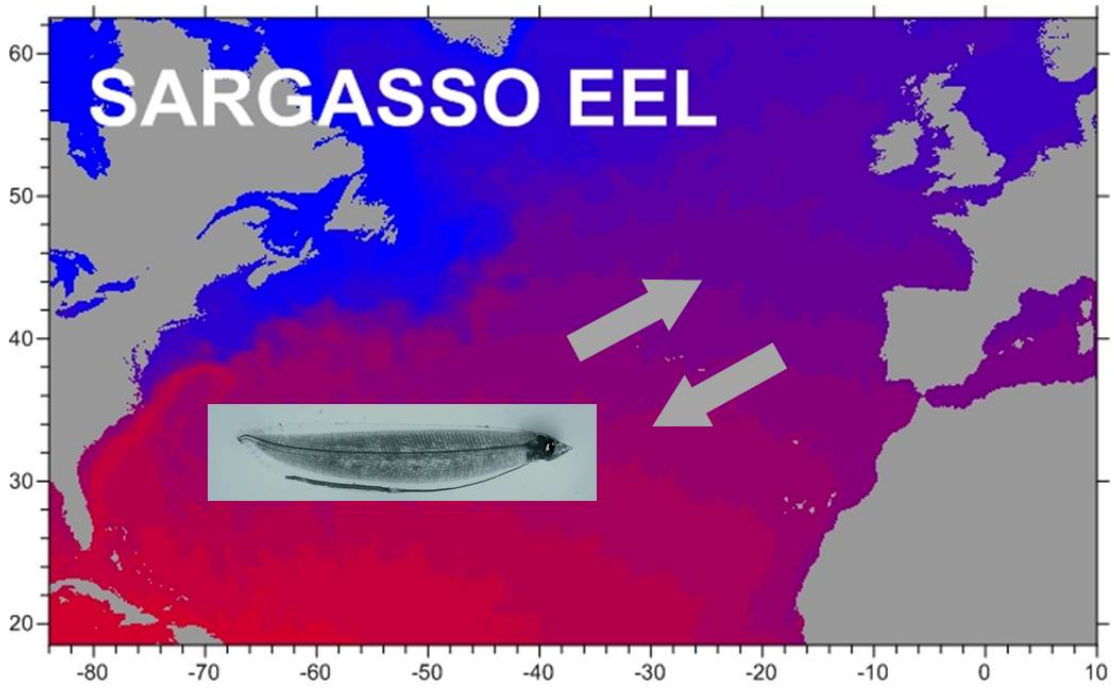
CRUISE PROGRAMME

The early life of eel in the Sargasso Sea – influence of oceanography and climate *(SARGASSO-EEL)*



Resume

Due to a dramatic decline in the recruitment and stock of European eel there is a need for improved insight into the lifecycle of eel, and an extensive research programme is carried out during a cruise to the spawning areas in the Sargasso Sea in 2014. A consortium of Danish scientists and many international collaborators will focus on the linkages between oceanography, biological production, eel spawning and the growth and drift of eel larvae. The research will provide insight into the physical and biological processes in the Sargasso Sea and improve our understanding of climate influence and possible causes for the decline in the eel population.

The cruise will be carried out using the research vessel DANA (Technical University of Denmark) which has lab facilities and a range of standard equipment: e.g. CTD's ADCP, hydroacoustics, plankton gear handling, and fishing experience.

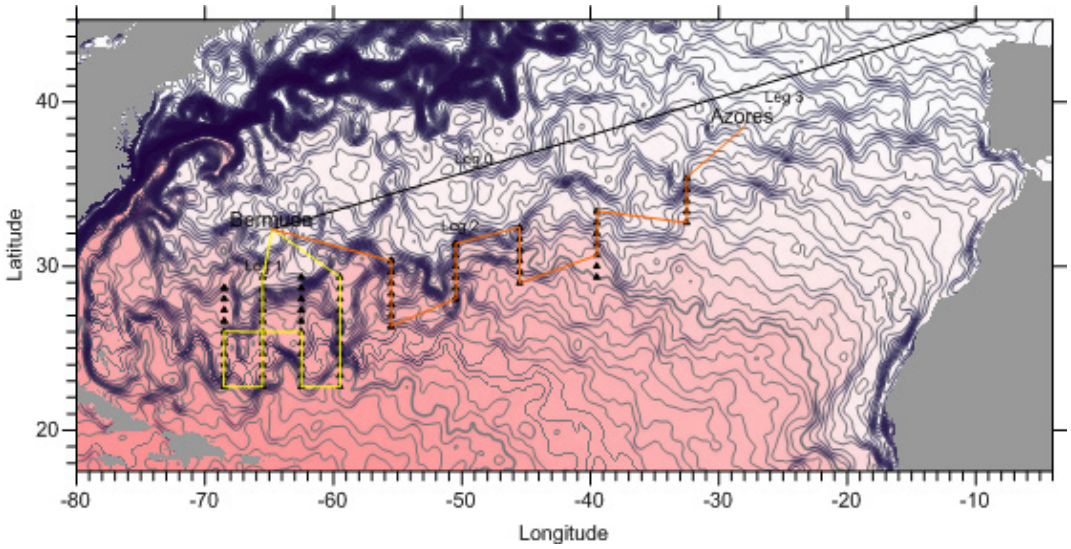
The investigations will focus on the key spawning grounds in the central Sargasso Sea and on the extension of larval distribution to the east, towards the Azores (see map below). Investigations are planned in two primary cruise legs (Leg 1 and Leg 2) which are separated by a short stay at Bermuda. (see time-line below), but sampling will also take place during the Leg3 from Azores to DK.

