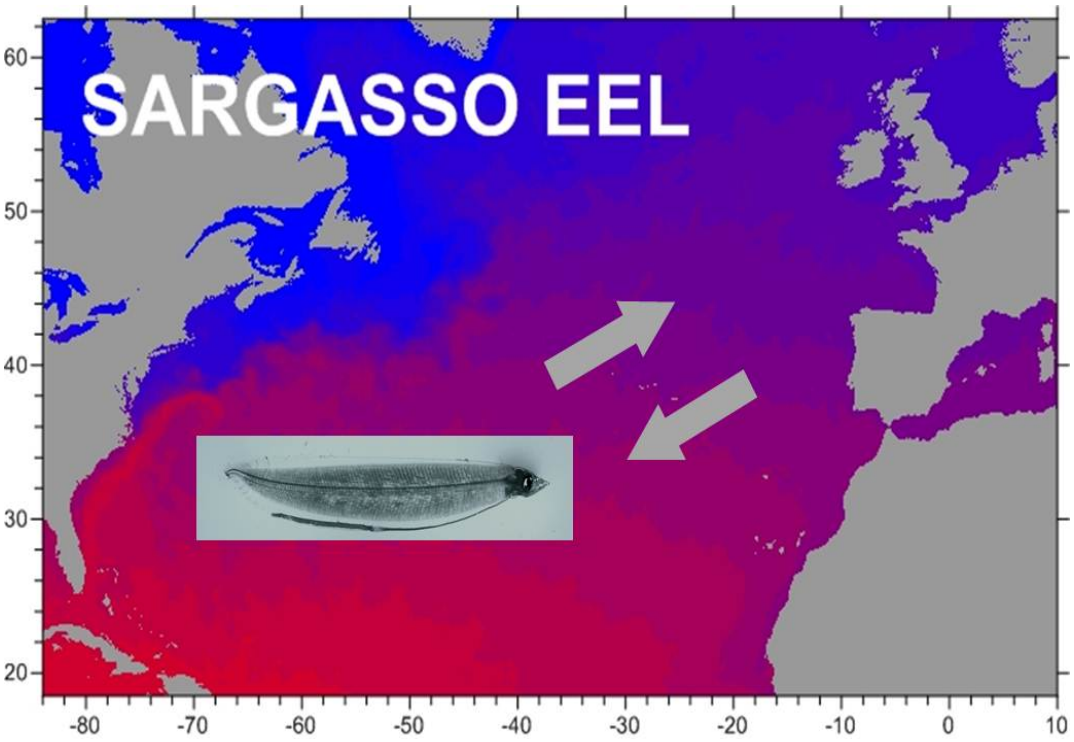

Presentations of ideas and expected results

1.. Spatial and temporal restriction of eel spawning. Large-scale spatial extension of spawning area and possible eastward drift.

Peter Munk/Daniel Ayala/ Evandro Malanski DTU Aqua

Background

Larval distributions are found delimited in north-south direction by sharp thermal fronts, and entrainment of larvae in eastward directed frontal currents is indicated. This suggests a key role of oceanic frontal processes, retaining eel larvae within a zone of enhanced feeding conditions and steering their drift. Further, new analysis of data indicate that spawning are distributed over a wider area, further to the east, and that the drift paths of American and European already separate in the Sargasso Sea, the A.r. drifting west, and the A.a. predominantly drifting east.

Primary research hypotheses

- Eels spawn predominantly during the new moon period at the southern front of STCZ, eggs/larvae drift northwards in the zone where after they are entrained in an eastward flowing Subtropical Counter Current.
- The spawning area of European eel extends far more easterly than assumed
- American and European eel larvae are vertically separated in areas of horizontally overlapping distributions leading to different life traits (drift) of the species

Methods

Basically by obtaining distributional data

- Sampling of larvae across frontal zone, sampling of eggs and early larvae during new moon (several transects, fine resolution, i.e.10 nm dist. across fronts)
- Extending the sampling eastward (of 58E) sampling along cross-frontal transects
- Dedicated vertical study 24-30h, continued stratified sampling at depths (hauling 3.5m MIK in series of constant depth)

Expected results (e.g. by titles of publications with major role)

- Spatial and temporal restriction of eel spawning and the drift pattern of eel eggs and larvae in frontal zones of the Sargasso Sea
- An eastward advection of European eel larvae from along-frontal spawning sites across the North Atlantic
- Vertical distribution differences of American and European eel larvae separate their life and drift.

2.. The early life of the European eel in the ichthyoplankton community of the Sargasso Sea

Daniel Jiro Ayala / Peter Munk DTU Aqua

Primary research hypotheses

- Larval dispersion and drift is strongly influenced by eddy- and frontal-processes, and dispersion is generally very high (estimated)
- Growth rates of eel larvae are high compared to other species, but vary with the physical environment.
- The growth of the larvae is linked to availability of given food items
- Information on the overall ichtyoplankton community of the STCZ will afford new understanding of the early life of eel.

Methods:

- Biodiversity investigations and identifications of larval fish captured in 2007 and 2014 will
- be accomplished via a combination of microscopy and broad-scale employment of molecular analysis. (DNA barcoding in concert with supplementary gene analysis: COI, mini-barcodes, 16S and cyt b).
- Otolith examinations will entail either traditional or scanning electron microscopes scrutiny.

Expected results (e.g. by titles of publications with major role)

- Abundance and distribution of leptocephali at frontal zones in the Sargasso Sea.
- Estimation of hatching locations and growth of leptocephali , based on otolith microstructure analysis.
- Quantitative assessment of the diet of European eel larvae resolved by next-generation molecular sequencing.
- Changes in dominant larval ichthyodiversity of the epi- and mesopelagic waters of the Sargasso Sea.

