

EXPERIMENTAL AND GENETIC STUDIES OF MEIOFAUNA ASSESS ENVIRONMENTAL QUALITY AND REVEAL MECHANISMS OF TOXICITY

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MEIOFAUNA
POLLUTION
MICROCOSMS
BIOASSAYS
SUBLETHAL EFFECTS
GENETIC RESPONSE

ABSTRACT. – Meiofauna have been used in numerous experiments to assess pollutant effects and to establish standards for water, soil and sediment quality. Many meiofauna can be reared in relatively easy-to-maintain cultures, and over 50 species have been used in laboratory-based bioassay experiments. Meiofauna species may be exceedingly sensitive to pollutants; reproduction and development are typically much more sensitive than adult survival. Sublethal effects of pollutants are becoming commonly studied with meiofauna because meiofauna lend themselves well to experiments that measure ingestion rate, and pollutant effects on fecundity and population density can be predicted from whole-life-cycle experiments with demographic techniques. Toxic responses of sediment-associated meiofauna generally reflect sediment and porewater chemistry. However, some meiofauna may form cryptic species complexes, and differences in tolerance within species and among cryptic species are possible, increasing uncertainty in toxicity tests. A model nematode species (i.e., *Caenorhabditis elegans*) and various model harpacticoid copepods (e.g., *Tigriopus*, *Tisbe*, *Amphiascus*) have been identified, and are increasingly being used in association with a growing baseline of biological, genetic and ecological data to elucidate the mechanisms of toxicity. Meiofauna have also been used in studies that determine acclimatory and genetic responses to contaminant exposure, and that examine gene expression profiles from contaminated environments. Field and microcosm experiments with hazardous substances suggest that meiofauna have many poorly understood species interactions with primary producers, predators and competitors that are manifested as indirect, ecological effects in the presence of contaminants. The continued use of meiofauna in assessment studies for newly discovered environmental concerns is justified.

INTRODUCTION

The seminal review by Coull & Chandler (1992) on meiofauna and pollution examined ~250 papers published before 1992, and summarized the known responses of meiofauna to many toxicants. Since then, the pace of publication has increased, and at least 350 papers have appeared in the peer-reviewed literature. Additional reviews that focus on taxon- (Bongers & Ferris 1999, Nipper & Carr 2003, Ruiz *et al.* 2005, Nigam *et al.* 2006, Sochova *et al.* 2006, Raisuddin *et al.* 2007), pollutant- (Danovaro 2000) and habitat-specific (Traunspurger & Drews 1996, Peterson *et al.* 1996) aspects of contaminant effects have been published. Furthermore, Giere (2009) summarized the responses of meiofauna to pollution in the field, and Kennedy & Jacoby (1999) reviewed the potential use of meiofauna in monitoring programs (see also Somerfield & Warwick, 1996). Relatively new classes of potential pollutants, including endocrine disruptors (Höss & Weltje 2007, Dahl & Breitholtz 2008), and manufactured substances such as nano-particles (Templeton *et al.* 2006, Ferguson *et al.* 2008, Ferry *et al.* 2009, Wang *et al.* 2009, Wong *et al.* 2010) have stimulated additional study. Advances in biotechnology have also inspired new

areas of research (e.g., toxicogenomics) to better understand gene and biochemical responses of meiofauna to pollutants. Here, we review recent laboratory, field and microcosm studies that manipulate the concentration of contaminants to which meiofauna are exposed, and we summarize how this body of research has advanced understanding of the mechanisms of toxicity. We also emphasize the value of meiofauna to assess pollution impacts and establish environmental quality standards. Studies of tolerance and the genetic responses of meiofauna to pollutants are also considered. Finally, we consider how the study of meiofauna may address future environmental concerns.

Why use or why not use meiofauna in pollution studies

Meiofauna are ubiquitous residents of aquatic sedimentary and terrestrial soil environments where they are routinely present in abundances of $\sim 10^6$ - 10^7 individuals per m² and biomasses of ~ 1 g per m². Meiofauna are rich in biodiversity with high species and major taxon diversity. Most of the animal phyla have representatives among the marine meiofauna, and many taxa (e.g., Kinorhyncha, Loricifera, Priapulida, Mystacocarida) are found

exclusively in sediments. These multi-cellular benthic invertebrates are small enough to pass through a 0.5 mm sieve, yet they perform a wide range of ecological roles and have important interactions with other biota. Meiofauna perform critical functions in soil and sedimentary ecosystems by interacting with the physical environment, by regulating microbes including microalgae, as predators of larval macrofauna, and as prey for higher trophic levels (Giere 2009). Energy consumption and secondary production is high; production/biomass ratios for meiofauna are typically higher than for macrofauna. Furthermore, meiofauna have a wide range of physiological capabilities, and responses to hazardous substances are varied. Some species are very tolerant while others are very sensitive to pollutants, especially during reproduction and development. Meiofauna acclimate to and, because of their short generation time, quickly respond to many pollutants by biochemical and genetic modification.

Meiofauna, therefore, offer many advantages as experimental subjects in aquatic ecotoxicology. Kusk & Woltenberger (2007) and Giere (2009) have summarized the advantages of meiofaunal research in research with environmental toxicants:

- Meiofauna are ubiquitous and ecologically important organisms.
- Meiofauna have intimate association with soil/sediment and porewater throughout the life cycle.
- Many highly sensitive species are found among the meiofauna.
- Standard protocols for laboratory bioassays are available for meiofauna.
- Meiofauna exhibit a high diversity of species available for community experiments (e.g., meiofauna are typically more diverse than macrofauna in estuaries or on beaches).
- The genomic sequence is available for the nematode *Caenorhabditis elegans* and is under development for the copepod *Tigriopus japonicus*.
- The life history features of meiofauna simplify laboratory culture and facilitate experimentation because:
 - a) some species thrive in culture without sediment and with low maintenance;
 - b) most are non-cannibalistic;
 - c) rapid population growth is possible in culture;
 - d) sexual reproduction is typical and sex determination possible at low magnification (for copepods);
 - e) larvae and juveniles are easily distinguished (for copepods).
- Meiofauna are cost and time effective research subjects because:
 - f) fewer laboratory supplies are required for routine culture and laboratory experiments;
 - g) little bench/incubator space is required for culture, laboratory, and microcosm experiments;

h) small quantities of toxicants and sediment are needed to expose meiofauna, minimizing use and disposal of hazardous materials;

i) rapid development (about 2 weeks) reduces the time needed to conduct developmental and inter-generational experiments.

The potential for the successful use of meiofauna in bioassays to investigate the effects of pollutants is greatly enhanced because model species have been established. *Caenorhabditis elegans* is a small (~ 1 mm in length and 0.3 µg in dry mass), soil-dwelling nematode that has been studied intensively because of its structural simplicity, ease of culture, habitat plasticity and because it was one of the first species targeted for complete genomic sequencing (Snape *et al.* 2004). Currently, *C. elegans* is being used to investigate a wide range of ecotoxicological issues from the elucidation of the mechanisms of toxicity to the rapid assessment of pollutant impacts in the laboratory and field. Genomic and biochemical responses to pollutants by *C. elegans* are being studied at an increasing rate with advanced biotechnology including gene expression microarrays, (e.g., Liao *et al.* 2002, Liao & Yu 2005, Cui *et al.* 2007, Wang *et al.* 2008, Hughes *et al.* 2009, Menzel *et al.* 2009, Jeong *et al.* 2010, Tvermoes *et al.* 2010). For example, transgenic strains of *C. elegans* have been produced that enhance the sensitivity to toxicants (Chu *et al.* 2005, Roh *et al.* 2006, Ma *et al.* 2009, Jeong *et al.* 2010, Swain *et al.* 2010), and Chu *et al.* (2005) developed a strain with a fluorescent reporter transgene coupled with a double mutant for stress detection resulting in a 10-fold enhancement of sensitivity to cadmium. Gene-expression profiles in *C. elegans* suggest that physiological-based models and transcriptomic analyses can link the mechanisms of action of toxic chemicals with resulting demographic effects (Swain *et al.* 2010). A sophisticated flow-sorting technology recently developed for use with *C. elegans* reproductive and developmental assays holds promise to reduce effort and increase the pace of assessment at contaminated sites (Boyd *et al.* 2009, Boyd *et al.* 2010).

Among the benthic copepods, several model species have been identified and a large body of research has assessed pollutant effects. Most bioassay protocols have been established for species in the harpacticoid genera *Tigriopus*, *Nitokra*, *Amphiascus*, and *Tisbe*. Adult copepods in these genera are about 1 mm in length and 1-2 µg in dry mass, and have been used in many innovative studies examining pollutant impacts. However, the most diverse studies among the copepods have been done with *Tigriopus* species. Unlike most other meiofauna, *Tigriopus* is not infaunal, however, as all life history stages (larvae, juveniles and adults) live in shallow tide pools without sediment on rocky coasts. The genomic toolkit for *Tigriopus* is growing; e.g., a gene microarray has been developed (Ki *et al.* 2009) and the mitochondrial genome has been sequenced (Burton *et al.* 2007). Extensive biochem-

ical information linked to pollutants is also available for *Tigriopus* (Seo *et al.* 2006a, Seo *et al.* 2006b, Lee *et al.* 2007, Lee *et al.* 2008b, Ki *et al.* 2009, Rhee *et al.* 2009, Wang & Wang 2009, Wang & Wang 2010).

Bioassays that incorporate reproductive and developmental endpoints have repeatedly shown that meiofauna may be extremely sensitive to environmental toxicants and compounds associated with the disruption of hormonal properties compared to other model organisms such as cladocerans, fish and amphipods (e.g., Forget *et al.* 1998). In a large comparative study, Greenstein *et al.* (2008) found that the benthic copepod *Amphiascus tenuiremis* was more sensitive than amphipods and 4 other macrobenthic species in toxicity tests with marine sediments and that sublethal toxicity endpoints in *A. tenuiremis* correlated with sediment chemical concentrations. These results suggest that meiofauna are exceptional sentinels of environmental quality and are well suited for assessment studies.

However, there are also many challenges associated with the use of meiofauna in ecotoxicological research. For example, the small body size of meiofauna limits tissue mass for biochemical, genetic and toxicant analysis, although sensitive analytical techniques overcome many of these concerns. Furthermore, genetic and genomic sequence data are unavailable for most species. Other disadvantages in the use of meiofauna as model species include:

Limited knowledge of basic biology and ecology:

The diet, interaction strength with prey, competitors and predators, the response to various environmental factors, and immigration and emigration rates of most species of meiofauna are poorly known. As a result, additional information may be required to properly devise bioassay studies or to interpret results from experimental research such as in microcosms.