The investigations will cover 1) the oceanography – importance of frontal phenomena, 2) the oligotrophic plankton ecosystem - its composition and rates, and 3) the eel larval life – distribution, species differences, feeding and growth, 4) the eel population structure(s) – environmental influence, panmixia and species differences, and 5) the adult eel – observation attempts and possibilities to catch these. For each element, focus will be put on defining and describing key characteristics and the multidisciplinary work will be further organized in a series of sub-projects. These includes: a) Frontal phenomena described by remotely sensed surface characteristics, b) General oceanography based on in-situ measurements, c) Nutrients, phytoplankton composition and primary productivity, d) Zooplankton composition and distribution, e) Grazing and feeding, f) Molecular determination of eel larval stomach contents, g) Distribution and separation between Atlantic eel larvae, h) Genomic basis of adaptive traits and speciation of the two species, i) Larval drift, and j) Condition of the spawning eel.

Cruise periods and legs

The first leg (Leg 0, 28/2 – 14/3) will be the transit to Bermuda, where the ship will stay a day and the scientists will embark. The first sampling leg (Leg 1, 15/3 – 6/4) will then focus on central areas of European eel spawning (approx. within 70W-62W, 23N-32N), and describe northern and southern limits of spawning. The first sampling will be carried out at least 140 nautical miles south of Bermuda, hence there will be at least 12 h steaming time before first station. The exact station distribution will be decided based on satellite information available shortly before the cruise, and the distribution will be

revised according to findings and new satellite information during the cruise legs. During the first part of Leg1 we will sample along a longitudinal transect until we meet the southern front where we expect to find relatively high concentrations of eel larvae. Close to here we will carry out intense, closely spaced sampling, horizontally and vertically. We will follow the front towards west and continue sampling along the route. Dependent on time available, we subsequently will cover three more north-south transects by measurements and sampling. This sampling will be planned and coordinated with the German RV Heincke, which is also sampling for eel larvae during this period. Search and fishing for adult will be carried out during 4 night periods during the last part of this Leg, when it is close to new moon.

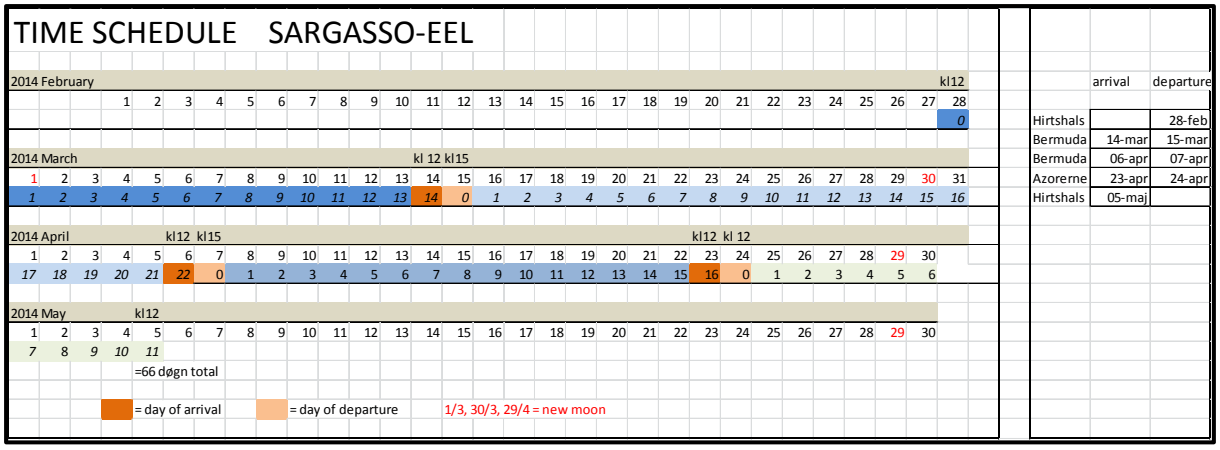


Tentative sampling scheme ill. on 2007 hydrography Length of transects, station distances etc. will be planned according to SST information during 2014 cruise and time available.

