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Crustacean cross-roads: Comparative analysis of population connectivity of copepods and euphausiids in the North Atlantic Ocean.

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ABSTRACT

Population connectivity (i.e., exchange of individuals) is an essential characteristic of species, which affects their resilience to external pressures, including climate change and anthropogenic impacts. Spatial structuring and population differentiation of key species in the diverse continental shelf, slope, and open ocean environments across the North Atlantic are critical foundations for ecosystem approaches to fisheries management. Use of a standard measure of connectivity, such as DNA sequence divergence of the mitochondrial cytochrome oxidase I (COI) barcode gene, allows comparisons of patterns of connectivity among species and over time. In this study, patterns and pathways of gene flow are characterized and compared for ecologically important North Atlantic species of copepods (e.g., *Calanus*, *Pseudocalanus*, *Clausocalanus*) and euphausiids (e.g., *Meganctiphanes norvegica*, *Stylocheiron* spp.) based on COI sequence variation. Results are discussed in terms of the effects of life history and behavior, biogeographical distribution, impacts of past climate effects (e.g., bottlenecks with glaciation), and possible responses to recent environmental changes. Future research directions include exploring conceptual and statistical approaches to using population connectivity in the context of ecosystem approaches to fisheries management.

Keywords: zooplankton, connectivity, gene flow, population genetics

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INTRODUCTION

Analysis of connectivity of zooplankton

Population connectivity (i.e., exchange of individuals among geographically separated subpopulations) is quantified by description and statistical analysis of the distribution of genetic variation within and between populations for a given species. Many important ecological processes are reflected in spatial and temporal patterns of genetic variation of species, including patterns of dispersal, reproduction, and mortality, as well as inferences of the evolutionary history of a population or species.

Although many zooplankton species exhibit broad geographic distributions and appear to have high dispersal potential, both biological and physical environmental processes may limit gene flow. Ocean processes that are thought to be significant for population connectivity of zooplankton are currents, persistent eddies, ocean gyres and other physical ocean structures at the mesoscale (10s to 100s km) to large scale (100s to 1000s km). The physical structure of the ocean can alter the timing of reproduction and mortality events, providing biological barriers to gene flow. Geological features – continents, islands and other land forms, continental shelves, seamounts, and ocean ridges – may form natural barriers to dispersal. Species may exhibit large-scale spatial patterns of connectivity consistent with isolation by distance models (i.e., reproductive isolation resulting when the geographic range of the species far exceeds the dispersal potential of an individual).

The expectation for high gene flow species such as zooplankton is that populations may be discriminated by significant differences in allele or genotype frequencies, but not by unique or private alleles restricted to a particular population or region. Thus, population connectivity analysis is unlikely to be useful to identify source regions, and tracing the transport of zooplankton in ocean currents has proven difficult. Also, active zooplankton behaviors (e.g., swimming, position-keeping, and vertical migration) and life history (e.g., differences among larval, juvenile, and adult stages) will impact the dispersal potential of a species. Since zooplankton species may respond to the same physical forces in very different ways, comparison among species with different behavioral and life history patterns is important to understand how the physical structure of the ocean affects both the dispersal of individuals and distribution of species.

Molecular characters for analysis of connectivity

Molecular markers are powerful tools for studying the genetic diversity and structure of natural populations. Selection of the appropriate marker is largely dependent on the scale of investigation that one intends to perform: the general rule is that more variable genetic markers are needed for investigations at finer temporal and spatial scales. In this context, there are several genetic markers that can be used for population analysis, including: DNA fingerprinting, mitochondrial DNA sequence variation, simple sequence repeats or microsatellites, and single nucleotide polymorphisms.

A growing database of DNA barcodes for marine zooplankton is providing the key for decoding species diversity by linking species names, morphology, images, and DNA sequence variation. DNA barcodes are also useful for comparisons among ocean regions and realms, analysis of large spatial scale population connectivity, global-scale phylogeography, and discovery of cryptic species. A taxonomically-comprehensive

barcode database for the zooplankton will facilitate rapid analysis of species diversity and distribution in the pelagic community, and will also provide a standard measure of connectivity to allow comparisons among species, considering phylogenetic constraints, life history and behavior, and other ecological and evolutionary conditions and processes. Next-generation high-throughput sequencing is making such approaches accurate, comprehensive, and cost-effective.

In light of the pivotal position of zooplankton in ocean food webs, their usefulness as rapid responders to environmental change, and the increasing scarcity of taxonomists, DNA barcodes offer many advantages for analysis of global patterns of pelagic biodiversity and population connectivity.

METHODOLOGICAL CONSIDERATIONS

Sampling strategies: A consideration for the analysis of population connectivity of zooplankton is the effect of sample size on the power of statistical analysis. Small collections over wide geographic areas may yield samples consisting mostly of genetically unique individuals because of under-sampling, thus drastically reducing the ability to accurately describe population genetic structure. Molecular analysis of many individuals and samples is likely needed, so technical approaches should allow assay of large numbers of individuals collected at sufficiently high spatial resolution to resolve structure across a wide range of spatial and temporal scales. Fortunately, rapid and inexpensive molecular techniques and protocols continue to be developed, and many are readily available as commercial kits.

