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The use of polychaetes (Annelida) as indicator species of marine pollution: a review

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Abstract: Polychaetes are usually the most abundant taxon in benthic communities and have been most often utilized as indicator species of environmental conditions. This review finds that, while the use of indicator species for a particular pollutant is not simple, polychaetes can provide a useful means of assessing the effects of poor environmental conditions. Polychaetes may be used as sensitive monitors of water quality especially in terms of the effects of pollutants on life history characteristics. They may also be utilized as general indicators of community diversity but those species indicative of lower diversity may differ geographically and temporally. While sewage is often a mixture of high organic material and other pollutants such as heavy metals and pesticides, high organic situations associated with aquaculture facilities indicates that members of the *Capitella capitata* species complex and the dorvilleid genus *Ophryotrocha* are often dominant. Some species of polychaetes are able to live in sediments very high in trace metal content and body burden of these metals often does not reflect sediment concentrations due to regulation by these species. Many species seem relatively resistant to organic contaminants and pesticides and the effects of these pollutants on life history characteristics of these species may provide a more sensitive assay method. Recent studies using biomarkers in polychaetes to indicate general heavy metal or pesticide contamination has shown some success. Polychaete species known to occur in appreciable densities in the Gulf of Nicoya, Costa Rica, and which have been most often used as indicator species of pollution, are listed as potential taxa for environmental monitoring in this tropical estuary. Rev. Biol. Trop. 56 (Suppl. 4): 11-38. Epub 2009 June 30.

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The polychaetes have long been an obvious choice to act as representative species in the analysis of the health of benthic communities as they are usually the most abundant taxon taken in benthic samples, both in terms of the number of species and numerical abundance. Additionally, unlike nektonic or reptant organism, the polychaetes usually live within the sediments or attached to hard surfaces and, while their larvae may be capable of long distance transport, the adults are relatively inert. This relative immobility ensures chronic exposure to any toxic materials in the environment

rather than the episodic exposures of a more vagile organism. Any long-term changes in the wellbeing of the benthos should be reflected in the polychaete community (Papageorgiou *et al.* 2006).

The life history characteristics of polychaetes also make them good candidates to act as indicator species. The epibenthic filter feeders maximize their exposure to any harmful materials within the water column as they process relatively large amounts of water during feeding. Deposit feeding and interstitial species, which are in intimate contact with

the sediments, are maximally exposed to any harmful materials both in the sediments and the pore water. The duration of the entire life cycle of many species of polychaetes is often on the order of days or weeks and reproductive rates may be very high, both of which allows a rapid population response to any changes in the environment such as the input of pollutants or organic material. Elias *et al.* (2005), for example, was able to distinguish a changeover of dominant polychaetes species in response to winter and summer pollutant input off Mar del Plata City, Argentina. These life history characteristics as well as their relatively small body size also make the polychaetes attractive as biomonitoring species. Cultures can be maintained over many generations in the laboratory allowing the monitoring of individual responses as well as any changes in life cycle characteristics due to changes in environmental factors.

If polychaetes species are assumed to be useful as indicators of the general health of benthic communities, what has been the success of this method of analysis? Has the analysis of the polychaetes fraction of the benthic community allowed us to somehow quantify the effects of harmful pollutants upon the benthos? Are there some widespread polychaetes species (such as the capitellid *Capitella capitata* that may act as warning signs of stressed marine environments or, conversely, may act as signals that the environment is relatively healthy? This review attempts to analyze the success of utilizing polychaetes as environmental monitors.

This review paper was prepared as part of a long term effort to assess the health of, and any possible effect of marine pollution on, the benthic community in the Gulf of Nicoya estuary on the Pacific coast of Costa Rica (Maurer & Vargas 1984, Vargas *et al.* 1985, De la Cruz & Vargas 1987, Maurer *et al.* 1988, Vargas 1987, 1988, Dean 1996, 2004). The use of polychaetes as indicators of environmental conditions for this estuary was apparent early on (Maurer *et al.* 1988, Dean 2001a, 2004) especially with the knowledge that the capitellids were a numerically important group (Vargas

1987, 1988, Dean, 1996, 2001a). Recent efforts have therefore been concentrated in updating the taxonomic position of polychaete species, especially those generally viewed as potential indicator species for the estuary (Dean 1996, 2001a,b, 2004, 2007, 2008). In this review of polychaete indicator species, many of those species known to be present in the Gulf of Nicoya or known to be tropical or semi-tropical in their distribution will be discussed. The species names of all polychaete species included in this review, along with the names of their original authors may be found in Appendix 1.

ECOTOXICOLOGICAL STUDIES

Reish & Bernard (1960) first used the polychaete species *C. capitata* in toxicological testing and many have continued this line of research using many other polychaete species as test organisms. In their review of this subject Reish & Gerlingher (1997) reported that up to that time 48 polychaete species had been utilized in the evaluation of toxic substances and their effects on marine organisms. They found that *Neanthes arenaceodentata* (Family Nereididae) was the most commonly used species with other often-used species including the nereids *Neanthes (Hediste) diversicolor* and *Nereis virens*, the dorvilleids *Dinophilus gyrociliatus*, *Ophryotrocha labronica*, and *Ophryotrocha diadema*, and *C. capitata*. Of these species all but *N. (H.) diversicolor* and *N. virens* had been maintained in laboratory culture. These tests for the effects of toxic materials included either acute LC50 tests lasting several days or chronic tests with exposures extending approximately 10 to 28 days (Reish & Gerlingher 1997). In chronic testing the effects of toxicants on additional factors as growth, feeding rate, number of eggs produced and generation time have been used as indicators of sublethal effects.

