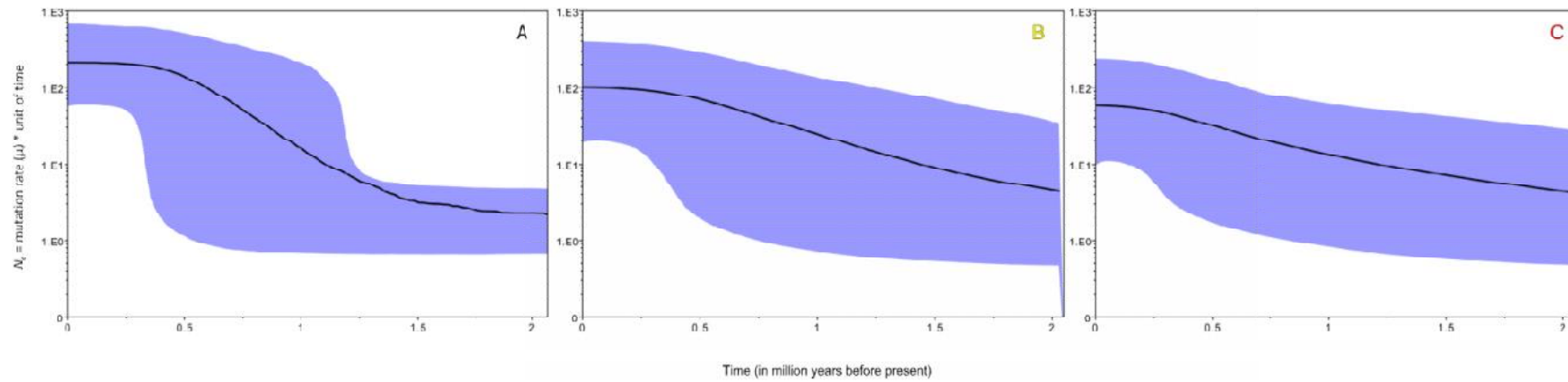
## Figures



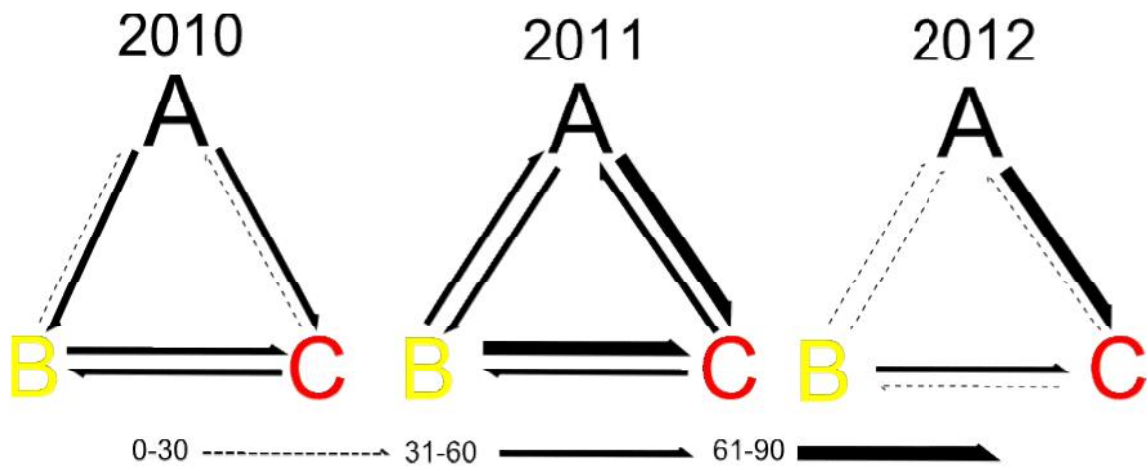
**Fig. 1 - Variation of condition index ( $K_n$ ) amongst cohorts.** The condition index at arrival ( $K_n$ ) was calculated with the slope and intercept of the  $\log(W)/\log(L)$  regression equation built upon the allometric growth of all specimens; \*\*\* represents pairwise relationships whose  $p < 0.001$ . The line at 1.0 represents the mean condition index, following (Le Cren 1951)



**Fig. 2 - Haplotype network depicting the mtDNA lineages.** Network obtained with maximum parsimony approach already containing all possible trees. Letters correspond to matrilineages. Mutation steps and median vectors are not represented.



**Fig. 3 – Demographic history of each lineage reconstructed with Bayesian Skyline Plot.** X-axis represents “time” and is defined in million years (my). Y-axis is the product of  $N_e$  (the female deme size) \* time, in million years. The black line represents the mean  $N_e$  and the blue shades the 95% high probability density interval.



**Fig. 1** – Effective number of migrants ( $N_e m$ ) amongst demes of each cohort. The arrows between mtDNA lineages represent flux and direction of migration. Different orders of magnitude are expressed in terms of thickness of the arrows.



## **Synthesis**

Overall, the outcomes of this thesis demonstrate the intrinsic relationship between ecological and evolutionary constraints that acting throughout the life cycle, define the population dynamics of the European eel. In this section, and after summing up the advances that this thesis contributed to, I will suggest directions for future research that might complement the specific results and improve our overall understanding on this enigmatic species.

For various reasons, the oceanic environment has often been advocated as a major player shaping the contemporary dynamics of the eel population (Friedland *et al.* 2007; Kettle *et al.* 2008a; Munk *et al.* 2010). By coupling ocean modeling and population genetics we here provided evidence that strongly supports the fact that the oceanic environment is indeed critical to the contemporary dynamics of the population, and most importantly, suggest that it might have shaped the evolution of the species. In this regard, the outcomes of this work are three-fold. First, we reported that the steep recruitment decline recorded in the beginning of the 1980s coincided with the weakening of the wind-driven oceanic pathway that connects the spawning grounds, in the Sargasso Sea, to the Gulf Stream, the main ocean current that transports eel's early stages towards Europe. Furthermore, modelling simulations revealed that the weak oceanic pathway lasted for several years. That time span had surely comprised several spawning events of the species, consecutively reducing the rate of recruits. Second, it allowed the formulation of an alternative hypothesis to the paradigm of panmixia, which aggregated under a plausible evolutionary hypothesis, deviations to a panmictic mode of reproduction reported in previous works (Dannewitz *et al.* 2005; Wirth & Bernatchez 2001). The exploratory perspective on which we were able to suggest the hypothesis of female philopatry stem from the versatility provided by the coupling of ocean models and population genetic theory. This framework showed the potential of biophysical modelling as *in silico* tools in evolutionary ecology. Lastly, the modelling simulation suggested that reasons other than oceanography must have precluded the eel recruitment to recover from the 1980s crash. Up to now, recruitment decline and the subsequent chronic low were interpreted and hypothesized to be a single event with a shared and common cause.

Here, it is important to mention that the role of overfishing cannot be neglected. The massive increase in fishing effort that had occurred in the 1950s-1960s - which has affected the sustainability of many fish stocks (Pauly *et al.* 2002) - may have indeed also impacted the European eel population's ability to recover from the successive unfavorable recruitment conditions. For instances,

it is known that small population sizes lead to low genetic diversities, which in turn may impose cryptic constraints to the adaptive potential of those same populations. This might have been particularly critical given the sudden introduction of the non-native nematode parasite *A. crassus* in European freshwaters in 1982-1983 (Taraschewski *et al.* 1987), which, by heavily damaging the swim bladder of infected eels, might have posed an acute selective pressure to the population. Analyses on two distinct generations of European eels, one closer to the time period on which the parasite was spread all over Europe (end of the 1990s) and other more recent (2009-2012), overall suggested that the parasite incidence, but also the recruitment decline might have impacted the eel population. First, we reported a recent - and common to both generations - loss of genetic diversity in the adaptive gene, an observation that resembles the genetic signature of a population bottleneck. However, this was not so evident in neutral evolving markers. This discrepancy reflects the increased resolution that screens of MHC genetic diversity may provide: due to its extreme standing genetic variation, the MHC is theoretically more sensitive to subtle population's fluctuations than the commonly used neutral evolving markers. However, the impact of the parasite introduction in this loss of diversity cannot be excluded. Between-generations analyses suggested that selection has indeed occurred. More precisely, the generation closer to the parasite introduction harbored fewer and more similar alleles than the recent generation, which already presented signs of recovery.

The third and last chapter was dedicated to a deeper investigation of the hypothesis of female philopatry that stemmed from the results presented in the first chapter of this doctoral thesis. To exist, this reproductive behavior may be relevant for the maintenance of the adaptive potential of the species, as was observed in turtles (Stiebens *et al.* 2013b). To test it, we assumed that the most frequent mitochondrial lineages (maternally inherited) observed in the population represented segregated female-specific breeding groups, while nuclear loci (biparentally inherited) were used to measure the gene flow amongst those matrilineages. This sort of genetic architecture is common in species where female philopatric behaviors have been observed (Kerth *et al.* 2000; Pardini *et al.* 2001; Stiebens *et al.* 2013b). Although not conclusive, results reported suggested that the mitochondrial lineages may indeed pose constraints to the evolution of full panmixia: not only a structured population model was statistically favored over a full panmixia model, but also we detected asymmetric gene flow amongst mitochondrial lineages. In a scenario of population recovery (as suggested to be the case by results of chapter two) such asymmetries in gene flow may be indicative of a potential replenishment process of the eel population, indicate that, to exist, female philopatry may be the mechanism that maintains the evolutionary potential of this species.



To summarize, major findings of this thesis encompasses the critical role of oceanic environment in the evolution of the species, the impact of the introduced parasite to its adaptive potential and female philopatric behavior as a hypothetical mechanism maintaining European eel's evolutionary potential. Altogether, the results of this work comply with the ongoing awareness on the intertwined nature of evolutionary and ecological processes shaping the dynamics of natural populations (Pelletier *et al.* 2007; Schoener 2011). Both the environmentally driven recruitment decline and the anthropogenic introduction of the parasite, i.e. ecological disturbances forced on the eel population, might have compromised its very recent evolution. Importantly, this study clearly exposed how distinct pressures are associated with specific stages of the life cycle of the European eel, an observation that might very well be extended to a broad range of marine fish species with similar life history strategies. Therefore, from an applied perspective, the results of this work reinforce the need to consider a species' evolutionary background while applying conservation or management measures (Allendorf *et al.* 2010; Hendry *et al.* 2011). This might be particularly relevant for the sustainability of exploited fish stocks (Conover & Munch 2002), whose recoveries in abundance may not necessarily reflect their viability due the negative impacts that exploitation produces on the fitness and genetic diversity of the populations (Kuparinen & Merila 2007; Pinsky & Palumbi 2014). Lastly, and although the European eel population might be recovering, the apparent fragile state of its adaptive potential together with a still unclear reproductive mode suggests that the species should still be considered critically endangered.



## ***Future research directions***

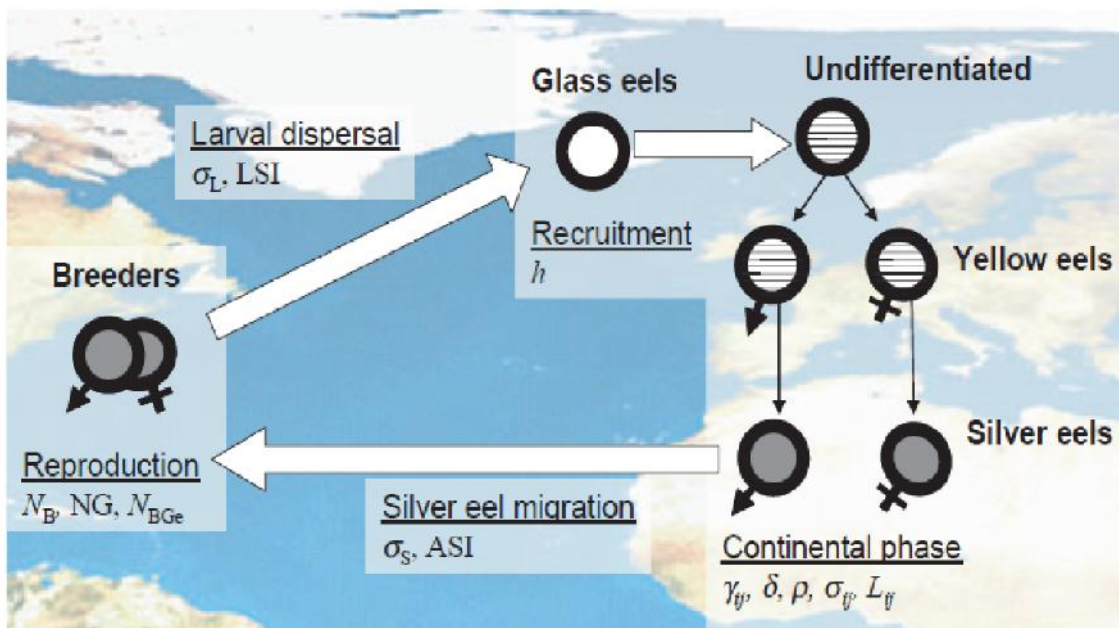
In this section, I will briefly describe two immediate perspectives - one related to biophysical modelling and other with genomics – that could be undertaken in order to complement or extend on the results presented in this thesis.

