

BEST Collaborative Research: The Trophic Role of Euphausiids in the eastern Bering Sea: Ecosystem Responses to Changing Sea-Ice Conditions

Introduction

The eastern Bering Sea supports productive marine ecosystems and extraordinarily rich marine resources, including vast numbers of marine birds and mammals and productive commercial fisheries (> 50% of US landings; BEST Science Plan, 2004). This sub-arctic marine environment is predicted to be one of the most severely affected region by global climate change (e.g., Sarmiento et al. 2004, Meier et al., 2005; Overpeck et al., 2005). Recent evidence shows that the eastern Bering Sea is warming, and that the extent and duration of seasonal sea ice cover is diminishing (Overland & Stabeno 2004). As the Bering Sea responds to variations in climate, its ability to support the resources upon which people depend may change (Best Social Science Plan, 2005). Warming will affect sea ice conditions (extent, coverage, thickness, and seasonality), water temperature and stratification in this region which will likely influence not only the timing, duration, and magnitude of the spring bloom but also the recruitment, growth, and nutritional condition of grazers, such as copepods and euphausiids. As a result, environmental responses by top predators, including humans, will be mediated by the responses of the key intermediate food web constituents, especially euphausiids. Euphausiids play a central role in high latitude ecosystems. In the eastern Bering Sea, they form a critical link between primary producers and seabirds, fish and mammals (Schneider et al., 1986; Hunt et al., 2002a). Compared to euphausiids in other high latitude ecosystems (e.g. Barents Sea, Antarctica), the life history, diet and feeding strategies of the major euphausiids species in the Bering Sea are poorly known. The major species found on the southeastern Bering Sea shelf are *Thysanoessa raschii* and *T. inermis* (Smith, 1990; Coyle and Pinchuk, 2002). *Euphausia pacifica* and *T. spinifera*, are more common in the Aleutian Passes (Coyle, 2005), but with warming, their distributions may shift northward. Longevity, age at maturity, and spawning season for these species are likely to be similar to those in the Barents Sea and Balsfjorden (Norway) and the Gulf of Alaska as discussed below, but these important characteristics have not been established for species in the Bering Sea.

The diets of euphausiids in other regions have been found to include a wide range of sea-ice and planktonic biota, depending on life stage and season. In the Antarctic in winter, larval and juvenile *Euphausia superba* mainly feed on sea-ice associated organisms (i.e. algae, heterotrophic protists; Meyer et al., 2002; Daly, 1990, 2004), while copepods and detritus in the water column are a major component of adult diets (Atkinson et al., 2002; Ju and Harvey, 2004). During summer, *E. superba* diets have been found to be quite variable, with heterotrophic dinoflagellates sometimes the main food source (Schmidt et al. 2006). Sea-ice communities can be a source of significant primary production (Arrigo and Thomas, 2004). In the Arctic and Antarctic, algae in sea-ice are diverse, and have associated protist and metazoan consumer communities (e.g. Garrison, 1991; Horner et al. 1992; Gradinger, 1999), all of which are potential prey for euphausiids. Much less is known about species assemblages of algae or heterotrophs in sea-ice in the eastern Bering Sea (Horner, 1976; Schandelmeier and Alexander, 1981). Sea-ice biota may be particularly important for overwintering survival and recruitment of young krill. In the southeastern Bering Sea, *T.inermis* and *T. raschi* probably overwinter as furcilia and juveniles and develop into adults and spawn in April and May (Smith, 1991). Therefore, variation in the timing and extent of sea-ice, and the development and composition of the associated microbiota, may have a considerable impact on the recruitment success of euphausiids.

Changing sea ice cover also affects the temperature, light levels, and stability of the water column, which in turn influence the timing, duration, and magnitude of the planktonic spring bloom. In the southeastern Bering Sea, when the ice remains past late March, the bloom occurs in March or April in association with the ice edge, when water temperatures are -1.5 to 2.0°C . When the ice retreats before late March, the spring bloom is delayed until thermal stratification of the water column, and the bloom occurs in relatively warm water ($6 - 8^{\circ}\text{C}$) in May or June (Stabeno et al., 1998, Hunt et al., 2002b). The changes likely result in differences in taxonomic composition of the blooms and the type of zooplankton

grazers (e.g. microzooplankton, copepods, and euphausiids) which can successfully consume them. These changes in bloom pattern during warm and cold years will have significant effects on food web pathways and the production of higher trophic levels (Walsh and McRoy, 1986; Hunt et al., 2002b).

This project directly addresses a central goal of the Bering Ecosystem Study (BEST) program to examine how changing sea-ice conditions directly or indirectly affect the ecology and population dynamics of the major euphausiid species. As euphausiids form an essential linkage regulating the distribution and abundance of upper trophic level organisms, this proposal will directly contribute to the goals of BEST's partner, the North Pacific Research Board's Bering Sea Integrated Ecosystem Research Program (NPRB BSIERP). Our primary hypothesis is that seasonal and interannual variation in timing and coverage of sea-ice and associated food resources for euphausiids will lead to differences in their demographic (i.e. age) structure, and their diet history and thus nutritional condition, both of which ultimately translate into differences in production rates and their availability as prey to higher trophic levels. We will apply recent developments in biochemical methods to determine population age structure of the major euphausiids over seasonal and interannual time scales together with parallel measures of nutritional condition at multiple sites (i.e. ice-covered, ice-edge, and open water zones). Feeding history and the role of trophic transfer of euphausiids will also be examined through detailed analysis of individual lipids and lipid classes to establish potential markers which reflect the spatial and temporal differences of ecological niches together with a combination of other techniques. Shipboard feeding and laboratory rearing experiments will allow prey to be identified and the retention of specific dietary markers to be tested as well as accurate estimates of absolute age in field populations of euphausiids to be validated. This study will be closely integrated with other BEST program investigations to better understand the potential impact of climate changes, particularly sea-ice conditions, on the eastern Bering Sea ecosystem.

Scientific Rationale

Lipids as indicators of nutritional status and reproductive potential of euphausiids

A number of studies have established that lipids are essential in the euphausiid life cycle, particularly in reproduction and as an overwintering strategy for euphausiids which must contend with seasonal food supply (Saether et al., 1986; Falk-Petersen et al., 2000; Hagen and Auel, 2001; Ju and Harvey, 2004; 2005). As one of the major constituents of euphausiids, particularly those species found in high latitudes, lipids show pronounced seasonal variations, typically ranging from 7 to 60% of dry mass (e.g. Falk-Petersen, 2000). Different species of euphausiids often show variable patterns of lipid storage in terms of the lipid structures accumulated and their metabolism which reflect the strong link between lipid dynamics and life cycle strategies (i.e. food availability, reproduction, prey selection, overwintering) and appear to enable different species to utilize multiple ecological niches (see Figure 1 below).

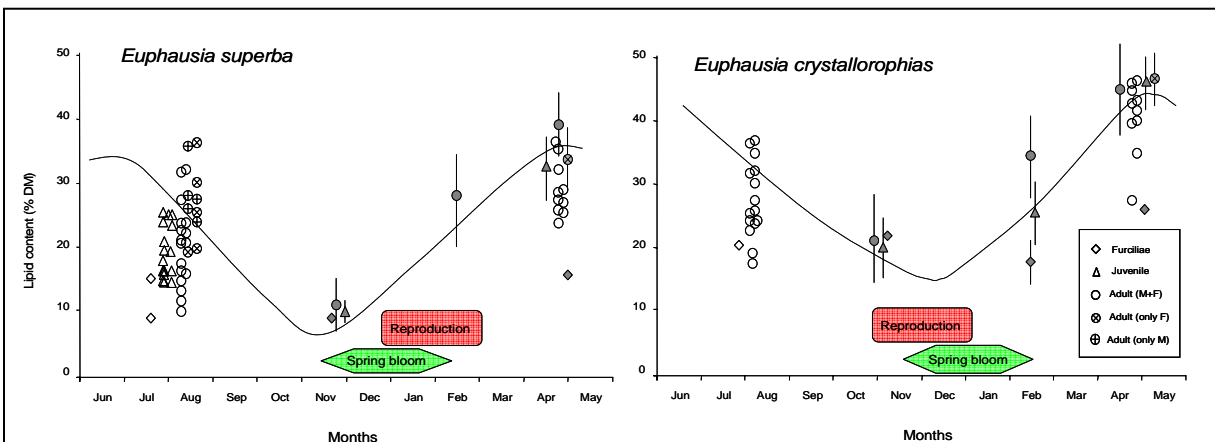


Figure 1. Seasonal lipid dynamics of Antarctic krill. Most of the data are taken from Ju and Harvey (2005) with the exception of spring and summer data (filled symbols) from Hagen et al. (2001) and Kattner and Hagen (1998). Dotted lines adopted from Falk-Petersen et al. (2000).