After a 2nd stay in Bermuda, Leg 2 (7/4 – 23/4) starts further east and sampling will be carried out along shorter cross-frontal transects to the east following the path of the fronts, as deduced from satellite observations. During this part another week of sampling time is added to the direct transit time.

There will be a 1 day stay at the Azores, and a new exchange of scientists. During the following Leg 3, a Phd course on benthic investigations will be carried out, combined with a specific sampling project close to the Azores, the SARGASSO-EEL has 10 stations of pelagic sampling along the direct route to Hirtshals, Denmark.

During the cruise we will mainly be in international waters. However, we obviously visit Bermuda (UK) EEZ, and Azores (Portugal) EEZ. We have also applied for allowed sampling in the UK sectors of Turk, British Virgin and Anguilla Islands.



Procedures on different legs

- Transit (leg 0): ADCP, acoustics and other on-the route measurements.
- Leg 1: ADCP, acoustics and other on the route measurements. Sampling basically in cross-frontal transects. Use of 15, 20 and 25 nm distances between stations, station distances are planned based on satellite information on actual SST distribution. Both Standard, Special and Fishing stations will be used. Transect longitudes planned in coordination with German vessel, so as transects are 1.5 degree apart. Transects used in 2007 will be revisited.
- Leg2: ADCP, acoustics and other on the route measurements (e.g. UnderWay CTD). Sampling in Standard stations along longitudinal transects, positions of sampling will be directed by satellite observations of frontal performance. General station distances 15 or 20 nm.
- Leg3: ADCP, acoustics and other on the route measurements(e.g. UnderWay CTD). A number of Standard pelagic (CTD, MIK) stations until the English Channel on a direct course towards Denmark

Stations

Sampling in three types of stations:

- **Standard station** during which a deep CTD to 400m is used for water sampling for water chemistry and biological parameters. Sampling for gelatinous zooplankton. Depth stratified sampling of zooplankton, using a Multinet opening closing net of 0.25 m2, equipped with 45 µm net. Depth integrated sampling for fish larvae using the 3.5 m MIK, lowered to 200 m and retrieved (an extra 3.5MIK haul at constant selected depth can be included in this standard station procedure)
- **Selected station**, with special sampling procedures, these could be 1) Vertical distribution of larvae. Procedures as for standard station but sampling for fish larvae is depth stratified, lowering the 3.5m MIK to given depth and hauling at constant depth for approx. 1h (5-6 strata). 2) Especially intensive sampling of plankton, rate measurements (primary, bacterio and zooplankton production)

are included at these stations. 3) Deep sampling for fish juveniles with special gears.

- **Fishing station**. At this station the major effort is directed at finding/catching fish. Only fishing during the night hours. Acoustic search and deployment of fishing gear. Also sampling for eggs is intensified in the area and time period of this fishing effort.

Standard equipment

Gears and equipment to be used

- Large small-meshed pelagic trawls, potentially to 500 m depth.
- A 3.5 m 0.5 mm mesh MIK to depths of standard 200, but also to min 400m. A 3.5 m 2 mm mesh MIK to max 800 m depth. These gears are handled using the central bar (løbekat), but hauled using the vessels trawl winches.
- A 0.25 m2 Multinet in both its vertical and horizontal setup. Mesh sizes standard 50 micron, but also 200 micron might be used.
- Miscellaneous ring nets, relatively easy to launch over the side of the ship (Hydrographic deck)
- CTD with fluorometer, standard to 400 m depth, some deeper casts. A camera will be mounted on the CTD (for plankton observations)
- On-route measurements ADCP, Acoustics and 5m physical measures
- UnderWay CTD
- Sediment core sampling is planned (Azores and along Leg 3)
- The use of daily satellite pictures requires a broad and stable internet connexion.