### ***1. Biophysical models in ecology and evolution***

#### *1.1. Towards a complete biophysical model of population dynamics*

Even though the hypothesis testing framework we following in the first chapter of this dissertation was novel, several additions could be made to that modeling framework to increase its biological realism, and therefore provide a clearer picture of the factors that determine the population dynamics of this species. First, it would be ideal to model the recruitment in a more realistic manner. For instances, the released v-eel cohorts could be linked by a generation time, upon which the successful recruits of generation I would correspond to the v-eels released in generation II. The generation time would be adjusted to an average of both sexes (~12-15 years).

Second, one could link the extensive data on ocean-mediated recruitment to models that account for factors other than the ocean currents to affect dynamics of the eel population, such as the one of Andrello *et al* (2011) (Figure 5). Andrello *et al* (2011) idealized a demographic model of the full life cycle of the eel which incorporated several parameters that predict the impacts of larval dispersal, recruitment success, fishing pressure and spawning migration to the dynamics of the eel population. Regarding larval dispersal and recruitment success, Andrello *et al* (2011) used an approximation to the survival rate based on the work of Bonhommeau *et al* (2009). Still, and since Bonhommeau and colleagues did not relate their simulations to natural recruitment indices, the outcomes of the first chapter of this thesis could instead be used to grant more realism to the model.



**Figure 5 – Diagram of a possible demographic model of the eel population (courtesy of Marco Andrello).** In this model, “larval dispersal” and “glass eels recruitment” parameters ( $\sigma_L$ ,  $h$ ) could, for instances, be provided by ocean model simulations of chapter I. The parameter  $\sigma_{ij}$  incorporates estimates of fishing mortality of yellow eels obtained from Dekker (2000) (Andrello *et al.* 2011). Briefly, the remaining parameters on the “continental phase” relate to sexual differentiation ( $\delta, \rho$ ) body growth ( $L$ ) and metamorphosis into silver eels ( $\gamma$ ). The parameters to consider for “silver eel migration” relate to chances of migration success, while the ones to consider for “reproduction” relate to number of breeders that successfully reach spawning areas ( $NG$ ) (Andrello *et al.* 2011).

### 1.2. Identification of cryptic strategies in natural populations: onset of speciation?

Challenges to the understanding of the biology of early life stages extend to the great majority of marine fishes. In the introduction of this dissertation, I attempted to describe how biophysical modelling emerged as a tool to characterize the ecological interactions early in life history. Those models are used to pinpoint the key factors that mediate the mass mortality that characterizes those stages (Miller 2007; Peck & Hufnagl 2012). Biophysical modeling approaches can become extremely valuable tools to investigate the evolutionary ecology of marine fishes. Those can be used, for instances, to clarify the role of natural selection in early life mortality or identify variable strategies in a population. The later can be of extreme importance to understand speciation in open ocean environments, which is still seen as paradoxical given the absence of conspicuous barriers to reproductive isolation (Bierne *et al.* 2003; Miglietta *et al.* 2011; Miya & Nishida 1997).

The speciation event that led to the two North Atlantic eel species (the European eel and the American eel) might be related to different strategies. For instances, the spatial distribution of each

species' early stages only overlapped in the central areas of the Sargasso Sea: American eel early stages are commonly found towards the Western boundaries of the Sargasso Sea, while European eel early stages are frequently observed towards the Eastern boundaries (Als *et al.* 2011). Here, biophysical modeling can be first employed to test whether particles released in the western boundaries have higher likelihood to be retained in the American coast while particles released on the western boundaries have higher likelihood to engage in a transatlantic dispersal. In addition, a parameter conditioning the time of metamorphosis into glass eels might also be incorporated. It has long been suggested that American and European eels differ in their developmental time (Tesch 2003), and recent evidence supports this perspective: first, gene expression analyses suggested differential timing of gene expression regulation during early development between the two species (Bernatchez *et al.* 2011) and second, species-specific polymorphism in genes related to developmental processes or energy synthesis through ATP phosphorylation have been identified (Jacobsen *et al.* 2014b). Therefore, developmental time differences may also be parametrized into the model as a biological parameter in order to refine the hypothesis-driven approach of distinct spawning grounds.

## **2. From genetics to genomics...and back**

### *2.1. Further investigations on the adaptive potential of the European eel*

The possible evolution of resistance against the parasite threat demands deeper investigations. Hence, future work will need to investigate whether an allele, or group of alleles, are associated with either resistance or susceptibility to the parasite infection. Resistance can be characterized by an association between individual heterozygosity, an optimum number of alleles or specific alleles and the absence or low incidence of parasite (Eizaguirre *et al.* 2012b; Wegner *et al.* 2003). This scenario is likely to be found in ecosystems where heterozygote advantage maintains MHC polymorphism (Eizaguirre *et al.* 2012a), and might be expected when populations are exposed to a broad range of parasites (Wegner *et al.* 2003). In turn, susceptibility can be characterized by an association between certain MHC alleles and presence or high incidence of parasites (Meyer-Lucht & Sommer 2005; Paterson *et al.* 1998; Radwan *et al.* 2012). The occurrence of such scenario is expected when rare allele advantage governs the maintenance of MHC polymorphism in a population (Sommer 2005), which might be the case when new parasites are introduced in a population (Spurgin & Richardson 2012). Therefore, a plausible follow-up to the work presented in the second chapter of this thesis would be to collect and screen silver eels for the presence/absence and intensity of infection of *A. crassus* and genotype the previously described region of the MHC. If the identification of resistance

or susceptibility allele or alleles turned out positive, one would proceed and compare their frequencies amongst the glass eels cohorts already genotyped. This could introduce, for instances, standard practices against stocking freshwater habitats known to harbor *A.crassus* while the frequency of susceptible individuals in the population is still high. Lastly, in order to provide a broader picture of the current state of the adaptive potential of the European eel to parasites or diseases, it is also important to characterize and screen the genetic diversity of MHC class I genes. This might be particularly informative given 1) the ongoing extensive eel farming activity that precedes stocking and 2) the occurrence of viruses (Esteve-Gassent *et al.* 2004; Haenen *et al.* 2014; Van Nieuwstadt *et al.* 2001) or bacteria (Alcaide *et al.* 2006; Esteve *et al.* 1993) in those farms.

### *2.2. Genome scans to identify loci under selection: the future of conservation biology*

As in the case of the MHC, to investigate the variability of genomic regions under selection can be extremely informative to evaluate the adaptive potential of endangered species. Techniques that allow the identification of candidate loci for selection (regions of the genome that deviate from a neutral mode of evolution) at the genome-wide scale have recently become available (Baird *et al.* 2008; Foll & Gaggiotti 2008; Joost *et al.* 2007) and its use in management and conservation is steadily becoming established (Allendorf *et al.* 2010; Eklom & Wolf 2014; McMahan *et al.* 2014). To identify those loci, performing periodic screens of their genetic variability within and amongst populations, and monitoring both the dynamics of the populations as well as potential selective pressures is probably the future of conservation biology.

### *2.3. Maintenance of evolutionary potential and population structure in the European eel*

With the transcriptome (Coppe *et al.* 2010) and a draft genome (Henkel *et al.* 2012) being available, questions regarding the population structure and maintenance of the adaptive potential of this species may soon be answered. Indeed, it is clear that finding conclusive evidence for whether female philopatry exists or not in the European eel is a critical but extremely challenging task. Evidence accumulates for and against it, and until an accurate documentation of the reproductive event is made, we will have to rely on indirect measurements and formulate hypotheses to be tested. Hence, the results presented in this thesis demand for a deeper investigation of the extent to what the hypothetical female philopatry behavior maintain the evolutionary potential of the species.

Here, a direct link to the second chapter of the thesis immediately emerges: does the gene flow amongst female lineages maintain the adaptive potential of the species? Although philopatric behaviors restrain gene flow - favoring the fixation of specific genetic variation within the geographic

areas to which sex-biased dispersal is confined to - it has recently been shown that female philopatry is critical to the maintenance of turtles adaptive potential (Stiebens *et al.* 2013b). Unequivocally, it is a research question worth to be investigated in the European eel. For that, it would be required to genotype the MHC of individuals of each respective matrilineage, and associate the loci diversities with differences in the rate and direction of gene flow, amongst matrilineages, measured with neutral makers. In addition, this work could be expanded to comparisons amongst generations of eels and one could focus in the identification of nuclear loci that segregates within each matrilineage to investigate their function. This framework would strengthen the inferential approach regarding the population of this species, especially recalling that neither reproduction nor spawning was ever observed in the European eel.





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## **Annexes**

Current Biology, Volume 24

### **Supplemental Information**

#### **Recruitment Collapse and Population**

#### **Structure of the European Eel**

#### **Shaped by Local Ocean Current Dynamics**

**Miguel Baltazar-Soares, Arne Biastoch, Chris Harrod, Reinhold Hanel, Lasse Marohn, Enno Prigge, Derek Evans, Kenneth Bodles, Erik Behrens, Claus W. Böning, and Christophe Eizaguirre**

#### **Supplemental Figures**

Figure S1. This figure has 4 panels. It relates to the association between oceanography and recruitment depicted in main figures 1 and 2, with a detailed description of the wind-ocean interaction.

Figure S2. This figure has 2 panels. These figures are a visual support for both methodology and results of the section “In silico population genetics” on main document.

Figure S3. This figure has 2 panels and presents deep phylogenetic analyses of the mitochondrial marker used in this study.

Figure S4. This figure has 3 panels. This additional information shows the validation of the ocean model used in this study. Results are compared with satellite and moored data.

#### **Supplemental Tables**

Table S1. Analyses of molecular variance (AMOVA) based on haplotype frequencies, performed on artificial v-eel populations.

Table S2. Test for population structure and isolation by time on in silico v-eel populations (*see separate Excel file*)

Table S3. Test for isolation by distance on in silico v-eel populations

Table S4. Relationship between ocean characteristics and artificial population genetics

Table S5. Ecological, geographical and molecular information of individuals sampled for the study (*see separate Excel file*)

Table S6. Population structure of natural populations: pairwise comparisons performed with mtDNA and microsatellite markers

Table S7. Test for neutral evolution of the mitochondrial marker

#### **Supplemental Experimental Procedures**

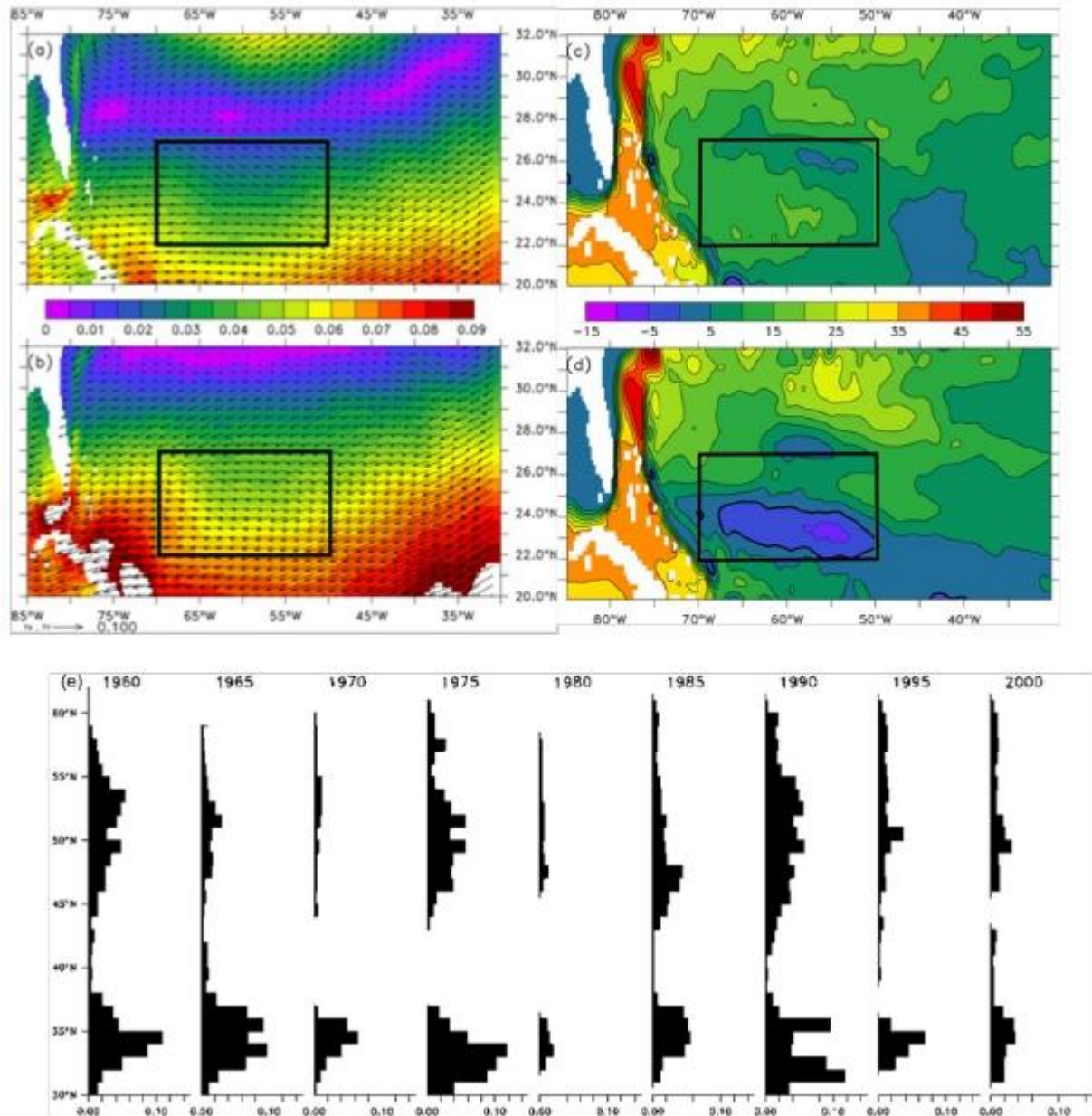
Text S1. Complements methodology of molecular analyses on natural populations