The storage lipids in krill are typically either wax esters or triacylglycerols depending on species (e.g. Falk-Peterson et al., 2000). For instance, while *E. crystallorophias*, *Thysanoessa macrura*, and *T. inermis*, appear to accumulate high amounts of wax esters as the primary storage lipid (Hagen et al., 1996; Kattner and Hagen, 1998; Falk-Peterson et al., 1982), *E. superba* and *T. raschii* use triacylglycerols (Hagen et al., 1996; 2001; Falk-Peterson et al., 1982). In addition, phospholipids, known principally as the major structural component of cell membranes are used as an additional storage lipid in some species. It has been suggested that phospholipids accumulation might be tightly linked with reproduction, particularly for ovary development and egg production (Hagen et al., 1996; Mayzaud et al., 2003; Ju et al. 2005). As a result, information on lipid storage and distribution in krill can provide much insight as both nutritional markers and well as indices of reproduction potential for krill. Given the high caloric density of lipids compared to either proteins or carbohydrates, the lipid content of krill act as an important prey resource for higher trophic levels. Climate effects such as changing physical features (i.e. sea-ice conditions, stratification) or food (i.e. phytoplankton, micro/mesozooplankton community structure) have the potential to directly impact krill nutritional status of krill and the coupling between trophic levels. Ultimately, these changes determine how much energy transfers into the higher trophic levels. It is not an exaggeration to consider the nutritional condition of krill as a key determinant in the distribution of top predators within the Bering Sea ecosystem.

Euphausiids as Trophic Links

Studies on the feeding ecology of krill are central to our understanding of their roles for trophic-transfer of nutrients and energy from primary producers to top predators in the Bering Sea ecosystem. Important for krill are also that food sources vary widely from phytoplankton, microzooplankton, detritus to copepods, depending on life stage, species, and food availability (see Table 2 below).

Table 2. A summary of the general life cycle characteristics of major euphausiid species in the Barent Sea and Bolsfjorden (for *T. inermis* and *T. rashii*) and Gulf of Alaska
(based on Falk-Petersen et al., 2000; Siegal, 2000; Coyle and Pinchuk, 2005).

Species	Longevity (years)	Age at maturity (years)	Spawning season	Feeding type	Habitat
<i>T. inermis</i>	3+	2+	April – May	Herbivorous (?)	Oceanic
<i>T. rashii</i>	2+	2+	Late May - June	Omnivorous	Neritic
<i>T. spinifera</i>	≥1+ (?)	1+ (?)	April - Oct. (?)	Omnivorous (?)	Neritic
<i>E. pacifica</i>	2+	1+ (?)	July – Oct.	Omnivorous	Oceanic

In case of Antarctic krill, larvae feed largely on organisms associated with ice during winter while adults are omnivorous, with copepods representing a significant dietary source (Ju and Harvey, 2004). Diet history is often a central question, and a range of approaches have been applied as shown in the generalized summary below (Table 3) with their strengths, constraints, and estimated time frames that each might be applicable. Central references to each are omitted for brevity. Among more recent approaches, the use of biochemical indicators, especially specific lipid markers have provided important insights into zooplankton feeding history. Controlled experiments in the late 80's provided detailed information on the ability of zooplankton to modify and incorporate algal lipids during feeding (e.g. Harvey et al., 1987, 1989). Since a number of individual structures (e.g. long-chain polyunsaturated fatty acids, specific sterols) are taxon-specific, they can be used to understand the history of consumption (see Dalsgaard et al. 2003, references therein).

Table 3. A summary of techniques used to assess diet history in zooplankton, their characteristics and estimated time frame over which each approach might integrate.

Method	Advantages	Disadvantages	Integration Time
Gut content analysis	<ul style="list-style-type: none"> - No incubation artifacts. - Information on size selectivity of diet. 	<ul style="list-style-type: none"> - Soft/rapidly digested items under represented. 	Hours to a day
Feeding incubations	<ul style="list-style-type: none"> - Allows direct estimation of feeding selectivity and ingestio rates. 	<ul style="list-style-type: none"> - Artifacts of confinement. - Hard to recreate realistic natural conditions 	Hours to days
Molecular techniques (nucleic acids/ antibodies)	<ul style="list-style-type: none"> - Identify assimilated prey species (highly taxon-specific) - No incubation artifacts. 	<ul style="list-style-type: none"> - Few tests in field. - Not easily quantified. - Sensitive to contamination. 	Hour to a day (?)
Fatty acids	<ul style="list-style-type: none"> - Indicates assimilated food. - No incubation artifacts. 	<ul style="list-style-type: none"> - Internal metabolism and modification can complicate interpretations. - Signatures not always diet-specific. 	Days to weeks
Other lipid markers (sterols/ alcohols)	<ul style="list-style-type: none"> - Indicates assimilated food. - Can take advantage of enzymatic limitations. - Highly taxon-specific. 	<ul style="list-style-type: none"> - Internal metabolism and modification possible. - Quantitative transfer uncertain. 	Weeks to months
Stable isotopes (C and N)	<ul style="list-style-type: none"> - Indicates trophic levels. - No incubation artifacts. 	<ul style="list-style-type: none"> - Not taxon-specific. - Variable values in food consumed. Especially in ice. 	Weeks to months

Stübing and Hagen (2003) have noted that in case of *E. superba*, dietary lipid components useful as trophic markers need careful interpretation since it might be possible for animals to preferentially retain essential components depending on physiological and environmental conditions. Planned feeding trials in this proposal will provide the needed validation to test this with certainty.

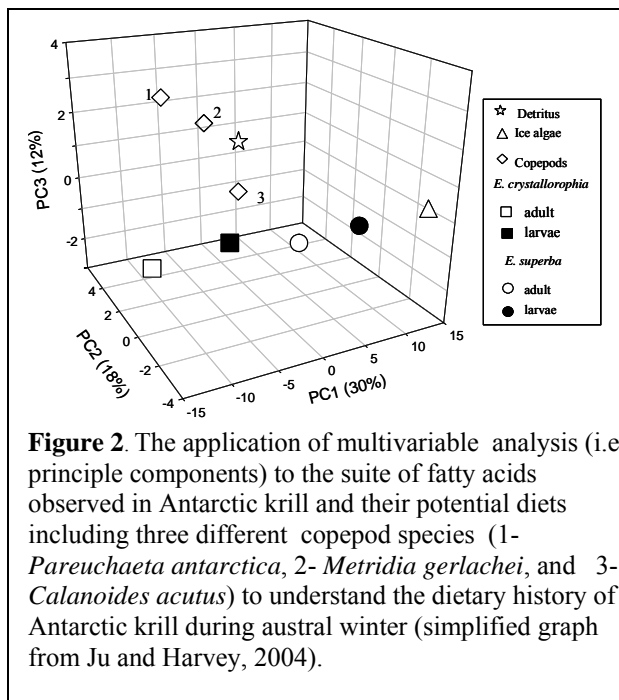
Given the potential to follow a large suite of individual lipid structures of varying sources, multivariate approaches have proven useful in defining major consumption patterns and diet history (e.g. Cripps et al., 1999). We have used this approach in the Antarctic to differentiate dietary patterns in *E. superba* (see Figure 2 and Ju and Harvey, 2004) which has allowed the diets of euphausiids and copepods to be distinguished. As with even this detailed analysis, however, questions of overlap among diets and the role of omnivory must be taken into consideration in their interpretation. Recent work has demonstrated that these biochemical markers in combination with other techniques (i.e. gut content analysis and feeding experiments) allow a comprehensive determination of feeding history of krill (Atkinson et al., 2002; Meyer et al., 2002; Stübing et al., 2003; Ju and Harvey, 2004; Schmidt et al. 2006). For this project we will analyze individual fatty acids, but also include additional lipid components that we have found to provide more specific dietary information. These include the alcohols and individual sterols which, due to their greater structural specificity and more limited taxonomic distribution, have a greater potential to trace consumption. In krill for example, the presence of unsaturated alkenes (e.g. 21:6 - Cripps & Hill, 1999) or alcohols (18:1 alcohols - Falk-Petersen et al., 1999; Ju & Harvey, 2004) has been used to implicate consumption of dinoflagellates or carnivory,

respectively. Virtue et al. (1993) has used the presence of the specific algal sterol, brassicasterol, in the digestive gland of *E. superba* to estimate consumption of the prymnesiophyte *Phaeocystis pouchetii*. Our own work with copepods has demonstrated that specific triterpenoid alcohols of bacterivorous marine ciliates (Harvey & McManus, 1991) can also be used as tracers of their consumption, and it can also be traced to the eggs of copepods after ciliate feeding (Ederington et al., 1995). Inclusion of these additional lipids, which are often less metabolically active than fatty acids, may allow a more quantitative measure of diet history of euphausiids, particularly the potential for following the expected shifts in prey availability associated with sea-ice changes.