These toxicological tests of polychaetes have shown that a species may vary in its LC50 responses to different toxicants and even closely related species may differ greatly in their sensitivities to toxicants. For example,

Reish & Gerlinger (1997) reported that of the species tested in their laboratory for heavy metal contaminants *O. labronica* was the most sensitive overall but eight species were found to be sensitive to at least one or more metals and nine species were characterized as tolerant to one or more metals. While *N. arenaceodentata* was found to be sensitive to chromium (Cr) it was apparently tolerant to elevated lead (Pb) concentrations. The nereid *N. virens* was identified as being a sensitive species to mercury (Hg) levels while *N. (H.) diversicolor* was found to be tolerant of this metal. Of the metals tested, mercury (Hg) and copper (Cu) were most toxic while zinc (Zn) and lead (Pb) were least toxic. Similar results to those of heavy metal contaminants were seen in toxicity studies involving petroleum hydrocarbons.

Chronic testing of polychaetes also shows variability in sensitivity to specific toxicants. Reish & Carr (1978) reported reproductive suppression in *O. labronica* when exposed to Cu, Zn and Pb at concentrations similar to or greater than LC50 values. Reish & Gerlinger (1997) reported that reproductive output of *Ctenodrilus serratus* (Family Ctenodrilidae) was affected at concentrations of Hg and Cu at concentrations similar to 96-h LC50 values. In the case of Cd, Pb and Zn, however, the concentrations found to negatively affect reproductive output were much lower than LC50 concentrations. Mauri *et al.* (2003) found that the concentrations of Zn used in their toxicity tests with *D. gyrociliatus* showed little effect upon survival but did result in decreased fecundity, growth rate and generation time. These latter results demonstrate that for some species the effects of toxicants on life history characteristics may be a more sensitive indicator of stress on the benthos than survivorship-based assays.

More recent work has focused upon the effects of pollutants on such characteristics as embryology, larval development, metamorphosis, and the like. Xie *et al.* (2005) found that adult *Hydroides elegans* (Family Serpulidae) exhibited significant mortality in lab tests only when the concentration of Cu was greater than

500 $\mu\text{g l}^{-1}$ but negative effects upon survivorship of larval forms occurred at 10-71 $\mu\text{g l}^{-1}$. Ross & Bidwell (2002) examined the effect of Cu on larval development of the serpulid *Galeolaria caespitosa* in Australia and found that levels which affected larval development were similar to the concentrations which resulted in a 50% inhibition of cell growth. Additionally they found that larval development of this polychaete was more sensitive to the effluent of a lead smelting plant than two species of microalgae as well as the gametes of the bivalve mollusc *Mytilus edulis* Linnaeus 1758 and could act as a sensitive bioindicator of mixtures of pollutants in the field. Gopalakrishnan *et al.* (2007) studied the effects of several heavy metals on fertilization, embryogenesis and larval development of *H. elegans* in laboratory culture and found that larval development and embryogenesis were the most sensitive stages. The developmental stages of *H. elegans* were ranked in their sensitivity to the metals tested in the following order: Cu>Al>Pb>Ni>Zn. Not only were the developmental stages of this species more sensitive to heavy metal concentrations when compared to LC50 values obtained using adults, these developmental stage tests take an appreciably shorter time to run.

While the exposure of polychaetes to water-borne toxicants has allowed a better understanding of the effects of these substances on benthic species, numerous complicating factors make direct application of these results to a field situation difficult. It is often the case that pollutants co-occur in nature and, as Reish & Gerlinger (1997) noted, there can be synergistic effects when test organisms are exposed to a mixture of two toxic metals. Temperature and salinity may also be a factor as Fernandez & Jones (1990) found that the toxicity of zinc to *N. (H.) diversicolor* increased with both increasing temperature and change in salinity above or below 17.5%. Yet another confounding factor may be oxygen availability as Neuhoff & Theede (1984) found that the toxic effects of copper were much greater on the polychaete *Pectinaria koreni* (Family Pectinariidae) when oxygen tension was reduced. Obviously these

factors must be controlled for in any laboratory assays but these factors make application of the results of such laboratory tests to field situations quite difficult.

Perhaps the greatest confounding factor in the application of laboratory toxicological tests to the “real world” situation is the effects of sediments and organics on the sensitivity of benthic organisms to the concentrations of pollutants in the water column. Pesch & Morgan (1978) found that when clean sediments were present in the test chamber with *N. arenaceo-dentata* the sensitivity to added copper was greatly reduced relative to the results of tests carried out in the absence of accompanying sediments. Toxic free cupric ions were readily adsorbed onto sediment particles and, although the sediments greatly increased in copper concentration, this metal was less bioavailable to the test organisms. The same situation has been noted in studies involving other species of nereid polychaetes for other metals (Maloney 1996, King *et al.* 2004) and polycyclic aromatic hydrocarbons (Cornelissen *et al.* 2006). This absorption of toxic material by the sediments, binding them and making them less available to organisms, may explain why some benthic species are capable of existing, or even thriving, in sediments with greatly elevated levels of toxicants.