Choice of molecular markers: DNA sequence variation of selected mitochondrial genes is a frequent molecular character used for population genetic studies of zooplankton. Advantages of mitochondrial DNA (mtDNA) over nuclear genes for animal species include higher mutation rates, maternal clonal inheritance, and the haploid genome. However, mtDNA haplotype frequency distributions are frequently highly skewed, with both numerous identical and numerous unique individuals – neither of which is informative of population connectivity.

Analytical approaches: There are several recent books and web-based summaries of the many statistical packages available for analysis of population connectivity (see e.g., <http://barcodingmarinelife.org>; <http://lgb.unige.ch/arlequin>; <http://www.rannala.org/gsf>). Among a number of widely-used software programs, T.C.S. (see <http://darwin.uvigo.es/software/tcs.html>) is a statistics program based on a parsimony approach to the analysis of population connectivity. DNA sequence data are used to reconstruct genealogical relationships among haplotypes using a network. The program outputs the sequences, the pairwise absolute distance matrix, probabilities of parsimony for mutational steps, a test listing of connections made and missing intermediates generated, and a graphical presentation of the resulting network. The network makes it possible to maintain different alternative connections between haplotypes. However, since ancestral and most frequent alleles generate the rarest according to coalescent theory, networks can be modified to reject connections between rare alleles. This presentation will provide a comparative analysis and assessment of population connectivity of selected copepod and euphausiid species based upon T.C.S. Ver. 1.2.1 (see Figure 1).

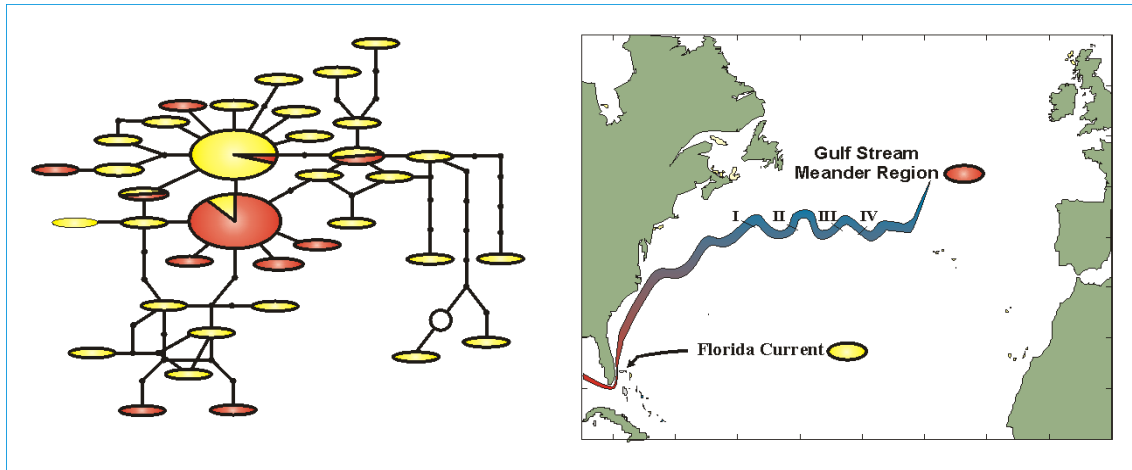


Figure 1. COI haplotype network of the euphausiid *Stylocheiron elongatum* constructed with TCS Ver. 1.2.1 to visualize diversity and phylogenetic relationships among different haplotypes (ovals) and provide qualitative assessment of their geographic distributions. Colors indicate geographic source of collections; sizes of ovals (pies) and pie slices are proportional to numbers of sampled individual with this haplotype; cross-bars on lines indicate numbers of base changes. Open circle indicates 10 base-pair changes.

CONCLUSIONS

Two general principles emerge from studies of connectivity of copepods and euphausiids in the North Atlantic Ocean. First, the species are quite variable at the protein and molecular level, and second, this variability is resolved into genetically-distinct, geographic populations for only some of the species and at some temporal and spatial scales. The degree of population connectivity varies widely among species depending upon habitat, behavior, spatial and temporal scales examined, and other factors. Some species show significant population genetic differentiation among samples – or patches – at smaller scales patches (10s to 100s km) and among regional populations at mesoscales (100s to 1000s of km). Some zooplankton species, especially those with coastal or continental shelf affinities, show marked genetic divergence between populations sampled on opposite sides of an ocean basin, separated by 1000s of km. A number of species with cosmopolitan and/or disjunct distributions have been shown to comprise genetically-divergent and taxonomically-distinct cryptic species with more limited geographic ranges.

There are many practical applications of connectivity analysis for zooplankton are issues associated with the management of living marine resources and ocean ecosystems. Several of these are:

- 1) Accurate estimates of zooplankton population connectivity – including appreciation for the range of variation among species – can contribute to assessments of ecosystem connectivity. This is useful to understand and predict ocean ecosystem function, wiser conservation of marine species, and recognition of impacts of global climate change.
- 2) Distinguishing between gene flow (i.e., interbreeding) and ocean mixing of distinct populations will help lead to quantitative descriptions – and eventually to predictions – of recruitment and abundance of zooplankton that are important in the food chain of commercially harvested species.