# Theme Session A

# Biochemical, biogeochemical, and molecular approaches to the study of plankton ecology and species diversity

## ICES CM 2009/A:01

### How many genes? Optimizing the molecular phylogeny of calanoid copepods

Leocadio Blanco Bercial, Lisa M. Nigro, and Ann Bucklin

The calanoid copepods (class Crustacea, subclass Copepoda, order Calanoida) comprise ~1700 species in 450 genera, 40 families, and 10 superfamilies. They are one of the most ecologically important and systematically complex groups of marine holozooplankton. We analysed the evolutionary relationships of calanoid families and superfamilies using diverse molecular markers, such as nuclear and mitochondrial ribosomal DNAs and protein-coding genes, including the "DNA barcode" region of mitochondrial cytochrome oxidase I (mtCOI). The gene sequences are analysed both separately and together, using a weighted and partitioned dataset, in likelihood and Bayesian frameworks. We evaluate the various genes for phylogenetic congruence, and seek to determine whether selection of a congruent set of nuclear and mitochondrial genes—as opposed to simply maximizing the sequence length—will yield an accurate and well-supported phylogeny. Our particular goals are to evaluate the contribution of the DNA barcode region to phylogenetic analysis of calanoid copepods, and to consider the evolutionary history of calanoid copepods in relation to hypothesized environmental and climatic conditions during the geological history of the oceans.

Keywords: calanoid copepods, phylogeny, bar-coding.

*Contact author: Leocadio Blanco Bercial, Department of Marine Sciences, Avery Point, University of Connecticut, 1080 Shennecossett Road, Groton, CT 06340, USA [e-mail: leocadio@uconn.edu].*

## ICES CM 2009/A:02 Withdrawn

## ICES CM 2009/A:03

### Gene expression patterns of the copepod *Calanus finmarchicus* in the Gulf of Maine (Northwest Atlantic Ocean)

Ebru Unal, Petra Lenz, David Towle, and Ann Bucklin

Genomic approaches, such as DNA microarray technology, allow rapid characterization of genome-wide patterns of gene expression, and greatly accelerate the hunt for metabolic genes that are indicators of environmental responses. Natural populations can be used successfully to determine differential gene expression patterns. In this study, we used DNA microarray analysis to investigate gene expression profiles of field-collected specimens of the planktonic calanoid copepod *Calanus finmarchicus* in the Gulf of Maine. Monthly vertically stratified time-series collections were made from Wilkinson Basin in the Gulf of Maine between March 2005 and April 2008. Samples were diluted and held for 24 hours, after which individual *C. finmarchicus* were placed in cryovials and flash frozen in liquid nitrogen. Eighteen samples were selected for examination of gene expression patterns using a "*Calanus* physiology microarray" printed with 1000 unique oligonucleotide probes designed from identified genes selected among 6000+ expressed sequence tags (EST). The preliminary results of nine different microarray hybridizations revealed marked differential up- or down-regulation (i.e. more than +1.0 log ratio) for >70 genes between samples collected at different times of year and from different depth zones. Genes of known function and likely significance for physiological condition were selected for detailed analysis using quantitative PCR. The detailed analysis of these gene expression profiles will help

reveal the genetic mechanisms that underlie the species' responses to changing environmental conditions.

Keywords: environmental genomics, DNA microarray, copepods, *Calanus finmarchicus*, Gulf of Maine.

*Contact author: Ebru Unal, Department of Marine Sciences, University of Connecticut, Avery Point, 1080 Shennecossett Road, Groton, CT 06340, USA [e-mail: ebru.unal@uconn.edu].*

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**ICES CM 2009/A:04**

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## Physiologic and genetic differentiation among seasonally separated clones of *Skeletonema marinoi* (Bacillariophyceae) in Gullmar Fjord, Sweden

Anna Godhe, Karolina Härnström, V. Saravanan, and Marianne Ellegaard

Water and sediment samples were collected from Gullmar Fjord during February, June, and September in 2004/2005 and 2007/2008. The abundance of planktonic *Skeletonema* in the water column was highest during the spring blooms every year. Isolates from respective season were examined by electron microscopy and large subunit (LSU) ribosomal DNA (rDNA) was sequenced from 23 clones, to confirm species identity and investigate intraspecific variation. Genotype diversity and population differentiation among the strains isolated at different season was confirmed by analysing six polymorphic microsatellite loci. Three strains from each season were selected for physiological experiments at different salinities and temperatures. The parameters examined were growth rate, maximum abundance, biovolume, and total RNA concentration per cell. The physiological response among the clones was partly attributed to the month of isolation. The February isolates had a higher division rate and the September clones reached higher abundances. The June clones were isolated during the time of the year when the natural abundance in the water column is lowest, and exhibited the smallest genetic and physiologic variation, which suggests that they are under strong selection pressure. Because phytoplankton cells divide asexually, approximately once per day, selection for individual clonal lineages could be quite rapid, resulting in population differentiation. When different populations are present at different seasons (in the same locality) this can be explained either by the complete replacement of one population by another or by strong directional selection in a continuously growing population. Either way, different physiological traits are likely the result of adaptation to particular microhabitats.

Keywords: *Skeletonema*, populations, differentiation, adaptation.