An additional source of confusion in bio-toxicity studies, as well as field studies, is the identification of species. Many species once considered to be cosmopolitan or at least widespread in their distribution have been found to consist of groups of sibling species. No more is this apparent than the species *C. capitata* which was formerly believed to be a cosmopolitan indicator of organic enrichment (Pearson & Rosenberg 1978) but has been shown to be a species complex of many morphologically similar species (Grassle & Grassle 1976). Members of this species complex may differ greatly in their reproductive mode and their larval development even when found to co-occur in the same region. Gamenick *et al.* (1998) studied the physiological response of four members of this complex and found that

they differ greatly in their abilities to respond to anoxic conditions. Three were found to be oxyregulators with different regulatory capabilities while one was an oxyconformer with little ability to cope with low oxygen conditions. Mendez *et al.* (2000) showed that *C. capitata* from three different regions differed greatly in their reproductive mode and developmental pattern. It is obviously vital that the identity of the test organism must be substantiated before any biotoxicity testing is attempted. This includes the recognition of any possible sibling species or differences in subpopulations of wide-ranging species. One alternative would be to establish a culture of a locally abundant species believed to be an appropriate indicator species or obtain specimens from already established and well identified cultures.

Polychaetes can serve as important ecotoxicological testing organisms due to their small size, relatively short life cycles, and general ease of maintenance in cultures. The use of developmental stages and life cycle characteristics has been found to provide much more sensitive monitoring of pollutants and often has an added advantage of requiring shorter exposure times compared to using adults. Potentially confounding factors include salinity, temperature, oxygen content and the presence of sediments or organic materials. As a result of these confounding factors, the quantification of the effects of toxicants on benthic animals any direct application of these laboratory toxicological results to field conditions is fraught with difficulty.

POLYCHAETES AS INDICATORS OF BENTHIC SPECIES DIVERSITY

When a benthic community is undergoing stress due to detrimental environmental conditions there are presumed to be notable changes in community parameters such as diversity, abundances, dominance, biomass, and so on (Pearson & Rosenberg 1978). Since the polychaetes are commonly a major component of any benthic community, these changes in community structure should be mirrored by the

polychaete community (although this was not always found to be true for the Mediterranean sandy beach community (Papageoriou *et al.* 2006). As a result polychaete species have been used as indicators of the general “health” of the overall community. A brief incomplete list of positive indicators of a stressed community due to pollution include the capitellids *C. capitata*, (Mendez *et al.* 1998, Belan 2003, Rivero 2005) and *Heteromastus filiformis* (Ahn *et al.* 1995), the spionids *Malacocerus fuliginosus*, *Paraprionospio pinnata*, and *Polydora ligni* (Mendez *et al.* 1998, Dix *et al.* 2005), the nereid *N. (H.) diversicolor*, the dorvilleid *Ophryotrocha adherens* (Bailey-Brock *et al.* 2000) and the cirratulidae *Chaetozone setosa* (Rygg 1985). Other polychaete species, such as members of the Lumbrineridae, Maldanidae (Belan 2003) and Terebellidae (Olsgard *et al.* 2003) have been used as negative indicators of poor benthic conditions, that is, their absence in a community is an indication of poor environmental conditions.

There are difficulties with the assignment of a particular polychaete species as an indicator of a degraded benthic environment. Each geographic region seems to respond in its own way to poor environmental conditions with its own set of dominant species acting as positive indicators and the absence of other species acting as negative indicators under stressed conditions. At one time species such as *C. capitata* and *C. setosa* were considered to be cosmopolitan in distribution and it was believed that perhaps their presence in an area could be a worldwide recognition signal of environmental degradation. These species, as well as others said to be cosmopolitan, have been found to be groups of morphologically distinct or sibling species differing in their life history characteristics (Grassle & Grassle 1976, Christie 1985, see discussion of *C. capitata* species complex above). Numerous species have been identified as being associated with stressed, or polluted, conditions but often these species are found to also occur in what are clearly nonpolluted environments (Bellan 1985, Rygg 1985, Hily & Glémarec 1990). Generalizations

about particular species acting as indicators of stressed, or low diversity, communities in different geographic areas are tenuous.

In an early study of the effects of pollutants on the benthic community in Oslofjord, Norway, Mirza & Gray (1981) noted a decrease in species diversity along a gradient from relatively clean areas to areas highly impacted by organic pollution. One important characteristic of this decrease in diversity was the increase in species dominance most notably by polychaetes. They found that what they identified as *C. capitata*, *Polydora* spp., and *Nereimyra punctata* were highly abundant at the most polluted sites and were therefore designated as indicators of polluted conditions. Of these three species, both *C. capitata* and *Polydora* spp. had been previously characterized by Pearson & Rosenberg (1978) as indicators of organic enrichment. In the Oslofjord, as well as nearby areas studied by Pearson (1975) and Rosenberg (1976), many polychaete species were characterized as indicators of unpolluted conditions and were correlated with high diversity including *Glycera alba* (Family Glyceridae), *Anabothrus* (= *Sosane*) *gracilis* (Family Ampharetidae), *Pholoe minuta* (Family Pholoidae), *C. setosa*, *Lumbrineris* spp. (Family Lumbrineridae), *Terebellides stroemi* (Family Terebellidae), and *Scalibregma inflatum* (Family Scalibregmatidae).