Inaccessible taxonomy for the non-specialist and cryptic species: Although data at the level of major taxon may provide sensitive information about the effects of contaminants (Raffaelli 1987, Warwick 1993, Lee & Correa 2005), many studies conclude that species-level data are most effective at detecting toxicant effects (Chandler *et al.* 1997, Millward *et al.* 2004). Species-specific identifications of meiofauna are therefore advantageous in field and microcosm studies but may be daunting to the non-specialist. Morphologically similar cryptic species (possibly with different tolerances) may be common among the meiofauna (Schizas *et al.* 1999, Rocha-Olivares *et al.* 2001), further increasing the importance of taxonomic expertise in contaminant research. Species-specific identification aids are becoming more assessable for many meiofaunal taxa with the advent of sophisticated web-based materials.

Possible high genetic variation within species and among cultures: A potential for bias exists when meiofauna are used in ecotoxicological research. For example,

species that grow easily in culture may not be representative of meiofauna because such “lab rats” may have atypical life history characteristics or tolerances that favor survival and growth in the laboratory. Losses of genetic diversity may occur in culture (e.g., due to population bottlenecks) and the response to pollutants for cultured species may differ over time. Natural genetic variation in geographically distinct populations has been shown to be very high in some species, including the genus *Tigriopus* (Edmands 2001). This high genetic variation could lead to unexpected variation in the response to pollutants (and in assessment of pollutant effects) if cultures are started from widely separate field collections or if cryptic species are present. Duan *et al.* (1997) expressed similar concerns about a freshwater amphipod species commonly used in bioassays. This largely unknown source of variation could be quantified by conducting genetic and routine toxicological screening under standardized conditions (for example, with a given metal species under defined aqueous conditions) within and between populations (and among laboratories) for species used in toxicity tests. Unfortunately, few species (except *C. elegans* and various *Tigriopus* species) have been genetically profiled, and few have been tested in routine assays. Miliou *et al.* (2000) suggested that the tolerances to metals were similar for different populations of *Tisbe holothuriae*; however, more tests should be done to establish baseline variation in genetic composition and tolerance within meiofaunal species used in toxicity testing.

The study of meiofauna has advanced the understanding of many issues associated with pollutant effects and environmental safeguards. Topics below reflect some of these important issues.

Single-species bioassays with meiofauna

Although field-collected meiofauna have been frequently used in bioassays, most of the research to assess pollutant effects relies on species maintained in culture. Many meiofaunal species can be cultured in the laboratory. Some have flexible requirements for food and tolerate a broad range of environmental stressors (e.g., low oxygen) associated with culture conditions (Fleeger 2005). Some are cultured with and, some without sediment, and several species have been identified that require low maintenance but produce the yield of individuals necessary to conduct bioassay experiments. About 50 meiofauna species from culture have been used in toxicity tests, including 15 harpacticoid copepod and 30 nematode species (Giere 2009). More species are being studied each year.

Many environmental factors, some subtle, some not so subtle, affect the response of meiofauna to pollutants; even ciliate epibiotic organisms associated with the cuticle may affect a copepod’s sensitivity to contaminants (Puckett & Carman 2002). Although time consuming,

determining relationships between toxicity and particle size, temperature, salinity, food availability etc. are relatively straightforward with meiofauna (Notenboom *et al.* 1992, Dave *et al.* 1993, Donkin & Williams, 1995, Larrain *et al.* 1998, Miliou *et al.* 2000, Staton *et al.* 2002, Kwok & Leung 2005, Dahl *et al.* 2009, Bollmohr *et al.* 2009, Wang *et al.* 2010), and results from such studies may suggest how best to standardize methods for bioassays. For example, Sibly *et al.* (2000) report that a stressor's effects at high population density may differ from its effects at low density, suggesting that density should be considered as a factor in population assays.

Laboratory bioassay protocols using mortality as an endpoint have been established for many aquatic species in water-only, soil, and sediment media (Rand 1995). Bioassays have been conducted both by adding pollutants to relatively clean (or artificial) sediment, and by testing soil or sediment collected at contaminated field sites. Meiofauna have been widely used in acute tests (e.g., Bongers *et al.* 2001, Ara *et al.* 2002, Lee *et al.* 2007, Pane *et al.* 2008) to generate toxicity data necessary to set standards for toxicant exposure. Meiofauna have also proven to be advantageous test organisms in many ways other than being good sentinels. For example, cultures of the copepod *Tisbe battagliai* have been used at sea in acute bioassays to assess seawater quality along cruise paths in real time (Williams 1992, Kirby *et al.* 1998). Species in the copepod genera *Tisbe*, *Schizopera* and *Tigriopus* are found in many parts of the world, and local species have been developed into regional standard tools for assessing toxicity (Lee *et al.* 2007, Medina *et al.* 2008, Araujo-Castro *et al.* 2009). Many standardized protocols have been promulgated (e.g., a method for *C. elegans* has been authorized by ASTM for acute toxicity tests, ASTM, 2008). Furthermore, toxicity tests with *C. elegans* may be conducted in various media including agar, soil, aquatic sediments and aqueous solutions (Donkin & Williams 1995); several meiofaunal species can be tested in both aqueous and sediment media.

Meiofauna are becoming especially significant and widely used subjects in chronic and sublethal tests. Sublethal toxicity tests usually measure behavior, feeding rate or endpoints based on reproduction and/or development. A wider array of endpoints has been developed for *C. elegans* (e.g., neurobiological endpoints, Xing *et al.* 2009). Meiofauna may be preferred over larger animals in feeding trials because many individuals may be held in a small space, and meiofauna behavior may not be as strongly modified by laboratory artifact relative to more behaviorally complex animals. A review of contaminant effects on feeding in meiofauna is found below. Another distinct advantage for the use of meiofauna is that many species complete their life cycle (from egg to egg) in about 2 weeks under laboratory conditions (although maximum life spans are much longer). This property facilitates the use of reproductive, early-life-history stag-

es, and developmental responses in sublethal tests, which usually increase the sensitivity of the metrics that measure toxicant effects. Early-life-history stages and fertility have been repeatedly shown to be more sensitive than adult survival to toxicants in benthic copepods. For example, Cary *et al.* (2004) noted that the insecticide fipronil at environmentally realistic concentrations caused no significant lethality to adult mating pairs of *Amphiascus tenuiremis* but inhibited reproduction by > 70 % by reducing male fertility. Copepod nauplii are particularly sensitive to toxicants (Green *et al.* 1996, Lotufo & Fleeger 1997, Hack *et al.* 2008a), perhaps because of stress associated with frequent molting. Developmental stages of *C. elegans* also appear to be more sensitive than adults, however not all reports agree (Donkin & Williams 1995, Guo *et al.* 2009); nevertheless, behavioral responses and reproductive endpoints correlate with mortality in toxicant exposures in *C. elegans* and all may be used as sensitive sublethal metrics (Dhawan *et al.* 1999, Anderson *et al.* 2001, Boyd *et al.* 2003, Anderson *et al.* 2004, Wang & Xing 2008). However, fecundity has been shown to be reduced in the marine nematode *Monhystera disjuncta* when exposed to cadmium (Vranken *et al.* 1991).

Reproductive tests with benthic copepods usually initiate exposure to toxicants with adult mating pairs and are completed after 10-28 days. Response variables include the number of offspring (eggs or hatched young), the number of broods, the rate or timing of brood production, sex ratio and egg size. Test protocols have been developed for field-collected meiofauna (Dipinto *et al.* 1993, Chandler & Green 1996), and for many species from culture. Species from culture include *Schizopera knabeni* (Lotufo 1997, Lotufo & Fleeger 1997), *Tigriopus* spp. (Misitano & Schiewe 1990, Forget *et al.* 1998, Pane *et al.* 2008, Bang *et al.* 2009), *Tisbe battagliai* (Hutchinson *et al.* 1999a, Barata *et al.* 2002), *Nitokra* spp. (Lotufo & Fleeger 1997, Breitholtz & Wollenberger 2003), and *Amphiascus tenuiremis* (Strawbridge *et al.* 1992, Green & Chandler 1996, Wirth *et al.* 1998, Kovatch *et al.* 1999, Bejarano *et al.* 2004). A well-defined protocol for a 14-day reproductive test for *A. tenuiremis* is detailed in Chandler & Green (1996), and a test for a freshwater harpacticoid species has been developed (Turesson *et al.* 2007).

Short-term (~4 day) reproductive/developmental tests have been developed for *C. elegans* (Anderson *et al.* 2001, Jonker *et al.* 2004a, Ibiem & Grant 2005, Martin *et al.* 2009, Höss *et al.* 2009, Boyd *et al.* 2010). Other freshwater or soil nematodes have also been used in reproductive assays (Boyd & Williams 2003, Boyle & Kakouli-Duarte 2008). Relatively few reproductive tests with marine species have been conducted even though nematodes frequently thrive in culture (Moens & Vincx 1998); most research has been done with three species, *Monhystera microphthalma*, *M. disjuncta* and *Pellioditis marina* (Vranken *et al.* 1985, Derycke *et al.* 2007). Among meiofaunal polychaetes, *Dinophilus gyrociliatus*

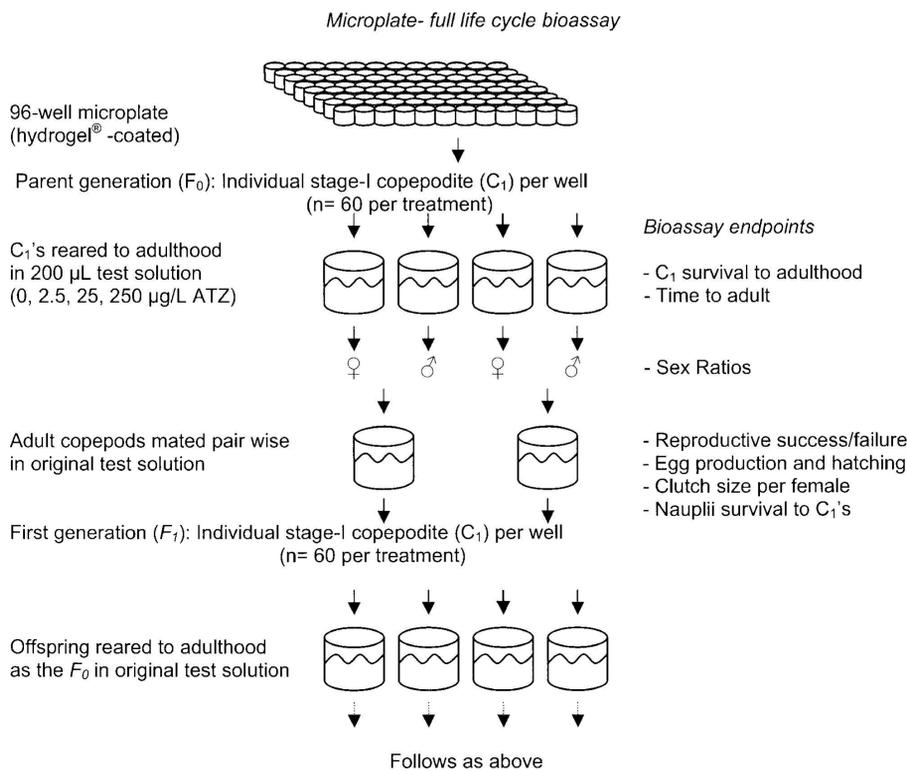


Fig. 1. – Illustration of a microplate, full life-cycle bioassay for use with *Amphiascus tenuiremis* (from Bejarano & Chandler 2003).

is a cosmopolitan species useful for toxicity testing with sediment porewater. Due to its short life cycle, it is suitable for sublethal toxicity tests using egg production by females as a sensitive endpoint, which can be assessed in a 7-day exposure period (Mauri *et al.* 2002, Mauri *et al.* 2003).