APPENDIX 1 List version of 23/1 2014

Participants list

SARGASSO-EEL

Leg 1	flight	22d flight 13/3-7(8)/4			
		Name	Institute	Main targets	e-mail
1		(Niels)Peter Munk	DTU Aqua	Distributional patterns front, vertical dist, adult eel	pm@aqua.dtu.dk
2		Daniel Jiro Ayala	DTU Aqua	feeding, growth, other larvae	djay@aqua.dtu.dk
3		Peter Rask Møller	Copenhagen University	Juvenile fish, adults, using eDNA techniques	PDRMoller@snm.ku.dk
4		Sune Riis Sørensen	DTU Aqua	eel eggs&larvae,infektion, morphology, adult eel	srs@aqua.dtu.dk
5		(Lars) Magnus W. Jacobsen	Aarhus University	Hybrids American and European, panmixia	
6		Henrik Sparholt	ICES	Larval abundance, catch of adult eel	henriks@ices.dk
7		Jon Christian Svendsen	University of Porto/CIIMAR	Leptocephali behaviour	jos@aqua.dtu.dk
8		Lasse Rieman	Copenhagen University	Bacteria, microplankton, larval feeding	LRiemann@bio.ku.dk
9		Fabian Lombard	Univ. P&M Curie FR	Marine snow	Lombard@obs-vlfr.fr
10		Hans Peter Grossart	Leibniz Inst. IGB GER	Marine snow	hgrossart@igb-berlin.de
11		Katherine Richardson	Copenhagen University	Primary production, reciculation, eddies	kari@science.ku.dk
12		Jørgen Bendtsen	CLIMATELAB	STCZ dynamic, eddies, drift	jb@climatelab.dk
13		Kam Tang	Virginia VIMS	Bacteria, microplankton	kamtang@vims.edu
14		Torkel Gissel Nielsen	DTU Aqua	Zooplankton	tgin@aqua.dtu.dk
15		Marja Koski	DTU Aqua	Zooplankton	mak@aqua.dtu.dk
16		Cornelia Jaspers	DTU Aqua	Jellyfish	coja@aqua.dtu.dk
17		Kristina Enders		Sargasso weed fauna, plastic contamination	kristina.enders@gmx.de
18		Robin Lenz	DTU Aqua	Sargasso weed fauna, plastic contamination	roble@aqua.dtu.dk
19		Line Reeh	DTU Aqua	Communication, PR, Video	lre@aqua.dtu.dk
20		Tommy Nielsen	DTU Aqua	Technical support	tn@aqua.dtu.dk
21		NN		Larvae and fish	

Leg 2		16d flight 5/4-25/4			
1		(Niels)Peter Munk	DTU Aqua	Distributional patterns front, drift	pm@aqua.dtu.dk
2		Daniel Jiro Ayala	DTU Aqua	feeding, growth, other larvae	djay@aqua.dtu.dk
3		Jon Christian Svendsen	DTU Aqua	Leptocephali behaviour	jos@aqua.dtu.dk
4		Evandro Malanski	DTU Aqua	Feeding of selected species	evma@aqua.dtu.dk
5		Dorte Bekkevold	DTU Aqua	Distribution of eel, eDNA technique	db@aqua.dtu.dk
6		Anna Zakrisson Braeunlich	Stockholm University	Marine snow (HPG)	anna.zakrisson@su.se
7		Martin Lilley	Univ. de la Mediterranée FR	Marine snow (FL)	lilley@obs-vlfr.fr
8		Helge Abilhauge Thomsen	DTU Aqua	Abundance of algal species , eDNA	hat@aqua.dtu.dk
9		Erik Askov Mousing	Copenhagen University	Primary production	eamousing@bio.ku.dk
10		Birgit Søborg	Aarhus University	Logistics, zooplankton (KT)	bis@dmu.dk
11		Torkel Gissel Nielsen	DTU Aqua	zooplankton	tgin@aqua.dtu.dk
12		Russ Hopcroft	Univ. Alaska Fairbanks	macroplankton, jellyfish	rrhopcroft@alaska.edu
13		Liv Louise Victoria Backhaus	Copenhagen University	Copepod ecology (carcasses)	nht919@alumni.ku.dk
14		Igor Belkin	Univ Rhode island US	Satellites, currents, fronts, physics	Igormbelkin@gmail.dk
15		Hjalte Parner	ICES DK	Hydrographic presentation, databases	hjalte@ices.dk
16		Priscilla Licandro	SAHFOS Plym. UK	jellyfish, plankton	prli@sahfos.ac.uk
17		Helene Bachmann	Copenhagen University	Primary production	helenebachmann7@gmail.com
18		Rasmus Bech	POLITIKEN	Newspaper articles	rasmus.bech@pol.dk
19		Daniel Flendt Dreesen	Blue Planet Aquarium	PR	dfd@denblaaplanet.dk
20		Ronny Sørensen	DTU Aqua	Technical support	roso@aqua.dtu.dk
21		Line Smedegaard Andersen	Aarhus University	Masters genetics hybrids	line_sunshine_89@hotmail.com