Rygg (1985) later analyzed pollution gradients in Norwegian fjords, including the Oslofjord, and found that his results showed that the absence of species such as the polynoid *Harmothoe imbricata* and the maldanid *Maldane sarsi* were indicative of poor environmental conditions while the absence of members of the genera *Paramphinome* (Family Amphinomidae), *Ceratocephale* (Family Nereididae), *Harmothoe* (Family Polynoidae) and *Lumbrineris* (Family Lumbrineridae) indicated low diversity due to the impact of highly detrimental environmental conditions. *C. setosa*, whose presence in earlier studies in the same region (Pearson 1975, Rosenberg 1976) indicated a highly diverse community, was found along the entire pollution gradient

and was numerically dominant at the most polluted stations thus serving as an indicator of low diversity. Obviously there are great differences in the species identified as indicators of stressed environments among these studies despite the fact that they were all conducted in a similar geographic range.

Mendez *et al.* (1998) considered *C. capitata*, *M. fuliginosus*, *Polydora ciliata* (Family Spionidae) and *Neanthes caudata* (Family Nereididae) to be indicators of organic pollution in the Mediterranean of Barcelona, Spain. Such species such as the capitellid *Mediomastus fragilis*, the spionid *Aonides oxycephala*, *Ophryotrocha hartmanni* and the syllid *Exogone verugera* were considered tolerant of pollution but not indicator species. While *C. capitata* and *M. fuliginosus* have been noted elsewhere as indicators of organic pollution (Pearson & Rosenberg 1978, Tsutsumi 1987), *P. ciliata* and *N. caudata* have been considered as being capable of existence in organically polluted sediments but not as being indicative of organic pollution. *N. caudata* and *P. ciliata* have been characterized from nearby regions as being capable of living in impacted areas but not exclusively so (Bellan 1985, Hily & Glémarec 1990). Additionally, the Oweniid *Owenia fusiformis* and the poecilochaetid *Poecilochaetus serpens*, both previously identified as being common in nearby non-polluted areas off Barcelona and considered to be indicators of non-polluted conditions, were collected only rarely in Mendez *et al.* (1998) study. Again, even in geographically adjacent regions generalizations as to which species are indicative of environmentally stressed conditions are difficult.

Pocklington & Wells (1992) reviewed the use of polychaetes in environmental quality monitoring and generalized that members of the families Capitellidae, and Spionidae seemed to be of particular value as pollution indicators. Rivero *et al.* (2005), in work on the Argentine coast, characterized *C. capitata* as an indicator of poor environmental conditions and low diversity but also characterized areas of intermediate disturbance by the presence

of the spionid *Polydora* sp., the cirratulid *Tharyx* sp., and the capitellids *Mediomastus* sp., *C. capitata* and *Capitella* sp. Areas with healthy environmental conditions were characterized by the capitellid *Mediomastus* sp., members of the Maldanidae, and the Nephtyid *Aglaophamus uruguayi*. In this example members of the Capitellidae are indicative of low diversity impacted, intermediately impacted (presumably moderately diverse), and clean (presumably highly diverse) areas while the spionid *Polydora* sp. indicates only intermediate impact. Generalizations such as those of Pocklington & Wells (1992) concerning indicators of diversity at higher taxonomic levels are oversimplifications given the wide ranges in life histories of even closely related polychaetes.

Elias *et al.* (2005) identified *Prionospio* spp. as an indicator species of organic enrichment in samples collected during the summer season off Mar del Plata City in Argentina. In winter the tourists leave the area and not only is organic input into the coastal waters greatly reduced but winter storms act to wash away the accumulated organic material. In the winter collections the dominant polychaetes species were members of the family Maldanidae, indicators of low organic input conditions. The cirratulid, *Caulleriella* sp., was almost entirely absent from the area but in a study conducted in the same region (Elias *et al.* 2004) during the previous summer this species occurred in high abundances and was identified as an indicator of a stressed community due to organic enrichment. The results of these studies illustrate the ability of the polychaetes community to respond quickly to changes in their environment and demonstrate that even dominant species cannot be counted on to act as enduring indicators of environmental conditions for the benthos.

Harkantra & Rodrigues (2004) assessed the utility of several methods, including analysis of the polychaetes community, in the recognition of pollution induced disturbance in tropical India. Based on earlier studies prior to industrialization in this region (Devassy *et al.* 1987), Harkantra & Rodrigues (2004) noted

the disappearance of some polychaetes species that had been commonly present. These species, which may have been negatively affected by increased industrial and sewage pollution, included the Onuphid *Diopatra neapolitana*, the spionid *Scolelepis squamata* (= *Nerine cirratulus*) and the pisionid *Pisionidens indica*. While there was numerical dominance by polychaetes species at some polluted sites there was no significant correlation of these polychaetes species with organic carbon input associated with sewage and industrial effluent, the presumed cause of stress to the benthos. In addition to a lack of identification of indicator species along a pollution gradient, there were no apparent gradients in species diversity, species richness, or abundances in their data with distance from the industrial effluent source.