#### **Supplemental References**

Procedures and software cited in Supplemental Information

## Supplemental Figures

**Figure S1 - Influence of Oceanic Currents on the Eel Dispersal**

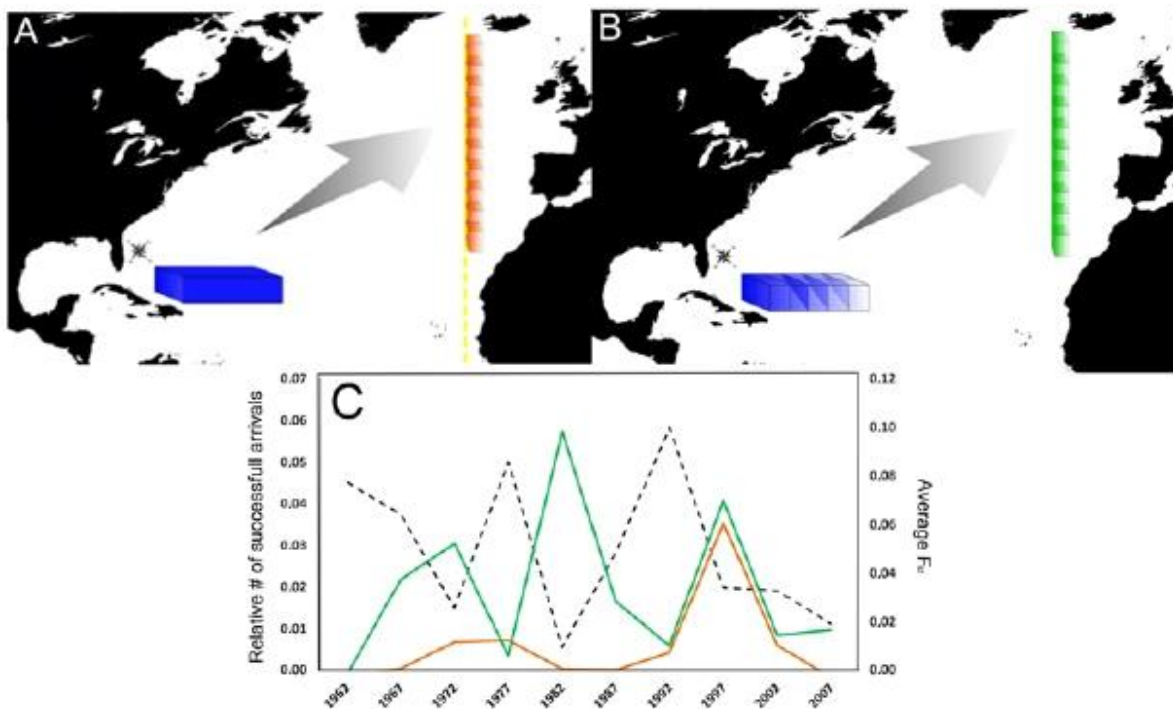


**Atmospheric conditions and oceanic response in the vicinity of the v-eel release.** Exemplarily v-eels for years of high (1990, a and c) and low (1980, b and d) dispersal. Shown are (a, b) magnitudes (in  $N/m^2$ ) and vectors of wind stress and (c, d) the streamfunction (in Sv) of the horizontal gyre circulation. Positive values represent an anticyclonic (clockwise) circulation, with strong gradients indicating strong flows. The box of the v-eel release is indicated in black. **Panel e:** Cumulated proportions of v-eels successfully arriving at  $25^\circ W$ , binned at  $1^\circ$ -resolution and summed up over the first 100 meters of the water column. Examples are given for 9 temporal simulations (every 5 years but simulations were run yearly). The numerical representation of the year corresponds to the beginning of simulation period. The particles were allowed to disperse for 2 years.

Atmospheric winds are one of the main drivers for the oceanic current variability. The large-scale wind pattern in the subtropical North Atlantic shapes the structure of the subtropical gyre, leading to prevailing westward but weak currents around  $20^\circ N$ . Crucial for the shortcut between the Sargasso Sea towards the Gulf Stream is the highly variable Antilles Current. Anomalous southward positions of the high-pressure systems (e.g., in the year

1980, Fig. S1b) lead to a southward shift of the currents in the subtropical gyre (Fig. S1d), not capturing the region northwest of the Sargasso Sea. In contrast, years of strong trades (Fig. S1a) feature strong westward currents in the Sargasso Sea and allow the direct migration of v-eels via a shortcut (see main document) towards the Gulf Stream. In addition, the upper ocean is subject to a direct influence from the local wind, the 'Ekman effect' that modulates currents with an angle between  $\sim 20^\circ$  and  $90^\circ$  [S1] to the right in the top 50 m of the water column. Wind systems like the one in year 1990 (Fig. S1b) additionally favor northwestward currents in the upper layers [S2].

**Figure S2 - In silico population genetics**

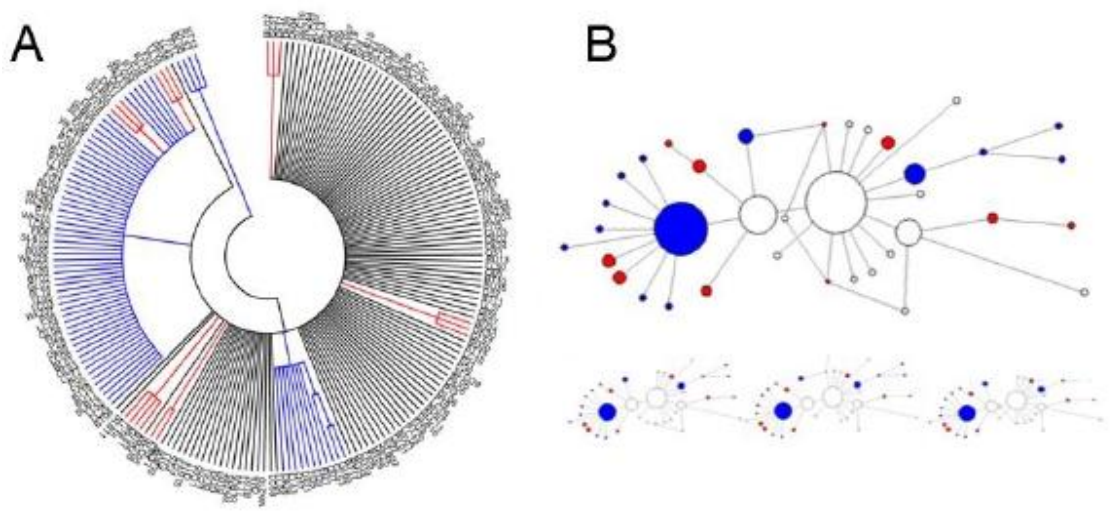


**Panel A and B:** Schematic representation of the simulation procedure undertaken to access the effects of a panmictic (A-left, blue polygon) and philopatric (B-right, blue sectioned polygon) spawning grounds in the distribution of genetic variability at continental locations -orange and green-shaded cubes. The yellow line, drawn at 25°W meridian, defines the “successfully” arriving v-eels used for further analyses. **Panel C:** Correlation between the relative number of successful arrivals (black line, left Y-axis) and  $F_{ST}$  values associated with each spawning scenario (colored line, right Y-axis) for each release event (X-axis). Orange stands for the panmixia scenario and green for female philopatry. Spearman rank correlations and p-value between v-eel recruitment and  $F_{ST}$  are, respectively,  $\rho_{\text{panmixia}} = 0.15$ , p-value=0.69 ;  $\rho_{\text{philopatry}} = -0.68$ ; p-value=0.04. Under the suspected mode of female philopatry reproductive strategy, years of low recruitment increase possibility for genetic structure, while only one simulated event (1997) would lead to significant  $F_{ST}$  under the panmictic mode of reproduction and no overall correlation with the simulated recruitment is observed.

Under the panmictic mode of reproduction, we created 10 genetic types (i.e. haplotypes) of unknown sequence. For each genetic type, 800 000 particles were released in the theoretical spawning ground randomly – thus reaching a total of 8 million v-eels to be tracked for 2 years. For the structured mode of evolution, the spawning ground was split into 10 regions, each consisting of one haplotype. 800 000 particles were released in each of the 10 regions reaching 8 million v-eels to also be tracked for 2 years. In both cases, v-eels were released in an area and depth range reflecting the putative spawning area of the European eel [S3,S4], following results and discussion for vertical distribution [S5]. Those parameters were chosen in order to allow comparison with previous studies but also to restrict already demanding simulations when possible. Varying depth of release and tracking period did not affect the overall described patterns for the tested simulations.

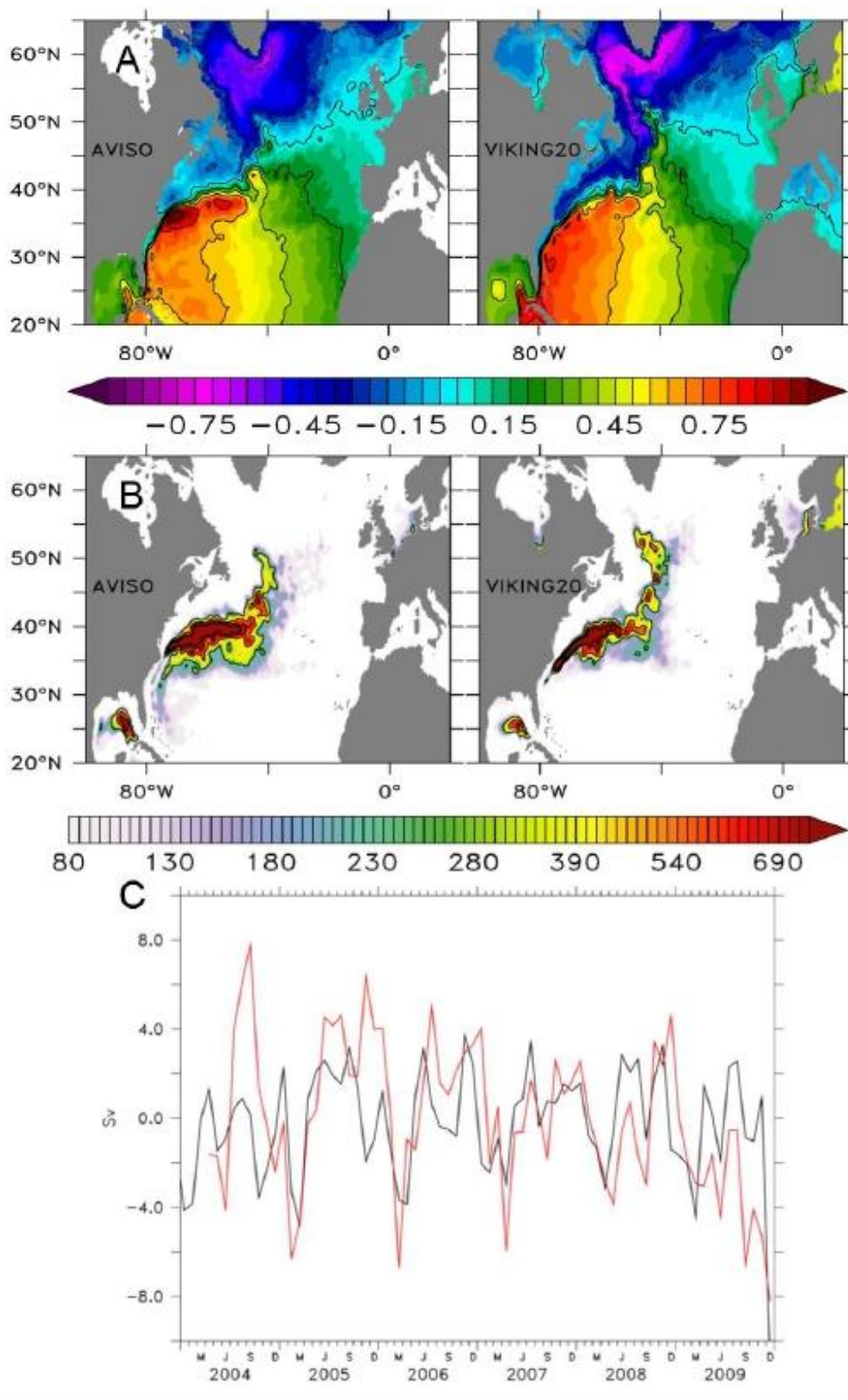


**Figure S3 - Intraspecific phylogeny of natural populations**



**Figure S3 Phylogenetic tree (panel a) and haplotype networks (panel b)** – We sampled yellow eels spanning 13 locations (Table S1), across both small (within Ireland) and large geographical (4 continental sites) scales. A total of 202 individuals were examined for a section of the *ND5* mitochondrial gene (355bp). The evolutionary history among sequences was inferred using the Neighbor-Joining method with 500 replications [S6]. The optimal tree with the sum of branch length = 0.109 is shown. The evolutionary distances were computed using the Maximum Composite Likelihood method [S7] and are given in number of base substitutions per site. The tree was condensed to show only bootstrap values  $>50$ , where branches in blue =  $50 < \text{bootstrap} < 60$  and in red =  $60 > \text{bootstrap}$ . **Haplotype networks (panel b)**. Median joining network: every circle represents a detected haplotype. Size differences represent frequency in the overall samples. Filled haplotypes represent the group of individuals comprised in branches of the Neighbor-Joining tree, with same color. Red diamond-shaped forms depict median vectors. Also represented are the shortest trees generated by optimal post-processing (MP) maximum parsimony algorithm implemented in NETWORK v4.6.1.0) [S8]. The main network shows three major haplotypes - a pattern that clearly deviates from the star-like shape emerging from a single central haplotype characteristic of a panmictic population [S9]. This result suggests maternally mediated cryptic population structure.