*Contact author: Anna Godhe, Department of Marine Ecology, Marine Botany, Göteborg University, Sweden [e-mail: Anna.godhe@marecol.gu.se].*

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**ICES CM 2009/A:05**

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## DNA bar-coding of North Atlantic zooplankton: applications for rapid analysis of marine diversity and ecosystem function

Ann Bucklin, Leocadio Blanco Bercial, Brian D. Ortman, Lisa M. Nigro, and Nancy J. Copley

DNA barcodes (short sequences for species identification and discrimination) are being determined for identified specimens of holozooplankton species collected from various regions of the North Atlantic Ocean. As part of the Census of Marine Zooplankton (CMarZ), we are working toward a taxonomically comprehensive DNA barcode database using a ~700-base region of the mitochondrial cytochrome oxidase subunit I (mtCOI) and other gene regions as necessary. The barcode database for holozooplankton will be useful to identify individual specimens, reveal cryptic species, describe biogeographical distributions, discover new species, and allow rapid characterization of species diversity. We report here on the current status of DNA bar-coding for groups within the 15 phyla of animals occurring in the North Atlantic Ocean holozooplankton assemblage. We then propose future applications of the database for rapid and automated taxonomic analysis of zooplankton samples, including identification and quantification of known species by comparison with available sequences, estimation of the numbers of unsampled or

undescribed species, and approach to ecosystem monitoring involving environmental sequencing and metagenomic analysis of holozooplankton diversity.

Keywords: zooplankton, DNA barcodes, marine biodiversity, ecosystem function.

*Contact author: Ann Bucklin, Department of Marine Sciences, University of Connecticut, Avery Point, 1080 Shennecossett Road, Groton, CT 06340, USA [e-mail: ann.bucklin@uconn.edu].*

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**ICES CM 2009/A:06**

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## Molecular sensors and the FerryBox-System—keys to high-resolution monitoring of marine phytoplankton

Katja Metfies, Sonja Diercks, Thomas Hanken, Wilhelm Petersen, and Friedhelm Schröder

In recent years, molecular-sensing technology has made a significant impact in the context of microbial surveillance. At the Alfred Wegener Institute, a DNA microarray (PHYLOCHIP) has been developed for phytoplankton assessment. Its power has been demonstrated by assessing the seasonality of selected phytoplankton groups around the island of Helgoland in the southern North Sea. A current project takes advantage of the power of the PHYLOCHIP and the FerryBox-System to monitor the phytoplankton structure in the North Sea with high spatio-temporal resolution. The FerryBox-system is an *in situ* measurement system for the marine environment (www.ferrybox.org), which is operated on board ships of opportunity that travel on regular routes. In the present project, samples have been taken on a monthly basis since April 2008 with a FerryBox-System installed on the ship "Tor Dania", travelling regularly between Cuxhaven (Germany) and Immingham (UK). The samples are analysed using PHYLOCHIP, which specifies the phytoplankton contributing to the photosynthetic biomass in the samples. In parallel, a portable semi-automated biosensor has been developed to facilitate surveillance of microalgae in the field. This electrochemical device allows the detection of microalgae in water samples in less than 2 h, without the need for expensive equipment. We are working on adaptation of the biosensor to the surveillance of key species in the North Sea and we aim to achieve full automation of the system, providing an autonomous monitoring tool for phytoplankton that can be combined with the FerryBox-System in future.

Keywords: molecular sensors, phytoplankton monitoring, FerryBox-System, North Sea.

*Contact author: Katja Metfies, Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany [tel: +49 471 48312083, fax: +49 471 48311425, e-mail: Katja.Metfies@awi.de].*

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**ICES CM 2009/A:07**

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## Secondary contact of two sibling species of *Eurytemora affinis*: distribution, diversity, and trophic role in the St Lawrence estuarine transition zone

Gesche Winkler

The St Lawrence estuarine transition zone (ETZ), characterized by strong environmental gradients, is a secondary contact zone for two sibling species (clades) of the dominant estuarine copepod *Eurytemora affinis*. This study analysed the pattern of distribution, genetic heterogeneity, and the trophic role of the species complex of *E. affinis*. Sequencing of 652 bp of the cytochrome oxidase I subunit (COI, mt-DNA) revealed a high degree of heterogeneity in genetic structure and habitat type along the salinity gradient. The two genetically distinct clades demonstrated a pattern of niche partitioning within the St Lawrence ETZ by separate geographic distribution. The North Atlantic clade occupied the central portion of the St Lawrence Middle Estuary, whereas the Atlantic clade was more prevalent along the margins, in the upstream reaches of the estuary and downstream saltmarshes. The genetic population structure observed at fine spatial scales may be responsible for differentiation of trophic roles between the two genetic clades when channelling carbon from primary producers up to higher trophic levels, because of the longitudinally organized distribution of primary producers and predators such as mysids and larval fish across the St Lawrence ETZ. Furthermore, this distribution pattern might result in a corresponding spatial structure in

secondary production patterns. Overall, this study illustrates the overriding importance of considering cryptic intraspecific diversity in studies on ecosystem functioning.

Keywords: copepod sibling species complex, mt-DNA, COI, St Lawrence estuary, trophic position, stable isotopes.