The Identification of Prey Taxa, Feeding Strategies and Validation of Lipid Biomarkers

Whether or not euphausiids are able to obtain adequate essential fatty acids for growth and reproduction will depend on the availability of appropriate prey. The availability and abundance of potential prey (algae, heterotrophic protists, copepods and detritus) are reflective of the temporal and spatial variations of sea-ice and planktonic communities and food webs. The nutritional value of prey is not only a function of taxa, but also the nutritional history of the prey (Dalsgaard et al. 2003), which may itself change with season, temperature, nutrients, light, and habitat (ice vs. water). As a result, both quantitative (i.e. abundance) and qualitative (i.e. nutritional value) differences in prey could alter the biochemical composition and physiology (i.e. growth, reproduction) of grazers and consumers (St. John and Lund 1996; Pond et al. 2005).

To quantify these potential variables, this study includes a systematic assessment of the identity of potential prey and their lipid status over season and different years with different ice conditions. By assessing the composition of natural communities and their lipid composition, we will be able to infer prey (and therefore feeding strategies) from assessment of lipid trophic markers in larvae, juveniles and adult krill. Also, concurrent lipid and microscopic analyses of seston and sea-ice biota may be able to identify specific biomarkers for heterotrophic, particularly herbivorous, protists (flagellates, dinoflagellates, ciliates) which have not been as well characterized as photosynthetic protists (e.g. Barrett et al. 1995; Harvey and McManus, 1991; Tang and Taal, 2005). When sufficiently abundant, we will also isolate and collect individual species of herbivorous protists (e.g., *Protoberidinium* spp., *Gyrodinium* spp., oligotrich ciliates) for direct assessment of specific biomarkers. When not naturally abundant, we will enrich for endemic herbivorous protists in shipboard cultures. By performing krill feeding experiments with natural prey communities, we will directly assess potential prey and lipid transfers to krill. Feeding experiments and gut content analysis will help to determine if trophic transfers are involved. To refine even further the information gained, we also have the potential to include compound specific isotopic measures (GC-IR-MS - e.g. Harvey & Macko 1997) of selected samples. We will target shipboard feeding experiments, which allow the more controlled conditions needed to distinguish direct consumption versus biosynthetic modification of lipids with identical structure. By combining detailed analysis of individual lipid compounds in euphausiids and prey assemblages in concert with traditional



techniques (i.e. shipboard feeding experiments, gut content analysis, plankton and sea-ice community descriptions) we can tightly constrain the feeding ecology of euphausiids under changing environmental conditions and their role as a trophic link to higher trophic levels.

Age Estimation in Crustacean Ecology

Knowledge of the demographic (i.e. age) structure of field populations is fundamental to understanding the population dynamics of marine organisms. For many species, age can be determined with reasonable certainty using hard parts which produce a characteristic growth increment over time, e.g. otoliths, scales, molluscan shell, teeth, or spines (e.g. Secor et al. 1995). These techniques have proven broadly successful in fishes and molluscs and are routinely used to gauge population structure. In crustaceans such as euphausiids, however, the lack of a growth record in a permanent hard structure has curtailed the determination of chronological age and thus demographic information is generally unknown. Most crustaceans periodically molt their exoskeleton to accommodate future growth, and in the process abandon any external evidence of age or previous size. As a result, key biological parameters of euphausiids, such as growth, longevity, and mortality, have relied on estimates from modal analysis of length frequency data many species (Harding, 1949; Macdonald and Pitcher, 1979; Rothschild et al., 1992). Unfortunately these methods are inherently imprecise in crustaceans due to their discontinuous growth patterns, a protracted spawning season, and necessary assumptions concerning molting frequency (e.g. Siegel, 1987). In some cases krill also undergo size reduction during periods of dormancy or food limitation as seen for *E. superba* (e.g. Ikeda and Dixon, 1982) and *E. pacifica* (Shaw and Peterson, 2005). As a result, individuals seen in a size-based cohort may vary in age by several months and perhaps even longer.

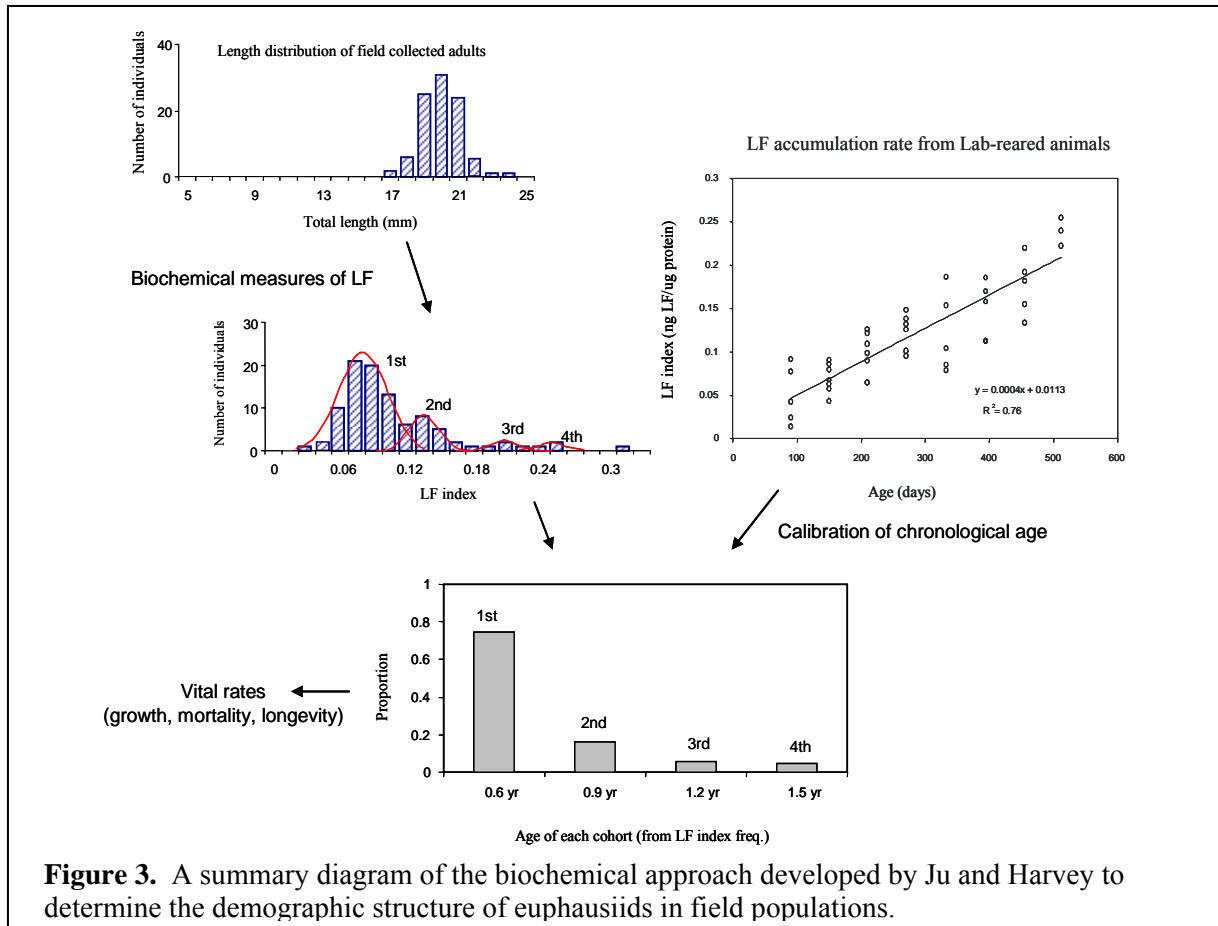
The critical need for age structure data on crustacean populations has led to a search for alternative methods for age determination. One we have investigated in multiple crustaceans is based on the accumulation of oxidation products in tissues which might provide a surrogate for chronological age. Every organism that relies on oxygen generates free radicals and aldehydes during normal cellular metabolism. As a scavenging mechanism to retard these highly reactive compounds in cells, organisms use a series of reactions with unsaturated lipids, nucleic acids and proteins as a sink, cross linking them through a series of reactions to form conjugated Schiff bases of the general structure, $-N=C-C=C-N-$. Collectively referred to as lipofuscins (LF) or age pigments, these products have unique fluorescent properties and been observed for decades in all aerobic organisms where they accumulate over the lifetime of every tissue. By using neural tissues with very long turnover times, they can provide a proxy for chronological age with proper calibration.

Our biochemical approach developed over the last decade has built upon many early efforts using LF, but has differed significantly in that we have investigated the fundamental structure of LF and its distribution among various tissues to better understand its formation. Our original model organism was the blue crab (*Callinectes sapidus*) and considerable effort was made to investigate methodological refinements allowing it to be an accurate predictor of age (Ju et al., 1999; 2001). We have learned that LF extracted from tissues is not a single compound, but rather a suite of fluorescent Schiff-base products of which only a subset accumulate with age in predictable rates. Its concentration is best followed in tissues which undergo limited post-mitotic division (e.g. brain, eye, and eyestalk) and we have developed procedures which base its concentration on the protein content of the tissue extracted to allow unbiased comparisons across large ranges in size and age. Results using this approach significantly improved our knowledge of the demographic structure of blue crab population in the field with precision within 2 months for crabs having over 5 year life spans (Ju et al., 2003). More recently we have miniaturized the approach to examine for individual euphausiids in higher latitudes as described below.