Whole-life-cycle tests: Meiofauna have frequently been adopted in whole-life-cycle (WLC) tests. WLC tests increase exposure to sensitive stages and are conducted such that larval or juvenile stages are first exposed to a toxicant, with exposure continuing through a second (F_1) or third generation (F_2) (see Fig.1). Exposure throughout multiple generations yields a sensitive assay with a high diversity of possible endpoints of potential toxicant effects. WLC protocols have been established for several species. Within marine and brackish benthic copepods, species in three copepod genera (*Amphiascus*, [Chandler *et al.* 2004a], *Tisbe*, [Bechmann 1999, Hutchinson *et al.* 1999b], *Nitokra*, [Breitholtz & Bengtsson 2001, Breitholtz & Wollenberger 2003]) are most commonly used in WLC tests. WLC bioassays have recently been developed for other marine copepods including *Robertsonia propinqua* (Hack *et al.* 2008b), *Tisbe biminiensis* (Araujo-Castro *et al.* 2009) and *Tigriopus japonicus* (Marcial *et al.* 2003, Lee *et al.* 2008a), as well as for *Bryocamp- tus zschokkei*, a freshwater copepod (Brown *et al.* 2005). *Tigriopus japonicus* in particular is a very promising species for WLC testing given the large amount of genetic and biochemical information that is available (Raisuddin *et al.* 2007).

Guidelines for WLC tests with copepod species have been developed by the Organization for Economic Cooperation and Development (Gourmelon & Ahtiainen 2007), and have been recently reviewed (Kusk & Wollenberger 2007). Significantly, ASTM guidelines have been generated for a WLC standardized test with *Amphiascus tenuiremis* (ASTM, 2004). *A. tenuiremis* WLC testing has been applied very successfully in several studies using this innovative protocol (Figure 1) (Bejarano & Chandler 2003, Chandler *et al.* 2004a, Chandler *et al.* 2004b, Bejarano *et al.* 2006a, Bejarano *et al.* 2006b).

Effects endpoints for WLC tests are more varied than for reproductive tests, and in addition to mortality, include development time, somatic growth (Dahl *et al.* 2006), various measures of fecundity, reproductive success and even lifespan (Harada *et al.* 2007). It is possible to follow meiofauna as individuals throughout their lifetime and to generate age-specific life tables that have powerful predictive abilities for population growth (Green *et al.* 1995). The population growth parameters r_m (maximum population growth rate) and λ (finite rate of increase of the population over time) have both been estimated in laboratory exposures and population models have been used to project contaminant-induced reductions in population size. Bechmann (1994) and Bechmann (1999) used *Tisbe furcata* life tables to estimate copper toxicity, Breitholtz & Wollenberger (2003), Breitholtz *et al.* (2007) and Lundstrom *et al.* (2010) used r_m to determine pollutant effects on *Nitokra spinipes*, and Chandler *et al.* (2004a), Chandler *et al.* (2004b) and Bejarano *et al.* (2006) pro-

jected the effects of different hydrophobic contaminants on population size in *Amphiascus tenuiremis* with Leslie models (a good discussion of Leslie models may be found in Lundstrom *et al.* 2010). WLC tests are strongly integrative of population responses, and life-table data may be used to address questions of fitness and exposure to contaminants (Kammenga *et al.* 1996).

Meiofauna have also been the subject of studies designed to evaluate the metrics used to assess effects, and have advanced the understanding of population responses to pollutants. For example, population parameters indicative of fitness have been shown not to be determined by the life-cycle trait most sensitive to a toxicant (Kammenga *et al.* 1996, Breitholtz & Wollenberger 2003), suggesting that responses to toxicants may best be described by r_m or λ . In addition, Rhodes *et al.* (2008) used *Tigriopus japonicus* in an inter-generational experiment and a sophisticated Bayesian mixing model to determine copper effects on reproductive output. They concluded that copper affects both ovisac maturation rate and the number of nauplii per ovisac, and that exposure to copper in the parent generation negatively affects current generation reproductive output.

Standardized toxicological tests that maintain meiofauna in microplates have been developed for reproductive and WLC tests (Höss *et al.* 2001, Chandler *et al.* 2004a, Brown *et al.* 2005, Bejarano *et al.* 2006a, Bejarano *et al.* 2006b, Templeton *et al.* 2006). These water-only, array-based tests are usually conducted in plates with from 24-96 wells, and $\sim 300 \mu\text{L}$ in volume. Figure 1 (from Bejarano & Chandler 2003) illustrates how multiple endpoints can be obtained from a microplate-based test by following individuals through development. Microplates function to simplify tracking of individuals, facilitate the visualization of meiofauna, increase the number of specimens exposed, and reduce laboratory and environmental chamber space necessary to conduct tests. In some bioassays, the same plate is used to rear copepod nauplii and then for mating trials from copepodites produced (Bejarano *et al.* 2006a). Cary *et al.* (2004) followed > 700 first-stage juveniles of *A. tenuiremis* in one experiment using microplates, highlighting their potential to facilitate exposure studies.

Sediment bioassays: Meiofauna are also becoming an important group of choice in toxicity bioassays using sediment, and protocols for sediment-based tests are available for several species (e.g., Chandler 1990, Lotufo & Fleeger 1997, Lotufo 1997, Kovatch *et al.* 1999, Kovatch *et al.* 2000, Chandler & Green 2001, Fleeger *et al.* 2007, Hack *et al.* 2008b). *Tigriopus* species have not been used in sediment bioassays, but may be tested with sediment-derived elutriates in water-only exposures. Sediment-dwelling meiofauna are in intimate contact with sediment and porewater without larval dispersal, and most non-annelid meiofauna consume microflora and detritus rather than bulk sediment (Green *et al.* 1993). Some meiofauna

have been shown to tolerate sandy and muddy particle sizes, suggesting that the use of single species across a wide range of sediment types is possible (Araujo-Castro *et al.* 2009). Relatively small amounts of contaminated sediment are needed for experiments, thus reducing the amount of hazardous material that must be disposed. Previous research suggests that sediment-quality guidelines developed from meiofauna will be protective of the environment (Pane *et al.* 2008), and meiofaunal assays in sediment will likely grow in prominence. For example, Hose *et al.* (2006) examined differences in the toxicity of sediment tested in the laboratory and *in situ* and concluded that toxicity of sediments in laboratory tests with macrofauna was substantially less than their toxicity *in situ*; overlying water may contribute to this relationship through additional contamination and toxicity. Toxicity to meiofauna may be highly correlated with sediment pollutant concentrations (see Greenstein *et al.* 2008) because of the increased importance of pollutant uptake via porewater. Carriers (e.g., silica gel) for toxicants and artificial soil or sediment have also been used to expose meiofauna to contaminants to reduce variability associated with variation in bioavailability among different sediments (Peredney & Williams 2000, Breitholtz *et al.* 2007, Karlsson *et al.* 2008).

The short generation time of meiofauna also aids in the examination of stage-specific acute toxicity. Stage-specific tests use different starting ages/stages in bioassays that extend to a whole generation (e.g., larvae to larvae, juvenile to juvenile, adult to adult) (Chandler & Green 2001). Such tests are important because hydrophobic organic contaminants differ in adsorption-desorption characteristics, and thus bioavailability can differ as contaminants age in association with sediment (Lu *et al.* 2003). If this occurs over the time of the test, different stages are will be exposed to different intensities of exposure, and tests with different starting points may be used to normalize effects of contaminant ageing. Such tests are impractical with many macrofaunal species given their long generation times.

Effects of pollutants on feeding in meiofauna

Feeding is among the most basic of physiological functions, and several researchers have measured feeding activity as a sublethal endpoint in toxicological assays or as an indicator of direct or indirect effects of contaminants in ecotoxicology experiments. Feeding assays can be used to assess short-term responses to contaminant exposure and provide a useful context for assays of lethality (e.g., LC50 experiments, which are commonly conducted over 96 hours) and WLC experiments (which commonly require weeks to complete). There are various ways that feeding assays can be conducted. Lotufo (1998b) fed ^{14}C -labeled algae to harpacticoid copepods exposed to individual poly-cyclic aromatic hydrocarbon (PAH)

compounds in laboratory assays and observed a 50 % decrease in feeding rate when exposed to fluoroanthrene for only 27 hours, and Lotufo (1997) found that grazing was inhibited at phenanthrene and fluoroanthrene concentrations approximately 4 times lower than lethal doses (Lotufo 1997). Silva *et al.* (2009) used ¹⁴C-labeled benthic diatoms to determine the toxic effects of sediments contaminated with mixtures of metals (lead, cadmium, and mercury) and phenanthrene. Their results indicated that cadmium-phenanthrene mixtures acted independently to inhibit grazing, whereas lead-phenanthrene and mercury-phenanthrene combinations had an additive influence on grazing reduction. Saiz *et al.* (2009) used clearance rates of protozoan prey to determine the influence of PAHs on a cyclopoid copepod. Similarly, Barata *et al.* (2002) used the “cell difference method” to examine the influence of fluoroanthrene, cypermethin and deltamethrin on *Tisbe battagliai*. Feeding rates can also be inferred from defecation rates. Lotufo & Fleeger (1996) used fecal production as a proxy for feeding rates in *Limnodrilus hoffmeisteri*, a deposit-feeding, macrofaunal oligochaete and again observed that feeding rates were significantly reduced by PAH (pyrene and phenanthrene). Hjorth & Dahllöf (2008) showed that the gut content of an arctic copepod harpacticoid copepod decreased with increasing pyrene concentration.