Figure S4 - Characteristics of the VIKING20 ocean model



**A** - Mean sea-surface height (1998-2007, in m) for AVISO satellite ([www.aviso.oceanobs.com](http://www.aviso.oceanobs.com)) and VIKING20 model data. **B** - Variance of sea-surface height (1998-2007, in  $\text{cm}^2$ ) for AVISO ([www.aviso.oceanobs.com](http://www.aviso.oceanobs.com)) satellite and VIKING20 model data. **C** - Inter-annual variability of the meridional overturning strength (in Sv) in RAPID observations (red) and the VIKING20 model (black).

VIKING20 is a  $1/20^\circ$  model of the subtropical-subpolar North Atlantic ( $30^\circ$ - $80^\circ$ N), nested into the  $1/4^\circ$  global ocean/sea-ice model (ORCA025). The oceanic currents and hydrography are simulated at great verisimilitude, both in mean and variability. Compared to satellite data, the model captures the path of the Gulf Stream and North Atlantic Current well, including the poleward turn into the Northwest Corner (panel **A**). The mesoscale representation (panel **B**) matches the correct level of variability and shows the two pathways of spreading towards Europe. The realism of interannual transport fluctuations is demonstrated by comparing the modelled transports at  $26^\circ$ N with observations from a moored RAPID array [S10] (panel **C**). Such ocean models, like HYCOM [S11] and NEMO [S12] accurately reproduce the trajectories of tracked drogues and so give a good representation of ocean flows. While the models may not reproduce every aspects of the real flows, they will give a good measure of flow variability and hence inter-annual patterns [S13]

## Supplemental Tables

**Table S1 – Population genetics on artificial populations: Analysis of molecular variance (AMOVA's) of artificial v-eel populations**

Partitioning of genetic variation was inferred both across release events (**A**) and across artificial populations (**B**), assuming each spawning scenario (panmixia vs. philopatry). Only artificial populations that were supplied with more than 9 v-eels were taken into account in all further calculations.

Source of variation	d.f.		variation %		Φ statistic		p-value	
	Panmixia	Philopatry	Panmixia	Philopatry	Panmixia	Philopatry	Panmixia	Philopatry
Among					<b>Φ<sub>ct</sub></b>			
Years	9	9	-0.04	8.57	-0.0004	0.0857	0.29	<0.001
Among sites					<b>Φ<sub>sc</sub></b>			
within years	53	53	0.3	1.16	0.003	0.0127	<0.001	<0.001
Within					<b>Φ<sub>st</sub></b>			
Sites	12003	11443	99.74	90.27	0.0026	0.0973	<0.001	<0.001

Source of variation	d.f.		variation %		Φ statistic		p-value	
	Panmixia	Philopatry	Panmixia	Philopatry	Panmixia	Philopatry	Panmixia	Philopatry
Among					<b>Φ<sub>ct</sub></b>			
Sites	5	6	-0.02	-1.14	-0.0002	-0.0114	0.33	0.87
Among years					<b>Φ<sub>sc</sub></b>			
within sites	52	55	0.25	9.84	0.0025	0.0973	<0.001	<0.001
Within					<b>Φ<sub>st</sub></b>			
Years	11893	11435	99.77	91.29	0.0023	0.0871	<0.001	<0.001

**A** - AMOVA across release events considering data created by simulating panmixia and female philopatry ; “years” = release events, “sites” = artificial continental sites. **B** - AMOVA across artificial populations considering data created by simulating panmixia and female philopatry; “years” = release events, “sites” = artificial continental sites.

**Table S2 – Pairwise comparisons of artificial populations (in excel format)**

**Table S3 – Isolation by distance between artificial populations**

To test for isolation by distance amongst artificially created v-eel populations, we first converted latitudinal degrees into kilometers in the following website:

<http://www.ncgia.ucsb.edu/education/curricula/giscc/units/u014/tables/table01.html>

We applied Rousset's methods [S14] to calculate isolation-by-distance, i.e. log transformed geographic distances matrices and  $F_{ST}/(1-F_{ST})$  transformed genetic distances matrices.

Release event		$R^2$	p-value
<b>Panmixia</b>			
1960-1962	$y = -0.1146x + 0.0334$	0.0178	>0.05
1965-1967	$y = 0.0791x - 0.0243$	0.0271	>0.05
1970-1972	$y = 0.2250x - 0.0625$	8.81E+03	>0.05
1975-1977	$y = 0.2826x - 0.0808$	2.16E+03	>0.05
1980-1982	$y = -0.05346x + 0.01735$	0.0829	>0.05
1985-1987	$y = 0.2339x - 0.0642$	0.0788	>0.05
1990-1992	$y = 1.002x - 0.2755$	0.0428	>0.05
1995-1997	$y = -0.1163x + 0.0364$	0.0432	>0.05
2000-2002	$y = 0.0519x - 0.0163$	0.0168	>0.05
2005-2007	$y = 0.1008x - 0.0270$	0.0405	>0.05
<b>Female philopatry</b>			
1960-1962	$y = 0.6641x - 0.1828$	0.6563	>0.05
1965-1967	$y = 0.2740x - 0.0810$	0.0768	>0.05
1970-1972	$y = 1.298x - 0.3691$	0.029	>0.05
1975-1977	$y = 0.6641x - 0.1828$	0.6563	>0.05

1980-1982	$y = 0.6160x - 0.1748$	0.8194	>0.05
1985-1987	$y = 1.266x - 0.3442$	0.0538	>0.05
1990-1992	$y = -1.988x + 0.6125$	0.154	0.05
1995-1997	$y = 0.1432x - 0.0391$	0.8166	>0.05
2000-2002	$y = -0.6037x + 0.1864$	0.0664	>0.05
<u>2005-2007</u>	<u><math>y = -0.2515x + 0.0796</math></u>	<u>0.0282</u>	<u>&gt;0.05</u>

Mantel test between transformed  $F_{ST}$ -values based haplotype frequencies produced by each release event, assuming **panmixia** and **female philopatry**. Equations shows the linear regression between the two matrices used for the Mantel test: pairwise genetic and geographic distances.

**Table S4 – Relationship between ocean currents and artificial population genetics**

Years	Relative proportion of successful v-eels	Relative proportion of successful v-eels	
		North	South
1960	0.045	0.020	0.025
1965	0.037	0.008	0.029
1970	0.015	0.003	0.012
1975	0.050	0.020	0.030
1980	0.005	0.002	0.003
1985	0.028	0.014	0.015
1990	0.058	0.029	0.029
1995	0.020	0.009	0.010
2000	0.019	0.009	0.010
2005	0.011	0.004	0.007

Relative (in relation to  $8 \times 10^6$  released v-eels) number of successful arriving v-eels at 25° W produced by each release event. Also relative numbers of successful arriving v-eels after the Mid Atlantic Ocean bifurcation: “North” and “South” were defined at 25° W by a perpendicular line superimposed in the 40°N meridian.





Data in Excel format

**Additional supplementary material Table S2 – Pairwise comparisons of artificial populations**

<b>1962</b>	C	D	E	F	G	H	I
C	0						
D	-0.003	0					
E	0.001	0.003	0				
F	-0.008	-0.002	-0.003	0			
G	0.005*	0.004*	0.002	-0.002	0		
H	-0.002	0.001	0.002	-0.005	0.001	0	
I	-0.019	-0.012	-0.001	-0.012	-0.008	-0.016	0
<b>PANEL A</b>							
<b>1967</b>	C	D	E	F	G	H	I
C	0						
D	0.003	0					
E	0.018*	-0.001	0				
F	0.018	-0.004	-0.008	0			
G	0.009	-0.002	-0.002	-0.006	0		
H	0.016*	0.001	-0.003	-0.005	0.000	0	
I	-0.021	0.000	0.003	0.005	-0.003	-0.002	0
<b>1972</b>	C	D	E	F	G	H	I
C	0						
D	-0.006	0					
E	-0.001	-0.002	0				
F	0.035	0.032	0.002	0			
G	0.008	0.000	0.000	0.055*	0		
H	0.009	0.001	0.000	0.043*	-0.002	0	
<b>1977</b>	C	D	E	F	G	H	I
C	0						

D	0.006*	0					
E	0.000	0.002	0				
F	0.054*	0.029	0.039*	0			
G	0.005	-0.001	-0.001	0.037*	0		
H	0.006*	0.000	0.003*	0.024*	0.001	0	
I	0.023	0.004	0.011	0.014	0.000	0.003	0
<b>1982</b>	C	D	E	G	H		
C	0						
D	0.011	0					
E	0.014	0.013	0				
G	0.008	0.002	0.057*	0			
H	0.001	0.007	0.023*	0.022*	0		
<b>1987</b>	C	D	E	F	G	H	
C	0						
D	-0.001	0					
E	0.000	0.006*	0				
F	0.012	0.001	0.009*	0			
G	0.010	-0.001	0.005*	0.004	0		
H	0.011	0.001	0.004*	0.007*	0.001	0	
<b>1992</b>	C	D	E	F	G	H	I
C	0						
D	0.002	0					
E	0.002	0.001	0				
F	0.002	0.004	0.003	0			
G	0.002	0.000	0.001	0.005	0		
H	0.000	-0.001	0.001	0.002	0.001	0	
I	0.016*	0.025*	0.020*	0.024*	0.024*	0.021*	0
<b>1997</b>	B	C	D	E	F	G	H
B	0						

C	0.104*	0					
D	0.064*	0.005	0				
E	0.092*	-0.004	0.006	0			
F	0.034	0.181*	0.122*	0.157*	0		
G	0.074*	0.002	0.012*	0.005	0.154*	0	
H	0.064*	0.003	0.002	0.004	0.129*	-0.002	0

**2002** C D E F G H

C	0						
D	0.016*	0					
E	0.011*	0.019*	0				
F	0.008	0.026*	0.004	0			
G	0.013*	0.011*	0.003	0.014	0		
H	0.003	0.010*	0.004	0.009	0.002	0	

**2007** C D E G H

C	0				
D	-0.008	0			
E	0.004	0.001	0		
G	-0.007	0.001	-0.005	0	
H	-0.009	-0.003	0.001	-0.005	0

**1962** C D E F G H I

C	0						
D	-0.001	0					
E	0.006	0.009*	0				
F	0.019	0.031*	0.005	0			
G	0.011	0.022*	0.010*	0.006	0		
H	0.002	0.001	0.003	0.019*	0.019*	0	
I	-0.034	-0.026	-0.034	-0.040	-0.036	-0.030	0

**1967** C D E F G H I

C	0
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D	0.009	0					
E	0.055*	0.129*	0				
F	0.017	0.091*	-0.019	0			
G	-0.013	0.019*	0.069*	0.043*	0		
H	-0.016	0.029*	0.058*	0.033*	-0.001	0	
I	0.058	0.136	0.013	0.000	0.064*	0.053*	0

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<b>1972</b>	C	D	E	F	G	H
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C	0					
D	-0.014	0				
E	-0.025	-0.008	0			
F	0.152*	0.160*	0.084	0		
G	0.021	0.005	0.028	0.206*	0	
H	0.013	0.001	0.006	0.152*	0.003	0

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<b>1977</b>	C	D	E	F	G	H	I
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C	0						
D	0.000	0					
E	0.003	0.003	0				
F	0.010	0.027	0.003	0			
G	0.021*	0.007	0.011*	0.034*	0		
H	-0.001	0.004*	0.002	0.006	0.015*	0	
I	-0.013	-0.003	0.000	-0.001	0.003	-0.002	0

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<b>1982</b>	C	D	E	F	G	H
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C	0					
D	0.170*	0				
E	0.178*	0.111*	0			
F	0.620	0.307	-0.216	0		
G	0.179*	0.040	-0.009	-0.053	0	
H	0.115*	0.056*	-0.003	-0.020	0.001	0

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<b>1987</b>	C	D	E	F	G	H
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C	0						
D	0.046*	0					
E	0.012	0.010*	0				
F	0.146*	0.019*	0.061*	0			
G	0.025	-0.001	0.009*	0.034*	0		
H	0.012	0.003	0.001	0.047*	-0.001	0	

<b>1992</b>	C	D	E	F	G	H	I
C	0						
D	0.014*	0					
E	0.009*	-0.001	0				
F	-0.001	0.031*	0.022*	0			
G	0.000	0.005*	0.005*	0.013*	0		
H	0.001	0.023*	0.021*	0.009*	0.003	0	
I	0.000	0.026*	0.019*	-0.005	0.012	0.008	0