*Contact author: Gesche Winkler, Institut des Sciences de la Mer, Université de Québec à Rimouski, Rimouski, Québec, Canada G5L 3A1 [tel: +1 418 724 1650 ext. 1703, e-mail: gesche_winkler@uqar.qc.ca].*

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## ICES CM 2009/A:08

### Trophic interactions and energy flow within the pelagic ecosystem in the Iceland Sea

Hildur Petursdottir and Astthor Gislason

A trophic study was performed in August 2007 on the pelagic ecosystem in the Subarctic Iceland Sea, north of Iceland, using carbon and nitrogen stable isotopes and fatty acid biomarkers. Stable isotope values and fatty acid profiles reflect dietary assimilation over longer periods than traditional stomach content analyses, which can be biased as soft prey or prey with easily digested hard parts can be underestimated. The aim was to study trophic linkages and positions of the most important pelagic species in this ecosystem with special emphasis on the trophic ecology of capelin. Particulate organic matter was collected to be used as a sample of trophic level one. In addition, four species of copepods were sampled, three species of euphausiids, three species of amphipods, one of chaetognaths, and several fish species. Multivariate statistical methods were performed on fatty acid compositional data, making it possible to detect relationships and patterns in the data. This study is a part of an ecological study in the Iceland Sea conducted from 2006 to 2008.

Keywords: trophic ecology, zooplankton, stable isotopes, fatty acids.

*Contact author: Hildur Petursdottir, Marine Research Institute, Box 1390, IS-121 Reykjavík, Iceland [e-mail: hildur@hafro.is].*

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## ICES CM 2009/A:09

### Trophic interactions in two estuaries with differing hydrological regimes in southwestern Australia

Thea Linke, Janna Peters, Luke Twomey, Mike StJohn, and Ian Potter

A sound knowledge of trophic interactions in aquatic ecosystems is crucial to managers and ecologists to understand the functioning of these systems and to provide a baseline for studying the effects of climate change in future. This study is aimed at identifying the key primary producers that support abundant fish species with different feeding modes in a permanently open (Swan-Canning) and a seasonally closed (Wilson Inlet) estuary in southwestern Australia. The species studied were the sparid *Acanthopagrus butcheri* (omnivore), the atherinid *Leptatherina wallacei* (pelagic feeder), and the gobiid *Pseudogobius olorum* (benthivore). Three complementary, quantitative approaches were used: (i) stomach content analyses to determine the dietary compositions of the above three species and how they vary with body size and season; (ii) stable isotope ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ for fish and their prey; and (iii) fatty acid biomarkers in fish and dietary items. Stomach content data demonstrated that size-related changes in diet were evident and that dietary compositions of each species differed among estuaries. Stable isotope ratios of $^{15}N/^{14}N$ demonstrated that the foodweb in each estuary contained three trophic levels, whereas the $^{13}C/^{12}C$ ratio allowed differentiation of the food chains based on detrital material and plankton. Fatty acid data revealed trophic markers for dinoflagellates in *A. butcheri* and *L. wallacei* and for diatoms in *P. olorum*. These findings suggest that (i) the food resources are partitioned within and among species, (ii) the dietary compositions differ between estuaries, and (iii) different sources of organic material support pelagic and benthic food chains.

Keywords: fish, microtidal estuary, foodweb, trophic relationships, biomarkers, stable isotopes.

*Contact author: Thea Linke, Murdoch University, Perth, Australia [e-mail: t.linke@murdoch.edu.au].*

## ICES CM 2009/A:10

### Coexistence in the pelagic deep sea: mechanisms of spatial and trophic niche separation

Silke Laakmann, Marc Kochzius, and Holger Auel

The coexistence of many closely related zooplankton species in the vast pelagic deep-sea realm raises the question of what mechanisms allow their co-occurrence without interspecific competition pressure. Patterns and mechanisms allowing the co-occurrence of abundant species of the copepod sister families Euchaetidae and Aetideidae in the polar deep sea were studied using an interdisciplinary approach combining ecology, biochemistry, and phylogenetics. Their ecological niches, in terms of vertical distribution, energy storage patterns (lipids), and general feeding habits (fatty acid trophic biomarkers and stable isotopes) are compared with phylogenetic relationships, determined on the nuclear non-coding genetic marker internal transcribed spacer 2 (ITS2). All *Paraeuchaeta* species (Euchaetidae) are very closely related, indicating a fast radiation within this genus. They feed carnivorously and store large amounts of lipids, mainly composed of the lipid class wax ester as long-term energy reservoirs and buoyancy aid. Aetideidae feed omnivorously and store moderate amounts of (*Gaetanus* spp.) or no (*Aetideopsis* spp. and *Chiridius obtusifrons*) wax esters. In general, closely related co-occurring species are rather similar in their ecological characteristics but differ in their spatial niches. In contrast, species occurring sympatrically in the same depth stratum usually differ in body size, prey-size spectra, feeding behaviour, and dietary preferences.

Keywords: euchaetidae, aetideidae, deep-sea zooplankton, lipids, fatty acids, stable isotopes, phylogeny.