leg 3		11 d flight single to azores 22/4			
1		Mads Ole Christoffersen	DTU Aqua	Large leptocephali	maoc@aqua.dtu.dk
2		Susanne Hansen	DTU Aqua	Large leptocephali	sh@aqua.dtu.dk
22		Ronny Sørensen	DTU Aqua	Technical support	roso@aqua.dtu.dk

Participants, not in cruises					
1		Jacob Høyer	DMI	Satellite pocessing and analysis	jlh@dmi.dk
2		Fritz Køster	DTU Aqua		fwk@aqua.dtu.dk
3		Bjarne Stage	DTU Aqua		bst@aqua.dtu.dk
4		Michael Ingemann Pedersen	DTU Aqua	Lifecycle of eel	mip@aqua.dtu.dk
5		Kim Aarestrup	DTU Aqua	Leptocephali behaviour	kaa@aqua.dtu.dk
6		Asbjørn Christensen	DTU Aqua	Individual behaviour, modeling drift	asc@aqua.dtu.dk
7		Mark Payne	DTU Aqua	Drift modeling, historical and cruise	mpa@aqua.dtu.dk
8		Bruno Buongiorno Nardelli		Mesoscale dynamics, drift modeling	
9		Patrizio Mariani	DTU Aqua	Mesoscale dynamics, drift modeling	pat@aqua.dtu.dk

APPENDIX 2

GENERAL PROJECT DESCRIPTION SARGASSO-EEL

The early life of eel in the Sargasso Sea – influence of oceanography and climate

Resume

Due to a dramatic decline in the recruitment and stock of European eel there is a need for improved insight into the lifecycle of eel, and an extensive research programme is proposed. The research will take place during a cruise to the spawning areas in the Sargasso Sea in 2014, one hundred years after the Danish scientist Johs. Schmidt led the first scientific cruise to investigate eels in the areas. A consortium of Danish scientists will focus on the linkages between oceanography, biological production, eel spawning and the growth and drift of eel larvae. This research will provide unique insight into the physical and biological processes in the Sargasso Sea and improve the understanding of climate influence and possible causes for the decline in the eel population.

Project background

The Sargasso Sea is situated more than 5,000 km from the European continent but nevertheless plays a tremendous role for one of the key fish species in European aquatic ecosystems, namely the European eel (*Anguilla anguilla*). In the southern Sargasso Sea, cold and warm water masses meet and mix, generating a more than 1000 km long frontal zone, the Subtropical Convergence Zone (STCZ), which extends as a narrow ribbon in an east-west direction. This zone might act as a cue for spawning of European eel and the closely related American eel (*A. rostrata*), and the early life of the newly hatched larvae takes place exclusively in the STCZ (Kleckner et al. 1983; Kleckner & McCleave 1987; Munk et al. 2010). Subsequently, the larvae drift towards the American (*A. rostrata*) and European and North African coasts (*A. anguilla*) by ocean currents, where they metamorphose into glass eels, which settle in the coastal zones or enter freshwater.

Until recently, European eel was one of the most common fishes in lakes, rivers and coastal zones. Here, it played a major ecological role as a predator of the benthic fauna and, in the case of larger individuals, a top predator feeding on fish, crayfish and amphibians. It has also sustained important fisheries from pre-historic times to the present. Recently, however, the species has declined drastically. Recruitment of glass eels is now less than 2% of the level prior to 1980 (Åström & Dekker 2007). It is listed by the International Union for the Conservation of Nature (IUCN) as “critically endangered”, the step immediately prior to “extinct in the wild”. The reasons for the decline are subject to intense debate, but undoubtedly involve overfishing, habitat destruction and introduction of new pathogens (van Ginneken & Maes 2005; Åström &

Dekker 2007). In addition, it is persuasively argued that changes in oceanographic features have likely contributed to the population decline (Friedland et al. 2007; Miller et al. 2009).

It was the Danish scientist Dr. Johannes Schmidt who, during a series of classic expeditions, identified the southern Sargasso Sea as the spawning place of both Atlantic eel species (Schmidt 1923). Since then, several other expeditions to the area took place, expanding on Schmidt's findings and adding further information to our understanding of the biology of this elusive species. Many unresolved questions remained, however, and in 2007, the Galathea 3 Eel project investigated important aspects of the eel biology from new angles using novel technological developments. This led to new discoveries of strong interest to the scientific community:.