<b>1997</b>	B	C	D	E	F	G	H
B	0						
C	0.100*	0					
D	0.107*	-0.005	0				
E	0.193*	0.005	0.008	0			
F	0.408*	0.080	0.072	0.023	0		
G	0.254*	0.019	0.018	-0.008	-0.006	0	
H	0.137*	0.002	0.000	0.002	0.053	0.011	0

<b>2002</b>	C	D	E	F	G	H
C	0					
D	0.00032	0				
E	-0.00505	0.00012	0			
F	0.02499	0.055*	0.02015	0		
G	0.01679	0.050*	0.0207*	-0.0089	0	
H	-0.00721	0.0124*	0.00184	0.02101	0.014*	0

2007	B	C	D	E	F	G	H				
B	0										
C	0.029	0									
D	-0.039	-0.007	0								
E	-0.016	-0.012	-0.010	0							
F	-0.223	-0.008	-0.033	-0.044	0						
G	0.022	-0.008	-0.002	-0.016	-0.017	0					
H	-0.173	0.094*	0.056*	0.068*	-0.039	0.092*					
C	1960	1965	1970	1975	1980	1985	1990	1995	2000	2005	
1960	0										PANEL B
1965	0.026*	0									
1970	0.009	0.009	0								
1975	-0.002	0.003	0.020	0							
1980	-0.011	0.033	-0.028	0.011	0						
1985	0.001	0.025	0.014	0.002	0.009	0					
1990	0.006	0.008	-0.002	0.005	0.002	-0.003	0				
1995	0.005	0.013	-0.002	0.008	-0.019	0.012	0.003	0			
2000	0.010	0.011	0.021	0.008	0.001	0.007	0.008	0.008	0		
2005	-0.008	0.000	-0.010	-0.007	-0.013	-0.005	-0.008	0.000	-0.002	0	
D	1960	1965	1970	1975	1980	1985	1990	1995	2000	2005	
1960	0										
1965	0.001	0									
1970	0.004	-0.003	0								
1975	0.001	-0.002	0.001	0							
1980	0.008	0.001	-0.010	0.006	0						
1985	0.002	0.000	-0.002	0.002	0.003	0					
1990	0.002	0.000	-0.003	0.000	0.002	-0.002	0				
1995	0.007*	0.003	-0.002	0.004*	-0.004	0.001	0.001	0			
2000	0.007*	0.004	0.008	0.006*	0.015*	0.012*	0.009*	0.040*	0		

2005	0.004	-0.003	-0.001	-0.002	0.006	0.006	0.001	0.009*	0.008*	0
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<b>E</b>	1960	1965	1970	1975	1980	1985	1990	1995	2000	2005
1960	0									
1965	0.001	0								
1970	0.002	0.000	0							
1975	0.002	0.000	-0.003	0						
1980	0.018*	0.008	-0.005	0.013	0					
1985	0.004	0.002	0.003	0.007	0.021	0				
1990	0.000	0.001	-0.003	0.003*	0.007	0.002	0			
1995	0.000	0.004	0.001	0.004	0.025*	0.004	0.001	0		
2000	0.008*	-0.002	-0.009	0.003	0.010	0.008*	0.005*	0.010*	0	
2005	0.003	0.011	-0.021	0.006	0.012	0.011	0.003	0.002	0.001	0

<b>F</b>	1960	1965	1970	1975	1985	1990	1995	2000
1960	0							
1965	-0.009	0						
1970	0.035	0.004	0					
1975	0.050*	0.036*	0.032	0				
1985	0.008	-0.001	0.038	0.010	0			
1990	0.002	-0.012	0.021	0.037*	0.013*	0		
1995	0.124*	0.132*	0.287*	0.260*	0.138*	0.143*	0	
2000	0.003	-0.008	0.033	0.055*	0.006	0.005	0.124*	0

<b>G</b>	1960	1965	1970	1975	1980	1985	1990	1995	2000	2005
1960	0									
1965	0.001	0								
1970	0.002	0.001	0							
1975	0.001	-0.001	0.004	0						
1980	0.019*	0.011	0.019*	0.009	0					
1985	0.002	-0.001	-0.002	0.001	0.011	0				
1990	0.002	0.000	0.001	-0.002	0.017*	0.001	0			

1995	0.000	0.003	-0.005	0.000	0.028*	-0.002	-0.001	0		
2000	0.009*	0.005*	0.003	0.001	0.019*	0.002	0.001	0.000	0	
2005	0.001	0.000	-0.002	-0.002	0.013	-0.004	0.000	-0.005	-0.003	0
<b>H</b>	1960	1965	1970	1975	1980	1985	1990	1995	2000	2005
1960	0									
1965	0.000	0								
1970	-0.001	0.000	0							
1975	0.001*	0.001*	0.001	0						
1980	0.002	0.001	-0.001	0.006*	0					
1985	0.002*	0.003*	0.003*	0.004*	0.004	0				
1990	0.000	0.001	0.002	0.002*	0.003	0.002*	0			
1995	0.000	0.000	-0.001	0.001	0.001	0.001	-0.001	0		
2000	-0.001	-0.002	-0.001	0.001	0.000	0.000	0.000	0.000	0	
2005	-0.003	-0.004	-0.002	-0.002	0.000	0.001	-0.003	-0.002	-0.005	0
<b>C</b>	1960	1965	1970	1975	1980	1985	1990	1995	2000	2005
1960	0									
1965	0.066*	0								
1970	0.127*	0.010	0							
1975	0.053*	0.079*	0.054*	0						
1980	0.388*	0.522*	0.506*	0.262*	0					
1985	0.125*	0.112*	0.036	0.005	0.268	0				
1990	0.080*	0.044*	0.054*	0.049*	0.355	0.073*	0			
1995	0.178*	0.152*	0.047	0.047*	0.391*	0.016	0.125*	0		
2000	0.091*	0.057*	0.013	0.004	0.295*	-0.006	0.027*	0.037*	0	
2005	0.100*	-0.010	-0.012	0.065*	0.445*	0.065*	0.040*	0.118*	0.028	0
<b>D</b>	1960	1965	1970	1975	1980	1985	1990	1995	2000	2005
1960	0									
1965	0.069*	0								
1970	0.124*	0.010	0							



1975	0.020*	0.028*	0.055*	0						
1980	0.249*	0.225*	0.175*	0.169*	0					
1985	0.222*	0.131*	0.079*	0.136*	0.106*	0				
1990	0.066*	0.066*	0.080*	0.036*	0.183*	0.169*	0			
1995	0.159*	0.061*	0.024*	0.078*	0.125*	0.017*	0.096*	0		
2000	0.100	0.049*	0.031*	0.041*	0.112*	0.077*	0.025*	0.026*	0	
2005	0.083*	-0.006	0.009	0.039*	0.247*	0.147*	0.080*	0.071*	0.061*	0

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<b>E</b>	1960	1965	1970	1975	1980	1985	1990	1995	2000	2005
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1960	0									
1965	0.125*	0								
1970	0.068*	0.151*	0							
1975	0.040*	0.209*	0.060*	0						
1980	0.228*	0.521*	0.220*	0.135*	0					
1985	0.162*	0.325*	0.095*	0.074*	0.146*	0				
1990	0.066*	0.175*	0.058*	0.066*	0.088*	0.119*	0			
1995	0.204*	0.406*	0.126*	0.120*	0.245*	0.019*	0.160*	0		
2000	0.077*	0.196*	0.019	0.054*	0.105*	0.063*	0.012*	0.094*	0	
2005	0.068*	0.116*	-0.008	0.088*	0.311*	0.128*	0.097*	0.143*	0.057*	0

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<b>F</b>	1960	1965	1970	1975	1985	1990	1995	2000
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1960	0							
1965	0.076*	0						
1970	0.086	0.023	0					
1975	0.165*	0.308*	0.220*	0				
1985	0.421*	0.526*	0.554*	0.255*	0			
1990	0.061*	0.068*	0.062	0.154*	0.350*	0		
1995	0.412*	0.578*	0.622*	0.247*	-0.034	0.344*	0	
2000	0.077*	0.069*	0.053	0.150*	0.375*	-0.013	0.359*	0

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<b>G</b>	1960	1965	1970	1975	1980	1985	1990	1995	2000	2005
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1960	0									
1965	0.046*	0								

1970	0.186*	0.106*	0							
1975	0.036*	0.048*	0.090*	0						
1980	0.346*	0.318*	0.128*	0.185*	0					
1985	0.288*	0.203*	0.021*	0.180*	0.149*	0				
1990	0.072*	0.057*	0.082*	0.029*	0.169*	0.180*	0			
1995	0.333*	0.247*	0.044*	0.217*	0.185*	0.002*	0.213*	0		
2000	0.113*	0.037*	0.070*	0.060*	0.224*	0.169*	0.023*	0.217*	0	
2005	0.118*	0.017*	0.077*	0.092*	0.332*	0.165*	0.082*	0.216*	0.034*	0
<b>H</b>	1960	1965	1970	1975	1980	1985	1990	1995	2000	2005
1960	0									
1965	0.054*	0								
1970	0.092*	0.098*	0							
1975	0.031*	0.096*	0.043*	0						
1980	0.187*	0.319*	0.132*	0.097*	0					
1985	0.167*	0.206*	0.036*	0.079*	0.145*	0				
1990	0.024*	0.040*	0.071*	0.050*	0.183*	0.168*	0			
1995	0.165*	0.176*	0.033*	0.090*	0.200*	0.011*	0.155*	0		
2000	0.061*	0.064*	0.004	0.036*	0.139*	0.067*	0.034*	0.058*	0	
2005	0.147*	0.155*	0.027*	0.084*	0.205*	0.013*	0.141*	-0.003	0.047*	0

Geographic isolation Years	mean $F_{ST}$ (SEM)	
	Panmixia	Female philopatry
1960	0.00 (0.001)	0.00 (0.004)
1965	0.00 (0.002)	0.04 (0.009)
1970	0.01 (0.005)	0.05 (0.019)
1975	0.01 (0.003)	0.01 (0.002)
1980	0.00 (0.005)	0.10 (0.024)
1985	0.00 (0.001)	0.03 (0.010)
1990	0.01 (0.002)	0.01 (0.002)
1995	0.06 (0.01)	0.07 (0.023)
2000	0.01 (0.001)	0.01 (0.005)
2005	0.00 (0.001)	0.02 (0.015)

isolation by time Populations	mean $F_{ST}$ (SEM)	
	Panmixia	Female philopatry
<b>C</b>	0.00 (0.002)	0.12 (0.021)
<b>D</b>	0.00 (0.001)	0.09 (0.010)
<b>E</b>	0.00 (-0.001)	0.13 (0.016)
<b>F</b>	0.06 (0.015)	0.23 (0.036)
<b>G</b>	0.00 (0.001)	0.14 (0.014)
<b>H</b>	0.00 (0.000)	0.10 (0.010)

**Panel A** shows pairwise comparisons between artificial populations - delimited every 4° latitude along the 25°W arrival line – produced by each release event. Each scenario is labeled as **Panmixia (orange)** and **Female Philopatry (green)** with respective year of arrival. **Panel B**, shows pairwise comparisons representing test for isolation by time amongst successful cohorts of v-eels in a given artificial population. Those populations were labeled from “A” to “J”, sharing same color code as the previous set of tables: **Panmixia (orange)** and **Female Philopatry (green)**. **Panel C**: Summary of the mean

average  $F_{ST}$  originated by matrices on panel A and B, respectively. Standard error of the mean was calculated according to the formula:  
$$\frac{stDEV}{\text{number of comparisons}}$$
. Populations absent from the matrices did not fulfill the >9 v-eels criteria. \*p<0.05 and year of arrival

Table S5 – Additional information on natural populations

Collection sites	Geographic location	Average full length (mm)	Average mass (g)	% males	% undif	Salinity	n	nHap	S	H	$\pi$	ts	tv	Tajima D	A	A (sd)
LC(LarneLagoon)	54°50'54.39"N; 5°48'51.02"W	654.35 (15.70)	662.43 (62.40)	0	0	25,2	19	5	5	0,743	0,004	4	1	-0.237	7.667 (4.179)	4,18
BT(BannToome)	54°45'23.06"N; 6°27'48.85"W	569.85 (61.93)	363.26 (35.47)	0	0	0,2	17	9	10	0,846	0,006	10	0	-1.241	7.667 (4.967)	4,97
Q(Quoile)	54°22'0.36"N; 5°40'46.83"W	500.20 (12.50)	238.21 (19.25)	0	0	14,5	15	5	5	0,638	0,003	5	0	-0.783	7.667 (4.274)	4,27
BU(Burrishole)	53°55'4.56"N; 9°34'20.56"W	436.75 (16.40)	159.11 (19.24)	0	0	NA	16	4	6	0,575	0,004	6	0	-0.962	8.833 (4.535)	4,54
BL(BannLower)	55° 9'15.57"N; 6°42'5.13"W	382.00 (23.42)	108.24 (20.78)	25%	38%	31,9	11	5	4	0,836	0,004	4	0	-0.152	7.833 (4.491)	4,49
SLC(LoughComber)	54°32'20.35"N; 5°42'6.98"W	381.27 (16.82)	109.95 (14.85)	33%	18%	31,4	9	5	6	0,806	0,005	6	0	-0.520	7.000 (4.336)	4,34
LL(Larne Lough)	54°49'26.17"N; 5°47'40.47"W	486.00 (7.37)	203.93 (12.73)	0	0	33,1	13	5	6	0,821	0,004	5	0	-0.501	7.833 (5.636)	5,64
SLB(Boretree)	54°26'39.79"N; 5°35'20.96"W	330.69 (23.85)	83.29 (24.74)	45%	31%	31,9	15	5	6	0,848	0,004	5	0	-0.072	8.500 (4.637)	4,64
GL(GlynnLagoon)	54°49'55.50"N; 5°48'40.57"W	468.95 (21.31)	233.54 (30.55)	5%	0	26,8	14	10	11	0,934	0,007	10	1	-1.036	7.833 (4.622)	4,62
Denmark	57°29' N; 10°36' E	541.00 (6.92)	284.10 (12.44)	0	0	28,7	20	13	15	0,932	0,007	14	1	-1.682	9.167 (6.338)	6,34
Finland	60°26' N; 26°57' E	849.35 (17.74)	1326.85 (92.47)	0	0	4,6	19	9	8	0,883	0,005	8	0	-0.711	9.333 (4.844)	4,84
Germany(Schwentine)	54°17'16.80"N; 10°14'54.89"E	687.83 (26.81)	633.58 (63.70)	5%	0	NA	17	6	5	0,779	0,004	5	0	-0.210	7.000 (3.689)	3,69
Portugal	38°46' N; 9°01' W	262.22 (7.82)	30.83 (2.45)	NA	NA	NA	17	7	7	0,831	0,005	5	2	-0.461	8.500 (5.924)	5,92