Bailey-Brock *et al.* (2002) also worked in the tropics and identified organic enrichment indicator species at an ocean outfall in Hawaii, recognizing several species as indicative of the “zone of dilution” of this outfall. The nereidid *N. arenaceodentata* and the dorvilleid *O. adherens* were the two dominants in this zone initially with *C. capitata* also abundant but additionally present in lesser numbers in the control stations. Three years into the nine year study *N. arenaceodentata* disappeared and was only rarely collected in the study again. Whatever the reason for this disappearance, *O. adherens* subsequently became more dominant in the zone of dilution and was the most robust positive indicator species of the sewage outfall dilution zone. The abiotic data, however, showed no evidence of the outfall as an important source of organic enrichment. In fact, the dilution zone was similar in diversity and species richness to the far field control sites and there were no obvious pollution level effects on the polychaete community. Apparently the nutrient poor waters and perhaps more rapid turnover rates in tropical species (Riddle *et al.* 1990) act to prevent the buildup of excessive amounts of organic matter in tropical regions. *O. adherens* may be an indicator species for the zone of dilution but it is not an indicator species of a low diversity community. The

dynamics of the benthos in tropical regions is less well known than that of temperate regions and perhaps the identification of indicator species for low diversity (stressed) environments may be a more difficult task.

One problem with species diversity measures is that it is difficult to attach statistical meaning to these values since there is usually large sample variance and a limited number of replicates. Additionally, species diversity often does not correlate directly with pollution gradients (Pearson & Rosenberg 1978). This led Bellan (1980) and Bellan *et al.* (1988) to establish an “annelid pollution index” to characterize polluted or disturbed communities versus non-polluted or undisturbed communities in the French Mediterranean. This index is based on the ratio of the pollution indicators *Platynereis dumerili* (Family Nereididae), *Protoaricia* (= *Theostomata*) *oerstedii* (Family Orbiniidae), and *Cirratulus cirratus* (Family Cirratulidae) to the indicators of non-pollution, members of the syllid genus *Syllis* spp. and the Sabellidae *Amphiglena mediterranea*. A group of “sentinel” species were first identified as those of highest numerical dominance in the samples. Those found to dominate in polluted areas were considered pollution indicators and those found to dominate in cleaner areas were considered indicators of non-polluted waters. The ratio of the sum of the dominance values of the pollution indicator species divided by the sum of the dominance values of the clean water indicator species is the annelid pollution index. Any value greater than one in any sample is characterized as an indication of pollution effects. This method has not been widely accepted and even Bellan *et al.* (1988) noted some anomalous results using this index. An obvious problem is the imprecision inherent in the assignment of pollution and non-pollution indicators.

More recently, Olsgard *et al.* (2003) analyzed polychaete data from the North Atlantic Norwegian coast in order to recognize possible indicator groups which could be used for faster analysis of polychaete diversity and also diversity of the entire benthic community. Their

data indicated that the greater the Terebellidae to total polychaete ratio in a sample the greater the species richness of the polychaetes community and, although not as highly correlated, the greater the overall benthic species richness. Since the members of this Order (Terebellidae, Ampharetidae, Trichobranchidae, Sabellariidae and Pectinariidae) are usually large and their taxonomy has been well studied they could be quite easily and quickly processed. While the abundances of Terebellidae may indicate the overall species richness of benthic communities on the Norwegian coast it is unlikely that this method would apply in regions where members of this taxon are not common. For example, in a review of the subtidal benthic polychaetes of the relatively unpolluted Gulf of Nicoya, Costa Rica by Dean (1996) only eight species of Terebellidae are reported to have been encountered and most of the records of these species were taken at single stations and in very small numbers.

In another attempt to simplify the characterization of benthic communities, Dix *et al.* (2005) reported that members of the family Spionidae could be used to characterize sub-regions of Tampa Bay, Florida (USA). Based upon analysis of species distributions in the bay they found *P. pinnata* to be associated with polluted areas, *Prionospio perkinsi* and *Streblospio gynobranchiata* associated with generally disturbed areas, *Polydora cornuta* associated with areas high in organics, and *Prionospio heterobranchia* associated with non-polluted areas. While this approach may have had some success in Tampa Bay, their species had great overlaps in their distributions so their characterization of sub-regions is imprecise. Also, other areas may not have as wide an array of spionids as was present in Tampa Bay, different taxa may fill similar niches to those filled by spionids.

Pagliosa (2005) found that non-polluted mangrove areas in Brazil were characterized by the magelonid *Magelona papillicornis*, the spionid *Polydora websteri*, and the paranoid *Aricidea (Aricidea) sp.*, all of which are either filter feeders and /or surface deposit feeders.

Areas of mangrove which had been affected by urbanization were characterized by the Nereid *Laeonereis acuta*, which is an omnivorous species. Pagliosa postulated that omnivorous species had a much wider range of feeding opportunities as compared to filter feeders and surface deposit feeders and were better able to respond to changes in their environment. What was proposed was to characterize polluted regions by a higher occurrence of omnivores relative to filter feeders and surface deposit feeders. This may be an avenue of future work but, as Pagliosa (2005) indicates there has been little work conducted on the recognition of feeding guilds and their use in ecological assessment.