*Contact author: Silke Laakmann, Marine Zoology (FB 2), University of Bremen, PO Box 330 440, 28334 Bremen, Germany [tel: +49 421 2189616, fax: +49 421 2182285, e-mail: laakmann@uni-bremen.de].*

## ICES CM 2009/A:11

### Seasonal variability of plankton community structure, productivity, and foodweb transfer along the salinity gradient of the Baltic Sea

Lutz Postel, Javier Arístegui, Santiago Hernández-Leon, May Gómez, Carlos Almeida, Agustin Portillo-Hahnefeld, Maria F. Montero, and Theodore T. Packard

The brackish water environment in the semi-enclosed Baltic Sea causes changes in plankton community structure closely associated with large-scale circulation patterns. In addition, the community structure is constantly modified by seasonal changes in nutrient levels and stoichiometric ratios, light, and stratification. Here we analysed a comprehensive dataset from the 1990s covering environmental properties, micro- and mesoplankton communities, their metabolic activity and productivity based on classical as well as enzymatic techniques. This analysis allowed us to reveal the interplay between community structure and foodweb transfer from the Kattegat to the Gulf of Finland. Ecosystem characteristics, such as the ratio between new and regenerated production, the percentage of primary productivity utilized by heterotrophs of different sizes, and the stoichiometry in nutrient regeneration by mesozooplankton were targeted in our analysis. For example, regions where new (primary) production was detectable were restricted to areas with river discharge after the spring bloom. New production never exceeded small percentages of gross production in these cases. Nitrogen and phosphorus were excreted by mesozooplankton in smaller N/P ratios in May than in August because of the larger percentage of parthenogenetically reproducing cladocerans in summer, and herbivorous plankton <100 μm utilized about 10 times more organic matter of autotrophic origin than larger plankton. We concluded that seasonality was as important as regional (salinity-driven) differences in determining community structures and foodweb transfer.

Keywords: plankton, brackish water environment, community structure, foodweb transfer, classical methods, enzymatic techniques.

*Contact author: Lutz Postel, Leibniz Institute for Baltic Sea Research Warnemünde, Germany [e-mail: lutz.postel@io-warnemuende.de].*

**ICES CM 2009/A:12**

## The dinoflagellate genus *Alexandrium* (Halim) in Scottish waters: a combined approach to assess diversity, toxin production, and response to environmental variables

E. Bresnan, C. Collins, E. Turrell, A-L. Amorim, J-P. Lacaze, L. Brown, J. Graham, and A. Koryzinska

The dinoflagellate genus *Alexandrium* (Halim) is of particular interest in Scottish waters. Selected species can produce potent paralytic shellfish poisoning (PSP) toxins that can result in severe human illness if contaminated shellfish are consumed. Some *Alexandrium* species can produce neurotoxic spirolides. Considerable interannual variation has been observed in both the concentration of PSP toxins detected in Scottish shellfish and *Alexandrium* cell densities in the water column. An in-depth study combining microscopic, molecular, and chemical analyses was performed to better understand the role of this genus in Scottish waters. Cell culture combined with microscopic and molecular identification revealed the presence of four species: *A. tamutum, A. minutum, A. ostenfeldii*, and *A. tamarense* (groups I and III). Chemical analysis revealed that *A. tamarense* (group I) and some *A. ostenfeldii* isolates produce PSP toxins, while *A. ostenfeldii* produces a range of spirolide toxins. Laboratory culture experiments demonstrated that toxin production increases with increasing phosphorous limitation and decreases with decreasing light intensity. *A. tamarense* (groups I and III) had the highest growth rate and cellular chlorophyll *a* content of the four species when grown at 15°C. This combined approach has considerably furthered our understanding of the diversity of *Alexandrium* in Scottish waters as well as the physiological response of different species to changing environmental variables.

Keywords: *Alexandrium*, PSP toxins, spirolides, growth, light, nutrients, diversity.

*Contact author: Eileen Bresnan, Marine Scotland Marine Laboratory, UK [e-mail: e.bresnan@marlab.ac.uk].*

**ICES CM 2009/A:13 Withdrawn**

**ICES CM 2009/A:14**

## Biochemical ecology of small pelagic fish: the linkage between environment and fisheries

Rui Rosa, Liliana Gonzalez, Bernardo Broitman, Susana Garrido, A. Miguel Santos, and Maria L. Nunes

Coastal upwelling ecosystems provide the bulk of the world's fishery yields but the biochemical ecology of the species that make up these fisheries has been surprisingly overlooked. Biochemical indicators can provide a mechanistic, ecosystem-based link between population and ecosystem dynamics. Here we investigated long-term interannual changes in the proximate composition and energetic condition of European sardine (*Sardina pilchardus*) and its relationship with oceanographic conditions in the Western Iberia Upwelling Ecosystem (WIUE). Energy density (ED) ranged between 4.0 and 14.2 kJ $g^{-1}$ and the seasonal cycle largely determined temporal variability, explaining >80% of the observed variation. ED variations were also closely linked to water (total $r^2$ = 99.0% in whole body; total $r^2$ = 95.0% in muscle) and lipid dynamics (total $r^2$ = 99.6% in whole body; total $r^2$ = 92.5% in muscle). After adjusting for seasonality (rED), and restricting the temporal analysis to the end of the feeding period (August–October), spring/early summer oceanographic conditions explained 67% of the late summer energetic peak. Interestingly, sardine rED peak on year ($t$) explained over 54.4% of the variation in catches in year ($t$+1), indicating that adult energetic condition during spawning is directly translated into the fishery through recruitment strength. Our results support earlier findings indicating that sardine population dynamics seem to be controlled by bottom–up effects. Also, they provide empirical evidence that biochemical assessments during critical periods of the fish life cycle are an essential approach to understanding population dynamics in upwelling ecosystems and developing a more solid basis for stock management and conservation.

Keywords: *Sardina pilchardus*, sardines, proximate composition, energy density, Western Iberia Upwelling Ecosystem.