Haplotype	Sequence information	Relative frequencies in populations											
		(LC)	(BT)	(Q)	(BU)	(BL)	(SLC)	(LL)	(SLB)	(GL)	(Dk)	(Fi)	(Wk)
1	TACCTAATTAAGGACCCGCGGATGCTATCGACA	<b>0,421</b>	<b>0,235</b>	0,000	<b>0,250</b>	<b>0,273</b>	0,000	<b>0,385</b>	<b>0,200</b>	<b>0,214</b>	<b>0,100</b>	<b>0,263</b>	<b>0,412</b>
2	TACCTAACTAAAGGACCCGCGGATGCTATCGACA	<b>0,263</b>	<b>0,353</b>	<b>0,600</b>	<b>0,625</b>	<b>0,273</b>	<b>0,444</b>	<b>0,154</b>	<b>0,267</b>	<b>0,214</b>	<b>0,250</b>	<b>0,211</b>	<b>0,235</b>
3	TACCTAATTAAGGACCCGCGGATGCTATCGACA	<b>0,211</b>	<b>0,059</b>	<b>0,133</b>	0,000	<b>0,273</b>	0,000	<b>0,231</b>	<b>0,267</b>	<b>0,071</b>	0,000	<b>0,105</b>	<b>0,176</b>
4	TACCTAACTAAAGGACCCGCGGATGCTACCGACA	<b>0,053</b>	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
5	TACTTAACCTAAAGGACCCGCGGGTGCTATCGACA	0,000	<b>0,059</b>	<b>0,067</b>	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
6	TACCTAACTAAAGGACCCGCGGGTGCTATCGACA	0,000	0,000	<b>0,133</b>	0,000	0,000	0,000	<b>0,077</b>	0,000	0,000	<b>0,100</b>	<b>0,158</b>	0,000
7	TACCTAATTAAGGACCTGCGGATGCCATCGACA	0,000	0,000	<b>0,067</b>	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
8	TACCTAACTGAAGGACCCGCGAATGCTATCGACG	0,000	0,000	0,000	<b>0,063</b>	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
9	TACCTAACTAAAGGACCCGCGAGATGCTATCGACA	0,000	0,000	0,000	<b>0,063</b>	0,000	0,000	0,000	0,000	<b>0,071</b>	<b>0,050</b>	0,000	0,000
10	TACCTAATTAAGGACCCACGGATGCTATCGGCA	0,000	<b>0,059</b>	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
11	TACCTAATTAAGGACCCGCGGATGCTATCGACA	0,000	<b>0,059</b>	0,000	0,000	<b>0,091</b>	0,000	0,000	0,000	<b>0,071</b>	0,000	<b>0,053</b>	0,000
12	TACCTAACTAAAGGACCCGCGGATGCTATCGACG	0,000	<b>0,059</b>	0,000	0,000	<b>0,091</b>	<b>0,222</b>	0,000	<b>0,133</b>	0,000	<b>0,100</b>	<b>0,053</b>	<b>0,059</b>
13	TACCTAATTAAGGACCCGCGGATGCCATCAACA	0,000	<b>0,059</b>	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
14	TACCTAATTAAGGATCCGCGGATGCCATCGACA	0,000	0,000	0,000	0,000	0,000	<b>0,111</b>	0,000	<b>0,067</b>	0,000	<b>0,050</b>	0,000	0,000
15	TACCTAATTAAGGACCCGCGGATACCATCGACA	0,000	0,000	0,000	0,000	0,000	<b>0,111</b>	0,000	0,000	0,000	0,000	0,000	0,000
16	TACCTAATTAAGGACCCACGGATGCTATCGACA	0,000	0,000	0,000	0,000	0,000	0,000	<b>0,077</b>	0,000	<b>0,071</b>	0,000	0,000	0,000
17	TGCCTAATTAAGGACCCGCGGATGCCATCGACA	0,000	0,000	0,000	0,000	0,000	0,000	<b>0,077</b>	0,000	0,000	<b>0,050</b>	<b>0,053</b>	0,000
18	TACCTAATTAAGGACCCGCGGATGCCATTGACA	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<b>0,067</b>	0,000	0,000	0,000	0,000
19	TACTTAATTAAGGACCCGCGGGTGCTATCGACA	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<b>0,071</b>	0,000	0,000	0,000
20	TACCTAACTGAAGGACCCGCGGATGCTATCGACG	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<b>0,071</b>	<b>0,050</b>	0,000	0,000
21	TACCTAACTAAAGGACCCGCGGATGCTATAGACA	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<b>0,071</b>	0,000	0,000	0,000
22	TACTTAACCTAAAGGACCCGCGGGTGCTATCGACA	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<b>0,071</b>	0,000	0,000	0,000
23	TACCTAACTAAAGGACCCGTGGATGCTATCGACA	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<b>0,050</b>	0,000	0,000
24	TACCTAACTAAAGGACCCGCGGATGTTATCGACA	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<b>0,050</b>	0,000	0,000
25	TACCTATCTAAAGGACCCGCGGATGCTATCGACA	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<b>0,050</b>	0,000	0,000
26	TACCTAACTAAAGGACTCGCGGATGCTATCGACA	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<b>0,050</b>	0,000	0,000
27	TACCTAATTAAGGACCCGCGGATGCTATCGATA	0,000	0,000	0,000	0,000	0,000	<b>0,111</b>	0,000	0,000	0,000	0,000	<b>0,053</b>	0,000
28	TATCTAATTAAGGACCCGCGGATGCCATCGACA	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<b>0,053</b>	0,000
29	CACCTAACTAAAGGACCCGCGGATGCTATCGACA	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<b>0,059</b>
30	TACCTAACTAAAGGACCCGCGGACGCTATCGACA	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<b>0,059</b>

31	TACCCAAC <b>T</b> AAAGGACCCGCGGAT <b>ACTG</b> TCGAC <b>G</b>	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<b>0,050</b>	0,000	0,000
32	TACCTAA <b>CGA</b> ACGGACCCGCGGATGCTATCGACA	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
33	TACCT <b>G</b> ATTAAGGACCCGCGGATGCCATCGACA	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
34	TACCTAATTAAG <b>A</b> ACCCGCGGATGCCATCGACA	0,000	<b>0,059</b>	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
35	TACCTAACTAAAGGACCCGCGGATGCTAT <b>AGACG</b>	<b>0,053</b>	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000

**A - Data collection information and molecular diversity indices:** Averaged values are given with standard error of the mean within brackets. Salinity values concerns the place where individuals were caught. NA= not available. Molecular diversity indices calculated individually for each sampled location, where n = number of samples, nHap = haplotypes, S = segregation sites,  $h$  = haplotype diversity,  $\pi$  = nucleotide diversity,  $T_i$  = transitions,  $T_v$  = transversions. Regarding Tajima's  $D < 0.05$ . The population size may be increasing or we may detect evidence for purifying selection at this locus, or existence of sub-populations, i.e negative correlation between number of populations pooled in a sample and Tajima's  $D$  (Ptak & Przeworski 2002). A = Allelic richness averaged over 17 microsatellites (mean number alleles/locus) and respective standard deviation for each population. **B - Haplotype-defining sequence list generated by DnaSP**, (not considering invariable sites). Single nucleotide polymorphisms (SNP) in comparison to Haplotype 1 are highlighted in blue. SNP Position in relation to 355bp fragment: 10; 13; 30; 31; 34; 38; 52; 61; 67; 73; 89; 100; 112; 119; 208; 223; 226; 235; 238; 239; 242; 261; 277; 280; 283; 286; 287; 298; 306; 323; 343; 347; 349; 352; 355. Relative haplotype frequencies in each population. Values in bold,  $>0$





## Supplemental experimental procedures

### Text S1

The 355bp ND5 mtDNA fragment was amplified in a reaction containing 1ul 10x Buffer, 1ul (10mM) dNTPs, 1ul [5 pmol/ul] each primer (Forward: GCCCCTCAGAATGATATTTGTCCTCA reverse: AATAGTTTATCCRTTGGTCTTAGG) [S16], 0.1 Taq, 4.9  $\mu$ l H<sub>2</sub>O and 1  $\mu$ l template. PCR conditions were as follow: 3min at 95 °C, 35sec at 95 °C, 40sec at 59 °C, 1sec at 72°C for 30cycles, 4min at 72°C. PCR products were cleaned with Exonuclease and FastAP (Fermentas) and directly sequenced from the reverse direction.

The nuclear loci were amplified in duplex (Aan01 and Aa02) [S16] and 3 multiplexes: 1 - B09, I14, M23, Aan03; 2- CT77,CT87, CA55,CA58,CA68, AjTR-37 ; 3- CT82, CT76, CT89, CT59, CA80, CT53 [S17-S19]. Duplex was performed in the following reaction conditions: 1  $\mu$ l 10x Buffer, 0.5  $\mu$ l (10mM) dNTPs, 1  $\mu$ l each primer 5pmol/ul, 0.05ul Taq 3.45 and 1ul DNA template. PCR conditions were the following: 3min at 95 °C, 35sec at 95 °C, 30 sec at 61 °C, 40 sec at 72°C for 30 cycles, 5min 72 °C; multiplex reaction was performed with QIAGEN© Multiplex PCR kit, following manufacturer instructions. Genotyping was then performed on a ABI ® 3100 Genetic Analyzer

Molecular and phylogenetic analyses using mtDNA were performed using the software DnaSP v5.10.01 [S20], NETWORK v4.6.1.0 [S8], and MEGA [S6]. To estimate the strength of neutral evolution, we added eight sequences of the ND5 fragment from *Anguilla rostrata* to the data set and performed a McDonald and Kreitman test [S21]. Departures from mutation/drift equilibrium were tested by means of Tajima's D test [S22]. Pairwise population differentiation comparisons were performed in Arlequin v3.5 [S23], by calculating Wright's index ( $F_{ST}$ ) based on haplotype frequencies (10.000 permutations). Patterns of isolation by distance were investigated using  $F_{ST}/(1-F_{ST})$  and log transformed geographic distances by the means of Mantel tests run on IBDWebService [S24].  $F_{ST}$  were chosen because they can be combined with oceanic modeling outcomes to test for multiple scenarios of genetic structure in the spawning ground.

Microsatellites were called using GeneMarker® software. Molecular indices were calculated in MStoolkit [S25] and GENETIX v4.5.02 [S26]. Pairwise population differentiation comparisons were performed in Arlequin v3.5 [S23] by calculating Wright's index ( $F_{ST}$ ) based on allele frequencies (10.000 permutations). Patterns of isolation by distance were investigated using  $F_{ST}/(1-F_{ST})$  and log transformed geographic distances by the means of Mantel Mantel tests run on IBD Web Service [S24].

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## **Supplementary material of Chapter II (as submitted)**

### **Supplementary Material and Methods**

#### *Supplementary text 1 – Amplification and genotyping protocols*

##### *Amplification and genotyping of microsatellite loci*

Here, amplification took place in four PCR multiplexes of four to six loci. Specifically: multiplex A – annealing 55°C (CT77; CT87; CA55; CA58; CT68; AJTR-37), multiplex B - annealing 55°C (CT82; CT76; CT89; CT59; CA80; CT53), multiplex C - annealing 60°C (C01; M23; AJTR-45; AJTR27; I14; O08), multiplex D - annealing 60°C (AJTR-42; B09; B22; N13). All reactions were performed in a total volume of 10 µl and followed the QIAGEN<sup>®</sup> Multiplex PCR kit's recommendations. Genotyping was performed on an ABI<sup>®</sup> 3100 Genetic Analyzer. Alleles were called in GENEMARKER<sup>®</sup> v. 1.91 (Softgenetics LLC, State College, PA).