In summary, while the use of polychaetes species as indicators of the diversity and general health of the benthic community has often been successful, the same species or even families cannot be expected to serve as indicators in different geographic regions or even, perhaps, at different times within the same region. Benthic communities are dynamic and the presence or absence of species may change as the environment varies adding great complexity to any attempts at recognition of species able to act as robust indicators of low diversity. While few studies have been conducted in tropical regions, there is some indication that it may be more difficult to identify species indicative of diversity gradients. Attempts to simplify analyses by using sub-groupings of the entire polychaete community, such as the Terebellidae, Spionidae or different feeding guilds, show some promise but may be of value only in limited geographic regions.

POLYCHAETES AS INDICATORS OF ORGANIC ENRICHMENT

Perhaps the most widespread type of pollution in coastal waters is the high input of organic matter as sewage. As the organic matter is broken down, oxygen levels may be reduced in the sediments leading to anoxic conditions. As was pointed out by Gray *et al.* (2002) it is not necessarily the amount of organic material

entering the system which is important but the degree of anoxia. High organic input into an area well swept by currents does not elicit great changes in the benthic community while lesser amounts input into a semi-enclosed basin may have a major effect on the benthos. The species composition and biomass of the benthos are highly sensitive to low oxygen conditions and are routinely used to detect the progress of eutrophication in an area.

In a highly influential paper Pearson & Rosenberg (1978) described a model of macrobenthic successional change in benthic communities both with distance from a source of organic matter and with time as organic matter increases in the environment. This enrichment model says there are changes in species number, abundance and biomass as the effect of organic enrichment diminishes. Their model included a high organic community made up of a few resistant species such as *C. capitata* and *M. fuliginosus* present in high abundances. With reduced organic input an intermediate zone was described characterized by species somewhat less pollution tolerant such as the nereidid *N. caudata*, the dorvilleid *Dorvillea (Schistomeringos) annulata* (= *Staurocephalus rudolphi*), and the cirratulid *Cirriformia tentaculata*. At some distance away from the source of organic input would be found species usually present in the non-polluted region.

Pearson & Rosenberg (1978) included an extensive list of organisms which had been associated with high organic situations and later Diaz & Rosenberg (1995) provided a list of species found to be resistant to hypoxic conditions. A few weakly competitive, opportunistic species which are tolerant of high organic sediments, such as *C. capitata*, are thought to be able to respond to large influxes of organic material and the resultant anoxia due to their release from competition by less pollution-tolerant species as well as their early maturation and high reproductive potential. The less pollution tolerant, but more competitive, species would occur in the regions of intermediate organic pollution with reduced oxygen. While

these lists of species are extensive and quite helpful, they are certainly not complete and should only act as a guide, especially in less extensively sampled regions.

Mendez *et al.* (1998) analyzed the benthic community along a pollution gradient from a sewage outfall area off Barcelona, Spain, and, based on the work of Pearson & Rosenberg (1978) and Tsutsumi (1987), expected to find indicator species of high organic matter such as *C. capitata*, *M. fuliginosus*, *P. pinnata* and *P. ligni*. While *C. capitata* and *M. fuliginosus* were found near the source of organic input, *N. caudata* and *P. ciliata* also occurred in high abundances at these sites. These two species had previously been considered indifferent to high organic conditions and were more likely to occur at intermediate levels of organic input. Also, based upon previous studies in this region they expected their indicator species for non-polluted areas to be *O. fusiformis* and/or *P. serpens* but they found few specimens of either of these species. Some previously identified indicator species were present, some species not known to be indicator species prior to this study were found to be so, and even the negative indicator species varied with time.

This ability of the benthic community to respond to changes in their environment may occur quickly and their response to what seems to be similar environmental conditions may differ temporally. In a study of coastal waters exposed to sewage off Mar del Plata City in Argentina, Elias *et al.* (2005) found that their main indicator species for organic enrichment during the summer tourist season was *Prionospio* sp. After winter storms had occurred, the community in this region changed to one dominated mainly by Maldanidae and Spionidae, indicative of low organic content sediments. In a previous study in the same region the year before Elias *et al.* (2004) reported the organic enrichment indicator species to be the cirratulid *Caulleriella* sp. but this species was rarely collected in the subsequent survey. The benthos changed from one of a low organic environment to one of a high organic environment but there were seasonal changes

in the responses of the individual species in these communities.

Studies of the response of benthic communities to sewage input is often assumed to be a response to organic matter but other pollutants such as heavy metals, hydrocarbons and other industrial wastes may also occur in sewage. The study of the effects of locally intensive aquaculture upon the benthos allows us to analyze the direct effects of organic matter without any associated effects due to other pollutants. Fish farms and other aquaculture facilities are often established in semi-enclosed, protected waters which do not flush rapidly allowing the buildup of fecal material as well as uneaten food on the bottom often causing marked enrichment of the bottom.