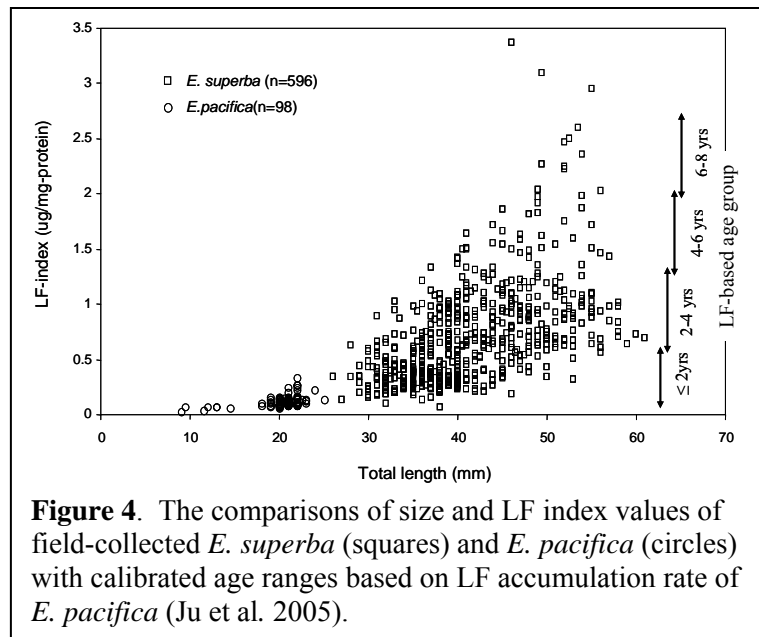
The case for age structure in euphausiids

Longevity and recruitment in euphausiids has been difficult to establish. The most extensive studies have been in the Southern Ocean, where two decades of work has examined the distribution and biology of *E. superba*. Despite this intense effort, it has not been possible to assess accurately the age structure of populations or to estimate their natural longevity (Nicols, 1990). Observations of high internal variability of krill densities in the Southern Ocean led to the suggestion that spawning success or failure during the preceding season and/or survival/mortality of recruits during the previous winter was a key factor (Siegel and Loeb, 1994; 1995). Using information from long-term net surveys (1977 to 1994), Siegel (1995) further examined the interannual variation of krill stock and recruitment with the associated environmental parameters during each survey. Although several important trends were observed, notably that sea ice condition (i.e. ice coverage) was an important factor in predicting recruitment success, no significant correlation was found between recruitment indices for any year and the previous year's overall stock density.

In an early attempt to use biochemical measures of LF to ascertain age of *E. superba*, Ettershank and George (1985) summarizes much of the initial work and difficulty in using length based measures of age in krill, including observation that overwintering populations of krill continue to molt and actually shrink in size. While this work did not allow ages to be established, 3 distinct groups of *E. superba* were seen in preserved specimens. Building on our work with the Blue crab, we modified our biochemical ageing method to examine euphausiid demographics. Firstly, we evaluated the potential of the biochemical approach using LF to determine the age structure of the major euphausiid *E. pacifica*, as a component of the Northeast Pacific GLOBEC programs. Concentrations of LF extracted from neural tissues of individual animals (eye and eye-stalk), were quantified and normalized to protein content to allow comparisons across sizes, life stage and species. A central tenet of lipofuscin age determination is the need for a calibrated population to account for organismal and metabolic differences which alter accumulation rates of age pigments. This was accomplished through collaborative rearings of *E. pacifica* from eggs for almost two years. This biochemical approach to determine the demographic structure of euphausiid is summarized below in figure 3. Multiple fluorescent components were observed, with LF content in known-age krill significantly correlated with chronological age ($r = 0.84$). Field-collected krill contained variable levels of LF dependent on size and age, with modal analysis indicating multiple age classes, even though size showed only two broad modes (juvenile and adult). These results support that this modified biochemical approach is feasible to determine the age structure of krill population in the field. An added advantage is that our approach of using optical neural tissue (in this case eye stalks), allow for detailed lipid analysis and age structure to be determined in *the same individual*.



Moreover, our recent comparisons of LF contents between high latitudinal (i.e. *E. superba*) analysed on board during Southern GLOBEC cruises and temperate (i.e. *E. pacifica*) species showed that high latitudinal species have much higher LF values than temperate species (figure 4). It could be related with the increased lifespan that high latitudinal krill species live longer than temperate species. Information on longevity could help to understand the environmental impact on the population of euphausiids. If each species of euphausiid have different longevity, their population should be affected by different time scale of environmental changes. For example, the population of long-lived species, particularly with one spawning season per year, will be significantly affected by interannual variation of environment, while for short-lived species, their biomass should be more affected by seasonal variation of their environment.



Research Objectives

This collaborative proposal entails three major objectives to understand the trophic role of euphausiids in the BEST study region. Combining our experience in marine biochemistry and plankton ecology will allow us to quantify the age structure and diet history of important euphausiids in the East Bering Sea with detailed information on their consumption and growth. We will link field collections and analysis with laboratory rearing for age calibration and shipboard feeding experiments, to test retention and validation of trophic lipid biomarkers as well as evaluate the quality and quantity of food resources. Our specific objectives include:

1. To determine the potential impact of climate-driven changes in sea-ice conditions on lipid content and lipid classes in major euphausiid species and thus nutritional condition and reproductive potential over seasonal and interannual scales.
2. To understand the diet history, feeding rates and grazing strategies of euphausiids under changing spatial (i.e. ice-covered, ice-edge, and open water zones) and temporal (i.e. seasonal and interannual) prey fields. Multiple approaches (i.e. feeding experiments, gut content analysis) will be used for validation and retention of specific lipid dietary markers observed in field collections.
3. To apply recent advances in biochemical approaches to determine the age structure in field populations of euphausiids and the potential effects of climate change on maintenance or disruption of cohort populations seasonally and interannually. Laboratory rearing conducted in parallel by Alaskan colleagues will allow calibrating precise ages in cohorts.

Research Plan

The BEST program anticipates two cruises per year in each of three field seasons, 2008-2010, an early spring ice-breaker cruise in March/April and a cruise in late spring/early summer (June/July). Although the actual station plan will be determined by consensus with other funded projects, a potential plan would include sampling along the middle domain from the Alaska Peninsula in the south, which now appears to be ice-free all year, to the south side of St. Lawrence Island, which is covered by sea ice until April – May (Figure 5). Because the effects of changing ice conditions may vary as a function of oceanographic domains (cross-shelf location and depth), sampling across the shelf will consist of four or five lines running from the inner domain (30 m isobath) through the middle and outer domains, and extending out to the middle of the slope (1000 m isobath). Thus, the onshore ends of these lines will sample coastal waters, and the offshore ends will be located over the basin, at or just beyond the maximal extent of the seasonal sea ice cover. We will participate in all cruises (icebreaker and open water cruises) to cover the temporal and spatial development of euphausiid populations and their prey.

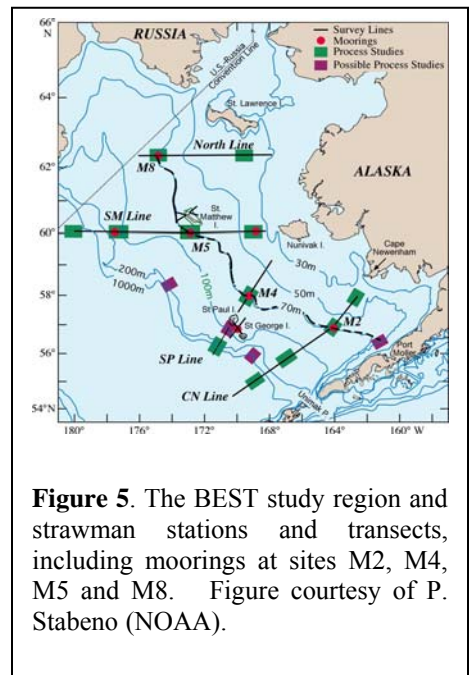


Figure 5. The BEST study region and strawman stations and transects, including moorings at sites M2, M4, M5 and M8. Figure courtesy of P. Stabeno (NOAA).