Firstly, by conducting bio-physical studies across the frontal zones in the Sargasso Sea it was demonstrated that the abundance of eel larvae is directly linked to the specific properties of the STCZ (Munk et al 2010). In particular, the 2007 data suggest that this STCZ frontal system is not a single zone of convergence where different water masses meet but a much more complex system. In the eastern parts of the investigated STCZ there was evidence of vertical mixing at the depth of the nutricline (approx.. 200 m) and it is estimated that the magnitude of new production was at least an order of magnitude higher than in the frontal zone further to the west, at the other two transects (Richardson et al submitted). Secondly, plankton investigations showed different plankton communities in the STCZ compared to surrounding areas (Andersen et al. 2011, Riemann et al. 2011), and DNA barcoding of gut contents of eel larvae showed that prey consisted of a surprising variety of zooplankton, but with a predominance of gelatinous plankton organisms (Riemann et al. 2010). Thirdly, microsatellite DNA analysis of Sargasso Sea eel larvae combined with similar analyses of glass eels from Europe and North Africa demonstrated that the species constitutes one, single panmictic population (Als et al. 2011). Fourthly, comparing gene expression of larvae of American and European eel collected in the Sargasso Sea lent support to the hypothesis that the major difference between the two species reflects timing of ontogeny, with European eel destined for a much longer larval phase than American eel.

The importance of gaining more knowledge about the biology of eel has increased considerably due to its recent, alarming decline. A more precise identification of spawning areas and conditions (depth, temperature etc.), along with a deeper knowledge of the ecosystem where spawning takes places (in particular plankton composition) would considerably improve the knowledge basis for understanding the causes of the decline, in particular the possibility of climatic factors being responsible. Moreover, this information could be used for developing artificial propagation of eel for aquaculture, which would be of considerable economic interest, would mitigate fishing pressure on wild eels and maybe prevent eel from extinction.

Project objectives

The overall goal is to reconsider the oceanic part of the eel life history to identify processes of key importance needed to obtain a more complete understanding of the life cycle. This concerns the spawning migration, the location of spawning sites, the physical and biological conditions during early life and the drift route of the larvae. The following hypotheses are guiding to our research:

- Eels spawn predominantly during the new moon period at the southern side of the convergence zone between subtropical and North Atlantic water masses (STCZ), and eggs/larvae drift northwards in the zone.
- This convergence zone can be regarded as an eddy field which generates small-scale variability in nutrient upwelling within the STCZ and this localised upwelling stimulates the food web supporting eel larvae.
- European eel larvae are entrained in the eastward flowing Subtropical Counter Current (SCC) and predominantly use this route towards the European and North African continents.
- The specific hydrography related to the STCZ and the SCC enhances productivity in these areas and thereby influences larval survival.
- The gut content of eel larvae reflects availability of prey and is linked to larval condition.
- The physical/biological conditions of the STCZ/SSC have changed during the last decades leading to higher larval mortality and a subsequent decline of European eel population
- Geographical differences in bio-physical properties, notably measures of productivity, coincide with differences in gene expression among eel larvae from different localities

Planned research

Subproject 1. Oceanography and productivity in the areas of eel spawning

In order to assess the spawning and distribution of eel it is imperative to analyse their oceanographic habitat and gain understanding of why the STCZ frontal zone is important to the eel spawning.. Hence, we hypothesize that specific characteristics in the oceanography and productivity are of ultimate importance to the early life of the Atlantic eels.

The physical and chemical environment will be characterized with regard to water masses properties, including currents, temperature, salinity, nutrient concentrations and optical properties. CTDs will be made routinely over the entire study area (station spacing on the order of 40nm). In regions where larvae are found, this spacing will be decreased (ca. 10 km) to obtain fine-scale resolution data in order to identify and describe possible upwelling processes. Investigations will be carried out to a) describe the physical mechanisms leading to upwelling, b) identify how widespread such phenomena are in the region and c) determine whether the larvae peak in abundance in

vicinity of such upwelling. Eddy positions will be identified using satellite detection of sea surface height. This will provide: i) description of frontal characteristics; clarification of current patterns and net-currents across and along the zone ii) an indication of the origins and mixing of major water masses; iii) a description of the light environment to improve estimates of phytoplankton productivity across the spawning places and iiii) ground-truthing data for the remote sensing measurements to be applied to extrapolate the geographical coverage.