Feeding rates have also been used in experiments involving microcosms of natural communities as an indication of direct and indirect contaminant effects. Carman *et al.* (1995, 1997, 2000b) used ¹⁴C-bicarbonate to synoptically label benthic microalgae (BMA) and measure grazing rates in a series of microcosm studies investigating the effects of petroleum hydrocarbons and metals on saltmarsh benthic communities. This technique can be used to determine per capita grazing rates as well as proportional grazing impact on the total BMA community. For example, Carman *et al.* (1997) showed that diesel fuel-contaminated sediment dramatically reduced meiofaunal grazing pressure on benthic microalgae which ultimately led to high BMA biomass; reduced grazing was primarily due to high copepod mortality. However, a tolerant copepod species and nematodes as a group exhibited transiently enhanced individual grazing rates on BMA, suggesting a competitive release. Sundback *et al.* (2010) conducted “physiological” grazing assays as part of their microcosm experiment examining the combined effects of PAH and nutrients. Naturally occurring BMA were pre-labeled with ¹⁴C then offered to meiofauna isolated from the microcosms. Sundback *et al.* (2010) observed reduced grazing rates in pyrene-contaminated sediments, and reduced grazing rates were correlated with enhanced BMA biomass in microcosms in which nutrients were enriched. These observations are consistent with Danovaro’s (2000) conclusion that oil spills may stimulate increases in BMA and that bottom-up effects occur on the meiofauna via the sediment-based microbial loop. How-

ever, Alsterberg *et al.* (2007) determined that, while copper pyrithionone negatively affected meiofaunal grazing on BMA in microcosm experiments, the reduced grazing did not lead to increased algal biomass.

When algae are consumed and digested, the chlorophyll *a* within them is degraded to pheopigments, and Chl *a*: pheopigment ratios have been used as an indirect indication of grazing impact in contaminant studies. For example, Carman *et al.* (1997) observed higher Chl *a*: pheopigment ratios in microcosms with low grazing pressure. Similarly, Bennett *et al.* (1999) found elevated Chl *a*: pheopigment ratios in sediment contaminated with produced water (a mixture of water, petroleum hydrocarbons and metals).

Bioaccumulation and bioavailability studies with meiofauna

Highly hydrophobic compounds such as pesticides, herbicides, polychlorinated biphenyls (PCB), PAH, and endocrine-disrupting chemicals bind to the organic carbon fraction of suspended particles and accumulate in the sediment. Many heavy metals bind to humic acids and clay particles and also accumulate in the sediment. Therefore, benthic animals may be exposed to higher concentrations of toxicants compared to pelagic species. However a chemical may or may not be available for uptake depending on the properties of the chemical and the medium in which it is found. This property is measured as contaminant bioavailability (Donkin & Dusenbery 1994, Traunspurger & Drews 1996, National Research Council Committee on Bioavailability of Contaminants in Soils and Sediments, 2003), and meiofauna have been used to better understand the relationship between bioavailability and the effects of hazardous substances. Meiofauna live either in the interstitial spaces or burrow through sediment, and infauna may bioaccumulate contaminants into tissues from porewater, overlying water, food and/or sediment. Knowledge of the pathways and rates of a chemical’s bioaccumulation into and elimination from tissues (i.e., toxicokinetics) provides important information regarding toxicity and toxic effects. For example, bioaccumulation data may be used to predict thresholds of toxic effects by the Critical Body Residue Theory (Lotufo 1998a) or used to test the efficacy of models (including Equilibrium Partitioning Theory, the Biotic Ligand Model or bioavailability models) that predict sediment-quality criteria from chemical data. Bioaccumulation data are also needed to evaluate the potential for trophic transfer of contaminants from meiofauna to their predators.

Tissue concentrations: The small body size and tissue mass of meiofauna has slowed but not prohibited the measurement of contaminant body burden, bioaccumulation, bioconcentration and biota-sediment accumulation factors (Lotufo 1998b, Klosterhaus *et al.* 2002). It is possible to directly measure tissue pollutant concentrations using

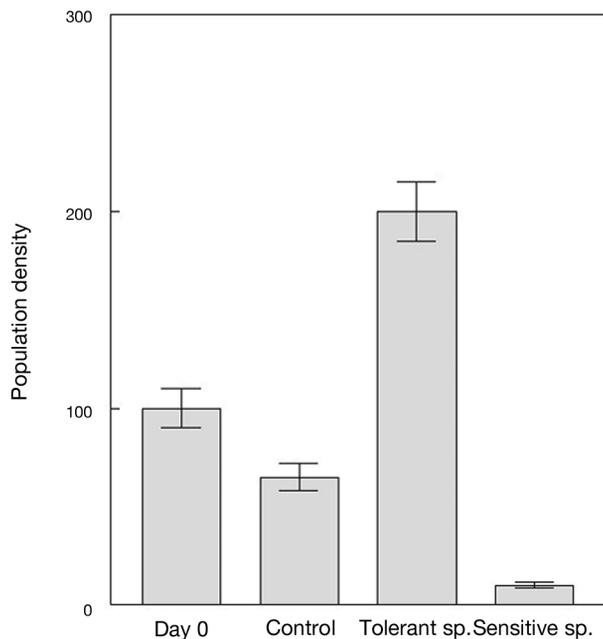


Fig. 2. – Hypothetical response of sensitive and tolerant meiofauna due to an indirect effect after application of a contaminant. Day 0 represents population density at the time of the experiment initiation for the tolerant species. Control is the abundance of the tolerant species at the end of the experiment in treatments without the addition of contaminants. Tolerant sp. refers to the abundance of the tolerant species at the end of the experiment in treatments with the addition of contaminants experiencing an indirect effect. The abundance of a sensitive species at the end of the experiment in treatments with the addition of contaminants is shown for comparison.

standard analytical techniques that require collection of a relatively low number of samples, e.g., all animals from a few sediment subsamples (Fichet *et al.* 1999). However, Wirth *et al.* (1994) used a very sensitive analytical technique to measure PCB in as few as 20 copepod specimens (about 25 μg of dry tissue), and Klosterhaus *et al.* (2002) succeeded in measuring PAH concentration in tissue samples with as little as 10 pg of PAH. Alternatively, single-compound tracers with radioactive labels may be used to measure the uptake and loss of specific compounds (Lotufo 1998a, Lotufo 1998b, Lu *et al.* 2003).

Studies with PAH (Lotufo 1998b, Klosterhaus *et al.* 2002), pesticides (Klosterhaus *et al.* 2003), and PCB (Wirth *et al.* 1994) suggest that meiofauna take up the majority of hydrophobic contaminants from porewater, that tissue concentrations increase with increasing tissue lipid content, and that bioaccumulation from sediments is closely related to chemical hydrophobicity. The reproductive cycle greatly affects tissue lipid content and therefore contaminant uptake in meiofauna. Reproduction may mobilize lipids and lipid-soluble compounds, possibly passing toxicants from females to eggs and thereby reducing toxicant concentrations in reproductive females. Males have no comparable mechanism of depuration and may thus be more sensitive to lipid-soluble

contaminants. Tissue bioaccumulation in meiofauna may be predicted from porewater concentration, although normalization for tissue lipid concentration and porewater dissolved organic carbon is required (Ferguson & Chandler 1998, Klosterhaus *et al.* 2002). The small body size of meiofauna contributes to a rapid rate of bioaccumulation, and tissue equilibrium may be reached in less than 12 h of exposure for compounds with low hydrophobicity (Lotufo 1998a). It is relevant to note that the factors that affect toxicokinetics in small annelids (mostly freshwater oligochaetes) have been studied intensively (Ankley *et al.* 1994, Kukkonen & Landrum 1995, Landrum *et al.* 2002, Leppanen & Kukkonen 2004). Many annelids are bulk deposit-feeders (compared to selective feeding nematodes and copepods) and have digestive processes that may greatly increase the assimilation efficiency of contaminants from sediment (Penry & Weston 1998). Such deposit feeders are sometimes tolerant of high tissue concentrations of contaminants (Millward *et al.* 2001a). Even though the rate of uptake by deposit feeders may be high, excretion or depuration may also be high, allowing Equilibrium Partitioning Models to adequately predict tissue levels and establish effects criteria from porewater concentrations (Kraaij *et al.* 2002, Lu *et al.* 2003, Lu *et al.* 2004a).

Sediment vs. water tests: Some pelagic species cannot tolerate sediment and some sediment-dwelling animals (e.g., tube dwellers) do not readily tolerate water-only conditions. Meiofauna are often capable of living in both media (see Traunspurger *et al.* 1997, Bejarano *et al.* 2004, Cary *et al.* 2004) which permits comparisons of responses of the same species in sediment and water. Direct comparisons between toxic responses in water-only and sediment exposures may provide important information about how contaminants partition between sediment, porewater and overlying water and suggest which mode of exposure contributes most to toxicity (Green *et al.* 1993, Chandler *et al.* 1994, Donkin & Dusenbery 1994, Höss *et al.* 2001, Klosterhaus *et al.* 2003, Araujo *et al.* 2009). Results suggest that although porewater exposure is generally most important, the relative importance of different sources depends on many factors, including feeding mode of the animal and the chemical properties of the toxicant; species specificity is common (Chandler *et al.* 1994). Studies with oligochaetes suggest that the importance of sediment ingestion as a route of exposure relative to porewater increases as the hydrophobicity of organic contaminants increases (Lu *et al.* 2004).

Effects of organic matter: One factor known to confound studies of contaminant bioavailability is dissolved organic matter (DOM) including dissolved organic carbon (DOC). Hydrophobic contaminants in porewater bind to DOC and metals react with organic substances such as humic acid. As a result, most studies suggest that increasing levels of DOM lead to reduced toxicity (Bresler & Yanko 1995, Haitzer *et al.* 1999a), although many coun-

ter-intuitive findings exist. For example, Bejarano *et al.* (2005a) found that DOM reduces toxicity of 2 pesticides but increases toxicity to male *Amphiascus tenuiremis* in a third pesticide (perhaps because DOM reduces light penetration altering pesticide photolysis). The chemical composition of DOM affects its ability to bind contaminants (Haitzer *et al.* 1999b) as does the time of contact (Haitzer *et al.* 1999b). Diet is also important as some contaminants may associate with food which may be an important route of exposure (Höss *et al.* 2001, Offermann *et al.* 2009).