Euphausiid and prey collections

Larval and adult euphausiids for lipid analyses and feeding experiments will be collected by BEST collaborators (S. Smith and R. Hopcroft/K. Coyle – see supporting letters) using multiple samplers: MOCNESS, multi-nets, and CALVET and plummet nets in open water, at the ice edge and under ice when ice cover permits. We (Harvey, Ju and Shaw) have significant experience from our work on Southern GLOBEC and NEP GLOBEC cruises and will assist with collections and sorting. Samples of co-occurring microbiota assemblages in the water column and ice will be taken with Niskin bottles (at or near depths of euphausiid collections) or with an ice corer, respectively, for lipid analysis and microscopic identification. These samples will provide a direct 'calibration' of lipid distributions with the taxonomic composition of the natural samples, as well as description of potential prey availability. A regular grid of stations for lipid analysis of *in situ* euphausiids and seston, and for LF analysis of collected euphausiids, will be sampled to provide spatial coverage (onshore, offshore, north and south). At a subset of stations where sufficient animals can be collected, feeding experiments and gut content analysis will also be carried out. For LF analysis, we hope for a minimum of 200 freshly collected and undamaged euphausiids, of which a subset can be used for lipid analysis. After morphological measurements (size, weight, eye diameter, etc.) are taken, eye and eyestalk tissues are carefully excised and dissected using a dissecting microscope. Tissues are transferred to amber vials and extracted for lipofuscin following procedures described in Harvey et al. (2005). The animal (minus eyes and eyestalks) is then transferred to a separated vial and immediately frozen in liquid nitrogen for lipid and gut content analysis. The ability to measure both age structure and diet history of the same individual animal is a significant advantage for interpretation of reproductive potential and growth history.

Feeding Experiments

Euphausiids will be collected at night either with a 1-m diameter plankton net, 60 cm Bongo nets or MOCNESS towed at low speeds (1-1.5 knots) using closed cod ends (Smith and Hopcroft/Coyle). After landing the nets, the contents of the cod end will be immediately poured into a picnic cooler filled with 30 L of surface seawater. Undamaged euphausiids will be sorted by species using plastic Chinese soup spoons with a focus on the most abundant *T. raschi* and *T. inermis*. Depending on availability, furcillia, juveniles and adults will be tested in separate experiments. Younger stages will be sorted with the aid of a dissecting microscope at 6X; otherwise, larger animals can be identified by eye. Feeding experiments will be conducted using seawater from the location of euphausiid collection; in the case of euphausiids collected under the ice, seawater can be supplemented with ice microbiota. Using acid-clean procedures, 50 L carboy(s) will be filled with seawater collected with Niskin bottles, and stored in a cold room prior to the experiments. For ice microbiota enrichments, chunks of ice will be melted according to Garrison and Buck (1986), to prevent the loss of delicate organisms, and added to seawater samples. In experiments with juvenile and adult euphausiids, a treatment with copepods added will be included. Feeding experiments will be set up and incubated in a cold room at ambient temperature. While gently mixing the 50L carboy with a clean plastic stirrer, subsamples will be siphoned for initial prey concentrations, chlorophyll a and lipid analyses and to fill control and replicate (4-8) treatment containers (2.5 - 10 L, depending on euphausiid stage). The numbers of euphausiids per treatment will be selected based on euphausiid stage and size, and expected clearance abilities. A subset of the test animals will be sacrificed at the beginning of the experiments to measure initial lipid content. After 24h incubations, samples will be siphoned out for prey enumeration, chlorophyll a, and lipid analyses. In the copepod enriched treatments, the rest of the sample will be filtered onto a 50 µm sieve and preserved in 4% formaldehyde for copepod enumeration. Euphausiids will be checked for mortality. Clearance and ingestion rates on different prey items will be calculated according to Frost (1972), and prey preference will be determined using electivity indices (Vanderploeg and Scavia 1979a, b; Olson et al 2006).

Egg Production and molting rate experiments. Measurements of euphausiid growth and reproduction are needed to relate nutritional status and prey selection to euphausiid health. Other PIs (Smith,

Hopcroft/Pinchuk) are proposing to do these measurements on some or all of the cruises, and we will collaborate with them to avoid duplication of effort and expense. Tracy Shaw, a technician on our proposal, has extensive experience measuring growth and EPR on euphausiids in the Antarctic and Northeast Pacific. Freshly collected, undamaged mature females with ripe ovaries will be placed in individual jars filled with filtered (< 200 µm) seawater from the area of collection. Ripe ovaries are easily identified as they turn blue when the females are ready to spawn. Jars will be incubated in the dark for 48 hours and checked every 12 hours to see if the female has released eggs. Females that spawn will be transferred to a new jar and maintained for the entire time period to check for multiple spawning events. Eggs will be counted at sea using a dissecting microscope. Females that spawn are measured at the end of the experiment. For molting rate experiments, freshly collected euphausiids will be placed in individual 500 ml jars filled with filtered (< 200 µm) seawater. Jars will be incubated in the dark for 48 hours and checked every 12 hours to see if the animal has molted. Animals that molt will be preserved together with their molt in 5% formalin and the telsons of the animal and molt will be measured using a dissecting microscope to determine the growth interval. All animals that do not molt during the experiment will be measured at the end of the experiment.

Isolation and culture of heterotrophic protists for identification of specific biomarkers.

Herbivorous protists (*Protoperdinium spp.*, *Gymnodinium spp.*, *Gyrodinium spp.*, oligotrich ciliates) will be isolated at sea under a Wild darkfield microscope, pipetted into filtered seawater, and filtered onto pre-combusted GF/F filters and frozen for lipid analysis (below). To ensure sufficient biomass, shipboard cultures will also be initiated by micropipetting protists into 70 ml tissue culture flasks filled with nutrient enriched filtered seawater (Lessard 1993). Algal prey, isolated at sea and/or transported from Lessard's laboratory collection, will be grown separately on f/2 medium. Aliquots of algal prey will be added weekly to the herbivore cultures. Enrichments will also be made of 200 µm-screened natural seawater. The cultures and enrichments will be incubated in the deckboard rotating plankton incubators and/or on a portable plankton wheel in a temperature controlled room.

Analytical Details

Lipid analysis. Lipid analysis will rely on familiar methods for the molecular level analysis of individual components in complex biological matrices. Total lipids are first extracted using dichloromethane:methanol (1:1) with probe sonication as described by Harvey et al. (1987, 1997). The total lipid extract is dried by rotary evaporation, redissolved in a smaller volume of solvent and split into sub-samples for further analysis of either lipid classes or individual components. Sub-samples of total lipid extract will be separated into each lipid class using normal phase extraction with strata NH2 cartridges (Phenomenex co.) and then collected each lipid class will be used for further structural analysis. For detailed structural analysis, internal standards (5α-cholestane for neutral and nonadecanoic acid for fatty acids) are added and the sample subjected to mild alkaline hydrolysis neutral and polar individual lipids are separated into fractions, with alcohols and sterols analyzed as their respective trimethylsilyl ethers and fatty acids as their methyl esters after BF₃ derivitization. Capillary GC is used for separation and quantification of individual compounds and structural identification by combined GC-mass spectrometry. Total lipids and lipid class are separated and quantified using an Iatroscan TLC-FID analyzer, which has been used in Harvey's lab for a decade to quantify lipid storage products, most recently being krill (Ju and Harvey, 2004). To confirm polar lipids structure, we have in house capability for liquid chromatography-mass spectrometry (LC-MS) which allows absolute structure of intact polar lipids to be determined with certainty (e.g. Sturt et al., 2004).

Isotopic composition of individual organic structures has proven to be very useful in the assessments of source and will provide information on selected fatty acids whose structure might have multiple origins. The GC/-IR-MS system (Micromass Optima) to be used for analyses of individual compounds is housed at the University of Virginia under Stephen Macko's direction, and is available at modest costs though

collaborative efforts. Prof. Macko is an international authority on isotopic measures in a variety of matrices with over 200 publications and a long time collaborator with Harvey (e.g. Harvey and Macko, 1997).

Lipofuscin analysis. The analysis of Schiff-Base oxidation products for age determination in euphausiids have been developed and refined over the last 8 years by Ju and Harvey with the blue crab as the initial model (Ju et al., 1999, 2001). Sampled tissues (eyes and eyestalks) are extracted and immediately analyzed to minimize potential problems of preservation and additional oxidation. Tissues are extracted with organic solvents with sonication followed by transfer and drying under N₂. Quantification of LF relies on flow injection HPLC, with concurrent fluorescence (excitation at 355nm, emission of 510nm for *E. pacifica*). The quantification of extractable LF is typically calibrated using quinine sulfate in 0.1 N H₂SO₄ (a maximum emission at 450 nm with excitation at 340nm). Quantitative measure of LF in tissue is normalized to protein content of extracted tissues measured by the modified fluorescence assay (Harvey et al., 2005).

In order to calibrate the absolute age of individual euphausiid using LF, laboratory rearing experiments are required to estimate LF accumulation rate with animals of known age over known increments of time. We have arranged a collaboration with Alexi Pinchuk (Univ. of Alaska – see letter) for rearing of animals over the three years needed for adequate calibration. Gravid females of euphausiids are identified from field collected animals using 1-m vertical live tow or 1m² MOCNESS and individually transferred to 750 ml tissue flasks filled with seawater collected simultaneously at the sampling site. The animals are maintained at the water temperature (i.e. 5°C in April-May and 8-12°C in July and August) in the dark and checked every 12 hours for eggs. Some of newly released eggs are harvested in amber vials and immediately frozen with liquid nitrogen for lipid analysis. Others are transferred to the 2L bottle and incubated at ambient seawater with a constant in situ temperature. Until they reach the calyptopis stage, no food is introduced. After the larvae reached the calyptopis stage, they will be fed a mixed phytoplankton diet over two years. Water and food were changed every 3-5 days. At 60 day intervals, 10-20 individuals will be sacrificed for LF analysis to quantify accumulation rates in known age individuals.