Ad i) Currents will be analysed by ADCP (Acoustic Doppler Current Profiler) measurements along cross-frontal transect, combined with remote sensing observations and hydrographic modelling. Ad ii) and iii) Special focus will be on the frontal zone and eddy mixing dynamics and on the impact on the planktonic food web upon which eel larvae are dependent. This will be done through measuring Fast Repetition Rate Fluorometry FRRF, primary production (14C method), stable isotope distributions (13C and 15N) in the plankton biomass, and determining phytoplankton speciation. A better description of the light environment will improve estimates of phytoplankton productivity across the spawning area. Ad iiii) Remote sensing observations will be validated and calibrated with the in situ observations and used to extend the in situ observed signals to larger scales and longer time periods. Special attention will be devoted to frontal detection from satellite SST observations and the seasonal, annual and inter-annual variability of the frontal strength and positions.

Subproject 2. Planktonic food web and the linkage to eel larval feeding and growth

Surveys have found the Atlantic eel larvae spatially restricted by the fronts of the Subtropical Convergence zone in the Sargasso Sea (Kleckner and McCleave 1988, Munk et al 2010). We hypothesize that diet, condition and growth of eel larvae are intimately linked to the availability of particular food items in the zone. This will be examined through: i) a novel gentle *in situ* sampling technique of plankton distribution and productivity, focused on likely food items for eel larvae (Riemann et al. 2010), molecular analyses of ii) larval gut contents and iii) larval condition status, and finally on iiii) larval growth revealed by otolith microstructure analysis. This will generate a profound understanding of how the biotic and abiotic factors in the STCZ affect the critical early larval phase of eels.

Ad i) We will apply a new sampling protocol where gelatinous zooplankton gently collected by small mesh nets and large volume water bottles are quantified live immediately after collection. Hereby, we quantitatively determine the distribution of the prey identified in the stomachs of the larvae. Video profiles will supplement the sampling to quantify the fragile zooplankton taxa as well as the marine snow particles *in situ*. Ad ii) Capture, sorting, and molecular species identification of European eel larvae will be done as previously described (Munk et al. 2010, Als et al. 2011). Larval guts will be excised using aseptic techniques (Riemann et al. 2010) and contents will be identified by 454 pyrosequencing using larval muscle tissue as control. The dietary analysis will be combined with sequencing of marine-snow-like material to examine

whether such already-degraded material is a food source for the larvae (Miller et al. 2009). Ad iii) Health status of eel larvae will be assessed using RNA/DNA ratios (Haines 1973), and most importantly, through transcriptome analysis of eel larvae from stations showing differences in productivity. Hereby, biomarkers indicative of the health of individuals and populations will be identified (Chaney & Gracey 2011; Miller et al. 2011). We expect that in areas with suboptimal productivity, eel larvae will up-regulate genes associated with starvation and stress and similarly down-regulate genes associated with growth and metabolism. Condition will also be assessed by analysing their contents of lipids, minerals, their enzyme activity (both digestive system and muscle) and their internal morphology (neural system, cardiovascular system, digestive system). Ad iiii) Larval growth will be analysed from microstructure analysis of their otoliths. This methods has been applied to Japanese eel larve (Umezawa et al 1989), but no systematic analysis of larval otolith's (and growth) of European eel has yet been performed.

Subproject 3. Extent of eel spawning and larval drift

While the observations during the 2007 survey show that larval distributions are strictly delimited in north-south direction by sharp thermal fronts, they also indicate an entrainment of European eel larvae in eastward directed frontal currents. This suggests a key role of oceanic frontal processes, retaining eel larvae within a zone of enhanced feeding conditions and steering their drift (Munk et al. 2010). In addition to these new observations, recent systematic inspection of the historical data on eel larval sampling in the Atlantic indicate that spawning might take place further to the east than hitherto assumed (Miller, Bonhommeau, Munk et al., in prep). Based on this new information, we suggest significant revision of earlier theories on the spawning of European eel. The common belief is a longitudinal restricted spawning, a subsequent drift of larvae in the Gulf Stream flow and consequently a drift and larval stage duration of approx. 2 years. Contrarily, we hypothesize that eel spawning is strongly linked to the extensive frontal systems across the mid-Atlantic, called the Subtropical Counter Current (SCC); hence, spawning would be more widespread to the east and most European eel larvae would drift immediately eastwards and be able to reach the European coasts within a year.

To test this hypothesis we will carry out: i) more fine-grained sampling of larvae in the STCZ concurrent with measurements of frontal processes, currents and system productivity, ii) stratified sampling in the intersection areas of American and European eel larvae to enhance our understanding of the separation between the larval distributions, iii) extension of sampling into the mid-Atlantic, searching for eastward limit of spawning and the continued flow of the SCC. These investigations will be based on both horizontally integrating, and vertically stratified sampling with large, fine-meshed ring nets.