*Contact author: Susana Garrido, Centre of Oceanography/Guia Marine Laboratory, Faculty of Sciences, University of Lisbon, Avenida Nossa Senhora do Cabo, no. 939, 2750-374 Cascais, Portugal [tel: +351 214869211, fax: +351 214869720, e-mail: garridosus@gmail.com].*

**ICES CM 2009/A:15**

## Influence of short-term nutritional variations on digestive enzyme and fatty acid patterns of the calanoid copepod *Temora longicornis*

Tobias Kreibich, Reinhard Saborowski, Wilhelm Hagen, and Barbara Niehoff

The calanoid copepod *Temora longicornis* is known to be a species with low lipid reserves and high biomass turnover rates. To survive and reproduce successfully, this species depends on a highly adaptive digestive system allowing it to exploit different food sources. The aim of our study was to investigate the capability of the digestive system of *T. longicornis* females to react to different nutritional conditions. In laboratory experiments, we kept females for 4 days at surplus food, changing the algal species in the food every day. As well as separating the digestive enzymes qualitatively by gel electrophoresis, we documented the copepods' ability to use the different foods by analysing the fatty acid compositions and fatty acid trophic biomarkers. *T. longicornis* females were sampled in the southern North Sea, off Helgoland, in May 2008 and incubated in beakers inoculated with different algal species. At the beginning and during the experiment, subsamples of copepods were frozen for biochemical analysis. On day 1, the copepods were offered the heterotrophic dinoflagellate *Oxyrrhis marina*. On day 2, they received *Ramalina baltica* followed by the diatom *Thalassiosira weissflogii* on day 3. Finally, on day 4 the copepods were again fed with *O. marina*. Digestive enzyme and fatty acid patterns revealed rapid changes after each day. The results demonstrate that both digestive enzyme and fatty acid patterns change rapidly in *T. longicornis*, proving that the species is highly adaptable to cope with a changing trophic environment.

Keywords: digestive enzymes, lipases, physiological adaptations.

*Contact author: Tobias Kreibich, Alfred Wegener Institute for Polar and Marine Research, Am Alten Hafen 26, 27568 Bremerhaven, Germany [tel: +49 (0)471 4831 1573, fax: +49 (0)471 4831 1918, e-mail: Tobias.Kreibich@awi.de].*

**ICES CM 2009/A:16**

## Pteropods (Gastropoda: Opisthobranchia) in the Southern Ocean: first results from fatty acid and stable isotope analyses on the SYSTCO material

Laura Würzberg, Janna Peters, Enrico Schwabe, Svetlana Rodkina, and Angelika Brandt

The Antarctic Ocean is a complex ecosystem including planktonic herbivores such as krill, salps, and copepods as well as carnivores such as amphipods, ctenophores, and cnidarians, which are fed upon by birds, fish, squid, seals, and baleen whales. Although there are many reports about the chemical components of the Antarctic species *Euphausia superba* and of calanoid copepods, which are key organisms in the planktonic foodweb of the Antarctic seas, information is scarce on the biochemical constituents of other common species, such as pteropods, which are frequently found in the Southern Ocean. To find out more about the feeding ecology and trophic position of these organisms, zooplankton samples were taken during the SYSTCO expedition and preserved for later biochemical analyses, more precise estimates of stable isotope ratios, and identification of fatty acid signatures. This approach is presented here on four pteropod species (*Clione limacina*, *Limacina helicina*, *Spongiobranchaea australis*, and *Clio pyramidata sulcata*). Some studies have detected very unusual depot lipids in zooplankton. This finding could indicate that this species has developed exceptional lipid biochemical adaptations in the Arctic and Antarctic. One aim of this study is to analyse and compare the lipid and fatty acid composition of these animals to discover possible adaptations to the polar environment as well as to identify the major food sources that are utilized. Furthermore, an approach is undertaken to define the trophic position of the studied animals. Preliminary results from stable isotope $^{13}C$ and $^{15}N$ analyses indicate a very basal position for all investigated pteropod species.

Keywords: Southern Ocean, pteropods, fatty acid signatures, stable isotope ratios.

*Contact author: Laura Würzberg, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany [tel: +49 172 8663820, e-mail: laura_wuerzberg@gmx.de].*

## ICES CM 2009/A:17

### Fatty acid biomarkers of Iberian sardine diet

Susana Garrido, Rui Rosa, Radhouan Ben-Hamadou, Maria Emilia Cunha, Maria Alexandra Chícharo, and Carl D. van der Lingen

The variability of the fatty acid composition of Iberian sardine prey was compared with the relative contribution made by the different prey types to dietary carbon, and with the fatty acid composition of sardine muscle, in order (i) to determine whether fatty acid biomarkers can be used as a complementary technique for the study of sardine diet and (ii) to relate the spatial and temporal variations of prey fatty acid content with sardine condition and reproduction. The study was conducted over more than one year on two areas of the Portuguese coast characterized by different feeding environments: the west coast of Portugal, where there are stronger and more frequent upwelling events, and the south coast, where these events are rarer and weaker. The most important prey for sardines were crustacean eggs, copepods, decapods, cirripedes, fish eggs, dinoflagellates, and diatoms, which together accounted for >90% of dietary carbon. Significant correlations were obtained between the relative contribution of specific prey types to dietary carbon and several fatty acid biomarkers. The higher prevalence of phytoplankton prey in sardines from the west coast is in accordance with variations of fatty acid biomarkers, including the Σ16:1/Σ16, EPA, and EPA/DHA, all of which reached significantly higher concentrations in the diet of sardines from the west coast. Spatial variation in sardine dietary fatty acid was also detected in their muscle composition, specifically for EPA, EPA/DHA, and (*n*-3)/(*n*-6), which were higher in sardines from the west coast. The spatial variability of plankton prey fatty acid composition may have a strong effect on the reproduction success of this species.