Phytoplankton and microzooplankton identification and enumeration. Water column, ice and experimental samples will be preserved with 0.5% glutaraldehyde, stained with DAPI and proflavin and filtered onto 0.2 µm and 0.8 µm polycarbonate filters, to enumerate and size nano- and microplankton using epifluorescence microscopy (Lessard and Murrell, 1996). Ciliates and large dinoflagellates and diatoms will be enumerated and sized in 5% Lugols preserved samples (Stoecker et al., 1994) using an inverted microscope. A computer-aided digitizing system and software (Roff and Hopcroft, 1986) will be used that automates data entry and calculates biovolumes. Biovolumes will be converted to carbon biomass using the equations of Menden-Deuer and Lessard (2000). Shipboard, we will also use a FlowCAM (Fluidimaging Technologies), a combination flow cytometer/imaging system to rapidly characterize the larger phytoplankton and microzooplankton in live samples.

Gut content analysis. Only limited information can be obtained from gut content analysis, as organisms with soft, digestible parts cannot be recognized. However, direct evidence for *in situ* feeding on diatom species, tintinnids, thecate heterotrophic dinoflagellates, copepods (through identification of mandibles) can be obtained. Freshly caught euphausiids will be sorted and frozen for later gut content analysis. Thawed specimens will be measured and dissected to remove their stomachs and guts, and gut contents will be determined with microscopy.

Statistical analysis. Multivariate statistical techniques (e.g. PCA) using lipid biomarker distributions of all euphausiid samples and potential diets will allow a detailed evaluation of the role of trophic linkages for euphausiids. Spatial and temporal differences of nutritional conditions (i.e. total lipid content, size, weight) of euphausiids will be tested by analysis of covariance. Regression analysis will be performed to

estimate the accumulation rate of LF and growth rate of lab-rearing animals. For modal analysis of frequency distributions of LF and size (i.e. total length) we have relied on statistical techniques developed for length-based population assessment (Gulland and Rosenberg, 1992). Frequency distributions will be analyzed using ENORMSEP (Gayanilo et al., 1996), which is a maximum likelihood method for identifying modes. A Chi-squared test will be used to test procedural assumption that frequencies are normally distributed for each mode. Each mode (cohort) separated from modal analysis is appropriately assigned an age class based upon the results of laboratory rearing experiment.

Project Personnel and Collaborations

We have assembled an interdisciplinary team of experts to carry out the goals of this project. Rodger Harvey has 2 decades of experience with the detailed analysis of lipids and their cycling in marine systems and Ju has a developing reputation in biochemical methods of age determination in crustaceans. Pinchuk is a junior scientist with strengths in rearing of euphausiids in the Bering Sea region and will provide essential calibration information at modest costs to the project. Evelyn Lessard is a protist ecologist with extensive experience in field studies of lower trophic level community structure and dynamics, including the southeastern Bering Sea and Gulf of Alaska. Tracy Shaw, a research technician, brings to the project many years of experience with euphausiid growth and egg production and feeding experiments in the Antarctic and Northeast Pacific.

Significance

This proposal will directly address a central goal of the Bering Ecosystem Study (BEST) program to examine how changing sea-ice conditions affect the ecology and population dynamics of the euphausiid species in the eastern Bering Sea. We know that the major euphausiids, primarily *Thysanoessa rascii* and *T. inermis* (but also *Euphausia pacifica* and *T. spinifera*), play a critical role in Bering Sea ecosystems as links between primary producers and top predators, but their responses to potential climatic impacts are unknown. The study will combine our expertise across several disciplinary areas to quantify the age structure and diet history of important euphausiids in the East Bering Sea together with detailed information on their consumption and growth. Linking field collections and analysis with laboratory rearing for age calibration and *in situ* feeding experiments, will allow the testing of retention and validation of specific lipid biomarkers as well as the quality and quantity of food resources. This study will contribute to the NPRB BSIERP by identifying specific trophic linkages under varying oceanographic conditions, determining the nutritional quality of euphausiids available to higher trophic levels and providing critical rates to parameterize and constrain BSIERP modeling efforts. The study will provide important opportunities for graduate students at both institutions as well as multidisciplinary training.

Broader Impacts

This project will take several approaches that will not only provide for student training in polar marine science, but also seek to disseminate results to a broad audience. In the more traditional role this project will support two graduate students in respective labs of Harvey and Lessard. Harvey has several graduate students in progress on Arctic related research projects (Laura Belicka and Karen Taylor) and hopes to recruit an additional graduate student specifically for this effort. Lessard will involve several undergraduates at UW in various aspects of the project.

We will also expand the impact of this work to the broader educational community through science teacher training. We all know that exciting scientific discoveries do not always translate to K-12 classroom; and educators and scientists alike recommend bringing science with real life examples to the classroom. The Maryland Sea Grant Extension Program (based at CBL) and UMCES are members of the Environmental Science Education Partnership (ESEP) (<http://www.esep.umces.edu/>), a team of university science education specialists that bring a wealth of experience in science education to environmental issues. Its mission is to provide opportunities for teachers to participate in science research, and to promote environmental science literacy. The ESEP bridges the gap between the research community and the public through innovative education and outreach programs and products that build directly on current science.

The *ESEP Teacher Research Fellowship* is a teacher professional development program that includes an intensive 6-week summer program, extensive follow-up academic year support, and comprehensive dissemination efforts. The fellowship program goals are: (1) to improve teachers' science content knowledge and inquiry skills within the context of the environment, (2) to help teachers develop and implement lessons that build on real world environmental data, and link to state and national standards, and (3) to disseminate teacher-developed and -tested lessons additional teachers. For this project, one teacher per year will be included in this program. Each fellow will begin the with a summer week-long workshop on science research and relating that experience to the classroom. A teacher fellow will take up residence at CBL, with Dr. R. Harvey as advisor. During this six-week research experience, they will also collaborate with ESEP specialists to develop student lessons on the Bering Sea ecosystem that build on their experience, compliment their current state curriculum, and support national standards. With direct interaction with the PI's and guidance from the ESEP team, they will test and refine their lessons the following academic year, conduct a field based experience with their students and share their lessons with peers at an ESEP-sponsored professional development workshop. These lessons will be distributed to countless more teachers on the ESEP website (www.esep.umces.edu). Over the last 5 years, over 100 teachers have participated in this program.