Nano-materials: Potentially novel contaminants have been studied with meiofauna to anticipate environmental consequences. Single-wall carbon nanotubes (SWNT) are very small particles (~1 nm in diameter but up to hundreds of micrometers in length) that are increasingly being used in industrial and biomedical applications. SWNT could someday be released into the environment in large quantities. Templeton *et al.* (2006) demonstrated that these small particles may cause toxicity to *Amphiascus tenuiremis* but only at environmentally unrealistically high concentrations. Ferguson *et al.* (2008) further demonstrated that SWNT are ingested but not assimilated by *A. tenuiremis*. However, carbon nanotubes have a high affinity for highly hydrophobic contaminants (HOCs) and the possibility exists that they could increase the assimilation of HOCs after ingestion, increasing toxicity to benthic organisms. Ferguson *et al.* (2008) found that carbon nanotubes did not increase the bioaccumulation of HOCs in *A. tenuiremis* and, in fact, significantly reduced bioaccumulation of HOCs in *Streblospio benedicti*, an estuarine polychaete.

Trophic transfer: Refractory hydrophobic contaminants and metals that accumulate in the tissues of meiofauna are able to be taken up and bioaccumulated in predators via trophic transfer. Dipinto (1996) and Dipinto & Coull (1997) studied the route of exposure of toxicants to a benthic-feeding fish (spot, *Leiostomus xanthurus*) that captures and consumes meiofauna from sediments. They collected meiofauna with high tissue levels of PCB and a pesticide from contaminated sediments, and they measured bioaccumulation in spot after consumption of only the contaminated meiofauna as well as from contaminated sediment without meiofauna. Bioaccumulation from contaminated meiofauna occurred but was low compared to the contaminant body burden acquired during the process of feeding. The fish's sediment-biting behavior brings the fish's mouth, gut and skin into contact with contaminated sediment and leads to a high bioaccumulation rate (amounting to a five-fold increase over that from contaminated meiofauna). However, predators may sense PAH and avoid feeding in contaminated sediments, and Marshall & Coull (1995), Hinkle-Conn *et al.* (1998) and Street *et al.* (1998a) examined whether fish feeding on meiofauna was influenced by PAH-contaminated sediment. Such avoidance behavior would reduce contaminant exposure. Hinkle-Conn *et al.* (1998) found

no evidence that spot alters its feeding behavior at moderate to high PAH concentrations in the laboratory. Marshall & Coull (1995) found greater removal of meiofauna by spot in uncontaminated sediments, but the difference was so small that it was probably not energetically significant. Street *et al.* (1998a) concluded that spot does not avoid predation on meiofauna at contaminated field sites. Juvenile spot consumes thousands of meiofauna individuals per day (Feller & Coull 1995) by concentrating feeding on high density patches of meiofauna (McCall & Fleegeer 1993), and is therefore at risk for both lethal and sublethal PAH effects because it does not avoid contact with contaminated sediment while feeding on meiofauna. However, feeding on suspended meiofauna by the darter goby (*Gobionellus boleosoma*) was reduced at very high levels of PAH probably due to the narcotic effect of PAH (Gregg *et al.* 1997). Less is known about the specifics of metal trophic transfer from meiofauna to predators, although research with oligochaetes has improved our understanding of metal availability to predators (Wallace and Lopez 1997). Because meiofauna are such important prey to many juvenile fish and shellfish, it seems likely that pollutants will affect many aspects of the predator-prey interaction.

Metals: Research with meiofauna has also enhanced our understanding of how metal speciation influences species relationships with toxicity to benthic species (Tatara *et al.* 1997, Tatara *et al.* 1998). Millward *et al.* (2001b) examined the effects of copper speciation on deposit-feeding macrofauna and meiofauna. Deposit-feeding macrofauna were more sensitive than meiofauna to metal pollution, probably because of exposure from ingested sediment. Copper effects on harpacticoid copepods were directly related to the fraction of free copper ions available in porewater. Hagopian-Schlekat *et al.* (2001) suggest that the bioavailability of metals in the oxygenated surface sediments where meiofauna live is probably controlled by organic-rich particles, porewater DOC, and reduction/oxidation reactions that occur among metals and among common binding compounds such as Fe and Mn oxides. Acid-volatile sulfides may also control porewater concentrations of metals but are most abundant in anoxic zones below the depths in which most meiofauna live. Metal bioaccumulation may differ among meiofaunal taxa; Fichet *et al.* (1999) found that nematodes accumulated higher body burdens of metals than copepods. Finally, meiofauna interactions with the sediment by bioturbation may increase the porewater metal concentration, increasing toxicity (Green & Chandler 1994).

Microcosms and indirect effects with meiofauna

Microcosms are model ecosystems ranging from small laboratory vessels to larger outdoor artificial habitats such as experimental streams or ponds (which are sometimes called mesocosms). Microcosms may be seeded with

specified communities or initiated with indigenous organisms, e.g., with sediment and its natural complement of biota obtained from the field. The natural environment is simulated in microcosms, to a greater or lesser degree, to mimic conditions typical for the habitat of interest. Factors such as toxicant concentration and frequency of application are easily controlled and many types of experimental manipulations are possible. Some microcosms have no direct connection to the environment, and changes in population size of the species of interest in microcosms are due to a combination of mortality and reproduction rather than migration. Investigators have tested for the adequacy of microcosm design or have used microcosm designs with established protocols (Suderman & Thistle 2003, Bejarano *et al.* 2005b). Although microcosm studies often examine responses at multiple trophic levels, meiofauna are particularly well suited to microcosm studies because they are relatively easy to manipulate and may thrive in such conditions. Quantities of sediment needed to establish meiofaunal microcosms are relatively small (~ 1 L or less) and manageable. Besides establishing risk from or sediment-quality criteria for a toxicant (e.g., tributyltin, Austen & McEvoy 1997a, or metals, Parmelee *et al.* 1993), microcosm experiments are also used to experimentally verify the causative agent of change in the field by mimicking the level and type of suspected pollutants. The investigator may note if effects on the community composition in contaminated microcosms result in a community that is similar to that at contaminated field sites. A good example is found in the study by Lee & Correa (2007) in which the effects of copper mine tailings on meiofauna were examined to establish copper porewater concentration as the causative agent of effects at field sites. Microcosms are also useful in determining the mechanisms by which pollutants influence community structure and function, which may provide basic insight into benthic ecology.

Although meaningful information is derived from microcosms, they have shortcomings. For example, microcosms cannot include all naturally occurring species, and at least some important predators, competitors or bioturbators will likely be excluded. Furthermore, some species do not thrive in microcosms, and environmental conditions cannot be perfectly mimicked (Carpenter 1996). As a result, changes in abundance sometimes occur in ways unrelated to toxicant concentration and "microcosm effects" are commonly observed. For example, Suderman & Thistle (2003) found that copepod abundance in experimental microcosms without toxicants increased while nematode abundance did not vary over time, and Carman *et al.* (1997) found that one species of copepod, *Coullana* sp., declined in abundance quickly while other species were not affected in saltmarsh microcosms without contaminants. Microcosm controls are thus essential so that changes between toxicant exposed and non-exposed microcosms can be compared to isolate

effects. For example, a doubling of a particular species may occur in controls during the course of the experiment and the effect of a toxicant may be to reduce this growth. Natural variability in meiofaunal populations is high and microcosm replication is essential. Another way to relate changes in density to toxicant exposure is to conduct concurrent bioassays of species from the microcosm. Such tests would allow one to relate change in abundance to toxicant exposure or to suggest that changes in abundance are unrelated to toxicants. This is rarely done (but see Traunspurger *et al.* 1996) and would benefit microcosm studies in which some species increase while others decrease in toxicant exposures (e.g., Carman *et al.* 1997, Chandler *et al.* 1997).

Sensitive vs. resistant species and indirect effects: Microcosm, field and laboratory studies have documented a great range in tolerance (spanning 1-3 orders of magnitude) among meiofauna in almost all communities, and these "toxicant-sensitive" and "toxicant-resistant" species frequently coexist. Genetic adaptation or biochemical differences associated with an acclimation response, or some combination of the two, are likely responsible for differences in sensitivity. The difference in tolerance contributes to indirect effects that propagate through communities. In addition, several studies suggest that a species tolerant of one type of contaminant may not be tolerant to others, e.g., tolerance to PAH might not be positively correlated with tolerance to metals within a species (Kamenga *et al.* 1994, Millward *et al.* 2004, Gyedu-Ababio & Baird 2006, Beyrem *et al.* 2007). Some meiofaunal communities are very tolerant, while others are much more sensitive to toxicants (Austen & McEvoy 1997b, Carman *et al.* 2000b), perhaps because tolerance patterns vary among species from community to community. Similarly, large variations in tolerances among species in nature have been found in response to events such as oil spills (Danovaro 2000).

When a sensitive species is affected by a toxicant, possible outcomes include local extirpation, reduced abundance or altered behavior (Fleeger *et al.* 2003). Tolerant species may in turn be affected not by the toxicant but by a resulting indirect ecological effect modulated by the sensitive species. Meiofauna communities are species rich and appear to have a great potential for species interactions that lead to indirect effects. Indirect effects initiated by the reduction in abundance in taxa that do not thrive in microcosms may alter the way toxicants influence other species, reducing the certainty of causation in microcosm studies. These indirect effects may extend to other trophic levels and contribute to trophic cascades that reach to primary producers (e.g., Carman *et al.* 1997). Impacts due to indirect toxicant effects may be greater than the direct effects of toxicants (Fleeger *et al.* 2003, Ekschmitt & Korthals 2006, Alsterberg *et al.* 2007).