Tsutsumi (1995), working in Japan, found that an area dominated by mollusks prior to creation of a fish net pen for red sea bream became oxygen depleted on a seasonal basis following establishment of the aquaculture facility. With the onset of fish culture the capitellid *Capitella* sp. I and some species of polydorids became the dominant species in the immediate area. Yokoyama (2002), working at a shallow water fish and pearl oyster facility in Japan, also found that the first species to colonize the anoxic bottom sediments at the fish farm site was what he called *Capitella* sp. followed closely by the spionid *Pseudopolydora paucibranchiata*. At the pearl oyster site, where there was less input of organic matter and no seasonal component to the organic input, the benthos at this site was similar to that of low organic input control sites. Apparently as long as the input of organic matter did not result in anoxia there seemed to be little effect on the benthos.

Lee *et al.* (2006) reported a temporal sequence to the colonization of the sediments beneath a Pacific threadfin fish farm in an open coastal area off Hawaii. They found that the oweniid *Myriochele oculata* occurring first as organic material began to be deposited on the bottom and the usual dominants, *Pionosyllis heterocirrata* (Family Syllidae) and *Euchone* sp. (Family Maldanidae) decreased in number.

C. capitata colonized the site when there were intermediate levels of organic matter but *O. adherens* became the numerical dominant when organic matter was present at high levels and the bottom became low in oxygen. Again, the degree of oxygen depletion resulting from the input of organic matter seems to be a major determinant of the response by benthic species to organic input. Recognition of indicator species due to high organics has to take into consideration the extent of oxygen depletion resulting from this organic input.

In an examination of the usefulness of indicator species in the assessment of organically enriched benthic communities Bustoz-Baez & Frid (2003) conducted a meta-analysis of benthic data from six impacted sites in the United Kingdom, including the study by Pearson & Rosenberg (1978). They found that of the 123 species included in those studies only 20% showed any response whatsoever to a gradient of organic enrichment. Only 16 species showed a positive response and four a negative response to organic content in two or more of these studies. These results call into question the utility of the species indicator approach or at least to the idea that there are certain widespread organic enrichment indicator species that should be expected to occur in any impacted area. Perhaps some of the inability to identify organic enrichment indicator species is due to the inattention paid to the degree of anoxia as a complicating factor.

In an interesting study, Jewett *et al.* (2005) found that in Chesapeake Bay the epifaunal serpulid species *Hydroides dianthus* doubled its coverage area if it had been exposed to periodic hypoxia. The hypoxic events apparently acted to remove other epifaunal competitors thus creating more free space for *H. dianthus*. These authors point out that the abundances of *H. dianthus* could be an indicator for exposure to stressful local conditions resulting from hypoxia.

In many of these studies of organic enrichment the most common species acting as an indicator of high organic matter is *C. capitata*. As another example, Klaoudatos *et al.* (2006),

working in the Aegean Sea, also reported colonization of anoxic sediments beneath fish pens by what was identified as *C. capitata* (along with *N. (H.) diversicolor* and *Scolelepis fuliginosa*). Is *C. capitata* a cosmopolitan indicator species for high organic input? Unfortunately, *C. capitata* is often a catch-all name for what has been shown to be many sibling species more correctly referred to as the *C. capitata* species complex (Grassle & Grassle 1976 and see discussion above). While members of the *C. capitata* complex may be useful as indicator “species” for high organic input, the differentiation of species types within the complex is required to ensure the indicator species is actually a single species type and not a summation of several species.

Polychaete indicator species have often been utilized to indicate stress on the benthic community at sewage outfalls. While sewage is made up of large amounts of organic wastes it often also includes other pollutants such as heavy metals, pesticides and polycyclic aromatic hydrocarbons. Studies of the effects of organic enrichment beneath aquaculture facilities present a much clearer picture of the response of the benthos to only organic input. Results of these studies illustrate that the degree of anoxia created by the excess organic material is the main determinant of the benthic response. Some of the variability between sites in the identification of polychaete indicator species may be traced to this inattention to the degree of oxygen depletion within the system.

POLYCHAETES AS INDICATOR SPECIES OF HEAVY METAL POLLUTION

Polychaetes have often been utilized as test organisms in the biotoxicity testing of heavy metals. In their review of such toxicological studies using polychaetes Reish & Gerlinger (1997) cited copper (Cu), along with mercury (Hg), as the most toxic metals tested. Less toxic were Chromium (Cr) and Cadmium (Cd) followed by Zinc (Zn) and Lead (Pb). Of the species that had been analyzed, *C. capitata* and *O. labronica* were sensitive, while *Cirriiformia*

luxuriosa (Family Cirratulidae) was tolerant of high Cu concentrations. *N. virens*, *C. capitata*, *C. serratus* and *Namalycastis abiuma* species group (= *Namenereis meukensis*) (Family Nereididae) were found to be very sensitive to Hg concentrations while the more Hg tolerant species included *N. (H.) diversicolor* and the polynoid *Halosydna johnsoni*. *C. capitata* and *D. gyrociliatus* were sensitive to high Cd levels while *C. luxuriosa* and the amphinomid *Eurythoe complanata* seemed to be fairly tolerant. The species that have been reported to be the most sensitive to Zn enrichment were *D. gyrociliatus* and *O. labronica* while *N. (H.) diversicolor* and *C. luxuriosa* were the most tolerant. More recently King *et al.* (2004) carried out toxicity tests on *Australonereis ehlersi* (Family Nereididae) and *Nephtys australiensis* (Family Nephtyidae) and found that both species were insensitive to Zn until it was present in very high concentrations (1mg/L). In short, polychaetes display great variability in their sensitivities to heavy metals both between species and with regards to a particular heavy metal.