Keywords: *Sardina pilchardus*, fatty acids, biomarkers, diet, spawning.

*Contact author: Susana Garrido, Centre of Oceanography/Guia Marine Laboratory, Faculty of Sciences, University of Lisbon, Avenida Nossa Senhora do Cabo, no. 939, 2750-374 Cascais, Portugal [tel: +351 214869211, fax: +351 214869720, e-mail: garridosus@gmail.com].*

## ICES CM 2009/A:18

### The response of *Centropages typicus* to changing food conditions: results from time-series sampling and laboratory experiments

Barbara Niehoff, Tobias Kreibich, Heidi Blank, Viviane Brüll, Pamela Ohlf, and Wilhelm Hagen

*Centropages typicus* is one of the dominant calanoid copepod species during late summer and autumn in the southern North Sea but its response to changing food sources is not well known. We have studied the condition of females, their stable isotope ratios, and fatty acid biomarker compositions as well as their reproductive activity during a time-series at Helgoland Roads. In addition, females were kept in the laboratory for 3 days with different food conditions. From August through October, the female $\delta^{15}N$ values indicated that the trophic level of *C. typicus* did not change significantly in the field, while the fatty acid compositions reflected feeding on different algal groups. Egg production related significantly to $\delta^{15}N$ values and to carbon, nitrogen, and lipid content of the seston. During incubation experiments, egg production increased at surplus food within 24 h and after only 3 days, the female fatty acid composition reflected that of the different food cultures. When females were fed with one algal species, the diatom *Thalassiosira weissflogii*, egg production and female mass changed with food concentration. When algae with C:N ratios of 5.8 and 7.2, respectively, were provided, egg production and female carbon content did not differ significantly but the N content was higher at the lower C:N ratio. Our results demonstrate that *C. typicus* responds quickly and flexibly to changes in food quality and concentration and is, therefore, well adapted to a varying food regime.

Keywords: copepods, feeding, stable isotopes, reproduction, fatty acid biomarkers.

*Contact author: Barbara Niehoff, Alfred Wegener Institute for Polar and Marine Research, Am Alten Hafen 26, 27568 Bremerhaven, Germany [tel: + 49 421 4831 1371, e-mail: Barbara.Niehoff@awi.de].*

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**ICES CM 2009/A:19**

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## Trophic relationships among zooplankton in the Weddell Sea with special emphasis on copepods

Sigrid B. Schnack-Schiel, Hillary Kennedy, Jan Michels, Henrike Mütze, Astrid Cornils, and David N. Thomas

We examined the $\delta^{15}N$ and $\delta^{13}C$ values of Antarctic zooplankton in spring 2003, 2004, 2006 in the eastern and western Weddell Sea. Zooplankton species for stable isotope analysis were obtained from samples taken with multi-nets and bongo nets within the upper 1000 m and from sea-ice samples. In each sample, between 1 and 50 individuals were pooled. Special emphasis was on copepods, including the pelagic species *Calanoides acutus, Calanus propinquus, Metridia gerlachei, Rhincalanus gigas, Euaugaptilus antarcticus*, and *Paraeuchaeta antarctica* and the sea-ice species *Stephos longipes* and *Drescheriella glacialis*. Among copepod species, $\delta^{15}N$ was lowest in *C. propinquus* (3–5), *M. gerlachei* (4–5) and both ice-associated species, *S. longipes* and *D. glacialis* (±5), indicating a low trophic level. Highest values occurred in the carnivorous *P. antarctica* (6–8). Interestingly, *R. gigas* had $\delta^{15}N$ values between 6 and 7, a trophic level similar to that of *P. antarcticus*. Highest variability of $\delta^{15}N$ values was found in *Calanoides acutus*, ranging from 3 to 9.9, probably indicating different states in overwintering. Based on these data combined with published data, the different feeding habits and life cycle strategies are discussed.

Keywords: Antarctica, Weddell Sea, *calanoid* feeding, stable isotopes.

*Contact author: Sigrid Schiel, Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany [e-mail: Sigrid.Schiel@awi.de].*

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**ICES CM 2009/A:20 Poster**

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## A general survey on plankton in the southern area of the Caspian Sea

A. Hedayati, V. Yavari, and T. Bagheri

Phytoplankton are the base of production in any aquatic ecosystem, so examination of the quality and quantity of phytoplankton is necessary to measure primary production in any area of sea. Zooplankton are the primary consumers in the sea and have an important role. Iran borders the southern shores of the Caspian Sea and it is important for us to improve our knowledge of plankton in order to obtain benefit without any harmful effects. In this study we examine important phyla and species in the Caspian Sea and then discuss in more detail two important phytoplankton groups (crysophytes and pyrrophytes) and one important zooplankton group (copepods). Our results demonstrate that the density of plankton decreases with distance from the coast. Phytoplankton species were mainly in the surface water. The density of phytoplankton in summer was higher than in the other seasons and there were more phytoplankton in the epilimnion than in the metalimnion or hypolimnion. The density of phytoplankton in the southern area of the Caspian Sea was four times that of zooplankton, and species were mainly crysophytes and Bacillariophyceae. Zooplankton were mainly protozoa, crustaceans, copepods, and calanoids. Of 92 identified species, 73 were crysophytes and 20 were pyrrophytes. Diatoms numbers were high throughout the year. Dominant species of crysophyte were *Rhizosolenia calcaravis* and the dominant species of pyrophyte was *Exuviealla cordata*. Crysophytes comprised 75% and pyrophytes 17% of the total phytoplankton. Copepods were most common group of zooplankton in the southern area of the Caspian Sea and numbers decreased with distance from the coast. Calanoids and nauplius were more common than cyclopoids in all stations, but nauplius was more common in the surface water and cyclopoids and calanoids more frequent in deeper waters.