A very commonly observed indirect effect occurs when a small number of tolerant species increase in abundance

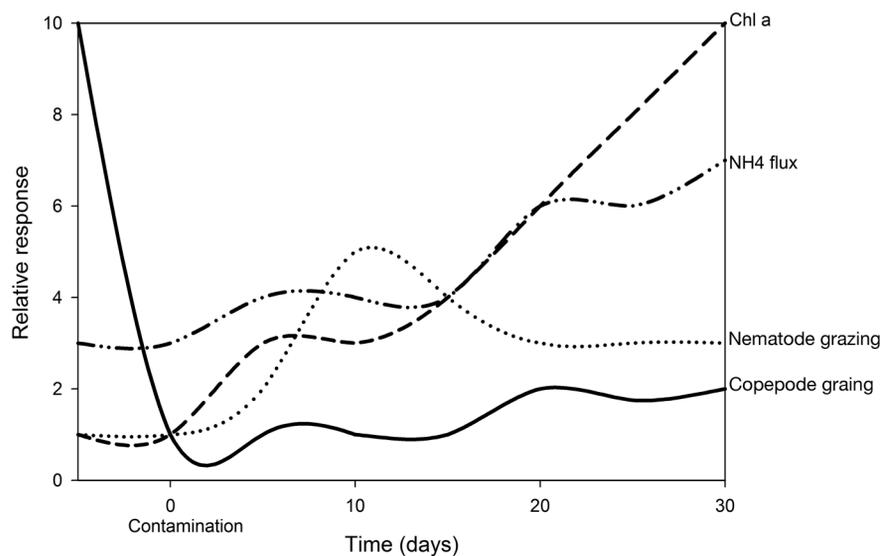


Fig. 3. – Time course of changes in chlorophyll a concentration, NH_4 flux and the grazing rates of nematodes and copepods following the introduction of a toxicant (on day 0) over 30 days.

when hazardous substances are added to microcosms (Sundelin & Elmgren 1991, Austen *et al.* 1994, Carman & Todaro 1996, Austen & McEvoy 1997a, Austen & McEvoy 1997b, Chandler *et al.* 1997, Carman *et al.* 1997, Carman *et al.* 2000b, Gustafsson *et al.* 2000, Schratzberger *et al.* 2002, Höss *et al.* 2004, Millward *et al.* 2004, Mahmoudi *et al.* 2005, Fleeger *et al.* 2006a, Hedfi *et al.* 2007, Mahmoudi *et al.* 2007, Hermi *et al.* 2009, Beyrem *et al.* 2010). Figure 2 shows a hypothetical example; note that abundance of the tolerant species can increase relative to controls and initial values, and compared to a sensitive species. Similar species-specific increases in abundance have been found in the field following events such as oil spills (Fleeger & Chandler 1983, Danovaro 2000), and large changes in relative abundance patterns of dominant species have been documented at field-contaminated sites (Giere 2009). The cause of such increases is rarely studied explicitly but could be due to top-down effects (e.g., decreases in predation), bottom-up effects (increases in food supply because sensitive grazers are reduced increasing primary producer biomass or because nutrients become more available stimulating primary producers), or reduced competition. Increases in abundance following pollutant application have been found in most taxa including harpacticoids (Carman *et al.* 1997), nematodes (Zhang *et al.* 2006, Hermi *et al.* 2009) and foraminiferans (Gustafsson *et al.* 2000), especially at low levels of contaminant addition.

An interesting example of an indirect effect was found in a microcosm study by Sundelin & Elmgren (1991). They found that sediment-dwelling amphipods are very sensitive to cadmium. Amphipods are abundant at the site studied and bioturbate the sediment which, by itself, has a strong influence on meiofauna communities. They also prey on some meiofauna. Sundelin & Elmgren added amphipods to microcosms with and without cadmium,

and results indicated that the effects of cadmium differed strongly in the presence and absence of amphipods because of the ecological effects exerted by amphipods. Similarly, Fleeger *et al.* (2006a) found that bioturbation by a fish species alters meiofaunal responses to hydrocarbon and metal contamination. Food-web-based indirect effects may also explain observations from field studies. After the initial mortality from hydrocarbons, microbial stimulation is likely (e.g., Hedrick *et al.* 2009, Carman *et al.* 1996). This may favor increases in single-celled organisms and meiofauna as more food becomes available. However, oil-spill responses are highly variable because the dose, exposure, bioavailability and fauna of the area vary from spill to spill.

Indirect effects are difficult to predict and quantify, and effects may differ in neighboring habitats (Fleeger *et al.* 2008). The cause of indirect effects among meiofauna is difficult to discern, and few experiments have been explicitly designed to examine causation (Fleeger *et al.* 2003). Carman *et al.* (2000a) isolated two kinds of contaminant-induced indirect effect on the BMA community (Fig. 3); a smaller, short-term effect from reduced grazing by meiofauna and a larger, longer-term effect by stimulation of the microfloral community that changed the cycling and transport of nutrients. Similar results were found by Petersen *et al.* (2009) and Sundback *et al.* (2010) who observed that BMA increased in association with pyrene application in shallow-water sediments. Alsterberg *et al.* (2007) also found that BMA increased when copper pyrithione was added to microcosms but that meiofaunal grazing was not reduced; toxicant-induced changes in nutrient cycling may contribute to the effect (Carman *et al.* 2000b, Sundback *et al.* 2007). Finally, toxicants offer the potential to better understand the basic ecology of meiofauna by removing toxicant-sensitive species selectively. For example, Carman *et al.* (1997) were able to

estimate the fraction of the BMA community consumed by grazing meiofauna because diesel fuel caused mortality to harpacticoid copepods, the most important grazers in that system.

Field manipulative experiments with meiofauna

Experiments conducted in the field allow for natural environmental variation and minimize unintended variation between treated and reference experimental units. Contaminant addition may be controlled by releasing known amounts of hazardous substances into the environment directly or by using azoic sediment amended with contaminants. The release of hazardous substances into the field (e.g., a controlled oil spill, Fleeger & Chandler 1983) has been conducted in a few instances. One recent example is the release of small quantities of liquid CO₂ into deep-sea benthic environments (Barry *et al.* 2004, Carman *et al.* 2004, Thistle *et al.* 2005, Fleeger *et al.* 2006b, Thistle *et al.* 2007, Sedlacek *et al.* 2009, Bernhard *et al.* 2009a, Ricketts *et al.* 2009, Bernhard *et al.* 2009b, Fleeger *et al.* 2010). Meiofauna are very useful subjects in such experiments because their small size facilitates sampling in close proximity to the release point, and they are typically poor dispersers with little ability to escape toxicants. Results suggest that CO₂ sequestered in this way to mitigate the Greenhouse Effect on a broad scale will have strong mortality effects on many taxa of meiofauna and single-celled eukaryotes. CO₂-rich seawater near the site of release experienced significantly reduced pH that probably contributed to mortality in release experiments. However, dose-response relationships between meiofauna and acidity are poorly known, and further research in this area would be useful in the evaluation of the potential impact of carbon sequestration in the deep sea.

Alternatively, azoic contaminated sediment may be placed into containers designed to monitor the colonization of meiofauna (Verdonschot & Braak 1994, Korthals *et al.* 1996a, Korthals *et al.* 1996b). Such studies usually find reductions in the rate of colonization into contaminated sediment (Christie & Berge 1995, Fleeger *et al.* 1996, Watzin & Roscigno 1997, Schratzberger *et al.* 2003, Saunders & Moore 2004, Gwyther *et al.* 2009), although species-specific responses may be highly variable (Decker & Fleeger 1984, Chandler *et al.* 1997). The exact reasons why toxicants typically slow colonization are unclear; colonizing meiofauna from the water or sediment may avoid contaminated sediments or individuals may suffer a higher mortality after colonization into toxicant-enriched sediments. Intermediate exposures have been shown to increase colonization rate (Alongi *et al.* 1983, Decker & Fleeger 1984), perhaps by indirect effects or hormesis. A different but interesting approach was developed by Mirto & Danovaro (2004) who placed faunal collectors (without toxicants) at contaminated and nearby non-contaminated areas. Colonization rates were

decreased at contaminated areas presumably because lower densities and species diversity at contaminated sites lead to lower rates of migration.

Carbon dioxide and meiofauna

CO₂ released by the burning of fossil fuels is dissolving into the oceans from the atmosphere at an increasing rate, and, because of the carbonate-bicarbonate chemistry of seawater, ocean acidity is currently increasing at unprecedented rates. How will increasing acidity over the next century affect meiofauna, and how will increased acidity alter contaminant bioavailability and pollutant effects? To date, studies of CO₂ and acidification effects on meiofauna are rare and somewhat contradictory. Takeuchi *et al.* (1997) found that a decrease of pH to 5.4 was necessary to cause significant effects (approximately 50 % mortality after 1 day of exposure and about 90 % mortality after 4 days of exposure for three species of shallow, subtidal nematodes). Widdicombe *et al.* (2009) suggest that coastal nematode communities will be altered after only 2 weeks of exposure at the pH (7.3) expected in the next century, but at pH 7.5, a subtidal nematode community was unaffected by high CO₂ after several days of exposure (Dashfield *et al.* 2008). Kurihara *et al.* (2007) observed no significant differences in the abundance of total meiofauna, nematodes, harpacticoid copepods (including adults and copepodites) and nauplii after exposure to concentrations of CO₂ 2000x higher than today. Pascal *et al.* (2010) measured the susceptibility of two coastal species of benthic copepods to changes in pH and predict that both should be able to tolerate the expected change in ocean pH by 2100. Natural variation in pH between habitats occurs and may help explain some differences in tolerance. Meiofauna from sandy environments appear to be more susceptible to acidification than from muddy bottoms (muddy sediments experience greater pH variation naturally, Widdicombe *et al.* 2009). The deep sea experiences almost constant pH conditions, and meiofauna there are expected to be more sensitive than coastal species. Effects on calcareous foraminiferans will likely be most severe among the non-metazoan meiofauna because calcium carbonate dissolution rates will increase at a lower pH (Bernhard *et al.* 2009b). Increased acidity may also have effects on metal pollutants because bioavailability is related to pH. Pascal *et al.* (2010) found that increasing acidity reduced cadmium and copper toxicity in *Amphiascoides atopus*, a benthic copepod, in seawater-only experiments. However, the effect of reduced pH may be most significant on metals in sediments because metals may bind with sulfides and other oxides; lower pH should increase the bioavailable fraction. The potential for indirect effects of acidification on meiofauna is great (Dashfield *et al.* 2008), but has not been studied extensively. Meiofauna offer many advantages in the study of ocean acidification, and should

provide answers to important questions regarding the potential for environmental effects on the oceans.

Contaminant mixtures and meiofauna

Many field sites are contaminated not with a single pollutant but with complex mixtures of many (even hundreds) of compounds belonging to different chemical classes (Daskalakis & O'Connor 1995). Meiofauna are good experimental subjects for research on the joint action of contaminant mixtures because they facilitate large experimental designs in laboratory (Martin *et al.* 2009) and microcosm studies. There is a long history of study of mixture effects with meiofauna dating back to the 1940's (Barnes & Stanburry 1948). The ultimate goal of such studies is to determine if toxicants interact in mixtures to affect the intensity of toxicity. Toxicants may have additive effects in mixtures in which the toxicity of two or more toxicants may be summed together to predict toxicity in mixtures (and, in effect, no interaction between the toxicants occurs). This is typical for toxicants, such as PAH, in the same chemical class with the same mode of toxic action. An alternative is that toxicants in joint exposures express independence in their toxic effect (in which the presence of one chemical will not have an impact upon the action of another chemical and the toxicity of the combination can be predicted, without interaction, from knowledge of the independent chemicals). Independent action is frequently assumed to occur between chemicals in different classes in which the mode of toxic action differs, e.g., metals and highly hydrophobic contaminants affect different physiological properties. Less-than-additive responses (i.e., antagonisms), in which the toxic effect the mixture is less than the summed effects of individual contaminants, is a rare form of interactive toxicology. The more-than-additive response, in which the effect of the mixture is greater than the sum of the individual toxicants (synergisms), causes the greatest concern because no-observed-effects-concentrations developed for individual compounds would be inadequate to safeguard the environment. Meiofaunal responses to mixtures have mostly been studied in the laboratory, where direct toxicant effects dominate responses, and in microcosm experiments where direct and indirect effects may both occur.