##### *Amplification and genotyping of the exon 2*

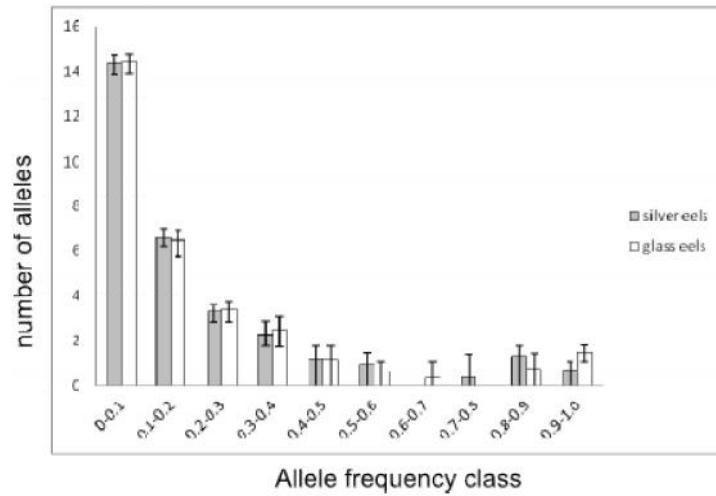
We followed protocols optimized for the European eels and used the forward AaMHCIIBE2F3 (5'-AGTGYCGTTTCAGYTCCAGMGAYCTG-3') and reverse AaMHCIIBE2R2 (5'-CTCACYTGRMTWATCCAGTATGG-3') primers. The use of degenerated nucleotides guarantees amplification of different allelic lineages. Sequencing was performed on a 454<sup>®</sup> platform at LGC genomics (Belgium) following (Stiebens *et al.* 2013a; Stiebens *et al.* 2013b). Briefly, two independent reactions were prepared for each individual. After a first PCR of 20 cycles, a reconditioning step (dilution 1:5) was performed, and the template was used for a second PCR of 20 cycles. The reconditioning step combined with independent reactions was shown to significantly decrease the number of PCR artifacts (Lenz & Becker 2008) and facilitate allele call (Stiebens *et al.* 2013a; Stiebens *et al.* 2013b). The second set of PCR was performed using the specific MHC primers extended by the 454 adaptors (F: CCATCTCATCCCTGCGTGTCTCGACTCAG, R: CCTATCCCCTGTGTGCCTTGGCAGTCTCAG) and a 10 bp individual tag. Allele calling and respective assignment to individuals followed (Stiebens *et al.* 2013a; Stiebens *et al.* 2013b) and primarily relied on matching alleles present in both independent reactions (Sommer *et al.* 2013). Even though variants may stem from different loci, we will refer to them as alleles hereafter.

#### **Supplementary text 2 – Brief description of CODEMEL and MEME procedures**

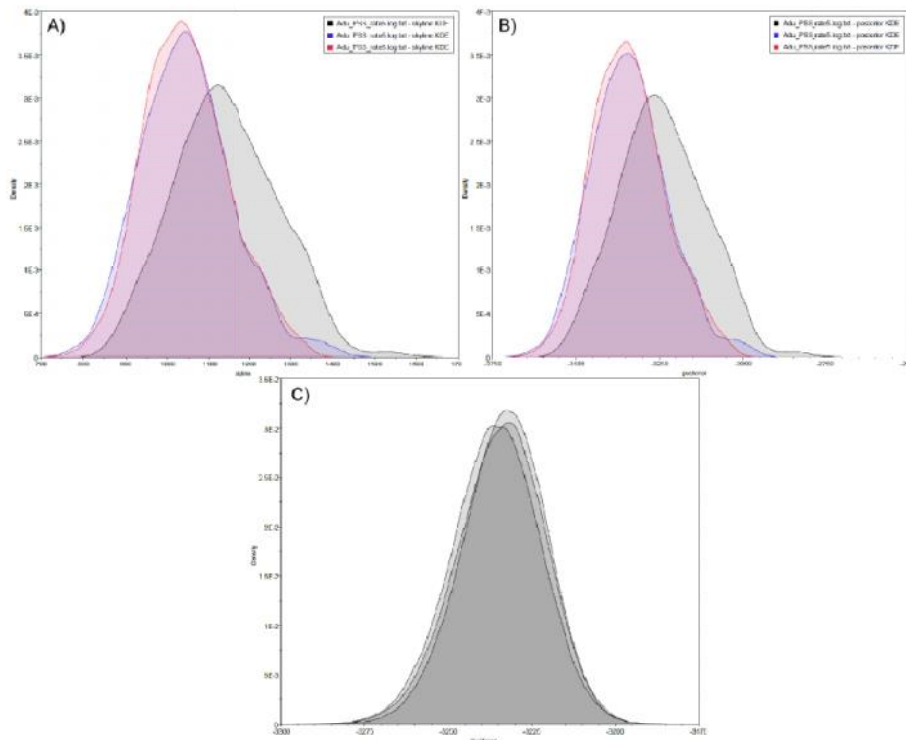
The maximum likelihood procedures evaluate heterogeneous rate ratios ( $\omega$ ) among sites by applying different models of codon evolution. Three likelihood-ratio tests of positive selection were

performed comparing the models M1a (nearly neutral) vs M2a (positive selection), M7 ( $\beta$ ) vs M8 ( $\beta + \omega$ ), and M8a ( $\beta + \omega = 1$ ) vs M8 (Yang 2007). In the models M2a and M8, positively selected sites are inferred from posterior probabilities calculated by the Bayes empirical Bayes inference method (Yang *et al.* 2005). We further tested for sites that experienced episodic events of positive selection by using a. This model considers that  $\omega$  varies between sites (fixed effect), and between branches at a site (random effect) (Murrell *et al.* 2012). The null expectation is that all branches have  $\omega < 1$ . In short, this model allows each site to have its own selection history, contrary to fixed effect models (as the ones implemented in CODEML) that assume constant selective pressures within a branch (Murrell *et al.* 2012).

## Supplemental Figures



**Supplementary Figure 1** – Average distribution of allele frequencies. Above is shown the average distributions of allele’s frequency classes for “silver eels” (grey bars) and “glass eels” (open bars). Error bars represents the maximum and minimum number of alleles observed amongst replicates. Values on the Y-axis were obtained by multiplying the number of alleles, ( $k$ ), with the frequency of the respective class. For purposes of visualization, all values were transformed to their square roots.



**Supplementary Figure 2** – Marginal probability densities of the three replicate (different random seed) Markov chain runs. Both the skyline a), posterior b) and likelihood c) overlap, conferring statistical support for the shape of the Bayesian plots produced with MHC data.



## **Supplementary material of Chapter III (as submitted)**

### **Supplementary Information:**

#### **1. Model selection and calculation of effective number of migrants**

Model selection between model I (Full panmixia) and model II (3 demes with symmetric migration rates). "Symmetry" accounts for the possibility of emigration and immigration. The effective number of migrants ( $N_e m$ ) can be calculated through the equation  $N_e m = M_{j \rightarrow i} * \theta_i$ . The priors of the parameters  $\theta$  and  $M$  were the same for both model 1 and model 2, which allowed direct comparisons of marginal likelihoods. We used a uniform prior of  $\theta$  ranging between 0 and 200, with mean = 200 and delta = 20, after improving over other posterior probability distributions. The uniform prior for  $M$  remained as default, i.e. range between 0 and 100, with mean = 500 and delta = 100. The running parameters comprised a long chain of 5000 recorded steps, with an increment every step of 100 over 3 identical replicates. A total of 1500000 parameters were visited. This methodology allowed the calculation of the harmonic mean of marginal log likelihoods (Raftery 1996).

#### **2. Inferences on allele frequency shifts and heterozygote excess of each deme within cohorts**

Results of BOTTELNECK (Cornuet & Luikart 1996) performed under a two-phase model of evolution (TPM), considering a 10% stepwise mutation and a 10% variance for the TPM. None of the hypothetical philopatric demes showed signature of bottleneck, i.e. significant heterozygosity (H) excess and/or mode-shift distribution.

#### **Cohort 2010 haplogroup A**

##### Wilcoxon test

Assumptions: all loci fit T.P.M., mutation-drift equilibrium.

Probability (one tail for H deficiency): 0.50000

Probability (one tail for H excess): 0.51269

Probability (two tails for H excess or deficiency): 1.00000

##### Allele frequency distribution

Allele frequency class: 0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

Frequency distribution: 0.789 0.137 0.033 0.020 0.003 0.007 0.000 0.000 0.000 0.010

Normal L-shaped distribution

**Cohort 2010 haplogroup B**

Wilcoxon test

Assumptions: all loci fit T.P.M., mutation-drift equilibrium.

Probability (one tail for H deficiency): 0.61274

Probability (one tail for H excess): 0.39952

Probability (two tails for H excess or deficiency): 0.79903

Allele frequency distribution

Allele frequency class: 0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

Frequency distribution: 0.738 0.169 0.048 0.020 0.008 0.000 0.004 0.000 0.000 0.012

Normal L-shaped distribution

**Cohort 2010 haplogroup C**

Wilcoxon test

Assumptions: all loci fit T.P.M., mutation-drift equilibrium.

Probability (one tail for H deficiency): 0.67218

Probability (one tail for H excess): 0.33943

Probability (two tails for H excess or deficiency): 0.67886

Allele frequency distribution

Allele frequency class: 0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

Frequency distribution: 0.742 0.164 0.049 0.020 0.008 0.000 0.004 0.000 0.000 0.012

Normal L-shaped distribution

**Cohort 2011 haplogroup A**

Wilcoxon test

Assumptions: all loci fit T.P.M., mutation-drift equilibrium.

Probability (one tail for H deficiency): 0.30508

Probability (one tail for H excess): 0.70604

Probability (two tails for H excess or deficiency): 0.61015



Allele frequency distribution

Allele frequency class: 0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

Frequency distribution: 0.797 0.129 0.031 0.017 0.010 0.003 0.000 0.000 0.000 0.010

Normal L-shaped distribution

**Cohort 2011 haplogroup B**

Wilcoxon test

Assumptions: all loci fit T.P.M., mutation-drift equilibrium.

Probability (one tail for H deficiency): 0.84735

Probability (one tail for H excess): 0.16044

Probability (two tails for H excess or deficiency): 0.32088

Allele frequency distribution

Allele frequency class: 0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

Frequency distribution: 0.736 0.155 0.081 0.000 0.016 0.000 0.000 0.000 0.000 0.012

Normal L-shaped distribution

**Cohort 2011 haplogroup C**

Wilcoxon test

Assumptions: all loci fit T.P.M., mutation-drift equilibrium.

Probability (one tail for H deficiency): 0.78776

Probability (one tail for H excess): 0.22170

Probability (two tails for H excess or deficiency): 0.44341

Allele frequency distribution

Allele frequency class: 0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

Frequency distribution: 0.711 0.180 0.066 0.013 0.009 0.009 0.000 0.000 0.013 0.000

Normal L-shaped distribution

**Cohort 2012 haplogroup A**

Wilcoxon test

Assumptions: all loci fit T.P.M., mutation-drift equilibrium.

Probability (one tail for H deficiency): 0.25142

Probability (one tail for H excess): 0.75870

Probability (two tails for H excess or deficiency): 0.50284

Allele frequency distribution

Allele frequency class: 0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

Frequency distribution: 0.780 0.143 0.040 0.017 0.007 0.000 0.003 0.000 0.007 0.003

Normal L-shaped distribution

**Cohort 2012 haplogroup B**

Wilcoxon test

Assumptions: all loci fit T.P.M., mutation-drift equilibrium.

Probability (one tail for H deficiency): 0.71697

Probability (one tail for H excess): 0.29396

Probability (two tails for H excess or deficiency): 0.58793

Allele frequency distribution

Allele frequency class: 0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

Frequency distribution: 0.738 0.167 0.048 0.024 0.004 0.008 0.000 0.000 0.000 0.012

Normal L-shaped distribution

**Cohort 2012 haplogroup C**

Wilcoxon test

Assumptions: all loci fit T.P.M., mutation-drift equilibrium.