Keywords: phytoplankton, zooplankton, biomass, density.

*Contact author: Ali Akbar Hedayati, Daneshjoo BLVD, Arghavan Alley, Hedayati Apartment, Second floor. Yazd, 89167-47483, Iran [e-mail: Marinebiology1@gmail.com].*

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### Analysis and separation of different types of nematocysts from *Cyanea capillata* and mass spectrometry of capsule contents

Annika Wiebring, Heike Helmholz, Stephan Lassen, Andreas Prange, and Gerhard Jarms

The impact of jellyfish as a component of the gelatinous zooplankton on marine ecology and trophic interactions is of increasing interest, but knowledge of their distribution, abundance, and adaptability is limited. One way to examine potential modifications caused by changing environmental conditions is to investigate the major characteristic of nematocysts and their toxins (venom). In this work, nematocysts from a dominant scyphozoan species (*Cyanea capillata*) with individuals of different size classes and habitats were analysed microscopically in order to investigate the compositions and sizes of the major types. A novel molecular technique called laser microdissection and pressure catapulting (LMPC) was applied to separate the nematocyst types, allowing type-specific collection and preparation of nematocysts for biochemical analysis. The isolated capsules containing a variety of toxins were directly analysed used matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). Protein and peptide mass spectra in the lower molecular weight range were compared and demonstrated remarkable differences. Capsule sizes in the *C. capillata* medusae from the North Sea differed from those from the Baltic Sea and although the resulting mass spectra of the separated nematocysts had some constituents in common, the overall patterns of the three distinct nematocyst types differed. This indicates a potential marker for physiological and adaptive processes on a biochemical level.

Keywords: nematocyst composition, toxin, mass spectrometry, adaptability.

*Contact author: Annika Wiebring, GKSS Research Centre, Institute for Coastal Research, Department for Marine Bioanalytical Chemistry, 21502 Geesthacht, Germany [e-mail: annika.wiebring@gkss.de].*

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### Toxicological and biochemical characterization of northern scyphozoan species

Heike Helmholz, Stephan Lassen, Christiane Ruhnau, and Andreas Prange

Jellyfish are a critical component of the pelagic zooplankton affecting the marine foodweb. They utilize a complex mixture of bioactive substances for prey capture and defence, but little is known about the chemical composition of whole venoms and distinct molecular modes of action and effects, in particular for northern scyphozoan species. A variety of *in vitro* assays were applied to obtain a comparative analysis of the toxic effects of the different scyphozoan species of various size classes and from different regions of the North and Baltic Sea, as well as their toxigenic organs. Cytotoxic effects against liver and gill cells, haemolytic and enzymatic activities were utilized to detect differences in the toxic potential of the jellyfish species. In addition to the toxicological characterization, a biochemical analysis of the whole venoms and isolated toxins was performed using gel electrophoresis and protein mass spectrometry. The proteinaceous toxins, which were isolated by multidimensional chromatographic processes, covered a broad molecular weight range. Cytotoxins were analysed structurally and partially sequenced. Sequence data could be used for homology searches. It was demonstrated that the proteomic analysis of unknown toxic proteins by means of *de novo* sequencing and subsequent homology searches within available databases (SwissProt, CnidBase) is a valuable tool for interspecies comparison. Species- and size-dependent differences in the biochemical composition of the venoms could also be demonstrated. It is thought that these biochemical parameters indicate physiological conditions and adaptation processes to environmental changes.

Keywords: Scyphozoa, jellyfish, *in vitro* toxicity, toxin.

*Contact author: Heike Helmholz, GKSS Research Centre, Institute for Coastal Research, Department for Marine Bioanalytical Chemistry, 21502 Geesthacht, Germany [tel: +49 4152/87-1844, fax: +49 4152/87-1875, e-mail: heike.helmholz@gkss.de].*

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## Environmental genomics of *Salpa thompsoni*: searching for molecular indicators of adaptation and bloom formation

P. G. Batta Lona, A. Bucklin, and P. H. Wiebe

Environmental genomics links the responses of living organisms to environmental change at the genetic level. One of the aims is to identify the key genes involved in the biochemical and physiological adaptation of organisms to changes in environmental conditions, including climate change. We report here recent progress on the genomic analysis of the Southern Ocean salp species, *Salpa thompsoni*. This species is thought to be an indicator of Antarctic climate change, and salp abundances have been reported to track low sea-ice coverage during austral winters. North Atlantic species of *Salpa* may exhibit analogous patterns of abundance in relation to climatic conditions. For this study, a cDNA library was created from specimens collected during the January 2009 cruise of the research vessel "Umitaka-Maru" in the Pacific sector of the Southern Ocean. Whole-genome sequencing of the cDNA library was done using 454 pyrosequencing to isolate genes encoding proteins associated with environmental stress responses, as well as genes thought to be related to salp bloom formation. Our goals include further analysis of the *S. thompsoni* cDNA library and development of selected genes as markers of bloom formation and adaptation to particular environmental conditions.