Most highly hydrophobic contaminants in the same chemical class (e.g., PAH) appear to follow additive toxicology in a broad range of taxa (Swartz *et al.* 1995), including *C. elegans* when exposed to pesticide mixtures (Svendsen *et al.* 2010). However, a test using brominated flame-retardants on *Nitokra spinipes* (Breitholtz *et al.* 2008) suggests that effects of individual compounds in mixtures are synergistic. Interactions among metals in binary combinations appear to be highly variable in general in benthic organisms including meiofauna. Metal-metal interactions were found to be less-than-additive in

43 % of studies, additive in 27 %, and more-than-additive in 29 % in a meta-analysis (Norwood *et al.* 2003). In laboratory binary exposures, metal-metal and metal-organic compound mixture studies rarely indicate additive or independent toxicology on meiofauna (Korthals *et al.* 2000), while both synergistic and antagonistic results are common (Barnes & Stanburry 1948, Verriopoulos & Moraitou-Apostolopoulou 1982, Vranken *et al.* 1988, Forget *et al.* 1999, Hagopian-Schlekat *et al.* 2001, Chu & Chow 2002, Jonker *et al.* 2004a, Jonker *et al.* 2004b, Fleege *et al.* 2007, Martin *et al.* 2009). Such results make it difficult to generalize findings, but suggest that current strategies for sediment-quality criteria are inadequate for contaminant mixtures.

Studies with microcosms also suggest there is a potential for synergistic and antagonistic toxicant effects in meiofauna but that it is not universal. Mahmoudi *et al.* (2007) found an antagonistic interaction between lead and zinc on the abundance of nematodes, and the relative abundance of individual nematode species responded differently in mixtures than in single compound exposures. Beyrem *et al.* (2007) found that total nematode abundance was synergistically reduced in cadmium-diesel exposures. Millward *et al.* (2004) and Fleege *et al.* (2006) examined the potential for direct and indirect effects on nematodes and the benthic copepod community in two large microcosm experiments designed to test for metal-diesel interactions. Both studies concluded that there was no evidence for synergistic or antagonistic interactions between metals and diesel in any taxon studied. However, contaminant-induced indirect effects differed in contaminant mixtures. For example, the presence of metals reduced an indirect increase in the abundance of some copepod species, probably mediated by metal interference in microalgal blooms common in diesel contaminated sediment (Fig. 4). These results again point to the very important role of species interactions and food-web effects in meiofauna. Several laboratory and microcosm studies emphasize the difficulty in measuring direct contaminant effects and attributing causation at field sites where mixtures are present (Kovatch *et al.* 2000, Schizas *et al.* 2001, Bejarano *et al.* 2004, van Vliet & de Goede 2008, Gardstrom *et al.* 2008). One promising approach to examine mixture effects may be to use gene-expression profiling to identify genomic transcriptional responses. Menzel *et al.* (2009) found that overrepresented functional gene categories and upregulated metabolic pathways in *C. elegans* exposed to river sediments varied in different sediments with unique contaminant mixtures.

True synergistic effects of toxicant mixtures are defined to occur as a result of physiological/ pharmacological interactions in tissues where toxic action is expressed. However, it is possible that pollutants in mixtures interact to alter bioavailability and/or affect toxicant concentrations in sediments in ways that effect toxicity. Millward *et al.* (2004) showed that the presence of die-

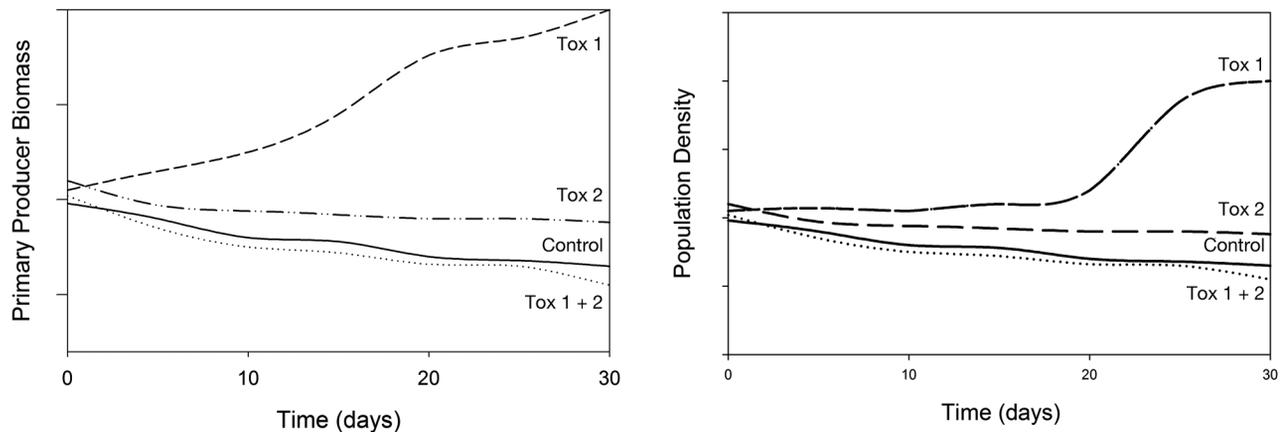


Fig. 4. – Left: Hypothetical response of primary producer biomass to two types of contaminants in a 30-day microcosm experiment in separate and binary mixture exposures. Right: Response of meiofauna tolerant of contaminant 1 and in which contaminant 2 modulates the response of contaminant 1 with combined contaminants in the same experiment. Legend: Control is the response without contaminant application, Tox 1 is the response to the first contaminant and Tox 2 is the response to the second but different contaminant. Tox 1 + 2 is the response to the combined contaminants.

sel fuel enhanced the retention of metals in sediments, and the presence of some metals may affect the porewater concentration of other metals in mixtures (Hagopian-Schlekat *et al.* 2001). Such effects may lead to a synergistic-like response of meiofauna in sediment because availability may be enhanced where multiple contaminants are found. Many compounds are directly bioavailable for uptake from water for animals the size of meiofauna but availability for uptake from sediment varies with sediment chemistry. Fleeger *et al.* (2007) showed that cadmium and phenanthrene expressed greater than additive toxicity in mixtures in the harpacticoid copepod *Schizopera knabeni* in both water-only and sediment exposures, suggesting that the cause of the synergism is associated with cellular or organismal responses. In contrast, Gust (2006) found that the joint toxicity of aqueous and sediment exposures of cadmium and phenanthrene differed in the freshwater amphipod, *Hyalella azteca*, suggesting that mixtures can influence contaminant uptake and enhance toxic effects. In another mixture study, phenanthrene decreased cadmium lethality antagonistically in the deposit-feeding oligochaete *Ilyodrilus templetoni* because phenanthrene induced a marked reduction in sediment ingestion, thereby reducing dietary exposure to cadmium (Gust & Fleeger 2006). Too little research has been conducted with pollutant mixtures to yield generalizations, and the area of contaminant mixture research represents a scientific and intellectual challenge.

Acclimatory and genetic responses of meiofauna to pollutants

The biochemical methods by which meiofauna detoxify and/or acclimate to pollutants have only recently begun to be studied intensively, and the research has been

conducted using *C. elegans* and *Tigriopus*. Metallothionein and related proteins have been identified in both taxa (Barka *et al.* 2001, Shimada *et al.* 2003, Hughes & Sturzenbaum, 2007, Jiang *et al.* 2009, Wang & Wang 2009, Zeitoun-Ghandour *et al.* 2010). These compounds bind and detoxify metals, although Hughes *et al.* (2009) found that cystathionine and phytochelatins in *C. elegans* responded to cadmium exposure while metallothionein did not. Recent work with *C. elegans* has also found the expression of cytochrome P450 enzymes that are known to detoxify many xenobiotics including PAHs (Menzel *et al.* 2001, Roos *et al.* 2004, Chakrapani *et al.* 2008, Schafer *et al.* 2009). Roh *et al.* (2007) found that the expression of cytochrome P450 and related compounds in *C. elegans* was increased by exposure to di(2-ethylhexyl)phthalate in a concentration-dependent manner. Similarly, exposure to copper was found to upregulate genes of some isoforms of cytochrome P450 in *Tigriopus* (Ki *et al.* 2009). Many biochemical and genetic indicators of exposure to neurotoxic pollutants, including insecticides and metals, are also being developed. For example, acetylcholine receptor gene families are now known in *C. elegans* (Sattelle 2009), and acetylcholinesterase inhibition has been assayed in *Tigriopus* (Forget *et al.* 1999). The identification of compounds that play a role in detoxification, as described above, and in response to environmental stressors (e.g., Roh *et al.* 2006, Lee *et al.* 2007, Rhee *et al.* 2009, Shen *et al.* 2009, Hwang *et al.* 2010, Tvermoe *et al.* 2010, Wang & Wang 2010) suggests that biomarkers of contaminant exposure in meiofauna can be developed and that it is possible to evaluate the mechanisms and rates (e.g., *via* inducible enzymes) at which meiofauna acclimate to contaminants.

Individuals persist and populations survive in polluted environments if they tolerate and reproduce in the condi-

tions present in their environment. Hazardous substances may reduce the fitness of sensitive species and, through selection, alter relative abundance patterns in communities. Sensitive species may be replaced with more tolerant species, or species may be extirpated at contaminated sites, reducing species diversity and altering community composition. Alternatively, individual species may become more tolerant at a contaminated site by acclimatory processes or adaptive genetic change (Morgan *et al.* 2007). Carman *et al.* (2000a) addressed these possibilities in a microcosm study conducted at two geographically similar sites but with differing histories of contamination. Meiofauna at the site with historically high levels of contamination were found to be less sensitive to contamination than were meiofauna from a site with historically low contamination levels, primarily by a community shift to more tolerant species. Changes in community tolerance as observed by Carman *et al.* may also be examined through the use of short-term acute toxicity tests of a subset of community members. This community test is referred to as Pollution-Induced Community Tolerance (the "PICT" test, Millward & Klerks 2002). PICT may be used to establish causal linkages between contaminants and effects. An increase in community tolerance compared to the baseline tolerance at reference sites suggests that the community has been adversely affected by toxicants. Results of studies with nematode communities suggest PICT is similar in sensitivity to community analysis but may require less effort (Millward & Grant 1995, Millward & Grant 2000).