Subproject 4. Spawning time and characteristics of the spawners

Recent findings of eggs and adults of the Japanese eel in the Pacific (Tsukamoto et al 2011) afford unique information on this species and points to an important synchrony in eel spawning. The Japanese results suggest that eel spawning is taking place during a short period around new moon, while both catches of eggs and spawners were made concentrated in the period of 4 days before new moon. Until now neither eel eggs nor adult eel have been caught in the Sargasso Sea or the mid-Atlantic. The only indication of an adult eel approaching the central Atlantic – possibly on its way to the Sargasso Sea – has been from the transmitting tag from one of the eels released during the mentioned 2007 investigation (Aarestrup et al 2009).

Encouraged by the success of the Japanese researchers, we will investigate the hypothesis of peak spawning around new moon by focused sampling for both eggs and adult eel when at the southern frontal zone and when we approach the period of new moon. We will use, i) intensive sampling for eggs by the ring net with on-board molecular identification of eel eggs, ii) search for adults by high frequency acoustics (ship mounted and towed side scan sonar), and iii) fishing for adult eels by use of a small-meshed, large-sized pelagic trawl. These activities will be directed by the satellite observations of frontal zones and findings of enhanced concentrations of eggs or small larvae.

If we are successful in catching adult eels we will assess the natural spawning condition which is completely unknown. We will carry out intensive analysis of body composition – lipid, minerals, enzyme activity, etc, bloodplasma for hormonal samples, pituitaries and brain, gonads for histology, hormonal analysis, otoliths for microstructure and mineral analysis.

Subproject 5. Hybridization between European and American eel

Newly spawned larvae of European and American eel show different overlapping spatial distributions in the Sargasso Sea, with European eel dominating in the East and American eel in the West, but also with extensive spatial overlap. Similarly, although peak spawning time differs between the species there is overlap. Hence, hybridisation is a possibility and hybrids have previously been identified using genetic markers, primarily in Iceland (Avise et al. 1990; Albert et al. 2006). Differences in proportions of hybrids among cohorts of Icelandic eels suggest that intensity of hybridization may differ among years. We hypothesize that differences in hybridization across years this may be due to oceanographic features, e.g. year-to-year variation in timing of establishment of thermal fronts and latitudinal position of the fronts. This could affect spawning time and cause more overlap in spawning time (and less reproductive isolation) between the species in some years than in others. Results from an ongoing study comparing ca. 1% of the genomes of the two species using RAD sequencing (Baird et al. 2008) suggest that gene flow between the two species may be considerably higher than previously assumed. There are, however. At the same time, there are clearly also genomic regions showing extensive divergence between the species, likely reflecting strong selection. We will investigate the dynamics further by developing a

set of ca. 50-100 species-diagnostic single nucleotide polymorphism markers based on the RAD sequence data . This will allow for precise estimation of the possible hybrid ancestry of individuals several generations back in time. We will 1) use historical sampling data to assess peak spawning time for the two species over many years, investigate if this correlates with hydrographical features (e.g. intensity of fronts and time of their emergence) and establish a possible causal mechanism for temporally fluctuating hybridization, 2) compare hybrid proportions among eel larvae collected in 2007 (Galathea 3) and 2014 (this cruise) to assess temporal differences in hybridization and 3) compare hybrid proportions among newly spawned larvae (from the Sargasso Sea) and glass eels from continental waters to assess selection against hybrids.

Subproject 6. Magnitude of larval abundance and variability in larval survival

One cause of the drastic reduction in eel recruitment could be a decline in larval survival during their drift across the Atlantic. We will therefore compare historical and contemporary estimates of larval abundances.

The area between 70W and 60W and 20N and 30N has been included in most historical surveys of the distribution of newly hatched European eel larvae. Assuming that these abundances in general represent the magnitude of spawning across the entire spawning area, we can assess temporal changes. From an abundance estimate of eel larvae from the spawning period 2014, we can estimate the proportion of larvae reaching the European coast in late 2014 and early 2015 (as it is found by ICES in the annual routine assessment of the eel stock). Comparison of this figure with proportions (i.e. survival estimates) from previous periods, would indicate whether some of the decline in eel stocks could be due to declining survival during the larval phase. Such analyses would also be useful in the consideration of potential routine eel larval surveys for tracking the development of the stock, and will contribute to the knowledge base for eel stock management.

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