3.. Eel spawning stock size

Henrik Sparholt, ICES

Primary research hypotheses

- In order to investigate the possible effect of climate and oceanic changes on the decline in the eel stock it is important to be able to quantify the abundance of eel at different life stages, including the larva stage in the Sargasso Sea, and the variability in larvae survival.

Methods

- The amount of eel larvae in the Sargasso Sea is a measure of the Eels spawning stock
- Catch rates reflect the density which when multiplied appropriately with the volume of the water filtered in the relevant depth strata can yield an index of amount of glass eel.
- By comparing this with eel larvae catches in 2007 and in previous expedition can give a relative measure of eel larvae amounts and thus indirectly eel spawning stock size.
- By comparing with glass eel index for the European continent we can deduct the mortality of eel larvae in the ocean phase, and thus judge this in relation to climate changes in a broad context.
- The sampling procedure will be done 1) as “V” shaped profiles in the water column from the surface down to 250m and up again as done at the 2007 cruise, 2) along the transects in the main spawning area, and 3) the eel larvae in the samples will be identified to species by genetic approaches and be length measured and counted.

Expected results (e.g. by titles of publications with major role)

- Trends in spawning stock biomass of European eel as estimated from larvae abundance in the Sargasso Sea since 1922.
- Is climate change the main course for the depletion of the European eel stock?

4.. Mesopelagic fishes

Peter Rask Møller, KU, SNM and possibly Cecília Corbella + Tom Gilbert

Primary research interests:

- Population genetics of globally distributed or widespread mesopelagic fishes – especially anglerfishes - Ceratiidae. Studies of some deep-water species (e.g. the giant squid) shows very limited variation and structure, suggesting a former bottle neck. Need to be confirmed with more species e.g. *Ceratias hoelboelii*.
- Assist other sub-projects in Fish- identification.
- Collection building – as mush of the Caught material should end at the Natutal History Museum of Denmark.

Methods

- The Sargasso eel cruise is a great chance to collect fresh material from a number for species.
- Sampling of larvae and juveniles fishes. A ring-net for slightly larger specimens is needed.

Expected results (e.g. by titles of publications with major role)

5.. Following the eel from cradle to the grave by eDNA water samples – and monitoring unknown fish diversity.

Peter Rask Møller, Philip Francis Thomsen, SNM, KU, Dorte Bekkevold DTU Aqua

Background

Recent progress in DNA-technology has shown a tremendous potential for aquatic biodiversity research. Environmental DNA (eDNA) can be used as an alternative approach for traditional bio-monitoring, research and environmental impact assessments i.e. faunistic investigations based on the isolation and analysis of genetic material obtained directly from water samples. The new methods are especially relevant in fragile and logistically challenging environments such as the deep sea and density poor marine areas such as the Sargasso Sea. The proposed project targets eels (Anguilliformes), and unknown fish diversity. It is assumed that a significant part of the fish fauna is not caught by traditional methods.

Primary research hypotheses

- Spatial distribution of eels (American and European eel + hybrids) can be mapped with eDNA water samples.
- A significant part of the fish fauna is not caught by traditional methods, but can be demonstrated by eDNA water samples.

Methods

Two litre of sea-water are sampled for eDNA use at all standard CTD stations (500 m depth). Additional deeper samples are taken (down to 2500 m) at selected stations – once a week. On the entire cruise from Hirtshals to Bermuda and back 2 litres are sampled from the surface (6-8 m) twice a day- at 24.00 and at 12.00 am. Water samples will be stored frozen onboard until the return to Denmark.DNA will be isolated from the water samples through filtration in laboratories in Copenhagen for subsequent analyses. DNA sequencing will be based on 2nd generation methods on available Illumina HiSeq/MiSeq and Roche 454 FLX. At the SNM based National High-throughput DNA Sequencing Centre we have the infrastructure for doing the DNA analyses. SNM also have a comprehensive comparative collection of most species of vertebrates from the Sargasso Sea.

Expected results (e.g. by titles of publications with major role)

- Abundance and distribution of eels based on eDNA in water samples in the Sargasso Sea.
- Cryptic fish diversity revealed by eDNA in water samples in the Sargasso Sea.

6.. Feeding of eel larvae

Lasse Riemann KU, Daniel Ayala, Peter Munk

Background

Our previous work on eel larvae indicated that they prey on a diverse assemblage of plankton organisms. These insights were obtained using gel-based analyses accompanied by sequencing of 18S rRNA genes and can merely be seen as a preliminary report of possible prey items. Nevertheless, the study indicated that gelatinous zooplankton appears to be of fundamental dietary importance to eel larvae (Riemann et al. 2010). Another shortcoming of the study was a lack of data on co-occurring gelatinous plankton.

In the coming project we plan to do in-depth sequencing of DNA from larval guts and from marine snow, and compare the sequences to address whether larvae actively prey on specific groups of organisms or non-selectively via marine snow. The selection of prey will be compared to plankton counts.

Primary research hypotheses

- The genetic composition of stomach contents is similar to that of marine snow, indicating that the diverse genes found in eel stomachs originate from marine snow, and not from a multitude of discrete organisms.