Meiofauna species have also been shown to become more tolerant to pollutants in heavily contaminated areas. Increased tolerance to metals at contaminated sites has been reported in copepods and nematodes (Millward & Grant 1995, Miliou *et al.* 2000, Kwok *et al.* 2009, Rubal *et al.* 2009) and to pesticides in nematodes in the laboratory (Lopes *et al.* 2008). However tolerance increases have not been found for all pollutants at heavily contaminated sites; we can find no reports of an increased tolerance in meiofauna to PAH. In some cases, meiofauna have been shown to develop an increased tolerance to very high levels of contaminants (Millward *et al.* 2000) in relatively few generations. Of relevance is the freshwater oligochaete *Limnodrilus hoffmeisteri* which developed resistance to highly elevated cadmium levels at a foundry site in less than 30 years (Klerks & Levinton 1989), and the development of a resistance to pesticides in *C. elegans*, which occurred in less than 20 generations (Lopes *et al.* 2008). However not all studies find increased tolerance at contaminated field sites. Kovatch *et al.* (2000) found that although there was a genetic difference among populations of the copepod *Microathridion littorale* from contaminated and clean sites, no difference in tolerance could be detected when individuals were exposed to sediment contaminated with a mixture of toxicants. There are many reasons why species-specific tolerance might not

increase at contaminated sites. Dispersal from surrounding non-contaminated area may enhance gene flow into the contaminated area and reduce the potential for or rate of adaptation. Alternatively, many sites are contaminated with toxicant mixtures, and mixtures may generally inhibit cellular or genetic responses, disrupting increases in tolerance compared to exposure to single contaminants.

Although studies in polluted environments often find increased species' tolerance, most do not determine if the mechanism causing the change is physiologically based acclimation or genetic adaptation by natural selection (Klerks & Weis 1987). Selection has been implicated in some (see Williams & Oleksiak 2008), but not all studies with meiofauna or other organisms. Most compellingly with meiofauna, Schizas *et al.* (2001) found differential survivorship of three mitochondrial lineages in the copepod *Microathridion littorale* to a pesticide mixture in a fashion consistent with selection. Street *et al.* (1998b), Gardestrom *et al.* (2006) and Gardestrom *et al.* (2008) exposed copepods in laboratory experiments to contaminants and examined genetic change in offspring; all found an inter-generational change in haplotype composition as some rare haplotypes were reduced after exposure, supporting selection as the cause. Rhodes *et al.* (2008) used laboratory-based, inter-generational exposures with copper in *Tigriopus* and found the number of offspring produced increased over time, again suggesting genetic adaptation. On the other hand, Derycke *et al.* (2007) found no change in haplotypes in the nematode *Pellioiditis marina* after exposure to cadmium, and Kwok *et al.* (2009) found that tolerance increased to copper in a multi-generational study in *Tigriopus japonicus* but experimentally attributed changes to acclimation. One complicating factor in these experiments is their short-term nature. The longest experiment for meiofauna is by Miliou *et al.* (2000) who maintained a population of *Tisbe holothuriae* from a polluted site for 40 generations. Results showed that copepods from the contaminated site remained more tolerant than those from a non-polluted area, suggesting a heritable genetic response. These results suggest that changes in genetic structure in laboratory experiments are due to selection rather than genetic drift; however, they may not suggest that the potential for genetic adaptation to pollutants is high for meiofauna. Selection for increased fitness in contaminated habitats may be associated with multi-gene responses rather than single-gene effects. Chaumot *et al.* (2009) showed that non-additive effects (interactions between genes) in multi-gene inheritance dominate the genetic response to cadmium in amphipods in the genus *Gammarus*, and that genetic resistance is not strongly heritable. If meiofauna are similar, the potential for genetic adaptation to some pollutants (e.g., PAH) may be low. Clearly, more research needs to be done to determine if selection or acclimation is responsible for changes in tolerance in meiofauna and other benthic organisms and how both apply to a broad range of contaminants.

As has been found in other organisms, meiofauna experience a fitness cost associated with increased tolerance to pollutants. Rhodes *et al.* (2008) found evidence for increased tolerance to parental copper exposures, but with an associated cost in reduced offspring production under copper concentrations that were different from the parental exposure in *Tigriopus japonicus*. Kwok *et al.* (2009) found that the intrinsic population growth rate of a copper-resistant lineage in *T. japonicus* was significantly lower than that in non-exposed copepods. Thus, tolerant strains should be expected to be at a fitness disadvantage at unpolluted sites. However, research on *Limnodrilus hoffmeisteri* at a remediated site suggests that a loss of tolerance was due to an invasion of genotypes from an adjacent population in the time since remediation, rather than cost-related reductions in fitness (Mackie *et al.* 2010).

Genetic diversity and pollutants: Pollution can have both positive and negative effects on genetic diversity although through different mechanisms (Depledge, 1996). On one hand, pollution may decrease population size (increasing genetic drift) or increase selection for homozygous genotypes, both of which decrease genetic variation. Indeed, some studies have clearly found reductions in genetic variation because of pollution (e.g., Street & Montagna 1996). Alternatively, pollution may increase mutation rates at marker loci or increase selection for heterozygotes (DiBattista 2008). The net effect of pollution on genetic variation should therefore reflect a balance between these various forces. The loss of genetic diversity in meiofauna has been shown to occur with different species and with different toxicants in the field and laboratory (Street & Montagna 1996, Street *et al.* 1998b, Schizas *et al.* 2001, Gardstrom *et al.* 2006, Gardstrom *et al.* 2008). The reduction has been found in short- and long-term exposures (Gardstrom *et al.* 2006, 2008), although some studies did not find a loss of genetic diversity (Kovatch *et al.* 2000, Derycke *et al.* 2007). Even though the causes of a reduction in genetic diversity have not been fully explored, the frequency with which it has been found suggests that genetic diversity may be a good indicator of pollutant effects in the field. Furthermore, the loss of genetic heterozygosity may have deleterious effects on population fitness (Reed & Frankham 2003).

Cryptic species: Studies of contaminant-associated shifts in genetic diversity have paid less attention to another potential complicating factor that may be relevant to meiofauna. Reductions in genetic diversity observed in some population samples from contaminated sites may represent a loss of species diversity through local contaminant-caused extinction of one or more members of a cryptic species complex rather than a within-species loss of less-tolerant genotypes or haplotypes. Cryptic species are morphologically similar but genetically distinct sibling species. Such complexes occur within some cosmopolitan meiofauna based on classical morphologically based systematics (Todaro *et al.* 1996, Rocha-Olivares *et*

al. 2001). In fact, many taxa that thrive in polluted habitats belong to complexes of cryptic species (Duan *et al.* 1997, Sturmbauer *et al.* 1999, Warwick & Robinson 2000). For cryptic species to contribute to losses in genetic diversity at contaminated sites, 1) the taxa must form a cryptic-species complex or be easily misidentified to, in effect, act as cryptic species, 2) cryptic species must co-occur at uncontaminated sites, and 3) cryptic species must exhibit different responses to contaminants such that differential mortality occurs at contaminated sites. Rocha-Olivares *et al.* (2004) found that a cryptic-species complex of benthic copepods has variable tolerance to metals and hydrocarbons suggesting that pollution-induced effects on cryptic species may occur. Observations by Derycke *et al.* (2007) supported the contention that cryptic species may contribute to reductions in genetic diversity under the influence of pollution.

Meiofauna and future environmental research

Many meiofauna species are widely distributed geographically. Within this context, phylogeography (biogeographic surveys of within species genetic variation to better understand microevolutionary patterns) may reduce spurious correlations and erroneous conclusions of studies of genetic change. Phylogeography has been underutilized to improve the understanding of relationships between pollution and genetic variation (Staton *et al.* 2001), especially as studies using population genetics and pollutants grow in frequency. This seems especially important for species in the genus *Tigriopus* that are used in numerous studies of contaminant fate and effects yet show high levels of intra-specific genetic variation. Common-garden experiments have also been underutilized in meiofauna. This type of experiment determines if changes in the response to pollutants are due to acclimation or genetic change, and if traits such as contaminant tolerance are heritable. Specimens from contaminated and non-contaminated locations would be brought into the laboratory and held under identical conditions, without contamination, to reduce differences in acclimation. The responses to contamination can then be tested in both populations (see Miliou *et al.* 2000). Heritability of traits that increase tolerance is especially poorly known for many contaminants (e.g., PAH and endocrine disruptors), and should be better understood.

Environmental genomics, and more recently toxicogenomics, proteomics and metabolomics, have been applied to organisms in order to better understand the hazardous effects of chemicals on individuals and ecosystems (Snape *et al.* 2004, Watanabe & Iguchi 2006, Bundy *et al.* 2009). Gene microarrays that quantify changes in gene expression over part of or even the entire genome after exposure to a pollutant are now available for a growing number of species. Meiofauna are increasingly being used as the subjects in gene expression studies (Liao &

Freedman 1998, Matsuno *et al.* 2002, Snape *et al.* 2004, Lee *et al.* 2006, Cui *et al.* 2007, Ki *et al.* 2009, Menzel *et al.* 2009, Jeong *et al.* 2010, Swain *et al.* 2010), and results are increasing our understanding of the toxic action of different pollutants. Most gene expression studies with meiofauna have been with metals; however, other toxicants should be studied more thoroughly. We feel that meiofauna will continue to contribute to future studies of environmental genomics and toxicant fate and effects because of their many desirable qualities – the ease of laboratory culture, toxic responses that reflects the chemistry of sediment and porewater, the existence of model species that facilitate experiments ranging from lethal and sublethal bioassays to gene expression, and because meiofauna have proven to be exceptional sentinels of the environment.

There are many contaminant effects in aquatic environments that are poorly understood, including those of pharmaceuticals (other than endocrine disruptors), mixtures of various contaminants (e.g., hydrocarbons, metals, pesticides, environmental estrogens, nutrients), and nanoparticles from new technologies. The 2010 oil spill in the Gulf of Mexico points to the need for an improved understanding of the population, community, and ecosystem impacts of major environmental events. These are but a few examples of the significant challenges that ecotoxicologists face. Innovative new approaches are needed to address these questions, and meiofauna provide useful model systems for their examination.

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Received October 5, 2010
 Accepted March 18, 2011
 Associate Editor: G Tita