Probability (one tail for H deficiency): 0.38726

Probability (one tail for H excess): 0.62488

Probability (two tails for H excess or deficiency): 0.77453

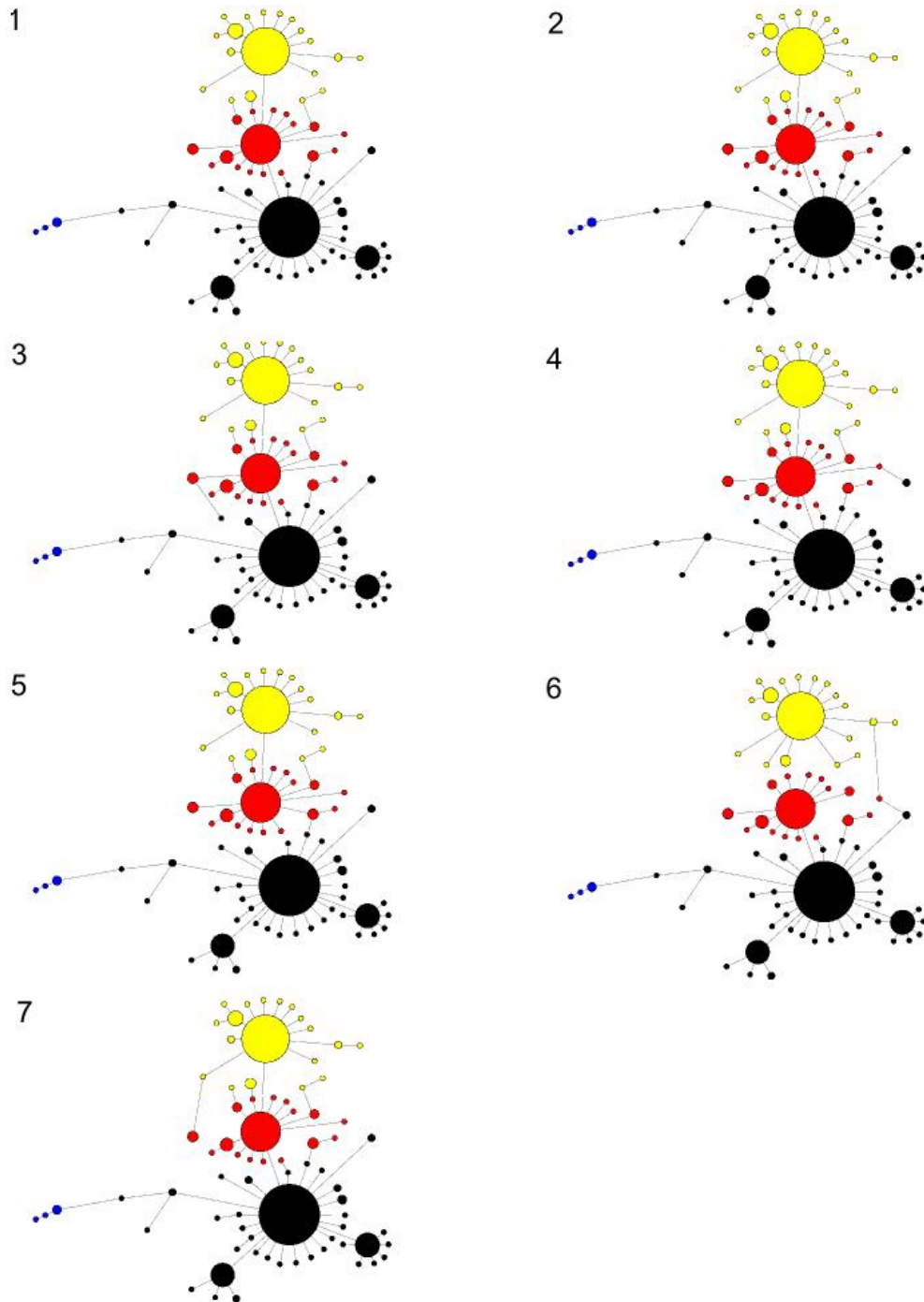
Allele frequency distribution

Allele frequency class: 0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

Frequency distribution: 0.734 0.154 0.066 0.025 0.004 0.004 0.000 0.000 0.008 0.004

Normal L-shaped distribution

**Supplemental figures**



**Figure S1** – All possible shortest trees calculated, through maximum parsimony (Bandelt *et al.* 1999), to generate the original network in figure 2 of the main text. In the case of ambiguous connections, the shortest link to the most frequent haplotype was chosen.

**Supplementary tables**

cohort	equation	R	p
2010	$y=2.817x-2.782$	0.73	<0.001
2011	$y=2.877x-2.864$	0.73	<0.001
2012	$y=3.003x-3.055$	0.75	<0.001
All	$y=3.397x-3.813$	0.8	<0.001

**Table S1-** Regression equations fitted for the  $\log(L) - \log(W)$  relationship in each cohort

$\log(W) \sim$	t	p
$\log(L)$	19.724	<0.001
cohort2011	-0.25	0.803
cohort2012	-0.758	0.449
$\log(L):$ cohort2011	0.328	0.743
$\log(L):$ cohort2012	0.934	0.351

**Table S2 -** ANCOVA testing for differences in the slope of  $\log(L) - \log(W)$  relationship amongst cohorts

Model	marginal log likelihood
3 demes with symmetric migration (model II)	-3740.630 (212.306)
Panmixia (model I)	-5400.113 (215.23)
Bayes Factor <sub>(MII-MI)</sub>	1659.483

**Table S3.** Marginal log likelihood estimates averaged for the three cohorts of each model. In parenthesis is reported the standard deviation.

### 2010

<u>Parameter</u>	<u>mode</u>	<u>2.50%</u>	<u>97.50%</u>
$\theta$ A	0.067	0	3.867
$\theta$ B	2.067	0	5.2
$\theta$ C	2.467	0	5.867
$N_e m_{B \rightarrow A}$	1.311	0	36.667
$N_e m_{C \rightarrow A}$	1.267	0	36
$N_e m_{A \rightarrow B}$	40.645	0	36.667
$N_e m_{C \rightarrow B}$	32.379	0	30.667
$N_e m_{A \rightarrow C}$	46.867	0	36
$N_e m_{B \rightarrow C}$	38.645	0	30.667

### 2011

<u>Parameter</u>	<u>mode</u>	<u>2.50%</u>	<u>97.50%</u>
$\theta$ A	2.6	0	5.733
$\theta$ B	2.067	0	5.2
$\theta$ C	4.6	0.4	8.533
$N_e m_{B \rightarrow A}$	56.334	0	221.69
$N_e m_{C \rightarrow A}$	49.4	0	206.4
$N_e m_{A \rightarrow B}$	44.779	0	201.07
$N_e m_{C \rightarrow B}$	35.133	0	173.33
$N_e m_{A \rightarrow C}$	87.4	0.8	307.2
$N_e m_{B \rightarrow C}$	78.2	0.267	284.44

### 2012

<u>Parameter</u>	<u>mode</u>	<u>2.50%</u>	<u>97.50%</u>
$\theta$ A	0.067	0	113.33
$\theta$ B	0.067	0	126.67
$\theta$ C	4.2	0.667	145.07
$N_e m_{B \rightarrow A}$	1.178	0	113.33
$N_e m_{C \rightarrow A}$	1.311	0	126.67
$N_e m_{A \rightarrow B}$	1.178	0	145.07
$N_e m_{C \rightarrow B}$	0.778	0	116.62
$N_e m_{A \rightarrow C}$	82.601	1.333	288.8
$N_e m_{B \rightarrow C}$	49.001	0	207.73

**Table S4.** Estimates for the modes and respective 95% confidence interval for  $\theta$  and  $N_e m$  in model II, where  $N_e m = M_{j \rightarrow i} * \theta_i$

## ***Acknowledgments***

„Posso ter defeitos, viver ansioso e ficar irritado algumas vezes,  
Mas não esqueço de que minha vida  
É a maior empresa do mundo...  
E que posso evitar que ela vá à falência.  
Ser feliz é reconhecer que vale a pena viver  
Apesar de todos os desafios, incompreensões e períodos de crise.  
Ser feliz é deixar de ser vítima dos problemas e  
Se tornar um autor da própria história...  
É atravessar desertos fora de si, mas ser capaz de encontrar  
Um oásis no recôndito da sua alma...  
É agradecer a Deus a cada manhã pelo milagre da vida.  
Ser feliz é não ter medo dos próprios sentimentos.  
É saber falar de si mesmo.  
É ter coragem para ouvir um “Não”!!!  
É ter segurança para receber uma crítica,  
Mesmo que injusta...  
  
Pedras no caminho?  
Guardo todas, um dia vou construir um castelo...”

***Fernando Pessoa***

*to Luis Alexandre Oliveira Soares (1957-2013) – I will see you in my dreams ☺*

Eu bem me parecia que te tinhas metido numa alhada, Pai. Recordei-te isso no teu penultimo dia e tenho a certeza que me ouviste. Tambem ouviste eu dizer-te que para ires onde tinhas que ir, que eu tratava das coisas por aqui. E foste. Mas nao fui so eu a tratar das coisas por aqui...a Mae, o Pedro, os Avos...onde quer que estejas, acho que podes estar orgulhoso deste ultimo ano. Eu estive aqui pensar, e cheguei a conclusao que esta tese nao e nada comparado com o que tu passaste no ultimo ano. Eu sei que te aguentaste estoicamente ate ao fim para nos dares tempo para nos adaptar-mos a tua perda. Para entendermos que a tua partida ia ser proxima, que nao havia volta a dar, e que num futuro muito proximo, tu nao estarias la. Foi um optimo plano. E foi, sem duvida alguma, uma grande licao para todos nos que ca ficamos. A mim pessoalmente, ensinou-me a ver a tua partida como algo que merece ser honrado, respeitado, e, talvez (as vezes, chorado). Mas nunca sentir pena. Ensinou-me tambem que todo o esforco que eu fizer doravante, nao sera nada comparado com o que tu fizeste. Isso e, unica e exclusivamente, a motivacao que preciso para continuar a fazer o que tenho que fazer ate ao fim da minha vida. Tu disseste me, mais que uma vez, para eu nao deixar a situacao afectar-me. "Pedras no meu caminho? Guardo todas, um dia vou construir um castelo..." Pois bem, a tua partida e a minha pedra angular.

A Mae, o Pedro...grandes. Tudo o que voces fizeram e teem feito em Portugal, com e sem o Pai, reflecte-se aqui comigo. Nunca conseguiria acabar isto se as coisas nao tivessem corrido como correram apos o Pai nos deixar. Tenho muito mais orgulho em voces do que voces teem que ter em mim. Estiveram ai para tudo. Para os meus Avos (Nana e Nano), e dificil arranjar palavras. Primeiro, porque, tal como a Mae e o Pedro, tambem passaram por tudo. Segundo, porque perderam um dos tres filhos. Ficaram ca dois. Agradeço o modo como reagiram e que muito me ajudou a acabar o doutoramento. Para os meus Avos (Quina e Ze): e dificil ver tudo a acontecer ao longe, agora entendo. Mais importante, entendo o sacrificio diario. E tambem um exemplo para mim.

Todo este trabalho, nao e so meu. E de todos nos. O meu nome de publicacao e Baltazar-Soares. E e assim que vou ficar conhecido no meu trabalho.



To “my” Anna Maria, who has been with me since the very first day of this “enterprise”. I am very happy to have found you and to have had your support during the pursuit of my doctoral degree. I have no idea how I would have balanced my personal life without you...

None of this work would have been possible without Christophe Eizaguirre. You have been more than a supervisor; I must consider you a friend for all of the support you provided during this 4 years. I have no words to express my gratitude, so I would rather state that has been a pleasure and an honor to be supervised and oriented by you.

Many others have contributed, in one way or another, to this achievement of mine. For a simple exercise of memory (in an attempt to not to forget anyone) I will briefly describe their contribution in the following lines, from the very beginning of the PhD program – and consequently, staying in Germany – up to now.

I would like to thank the International Max Planck Research School for accepting me in their doctoral program.

I would like to thank Dr. Kerstin Mehnert, whose help was critical not only for my successful settlement in German territory but also for being a dedicated – always there - scientific coordinator of the IMPRS.

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To Robert, Seun, Julian, and Ana, for the nights out in Kiel! (Ok, I know some of you are Dr, but I skip the formalities to avoid embarrassment)

To Dr. Jan Dierking for his help in the very early stages of my staying at GEOMAR. Both in a scientific sense, but also from a personal sense by introducing me to his football group! Sometimes, the only reason to look forward weeks to come...

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## ***Author's contributions***

All chapters of this thesis were designed and written for peer-reviewed publications. The contribution of each author, including my own, is specified in this section

### ***Recruitment Collapse and Population Structure of the European Eel Shaped by Local Ocean Current Dynamics***

Christophe Eizaguirre, Miguel Baltazar-Soares and Arne Biastoch designed the study experiment and wrote the manuscript. Miguel Baltazar-Soares coupled the oceanographic data with population genetics, performed the *in silico* population genetic analyses, extracted and amplified DNA from eel tissue and performed the *in vivo* population genetics analyses. Arne Biastoch, Erik Behrens and Claus W. Böning performed the ocean model simulations and processed the data prior to its use by Miguel Baltazar-Soares. Reinhold Hanel, Lasse Marohn, Enno Prigge, Derek Evans, Kenneth Bodles provided tissue samples as well as Chris Harrod who also contributed to editing the manuscript. All authors provided insightful amendments that greatly increased the quality of the manuscript.

### ***Evaluation of adaptive potential of the European eel population suggests a recovery of its genetic status***

Miguel Baltazar-Soares and Christophe Eizaguirre designed the study and wrote the manuscript. MBS performed all the population genetic analyses. Seraina E. Bracamonte (SEB) developed all the laboratory work for the MHC. Frédéric J.J. Chain and Christophe Eizaguirre analysed and filtered 454 data for the MHC. Till Bayer provided help for the phylogenetic analyses. Chris Harrod and Reinhold Hanel provided tissue samples. All authors greatly contributed for the final version of this manuscript.

### ***Asymmetric gene flow amongst matrilineages maintains the evolutionary potential in the endangered European eel***

Miguel Baltazar-Soares (MBS) designed the study and performed all the analyses. Miguel Baltazar-Soares and Christophe Eizaguirre wrote the manuscript.

## ***Declaration***

Hereby I declare that,

i. apart from my supervisor's guidance, the content and design of this dissertation is product of my own work. Co-author's contributions to specific paragraphs are listed in the following section

ii. this thesis has not been submitted either partially or wholly as part of a doctoral degree to another examination body, and no other materials are published or submitted for publication than indicated in the thesis

iii. the preparation of the thesis has been subjected to the Rules of Good Scientific Practice of the German Research Foundation.

Kiel

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Miguel Alexandre Baltazar Soares