Methods

- Preservation of individual eel larvae from various regions/stations/depths (extensive washing procedure of larvae before preservation)
- Freezing of hand-picked marine snow particles in buffer (how?)
- Illumina sequencing of excised eel intestines and of marine snow particles (18S rRNA gene or COI?)
- Coupling of larval intestine sequence data to abundance data on marine snow and gelatinous plankton, and to sequence data from discrete marine snow particles

Expected results (e.g. by titles of publications with major role)

- Eel larvae prey on marine snow particles in the Sargasso Sea

7.. Hybridization of eel

Michael M. Hansen 1 , (+ Marti Pujolar, Magnus Jacobsen)
Bioscience, AU

Background

- European and American eel are known to hybridize
- Hybrids found almost exclusively in Iceland, thought to constitute a hybrid swarm
- Numbers of hybrids differ between age classes
- Poor resolution with available markers – erroneous results?
- Novel results (Pujolar et al. MS): RAD sequencing of both species
- Ca. 500,000 SNPs, ca. 3,000 diagnostic SNPs (fixed differences between species)
- Screening of larvae from the Sargasso Sea, Iceland, Faroe Islands using 92 diagnostic SNPs
- All hybrids either F1 (European female x American male) or F2 backcrosses (European female x F1 male)
- Backcrosses unusual genetic composition, possibly outbreeding depression

Primary research hypotheses

- Does intensity of hybridization differ between years?
- Do hybrids beyond F2 occur in the Sargasso Sea
- Does selection/incompatibility act in backcrosses due to outbreeding depression?

Methods

- Analyzing as many larvae as possible using our battery of diagnostic SNPs
- Various types of admixture analysis, estimating likelihood of specific hybrid genotypes

Expected results (e.g. by titles of publications with major role)

- A genome-wide spatiotemporal assessment of hybridization between Atlantic eels

8.. The panmixia hypothesis of eel populations

Michael M. Hansen 2 , (+ Marti Pujolar, Magnus Jacobsen)
Bioscience, AU

Background

- European and American eel are assumed to be panmictic
- Nevertheless, in American eel a tiny proportion of the genome seems to be under temperature-related directional selection among eels from North to South -> significant genetic differentiation at these loci -> obliterated during each generation of random mating
- RAD sequencing of 290 European eel from 8 localities from Iceland to Morrocco
- > 500,000 SNPs, ca. 1,000 may be under directional selection

Primary research hypotheses

- Testing the panmixia hypothesis (again-again-again-again….) but in a **reverse** way
- Do SNPs under selection in the continent also show genetic differentiation in the Sargasso Sea?

Methods

- Genotyping a subset of SNPs found to be under selection in continental samples – but in the Sargasso Sea eel larvae
- Various pop.genetic analyses

Expected results (e.g. by titles of publications with major role)

- Testing the panmixia hypothesis in European eel using SNPs under selection

8.. Adult eels: Hydroacoustics and fisheries

Fritz Köster and Bjarne Stage and others

Primary research hypotheses

- Aggregation patterns of eels are defined by hydrography and ocean currents, lunar phase and diurnal migration.
- European eel is a capital breeder with female indeterminate fecundity and batch spawning, each batch synchronised by lunar phase, and male continuous sperm production.
- Endocrinological analysis will provide answers to the yet unsolved hormonal regulation of gonadal maturation and ovulation.
- Stored resources of eels are used differentially with muscle neutral lipids used primarily for swimming and muscle proteins and phospholipids used for gamete development.

Methods

Localisation of adult spawning eels using hydrographic cues and acoustics.

Fisheries and sampling of eels using a large midwater trawl during nights around new moon.

2-4.Intensive sampling of individual eels in different maturation stages: morphometric records and up to 35 samples per specimen for various analyses.

Expected results (e.g. by titles of publications with major role)

- Aggregation pattern and cues of spawning eels in the Sargasso Sea.
- European eel: a capital breeder with female indeterminate fecundity and batch spawning, and male continuous sperm production.
- New insight in the hormonal regulation of gonadal maturation and ovulation in European eel from wild caught European eel in the Sargasso Sea.
- Differential use of lipids and proteins for swimming and gamete production of European eel caught in the Sargasso Sea (as compared to captive reproduced eels).

9.. Leptocephali: Swimming ability and oxygen uptake

Kim Aarestrup, Jon Svendsen & Anders Koed, DTU Aqua.

Primary research hypotheses

1. Anguilla leptocephali are capable of active swimming to a degree that may aid in the migration to the growth habitat- *thereby resolving uncertainties in stock-recruitment modelling*
2. Swimming and oxygen consumption is linearly related to size- may help resolve hypotheses re: impact of climate change on survival and growth in Sargasso
3. Swimming depends on physiological and nutritional status of the animal- may help resolve hypotheses re: impact of climate change on survival and growth in Sargasso
4. Anguilla leptocephali are receptive to magnetic cues in their swimming direction- resolving uncertainty in hypotheses relating to navigational cues and dependency upon imprinting (stocking)

Methods

1. Capture and securing live leptocephali
2. Apply a swimming protocol within a mini swim tunnel (Loligo Systems APS, Tjele, Denmark) to measure U_{crit} (cm s^{-1}) and oxygen consumption rates (mg O_2 kg^{-1} h^{-1}) at different swimming speeds in Anguilla leptocephali, preferably European eel (*A. anguilla*) .
3. Measure physiological status of the larvae using in-situ data, followed by biochemical analysis

Expected results (e.g. by titles of publications with major role)

1. Estimating sustained and preferred swimming speeds of Anguilla leptocephali using U_{crit} and U_{opt}, respectively.
2. Swimming energetics of *A. anguilla* leptocephali captured in the wild.
3. Influence of anaerobic metabolism at increasing swimming speeds as measured by EPOC in Anguilla leptocephali
4. New larvae drift model including swimming ability

10.. European eel egg and larval development and characterization of their ambient environment in the Sargasso Sea

Sune Riis Sørensen, Peter De Schryver, Elin Kjørsvik, Peter Munk Jonna Tomkiewicz

Background

Research in captive reproduction of European eel has currently enabled mass production of eel larvae. Egg quality is still challenging offspring production and we lack knowledge on the natural development of embryos as well as water quality parameters in the natural environment. Of key importance in larval rearing is tolerance to microbial colonization, which is a major obstacle in captive production. Furthermore, knowledge on natural development of the early life stages is valuable to benchmark captive rearing success. Characterization of the natural growth pattern, development of the digestive system, and nutritional requirements before and after external food intake would provide information fundamental to enhance European eel larvi-culture.