Bibliography

- Arrigo AR, Thomas DN (2004) Large scale importance of sea-ice biology in the Southern Ocean. *Antarct Sci* 16(4): 471-486
- Atkinson A, Meyer B, Stübing D, Hagen W, Schmidt K, Bathmann UV (2002) Feeding and energy budgets of Antarctic krill *Euphausia superba* at the onset of winter-II. Juveniles and adults. *Limnol Oceanogr* 47: 953-966
- Barret SM, Volkman JK, Dunstan GA (1995) Sterols of 14 species of marine diatoms (Bacillariophyta). *J Phycol* 31 360-369.
- *Belicka LL, Macdonald RW, Harvey HR (2002) Sources and transport of organic carbon to shelf, slope and basin surface sediments of the Arctic Ocean. *Deep Sea Res* 49: 1463-1483
- *Belicka LL, Macdonald RW, Yunker MB, Harvey HR (2004) The role of depositional regime on carbon transport and preservation in Arctic Ocean sediments. *Mar Chem* 86: 65-88
- BEST (Bering Sea Ecosystem Study) Science Plan (2004) Arctic Research Consortium of the U.S., Anchorage, Alaska. 82 pp.
- BEST (Bering Sea Ecosystem Study) Social Science Plan. 2005. Sustaining the Bering Ecosystem - A Social Sciences Plan. Arctic Research Consortium of the U.S., Available online at: <http://arcus.org/Bering/hbest/index.html>
- Coyle KO (2005) Zooplankton distribution, abundance and biomass relative to water masses in eastern and central Aleutian Island passes. *Fish Oceanogr* 14 (suppl 1): 77-92
- Coyle KO, Pinchuk AI (2002) The abundance and distribution of euphausiids and zero-age pollock on the inner shelf of the southeast Bering Sea near the Inner Front in 1997-1999. *Deep Sea Res II* 49: 6009-6030
- Coyle KO, Pinchuk AI (2005) Seasonal cross-shelf distribution of major zooplankton taxa on the northern Gulf of Alaska shelf relative to water mass properties, species depth preferences and vertical migration behavior. *Deep Sea Res II* 52: 217-245
- Cripps GC, Hill HJ (1998) Changes in lipid composition of copepods and *Euphausia superba* associated with diet and environmental conditions in the marginal ice zone, Bellingshausen Sea, Antarctica. *Deep-Sea Res* 45:1357-1381.
- Cripps GC, Watkins JL, Hill HJ, Atkinson A (1999) Fatty acid content of Antarctic krill, *Euphausia superba*, at South Georgia related to regional populations and variations in diet. *Mar Ecol Prog Ser* 181: 177-188
- Dalsgaard J, St. John M, Kattner G, Müller-Navarra D, Hagen, W (2003) Fatty acid trophic markers in the pelagic marine environment. *Adv in Marine Biology* 46: 225-340
- Daly KL (1990) Overwintering development, growth and feeding of larval *Euphausia superba* in the Antarctic marginal ice-zone. *Limnol Oceanogr* 35 (7): 1564-1576
- Daly KL (2004) Overwintering growth and development of larval *Euphausia superba*: an interannual comparison under varying environmental conditions west of the Antarctic Peninsula. *Deep Sea Res II* 51: 2139-2168
- Ederington MC, McManus GB, Harvey HR (1995) Trophic transfer of fatty acids, sterols and a triterpenoid alcohol between bacteria, a ciliate and the copepod *Acartia tonsa*. *Limnol Oceanogr* 40: 860-867
- Ettershank G (1985) Population age structure in males and juveniles of the Antarctic krill, *Euphausia superba*. *Polar Biol* 4: 199-201
- Falk-Petersen S, Hagen W, Kattner G, Clarke A, Sargent J (2000) Lipids, trophic relationships, and biodiversity in Arctic and Antarctic krill. *Can J Fish Aquat Sci* 57: 178-191
- Falk-Petersen S, Sargent JR, Hopkins CCE (1982) Ecological investigations on the zooplankton community in Balsfjorden, Northern Norway: lipids in euphausiids *Thysanoessa rashii* and *T. inermis* during spring. *Mar Biol* 68: 97-102
- Falk-Petersen S, JR Sargent, OJ Loenne & S Timofeev (1999) Functional biodiversity of lipids in Antarctic zooplankton: *Calanoides acutus*, *Calanus propinquus*, *Thysanoessa macrura* and *Euphausia crystallorophias*. *Polar Biol* 21: 37-47
- Frost BW (1972) Effects of Size and Concentration of Food Particles on Feeding Behavior of Marine Planktonic Copepod *Calanus pacificus*. *Limnol Oceanogr* 17: 805-815
- Garrison DL, Buck KR (1991) Surface-layer sea ice assemblages in Antarctic pack ice during austral spring-environmental conditions, primary productions and community structure. *Mar Ecol Prog Ser* 75: 161-172

- Gayani FC, Sparre P Jr, Pauly P (1996) FAO/ICARM stock assessment tools (FISAT). FAO, Rome, Italy.
- Gulland JA, Rosenberg AA (1992) A review of length-based approaches to assessing fish stocks. FAO Fish Tech Paper, Rome, Italy, pp323
- Gradinger R (1999) Integrated abundance and biomass of sympagic meiofauna in Arctic and Antarctic pack ice. *Polar Biol.* 22(3): 169-177
- *Grebmeier, J.M. and H.R. Harvey 2005. The western Arctic-shelf Basin Interactions project: An overview. *Deep Sea. Res II.* 52,3109-3115.
- Hagen W, Auel H (2001) Seasonal adaptations and the role of lipids in oceanic zooplankton. *Zoology* 104: 313-326
- Hagen W, Kattner G, Terbruggen A, Van Vleet ES (2001) Lipid metabolism of the Antarctic krill *Euphausia superba* and its ecological implications. *Mar Biol* 139: 95-104
- Hagen W, VanVleet ES, Kattner G (1996) Seasonal lipid storage as overwintering strategy of Antarctic krill. *Mar Ecol Prog Ser* 134: 85-89
- Harding JPO (1949) The use of probability paper for graphical analysis of polymodal frequency distributions. *J Mar Biol Ass UK* 28: 141-153
- *Harvey, H.R. 2005. Sources and Cycling of Organic Matter in the Marine Water Column. *In: J. Volkman, [Ed.], The Handbook of Environmental Chemistry. Marine Organic Matter: Biomarkers, Isotopes and DNA.* Chapter 1. Springer pp 1-27.
- *Harvey HR, Ju SJ, Feinberg L, Peterson WT (2007) Biochemical determination of population age structure in euphausiids, a regional comparison (in review)
- Harvey HR, Ederington MC, McManus GB (1997) Lipid composition of the marine ciliates *Pleuronema* sp. and *Fabrea salina*: Shifts in response to changes in diet. *J Eukaryotic Microbiol* 44 (3): 189-193
- Harvey HR, Eglinton G, Ohara SCM, Corner EDS (1987) Biotransformation and assimilation of dietary lipids by *Calanus* feeding on a dinoflagellate. *Geochem Cosmochim Acta* 51 (11): 3031-3040
- Harvey HR, Macko SA (1997) Kinetics of phytoplankton decay during simulated sedimentation: changes in lipids under oxic and anoxic conditions tracking bacterial mediation of early diagenesis in the marine water column. *Org Geochem* 27: 129-140
- *Harvey HR, Mannino A (2001) The chemical composition and cycling of particulate and macromolecular dissolved organic matter in temperate estuaries as revealed by molecular organic tracers. *Org Geochem, Special Issue on Estuaries* 32: 527-542
- Harvey HR, McManus GB (1991) Marine ciliates as a widespread source of tetrahymanol and hopan-3 β -ol in sediments. *Geochim Cosmochim Acta* 55: 3387-3390
- Harvey HR, Ohara SCM, Eglinton G, Corner EDS (1989) The comparative fate of dinosterol and cholesterol in copepod feeding – implications for a conservative molecular biomarker in the marine water column. *Org Geochem* 14(6): 635-641
- Horner R (1976) Sea ice organisms. *Oceanogr Mar Biol Annu Rev* 14:167-182.
- Horner R, Ackley SF, Dieckmann GS, Gulliksen B, Hoshiai T, Legendre L, Melnikov IA, Reeburgh WS, Spindler M, Sullivan CW (1992) Ecology of sea ice biota. I. Habitat, terminology, and methodology. *Polar Biol* 12 (3-4): 417-427
- Hunt GL Jr, Baduini RD, Jahncke J (2002a) Diets of short-tailed shearwaters in the southeastern Bering Sea. *Deep Sea Res II* 49: 6147-6156
- Hunt GL Jr, Stabeno P, Walters G, Sinclair E, Brodeur RD, Napp JM, Bond NA (2002b) Climate change and control of the southeastern Bering Sea pelagic ecosystem. *Deep Sea Res II* 49 (26): 5821-5853
- Ikeda T, Dixon P (1982) Body shrinkage as a possible overwintering mechanism of the Antarctic krill, *Euphausia superba* Dana. *J Exp Mar Biol Ecol* 62: 143-151.
- *Ju, S.-J, H.R. Harvey, J.G. Gutierrez and W. T. Peterson. 2006. The role of lipids during embryonic development of the euphausiids *Euphausia pacifica* and *Thysanoessa spinifera*. *Limnol. Oceanogr.* 51:2398-2408.
- *Ju SJ, Harvey HR (2004) Lipids as markers of nutritional condition and diet in the Antarctic krill *Euphausia superba* and *Euphausia crystallorophias* during austral winter. *Deep Sea Res II* 51: 2199-2214
- Ju SJ, Harvey HR (2005) Lipid ecology of Euphausiids in the Antarctic and North East Pacific Oceans. (in review)
- Ju SJ, Scolardi K, Daly KL, Harvey HR (2004) Understanding the trophic role of the Antarctic ctenophore, *Callianira antarctica*, using lipid biomarkers. *Polar Biol* 27: 782-792