Keywords: environmental genomics, salps, bloom formation.

*Contact author: Paola Batta Lona, University of Connecticut, USA [e-mail: paola.batta_lona@uconn.edu].*

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## Temporal and spatial variation of egg production, RNA:DNA ratio, and fatty acid composition of the calanoid copepod *Acartia clausi* in the Gulf of Cadiz

J. Cruz, A. M. Chícharo, S. Garrido, R. Ben-Hamadou, L. Chícharo, P. Ré, and A. M. P. Santos

Temporal and spatial patterns of copepod secondary productivity have been studied since December 2008 in three different marine systems off the southern Portuguese coast: the Guadiana River estuary and adjacent coastal waters, the Ria Formosa lagoon system (river input enrichment), and the coastal pelagic ecosystem off Cape S. Vicente, the latter strongly influenced by coastal upwelling events. The variability of copepod production, assessed by *in vivo* incubation experiments, is related to the growth and nutritional condition of copepods, using nucleic acid based indicators (i.e. RNA:DNA) and fatty acid biomarkers. The interest in RNA:DNA ratio as an index of growth rates and reproductive capacity of marine copepods has increased rapidly in recent years. Such an index would allow simplification of the time- and labour-consuming measurement techniques currently used, permitting more extensive sampling in the field. Moreover, recent studies have emphasized the importance of food quality for copepods reproductive success, in which fatty acids have a crucial role. Egg production rates (EPR) and hatching success (HS) have been measured for the broadcast spawner calanoid copepod *Acartia clausi*, one of the dominant mesozooplankton species found in Portuguese coastal waters. In addition, RNA:DNA ratios and fatty acid contents of the females used in the EPR experiments have been analysed to investigate their relationship with the reproductive traits measured (EPR and HS). The temporal variability of all these measurements is being assessed with high-frequency sampling aiming to describe the variability of zooplankton productivity in relation to environmental conditions.

Keywords: *Acartia clausi*, egg production, RNA:DNA ratio, fatty acids.

*Contact author: Joana Cruz, Instituto Nacional de Recursos Biológicos (INRB-IPIMAR), Avenida Brasília s/n, 1449-006 Lisboa, Portugal and Centro de Ciências do Mar do Algarve, Universidade do Algarve. Campus de Gambelas, 8005-139 Faro, Portugal [e-mail: jcruz@ipimar.pt].*

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### Sensitive assays for studying digestive enzymes in individual copepods

Tobias Kreibich and Reinhard Saborowski

Copepods are significantly involved in the transfer of matter and energy between trophic levels. They utilize food by a set of digestive enzymes from the midgut region. The activities of such enzymes reflect the potential to digest different organic compounds and may indicate adaptations to different nutritional conditions. Unfortunately, the sensitivities of the enzyme assays available are low and hence applicable to pooled samples only. Therefore, we established sensitive enzyme assays suitable for analysing individual pelagic copepods. These assays allow the measurement of activities and the presentation of zymograms, allowing us to analyse important biochemical parameters in individual animals they may contribute to our understanding of the physiological reactions and ecological functions of copepods within a complex and changing marine environment.

Keywords: digestive enzymes, fluorogenic substrates, SDS–PAGE.

*Contact author: Tobias Kreibich, Alfred Wegener Institute for Polar and Marine Research, Am Alten Hafen 26, 27568 Bremerhaven, Germany [tel: +49 471 4831 1573, fax: +49 471 4831 1918, e-mail: Tobias.Kreibich@awi.de].*

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### Aminoacyl-tRNA synthetases (AARS) and RNA:DNA methods as an index of *in situ* growth rate for Atlantic herring larvae

Inmaculada Herrera-Rivero, Stephanie Borchardt, Philipp Kanstinger, Myron A. Peck, Lidia Yebra, and Santiago Hernández-León

Aminoacyl-tRNA synthetases (AARS) have been used to examine the condition of marine zooplankton but their suitability as an indicator of starvation in marine fish larvae has never been tested. Here, changes in the activity of AARS and RNA:DNA ratios in yolk-sac and early feeding Atlantic herring larvae were quantified at different temperatures (7°C, 12°C, and 17°C) and different feeding levels (starvation, low, medium, and high) in the laboratory. RNA:DNA ratio has proven to be a sensitive indicator of nutritional condition but involves a complicated fluorescent assay. In contrast, the AARS method is a simple, inexpensive photometric assay that may be more suitable for large-scale sample processing from oceanographic studies. These laboratory trials conducted using natural diets (copepods, *Acartia tonsa*) indicated that changes in AARS compared well with changes in RNA:DNA ratio and that the former is an alternative biochemical-based indicator of starvation potential because of its rapid response time with changes in feeding conditions.

Keywords: none.

*Contact author: Inmaculada Herrera-Rivero Biological Oceanography Laboratory, Facultad de Ciencias del Mar, Campus Universitario de Tafira, 35017, Las Palmas de GC, Canary Islands, Spain [e-mail: inmaculda.herrera102@doctorandos.ulpgc.es].*