Methods

Water sampling at different depths for analysis of salt composition, pH, and measurement of related hydrography (oxygen, salinity, temperature). Furthermore, water samples are used to isolate living microbial members from the natural environment. Microbial community is characterized onboard, regarding r and K-strategists by agar plating while the microbial samples are viably stored in 20% glycerol at –80°C. Egg (5-10) and yolk sac larvae (5-10 specimens) are sampled for analysis of associated microbes (RNAlater or -80˚C). Eggs from other fish species are also sampled in case no eel eggs are caught. Larvae samples within different size group optimally 5, 10, 15, 25 mm (3-5 specimens per group–alt. only digestive tract) for analysis of digestive enzymes (RNAlater). Otolith are sampled for age determination. Potentially samples for histology – optimally few specimens diff. size.

Expected results

- Sea salt composition as contribution to a publication on optimization of fertilization conditions for experimentally produced gametes.
- A publication on microbial community characteristics, as well as egg and larval coverage. Community characteristics will be related to microbial conditions in experimental larval rearing systems.
- Publication of larval growth morphology focused on growth and development, digestive system and nutritional requirements before and after external food intake.

11.. Nanoflagellate flora in Sargasso and central Atlantic

Helge Thomsen, DTU Aqua

Primary research hypotheses

- The nanoflagellate flora and fauna of the central Atlantic ocean is basically similar to the Indian Ocean (which has been studied to a much larger extent).
- The water masses sampled will not differ substantially when contrasting the observed nanoflagellate biodiversity and abundance data

Methods

- Sampling of nanoflagellates with max. resolution in time and space
- Complete vertical coverage at selected stations
- Single cell isolation of selected organisms for DNA sequencing
- Sampling for environmental DNA analysis
- Selected samples will be quantified based on scanning electronmicroscopical analysis of filters
- Samples will be qualitatively described based on a combination of light and transmission electron microscopy

Expected results (e.g. by titles of publications with major role)

- A contribution to substantially increasing our knowledge of nanoflagellate biodiversity in an under sampled area
- A contribution to unravelling the phylogeny within selected groups of organisms, eg the loricate choanoflagellates

12.. Localised upwelling, mixotrophy and primary production

Katherine Richardson, KU & Jørgen Bendtsen Climatelab

Background

- Eel larvae known to be concentrated in frontal regions in the STCZ
- Suggestion from Galathea study (G3) that it is not frontal regions per se that may be important but, rather, patches where there is localised upwelling that stimulates the food web
- Sargasso Sea considered a "desert" in terms of food availability because of low PP measurements but data from G3 suggest mixotrophy may be quantitatively important to increase in carbon at base of food web relevant for larvae.

Primary research hypotheses

- Localised upwelling stimulates the food web at certain regions of the frontal zone (where and why?)
- Mixotrophy is important for carbon incorporation at the base of the (for larvae relevant) food web

Methods

- CTD (multiple sensors), ADCP, water collection for PP, species analysis, variable fluorescence (Fv/Fm), nutrient analysis, etc. (+ Underway CTD/XBT?)
- On deck incubations for determination of plankton carbon increase

Expected results (e.g. by titles of publications with major role)

- Description of where and when localised upwelling occurs and its importance for plankton food web
- Quantification of importance of non-autrophic processes in carbon build-up in plankton food web

13.. Zooplankton carcasses in the Sargasso Sea and across the Atlantic

Kam, HP, Lasse, Liv &TGN

Background

Traditionally zooplankton sampling disregards the live/dead status of the specimens. This oversight leads to erroneous understanding of zooplankton population dynamics and related ecological processes. Zooplankton carcasses are mainly the result of non-predation mortality such as starvation and diseases, and starvation is particularly important in oligotrophic waters. Carcasses themselves can become vehicles for organic matter sinking fluxes or hotspots for microbial biodiversity and biogeochemical processes.

Primary research hypotheses

- Zooplankton carcasses are prevalent and increases with depth in the Sargasso Sea
- Live/dead composition varies among zooplankton taxa
- Live and dead zooplankton harbour different microbial communities and support different microbial processes
- Zooplankton carcasses significantly contribute to organic matter sinking fluxes and hence the biological pump

Methods

- Depth-specific net sampling of zooplankton; remove subsamples for live/dead sorting (Neutral Red staining and microscopy)
- Selectively preserve (freeze) live and dead individuals for molecular analysis of their associated microbial compositions (DGGE + 16S RNA sequencing) and processes (Illumina metagenome sequencing); collect parallel water samples for comparison
- Sediment trap deployment to quantify sinking fluxes of zooplankton carcasses vs. other organic matter

Expected results (e.g. by titles of publications with major role)

- Prevalence and taxonomic composition of zooplankton carcasses in the oligotrophic Sargasso Sea
- Live and dead zooplankton as distinctive hotspots of microbial diversity and processes in the Sargasso Sea
- Live and dead zooplankton as important vectors for organic matter sinking flux in the oligotrophic ocean

14.. The importance of Copepod fecal pellets for the vertical flux of organic matter from the surface layers of the Sargasso

Torkel, Marja, Lasse, Kam

Background

The plankton community of the Sargasso Sea is dominated by picoplankton; consequently sedimentation of fresh phytoplankton material out of the euphotic zone is very limited and an important vehicle for vertical export of organic matter is copepod fecal pellets. During grazing the copepods are packing the picoplankton in large fast sinking fecal pellets. Measurements of grazing rates are very time consuming to conduct therefore measurement of fecal pellet production can be used as a proxy for grazing rate. Because of the short response time between changed feeding conditions and pellet production, measurement of pellet production can provide a very sensitive proxy for grazing conditions. Moreover, molecular analysis of the produced pellets can provide information about the prey items ***in situ***.