- Ju SJ, Secor DH, Harvey HR (1999) Use of extractable lipofuscin for age determination of blue crab *Callinectes sapidus*. *Mar Ecol Prog Ser* 185: 171-179
- Ju SJ, Secor DH, Harvey HR (2001) Growth rate variability and lipofuscin accumulation rates in the blue crab *Callinectes sapidus*. *Mar Ecol Prog Ser* 224: 197-205
- Ju SJ, Secor DH, Harvey HR (2003) Demographic assessment of the blue crab (*Callinectes sapidus*) in Chesapeake Bay using extractable lipofuscins as age markers. *Fishery Bull* 101: 312-320
- Kattner G, Hagen W (1998) Lipid metabolism of the Antarctic euphausiid *Euphausia crystallorophias* and its ecological implication. *Mar Ecol Prog Ser* 170: 203-213
- Lessard, E.J (1993) Culturing marine phagotrophic dinoflagellates. *Handbook of Methods in Aquatic Microbial Ecology* (P.F.Kemp, B.F. Sherr, E.B. Sherr and J. Cole, eds), pp 67-75.
- Lessard, E. J., and M. Murrell (1996) Distribution, abundance, and size composition of heterotrophic dinoflagellates and ciliates in the subtropical Sargasso Sea near Bermuda. *Deep-Sea Res.* 43: 1045-1065.
- Macdonald PDM, Pitcher TJ (1979) Age-groups from size-frequency data: a versatile and efficient method of analyzing distribution mixtures. *J Fish Res Board Can.* 36: 987-1001
- *Mannino A, Harvey HR (2000) Terrigenous dissolved organic matter along an estuarine gradient and its flux to the coastal ocean. *Org Geochem* 31:1611-1625
- *Mannino A, Harvey HR (2004) Black Carbon in estuarine and coastal ocean dissolved organic matter *Limnol Oceanogr* 49: 735-740
- Mayzaud P, Boutoute M, Alonzo F (2003) Lipid composition of the euphausiids *Euphausia vallentini* and *Thysanoessa macrura* during summer in the Southern Indian Ocean. *Antarctic Sci* 15(4): 463-475
- Meier W, Stroeve J, Fetterer F, Knowles K (2005) Reductions in Arctic sea ice cover no longer limited to summer. *Eos Trans AGU* 86(36): 316-317
- Menden-Deuer S, Lessard EJ (2000) Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. *Limnol Oceanogr* 45: 569-579
- Meyer B, Atkinson A, Stübing D, Oetl B, Hagen W, Bathmann UV (2002) Feeding and energy budgets of Antarctic krill *Euphausia superba* at the onset of winter-I. Furcilia III larvae. *Limnol Oceanogr* 47: 943-952
- Nicol S (1990) The age-old problem of krill longevity. *Biosci* 40: 833-836
- Nicol S, Stolp M, Hosie GW (1991) Accumulation of fluorescent age pigments in a laboratory population of Antarctic krill *Euphausia superba* Dana. *J Exp Mar Biol Ecol* 146: 153-161
- *Nguyen RT, Harvey HR (2003) Macromolecular associations preserve proteinaceous material during *B.braunii* decay: evidence from amino acids, SEC and 2D electrophoresis. *Org Geochem* 34: 1391-1403
- *Olson M., Lessard EJ, Wong CJ, Bernhardt MJ (2006) Copepod feeding selectivity on microplankton, including the toxigenic diatom, *Pseudo-nitzschia* spp., in the coastal Pacific Northwest. *Mar. Ecol. Prog. Ser.* 326:207-220.
- Overland JE, Stabeno PJ (2004) Is the climate of the Bering Sea warming and impacting the ecosystem? *Eos Trans AGU* 85(33): 309-310, 312
- Overpeck JT, Sturm M, Francis JA, Perovich DK, Serreze MC, Benner R, Carmack EC, Chapin FS, Gerlach SC, Hamilton LC, Hinzman LD, Holland M, Huntington HP, Key JR, Lloyd AH, MacDonald GM, McFadden J, Prowse TD, Schlosser P, Vörösmarty C (2005) Arctic system on trajectory to new, seasonally ice-free state. *Eos Trans AGU* 86(34): 309, 312-313
- Pond DW, Atkinson A, Shreeve RS, Tarling G, Ward P (2005) Diatom fatty acid biomarkers indicate recent growth rates in Antarctic krill. *Limnol Oceanogr* 50(2): 732-736
- Roff JC, Hopcroft RR (1986) High precision microcomputer based measuring system for ecological research. *Can J Fish Aquat Sci* 43: 2044-2048
- *Roth, L.C. and H.R. Harvey. 2006. Intact protein modification and degradation in estuarine environments. *Mar. Chem.* 102:33-45.
- Rothschild B, Ault J, Patrick E, Smith S, Li H, Maurer T, Daugherty B, Davis G, Zhang C, McGarvey R (1992) Assessment of the Chesapeake Bay Blue Crab Stock. Univ. of Maryland, Chesapeake Bay Biological Lab. CB92-003-036, CEES 07-4-30307, Solomons, Maryland.
- Saether O, Ellingsen TE, Mohr V (1986) Lipids of North Atlantic krill. *J Lipid Res* 27: 274-285

- Sarmiento JL, Slater R, Barber R, Bopp L, Doney SC, Hirst AC, Kleypas J, Matear R, Mikolajewicz U, Monfray P, Soldatov V, Spall SA, Stouffer R (2004) Response of ocean ecosystems to climate warming. *Global Biogeochem Cycles* 18(3): Art. No. GB3003
- Schandelmeier L, Alexander V (1981) An analysis of the influence of ice on spring phytoplankton population structure in the southeastern Bering Sea. *Limnol Oceanogr* 26:935-943.
- Schmidt K, Atkinson A, Petzke K-J, Voss M, Pond DW (2006). Protozoans as a food source for Antarctic krill, *Euphausia superba*: complementary insights from stomach content, fatty acids, and stable isotopes. *Limnol Oceanogr* 51:2409-2427.
- Schneider DC, Hunt Jr. GL, , Harrison NM (1986) Mass and energy transfer to seabirds in the southeastern Bering Sea. *Continental Shelf Res* 5: 241-257
- Secor DH, Dean JM, Campana SE (1995) Recent developments in fish otolith research. Belle W. Baruch Library in Marine Sciences Number 19. Univ of South Carolina Press, Columbia, South Carolina.
- Shaw CT, Feinberg LR, Peterson WT (2004) Molting and growth rates of two species of euphausiids off the Oregon Coast: seasonal, spatial and life stage differences. *ASLO/TOS Ocean Research Conference*. Honolulu, HI.
- Siegel V (1987) Age and growth of Antarctic Euphausiacea (Crustacea) under natural conditions. *Mar Biol* 96: 483-495
- Siegel V (2000) Krill (Euphausiacea) life history and aspects of population dynamics. *Can J Fish Aquat Sci* 57: 130-150
- Siegel V, Loeb V (1994) Length and Age at Maturity of Antarctic Krill. *Antarct Sci* 6: 479-482
- Siegel V, Loeb V (1995) Recruitment of Antarctic Krill *Euphausia superba* and Possible Causes for Its Variability. *Mar Ecol Prog Ser* 123: 45-56
- Smith SL (1991) Growth, development and distribution of the euphausiids *Thysanoessa raschi* (M. Sars) and *Thysanoessa inermis* (Krøyer) in the southeastern Bering Sea. In: Sakshaug E, Hopkins CCE, Øritsland NA (eds) Proceedings of the Pro Mare Symposium on Polar Marine Ecology, Trondheim 12-16 May 1990. *Polar Res* 10: 461-478
- Stabeno PJ, Schumacher JD, Davis RF, Napp JM (1998) Under-ice observations of water column temperature, salinity and spring phytoplankton dynamics: Eastern Bering Sea shelf. *J Mar Res* 56 (1): 239-255
- St. John MA, Lund T (1996) Lipid biomarkers: Linking the utilization of frontal plankton biomass to enhanced condition of juvenile North Sea cod. *Mar Ecol Prog Ser* 131: 75-85
- Stoecker, D. K, D. J. Gifford, and M. Putt (1994) Preservation of marine planktonic ciliates: Losses and cell shrinkage during fixation. *Mar. Ecol. Prog. Ser.* 110: 293-299.
- Stübing D, Hagen W (2003) Fatty acid biomarker ratios-suitable trophic indicators in Antarctic euphausiids? *Polar Biol* 26: 774-782
- Stübing D, Hagen W, Schmidt K (2003) On the use of lipid biomarkers in marine food web analysis: an experimental case study on the Antarctic krill, *Euphausia superba*. *Limnol Oceanogr* 48(4): 1685-1700
- Sturt HF, Summons RE, Smith K, Elvert M, Hinrichs K.-U. (2004) Intact polar membrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry-new biomarkers for biogeochemistry and microbial ecology. *Rapid Commun Mass Spectrom* 18: 617-628
- Tang KW, Taal M (2005) Trophic modification of food quality by heterotrophic protists: species-specific effects on copepod egg production and egg hatching. *J Mar Biol Ecol* 318:85-98
- Vanderploeg HA, Scavia D (1979a) Calculation and Use of Selectivity Coefficients of Feeding – Zooplankton Grazing. *Ecol Model* 7: 135-149
- Vanderploeg HA, Scavia D (1979b) Two electivity indexes for feeding with special reference to zooplankton grazing. *J Fish Res Board Can* 36: 362-365
- Virtue P, Nicol S , Nichols PD (1993) Changes in the digestive gland of *Euphausia superba* during short-term starvation: lipid class, fatty acid and sterol content and composition. *Mar Biol* 117: 441-448
- Walsh JJ, Mcroy CP (1986) Ecosystem analysis in the Southeastern Bering Sea. *Continental Shelf Res* 5: 259-288
- *Yunker, M.B., L.L. Belicka, H.R. Harvey and R.W. Macdonald 2005. Tracing the inputs and fate of marine and terrigenous organic matter in Arctic Ocean sediments: A multivariate analysis of lipid biomarkers. *Deep-Sea Res. II* 52, 3478-3508.