As feacal pellets sink through the water column they are gradually degraded by bacteria. Consequently, the microenvironment in the pellets as well as the surrounding chemical and physical macroenvironment changes with depth supposedly leading to gradual changes in microbial activity as well as a microbial community succession. This is, however, unknown.

Primary research hypotheses

- Community pellet production will change across the frontal system as consequence of change in grazing rate caused by phytoplankton cell size and primary production
- Although the pellet production is typically low due to food limitation, increase in food concentration will quickly result in increased pellet production
- Copepod fecal pellets are a significant component in the vertical flux of organic matter in the Sargasso sea
- 18S/COI gene sequencing of pellets generated onboard by copepods obtained from various depth strata shows that copepod selection of eukaryotic prey changes dramatically with depth

Methods

Depth distribution of fecal pellets: The concentration and biomass of fecal pellets will be quantifies in vertical profiles together with the standard water sampling program. On selected stations pellets will be sorted out fresh and saved for CN analysis.

Production of fecal pellets: Copepods collected by a fast vertical net haul from 100 m to the surface using a 45 µm WP-2 net with a non-filtering Cod end. Immediately after retrieval the content of the cod will be diluted in a 2 liters jug with 5 µm filtered seawater. A subsample will be distributed between four fecatrons and a bucked with logols serve asstart sample. Thereafter the samples are incubated in dim light in a thermo box with 50 liters of surface water for approximately 60 min where after the content of the samples will be concentrated on a 20 µm sieve and fixed in Lugols. After fixation the samples are counted under dissection microscope and a sub sample of 30 copepods and 50 fecal pellets are measured for each sample under inverted microscope. 50 pellets will be sorted out per sample, and pooled for subsequent DNA extraction, PCR amplification and 18S rRNA or COI gene sequencing (probably Illumina).

The response of pellet production to food concentration: Copepods collected with WP2 net will be incubated in *in situ* water as well as in increasing concentration of phytoplankton (*Thalassiosira weissflogii*) culture to investigate 1) the potential of pellet production and its relation to food concentration and 2) the degree and type (quality vs. quantity) of food limitation in the area. The set up will consist of 24-h incubations with 5 concentrations of *Thalassiosira* + filtered seawater and in situ water, with counts and measurements of pellets as above.

Expected results

Quantitative estimate of the importance of copepod fecal pellets on flux attenuation (efficiency of the biological pump)
Patterns in grazing, prey selectivity, and pellet-mediated vertical export in relation to oceanography

15.. Vertical distribution, flux, composition and structuring role of marine snow of the Sargasso Sea

Fabien, Lasse, Marja, TGN et all

Background

Marine snow is the principal vector for carbon flux in the ocean. Flux of particulate organic carbon to the sea floor is one of the major fields in contemporary oceanography which focuses on the origin of this flux, how properties of particles control sinking speed, and how the flux decreases with depth. Marine snow is generated in the epipelagic layer through coagulation of phytoplankton cells or activity of zooplankton (fecal pellets, nets of filter feeders animals). Its sinking characteristics strongly depend on its composition (inclusion of ballast materials, diatoms…), degree of compaction or porosity, and size. Those features are mostly determined by the process generating the aggregate: if coagulation is a major source of marine snow formation, it mostly results in fluffy aggregates with high porosity which therefore sinks slowly. On the contrary, if aggregates are mainly formed by the action of zooplankton they are highly compacted and sink at very high rates.

Once produced, the flux of marine snow decreases progressively down through the water column due to biological activity since it serves as food source for various trophic levels, from bacteria to fish. Eel larvae are among the organisms that are believed to use marine snow as a food source, but evidence is rather anecdotal.

Primary research hypotheses

-Marine snow composition, abundance and flux will change across the frontal system as a consequence of physical structures and primary production.

Marine snow composition will change according to the prevalence of particular zooplankton groups (e.g., copepods vs appendicularians)

Pelagic retention zones of marine snow may serve as hot spots for eels larvae feeding and abundance but also for other types of mesopelagic organisms.

-Marine snow could serve as food for ell larvae.

Methods

The Underwater Video Profiler (UVP) is an image acquisition device allowing visual and automatic quantification of marine snow during standard sampling

(added to the CTD rosette). It allows high-resolution sampling of marine snow (typically 12 sampling per meter), quantification of its abundance and size spectra together with usual monitoring of physical and biological sampling (T°C, salinity, fluorescence, water samples), but also allows observation of zooplankton and especially fragile gelatinous zooplankton, which impossible to collect using conventional nets. This UVP sampling will characterize the vertical distribution of marine snow and large zooplankton. From vertical profiles, total flux of carbon and flux decay with depth will be calculated and correlated with physical and biological parameters. The UVP sampling will give crude estimates of the vertical distribution of marine snow, and will therefore be used to identify depths where marine snow and zooplankton peak in abundance. These depths will be sampled specifically the marine snow catcher.

Snow catcher: The size and composition of the snow particles will be evaluated from collections by a snow catcher (70 liter water sampler). The fragile snow particles will be gently collected by pipetting. These individual particles will be preserved for molecular analysis (see description in project on ell larvae gut contents).

Marine snow production by zooplankton: Appendicularians are one major zooplankton group, which is responsible for the formation of a high proportion of the marine snow. From zooplankton samples, appendicularians will be incubated onboard and their houses (marine snow) and fecal pellet production will be quantified.

Predation of eels on marine snow and appendicularians. If good samples of marine snow and live ell larvae can be obtained sampled, marine snow and appendicularians will be provided as food to determine if ell larvae eat only on appendicularians (therefore ingesting marine snow in formation) or can eat marine snow after production by appendicularians.

Expected results

-Quantitative estimate of marine snow standing stock and flux over the entire survey in relation to hydrographic properties and biological activity

-Quantitative estimate of fragile deep water zooplankton

16.. The importance of small copepods for the trophic transfer efficiency and (zooplankton) production

Marja Koski, Kam Tang & Torkel Gissel Nielsen

Background

Small copepods, such as the pelagic harpacticoid *Microsetella* sp. and poecilamostoid *Oncaea* spp. numerically dominate zooplankton in Sargasso Sea (Böttger 1982). These species have been shown to colonise and feed on marine snow particles (Green & Dagg 1997), though the exact composition of their diet is unknown. *Microsetella* and *Oncaea* are not quantitatively sampled with the traditionally-used 180 µm plankton nets, and since they are fragile and difficult to experiment with, we do not know particularly much about their ecology, abundance, vertical distribution or importance for the productivity of the plankton community. However, they might be important for the nutrition of eel larvae, both as individual prey items or as part of the marine snow community.

Primary research hypotheses

- *Microsetella* and *Oncaea* in Sargasso Sea feed primarily on marine snow (e.g., appedicularian houses), and form part of the marine snow community which might be ingested by eel larvae
- *Microsetella* and *Oncaea* obtain high production rates and dominate the zooplankton community, depending on environmental conditions
- *Microsetella* and *Oncaea* primarily reside at the base of the euphotic zone where they wait for the sinking marine snow particles; by feeding on these, *Microsetella* and *Oncaea* can largely clear up the water column from sinking particles resulting in high flux attenuation.

Methods

Diet: We will investigate the feeding of *Microsetella* and *Oncaea* by collecting samples for gut chl-*a* (indication of feeding on suspended phytoplankton) and molecular gut analysis. We will also conduct incubations on functional response of feeding on diverse types of marine snow particles (using pellet production as indication of feeding). Functional responses give information on encounter rate, handling time and maximum ingestion rates on different types

of marine snow particles. The marine snow particles will include appendicularian houses and marine snow particles collected with the SeaCore sampler; copepods will be collected by a 90 µm WP2-type plankton net.

Production: Egg production and egg hatching success will be investigated by individual incubations conducted at the *in situ* temperature, where eggs and nauplii are counted at regular intervals. Egg production will also be estimated based on plankton samples (see below) and egg-ratio method. In addition we will measure respiration rate (using microelectrodes) and pellet production of *Microsetella* and *Oncaea* at different stations – this will indicate the changes in feeding and minimum carbon consumption rates due to changes in environmental conditions.

Abundance and vertical distribution: Zooplankton will be sampled using Multinet (type Midi) with 50 µm nets, with regular day / night casts and with 10 depth layers.

4. Expected results (e.g. as titles on potential publications)

We expect to obtain:

- Abundance, biomass and vertical distribution of zooplankton (including small species)
- Rates of feeding, secondary production and respiration (individual carbon budget) of *Microsetella* and *Oncaea* as well as their gross growth efficiency
- Qualitative information of their diet, which gives an indication of their trophic position
- Quantitative estimate of their effect on flux attenuation (efficiency of the biological pump) as well as an idea of their contribution to the nutrition of eel larvae

17.. Potential growth and pellet and house production of the Sargasso larvaceans

Russ, Conny, Fabien, Marja & TGN

Background

Larvaceans are abundant in marine ecosystems (Hopcroft et al. 1998) and at times can consume major portions (50-100%) of the daily production of phytoplankton (e.g., Deibel, 1998; López-Urrutia et al., 2003b). Larvaceans feed by filtering water and particles through a delicate and elaborate mucoid structure called a house that encapsulates the animal and drifts suspended in the water column (Flood and Deibel, 1998; Flood, 2003). Their complex filtration system allows larvaceans to effectively graze on particles much smaller than can be exploited by most other multi-cellular zooplankton (Deibel, 1998; Fernandez et al., 2004), with prey ranging from bacteria to micro-zooplankton (Gorsky and Fenaux, 1998). New houses are secreted continually as they must be abandoned periodically when their filters clog, with surface-dwelling species generating up to 40 houses/day/individual (Sato et al., 2001, 2003). Discarded houses, rich in clogged particles (e.g. Deibel, 1998), eventually collapse and become marine snow aggregates as they parachute toward the ocean floor, scavenging particulate debris including fecal pellets and crustacean molts as they fall (Hansen et al., 1996; Gorsky and Fenaux, 1998; Vargas et al., 2002). Such particle-laden material also provides a rich substratum on which active microbial communities develop (Silver et al., 1998). The potential contribution of houses to food webs as a carbon resource can be substantial (Hansen et al., 1996; Gorsky and Fenaux, 1998; Silver et al., 1998; Vargas et al., 2002).

Primary research hypotheses

Larvacean growth rates in oligotrophic water will be high despite apparent low concentrations of food. Rates of growth and reproductive rates will exceed those of the copepods.

Methods

To determine larvacean growth rates, we propose using ship-board techniques similar to those employed by Hopcroft et al. (1998), but of larger total capacity. We will employ 30L Niskin bottles to collect water from a depth were larvaceans are shown to be abundant using the UVP. Bottles contents will be screened through 64 or 100 µm Nitex to remove most mesozooplankton while leaving phytoplankton and larvaceans eggs behind. In total 25 20L carboys will be filled an the contents of 5 carboys will be screened onto 45 µm filtered each day for a total of 5 days. If water conditions are calm and larvaceans can be

collected in good condition using large cod-end nets, individuals will be incubated in 250-500 ml beakers and observed for house and fecal pellet production regularly.
Growth of the larvaceans will be assessed as the change in trunk length, and/or number of external mucus-feeding filters produced over time. Fecundity will be determined by counting eggs in mature females, or through increase in population size over the course of the experiment.

Expected results

Estimates of larvacean growth rates to be used in calculation of their production. Production estimates represent the resources available to their predators (i.e. eel larvae).

18.. Frontal dynamics and meso-scale activity using satellite and in situ observations (1),

Jacob Høyer, DMI

Background:

Several satellite data sets exists today of SST and ocean colour. These have been used in various publications to produce frontal statistics. Due to the spatial and temporal variability of the fronts, together with the spatial nature of fronts, it is very hard to quantify the findings from satellite observations using the typical reference networks, such as drifters and Argo floats to assess the performance of the frontal algorithms and satellite data sets. Infrared radiometers provide traceable and accurate underway SST measurements, which can be used together with in situ observations to calibrate and validate and the satellite observations and the algorithms used for frontal detections.

The validation results will be used together with the in situ observations to assess the frontal dynamics and mesoscale activities and link interannual variability to the variability in the Eel larvae catches.

Methods

- State of the art self-calibrating infrared radiometer will be used to validate satellite sea surface temperature products.
- In situ Chl_a + light attenuation used to calibrate satellite ocean colour products.
- Frontal zones will be identified using statistical processing of satellite sea surface temperature, sea level anomalies and ocean colour.
- Frontal algorithms will be validated and calibrated using in situ observations from an Infrared radiometer and a fluorometer
- Derive seasonal and inter-annual patterns in the frontal behaviour.
- Link the findings with the Eel larvae catches?

Expected results

- Validation of satellite observations and detected fronts with in situ radiometer observations
- Interannual variations in the frontal structures in the Sargasso Sea using satellite and in situ observations.
- Relationship between meso scale ocean currents, frontal variations and eel larvae distribution

19.. Mesoscale dynamics and effects on eel larvae transport in the Sargasso sea

Bruno Buongiorno Nardelli and Patrizio Mariani

Background

The ocean dynamics at the mesoscale (1 – 100 Km, 1 – 10 days) is regulated by a series of coherent structures such as eddies, isolated vortices, filament or fronts. Although very important for the marine system the mesoscale dynamics are difficult to describe. Indeed, vertical exchanges driven by mesoscale eddies cannot be directly measured due to a lack of synoptic 3D observations but also due to limits in the conceptual framework used to describe the mesoscale dynamics (Buongiorno Nardelli in press).

Methods

- Integration of in-situ CTD casts, ARGO floats and satellite data to describe the 3D mesoscale dynamics (Figure 1) and to simulate transport of eel larvae within mesoscale structures in the Sargasso Sea.
- This result is achieved in three successive steps (Buongiorno Nardelli in press): first, by applying a technique that allows to merge satellite and in situ measurements to reconstruct a time series of 3D tracer fields at mesoscale, then by using these fields to diagnose the vertical velocities in the inviscid, adiabatic, semi-geostrophic approximation, and, finally, by simulating Lagrangian trajectories from the synthetic dynamical fields.

Requirements:

- Temperature and salinity profiles from surface to the base of the main thermocline (500 – 1000m)
- Continuous surface temperature and salinity data
- SST data from satellite (free)
- ARGO floats data (free)
- Absolute dynamic topography from satellite (free)
- ACDP data: best if collected when the ship is not moving
- Data storage and high performance computing (HPC)

Expected results

The Lagrangian dynamic will provide the 3D displacement of eel larvae at the mesoscale and will be used to assess the variability in larval growth and survival as depending from the physical structure they live in.

20.. Fronts watermasses and LME's

Igor Belkin, GSO, University of Rhode Island

Methods/results

- Reconstructing surface frontal paths from all available historical in situ data (World Ocean Database 2013) as, for example, in Belkin and Gordon (1996, attached);
- Using all available satellite data to map SST and Chl fronts. Applying front detection algorithm (Belkin and O'Reilly, 2009), now officially accepted by NOAA.
- Combining in situ and satellite data to infer frontal paths toward the Azores;
- A historical retrospective aimed at elucidation of climatic (secular/centennial) variability of the frontal path(s);
- Performance of water masses, salinity (using Aquarius satellite data), and climate-related changes (Belkin, 2009).

21.. Establishment of individual-based modelling infrastructure for studying eel larval drift

Asbjorn Christensen, Mark Payne, Patrizio Mariani

Background:

To perform individual-based modelling in sub projects, a modelling platform is needed. To save time and ensure scientific quality, a common platform should be available.

Methods:

The physical-biological interface of the individual-based framework IBMlib is setup for a mini ensemble of hydrodynamical data sets:

1.NEMO output (obtained from Euro-BASIN)

2.Reconstruction of 3D local mesoscale dynamics (in collaboration with Buongiorno-Nardelli, involved in this project)

3.Geostrophic currents from MyOcean (free download: GLOBAL_REP_PHYS_001_012) Access to three hydrodynamic data sets allows assessment of the envelope of uncertainty from physical forcing as well as basic cross-validation. Establishment of the basic physical-biological interface allows to perform basic drift modelling of eel larvae and assess cohort transport patterns at arbitrary spatio-temporal scales.

Outcome:

- Provide tools for conducting research on eel larval drift
- Provide tools for testing hypotheses requiring access to 3D hydrography in other sub projects, if funding is available to do this.

22.. Modeling studies of growth and survival of European eel larvae

Asbjorn Christensen, Henrik Mosegaard and Jonna Tomkiewicz

Background:

Over the last 30 years numerous modelling studies have appeared addressing the physical drift of early life stages of European eel, using state-of-art hydrodynamic ocean circulation models; by now the basic transport patterns seems well understood and the controversy between otolith microstructure based transport time estimates and other approaches seems resolved by including temperature modulation of otolith microstructure growth (Zenimoto et al 2011). Statistical analyses (Friedland et al, 2007, Bonhommeau et al. 2008) of European eel recruitment links recruitment variability to climatic drivers. Apparently, this link is not via changes in mesoscale or basin scale current pattern, since this is not reflected in previous drift studies. However climate drivers may change ambient conditions for eel larvae, e.g. food supply and temperature, which has not been examined in previous drift studies.

Hypotheses: the observed recruitment decline reflects a decline in recruitment potential due to reduced growth potential caused by changed climatic patterns.

Methods: We propose to close the gap between drift modelling and recruitment observations by formulating a bioenergetic model for growth and survival of eel larvae in relation to ambient conditions; there are several challenges in this: one is that the diet is not well characterized, even though gelatinous species have been observed in larval stomachs. Alternatively, as for Japanese eel, marine snow may be part of the diet; anyway, primary and/or secondary production accessible from coupled hydrodynamic models in WP1 may act as proxies for food supply to support a bioenergetic model. Further, we plan to draw on biological observations for early life stages of Japanese eel, which are more well studied, as well as data from aquaculture experiments in e.g. projects like PRO-EEL.

Outcome:

- A better mechanistic understanding of eel recruitment decline: what can we expect in the future (given climatic scenarios), is there any thing at all humans can do to change this and is there any scope for natural trait adaptation to release pressure on the eel life strategy imposed by climate changes.
- The ability to quantify the relative influence of fishing and climatic drivers on eel recruitment decline on a mechanistic basis.

23.. Reconstruction of spatio-temporal spawning distributions from historical data on European eel distributions

Mark Payne

Background

The current understanding of the spatial and temporal distribution of eel spawning is based solely on observations of the very smallest larvae. However, these larvae represent a relatively minor fraction of the total number of larvae that have captured throughout time. Relying on these relatively small number of larvae potentially biases our understanding towards the temporal and spatial regions where we have these particular samples and greatly underutilizes the tremendous potential of the eel larval database. Here we propose to maximize the utility of all eel larval observations collected both as part of the SARGASSO-EEL cruise, and also those collected previously.

Methods

We propose to use a new statistical approach to hydrographic back-tracking of the larvae from their capture position to recreate the spatial and temporal spawning distribution. In addition to the time and place where a haul was performed, we also propose to use both the length distribution of any larvae captured and the zero hauls directly in this analysis. Furthermore, we also consider all observations simultaneously in a unified inverse-modelling approach and thereby gain “strength in numbers” by effectively synthesizing the entire body of information available in a coherent manner. Finally, by analyzing the entire data set, we gain a greatly improved coverage, both in space and time, of the potential spawning areas for this species.

Expected results

- A methodological paper, outlining the general concepts of the approach and some proof-of-concept analyses. European Eel will be used as a case study to demonstrate the technique and examine its robustness. It is expected that this method will be broadly applicable to many studies of icthyoplankton, and other case studies (e.g. North Sea herring) will also be developed within this framework.
- Direct application of the framework to Sargasso Eel, to characterize the distribution of spawning in time and space. The importance of environmental correlates, such as temperature, salinity and phase of the moon will also be considered in this analysis, where possible, in an attempt to better characterize the spawning domain.

24.. Microplastic debris in Sargasso sea and toxicity to copepods

Kristina Enders / Robin Lenz DTU Aqua

Backround

- The world's oceans are contaminated by synthetic non-degradable debris. Larger visible items of plastic split by UV radiation and mechanical abrasion by waves into ever smaller micro- and nanoscopic plastic fragments. Many particles origin from small fibres and microbeads slipped though water treatment plants and add up to the fragmented plastic pool.
- Recent studies (Morét-Ferguson et al., 2010) report an indication for a decrease in size of plastic particles. It is of increasing importance to study their impact within trophic systems and ecosystems since they are bioavailable to a wide range of organisms. Microplastics (<5mm) can be ingested by filter and deposit feeders, detrivores and planktivores. Accumulation causes physical harm such as internal abrasions and blockages. Besides that, leaching constituents such as plastic additives are under suspicion to cause carcinogenesis and endocrine disruption. Additionally due to their hydrophobic surface microplastics concentrate waterborne POPs possibly travelling further up the food chain leading to bioaccumulation and biomagnification.
- Given the continuous fragmentation, numbers of plastic particles are likely to increase with decreasing size. Previous studies rarely focused on particles smaller than 300 µm.

Primary research hypotheses

- The new sampling method used shows a representative composition of surface micro plastics compared to traditional neuston net tows.
- In Sargasso sea and at the Bermuda/Azores leg micro plastic debris occurs in numbers and sizes that can impact copepods (potentially also other planktonic organisms / eel larvae) when ingested.
- Observed particles are enriched with persistent organic pollutants / metals → increase bioavailability + bioaccumulation

Methods

- Continuous microplastic sampling via ship's underway seawater intake (volume: 3 L/min) equipped with a self-built staggered filter system (down to 30 µm mesh, replaced every 8 h, leg 1 & 2). Filters are stored in sealed petri dishes to avoid contamination.
- Parallel traditional sampling with plankton nets (open questions: direct analysis? How conservation?)
- Collection of particles (at home): Resuspension with filtered seawater and vacuum filtered onto glass microfibre filters.
- Analysis under the microscope, measurement and characterization via Matlab and Image processing program, possibly FT-IR analysis

Expected results

- Knowledge about sizes, abundancy and materials of microplastic particles in surface water → mapping of the transects in Sargasso sea and Bermuda / Azores
- Estimates of ingestion of microplastic by plankton in these areas
- Developments in microplastic sampling method via continuous sampling from underway-ship intake
- Follow-up lab studies on the impact of nano- and microplastic particles on survival, development and fecundity via toxicity tests of copepods and blue mussels.