Heavy metals such as Cu, Cd, Zn, and Pb enter coastal waters as runoff from sources such as mine tailings, industrial and agricultural wastes, sewage, and others. Most of these metals are rapidly adsorbed onto sediment particles and become concentrated in the sediments. Bryan & Langston (1992), for example, reported Cu concentrations in a polluted creek in the United Kingdom of about 3 000µg per gram of sediment while concentrations in the overlying water were 3-176µg per liter. Presumably, the Cu was being adsorbed from the water and concentrated in the sediments by iron oxides and humic substances. Adsorbed metals may then be released back into the water column or, perhaps more importantly to the benthos, into the interstitial pore waters of the sediments (Comans & van Dije 1988).

This uptake of metals by sediment particles and organic matter greatly affects the availability of these pollutants making extrapolation of the results of laboratory toxicology studies to field conditions very complex. Vázquez-Núñez

et al. (2007) noted that *E. complanata* from Mazatlán, Mexico, absorbed smaller amounts of Hg when sediments were present in their test chamber than if the worms were fully exposed to the surface waters. In the lab Selck *et al.* (1998) found that when *C. capitata* was exposed to Cd dissolved either in water, in sediments, or both, only those exposed to Cd present dissolved in water resulted in a slowing of the growth rate of this species. When sediments were present the worms took up Cd with little or no demonstrable effects despite sediment loads being similar to those of known polluted sediments (Bryan 1984). Field studies of the effects of Cu on polychaetes seemed to show that organic matter in the sediments may tie up the most toxic cupric ions thus reducing their bioavailability to the benthos (Athalye & Gokhale 1991, Bryan & Langston 1992). Pesch (1979) and Austen *et al.* (1994) found that Cu was more toxic to *N. arenaceodentata* in sand than mud perhaps as a result of the smaller sediment particles and associated organic matter of mud adsorbing greater amounts of the Cu.

Ray *et al.* (1980) and Ray & McLeese (1983) demonstrated that Cd was not being absorbed from the overlying water or sediments but from interstitial water and that Cd concentrations were actually related to the relative amounts of acid volatile sulfide in the sediments. Cd in the sediments was apparently unavailable to *N. virens* even at high concentrations while the animals were absorbing much lower levels of remobilized Cd from the pore waters. Similarly Ahn *et al.* (1995) found that the polychaetes *H. filiformis* and the Nereid *Nereis* (= *Perinereis*) *aibuhitensis* were capable of living in coastal areas of Korea despite elevated levels of metals such as Pb, Cd, Zn, and Cr due to the buildup of organic matter from industrial sewage. Méndez & Paez-Osuna (1998), working in Mazatlán Bay, Mexico, found the highest body levels of Cd in the tropical Amphinomid *E. complanata* were surprisingly not at the sewage outfall but in the relatively clean control sites. The explanation for this was that the sediments at the clean site were low in organic matter while there was

high organic input at the sewage outfall site and these organics tied up the Cd making it much less available to the worm. This could also explain the results of Kelaher *et al.* (2003) from the Hudson River, USA and Trannum *et al.* (2004) from the Oslofjord, Norway, who found no significant effect of high sediment Cd levels on abundances of species, community character or recruitment. While polychaetes may be useful in the detection of elevated heavy metals in the water column using laboratory toxicology studies (with no sediments present) translation of these results to field conditions is complicated by the effects of sediment particles and organic matter.

While absorption by the sediments may offer some degree of protection from exposure to sediments with elevated total heavy metal content, some deposit feeding polychaetes are known to take up heavy metals in their food. Maloney (1996), using *N. (H.) diversicolor*, and Selck *et al.* (1999), using *C. capitata* sp 1, both found that while Cd uptake was partially correlated with pore water concentrations it was mainly correlated with the Cd content of the food sources. In the case of Zn, Windom *et al.* (1982) saw a direct relationship between the levels of Zn in *C. capitata* and the levels of Zn in their detrital food source. Gibbs *et al.* (2000) explained the wide range of Cu and Zn concentrations seen in *O. fusiformis* as most likely the result of the ability of these animals to switch between a filter feeding and a detritus-feeding diet. The greater the reliance on deposit feeding by an individual the higher its uptake of heavy metals was from the sediments. Often the feeding strategies of polychaetes are poorly known but this may be an important factor in any interpretation of results of heavy metal uptake studies.

While polychaetes may take up appreciable amounts of metals they often have capabilities to detoxify these metals and store them in a less toxic form. Many polychaetes take up high levels of heavy metals associated with their food but these metals are converted to less toxic forms in the gut and are less hazardous than absorption through the body wall (Selck

et al. 1998, Berthet *et al.* 2003). Koechlin & Grasset (1988) found that silver (Ag), which is highly toxic, could be stored in the sabellid *Sabella pavonina*, in a biologically inactive form allowing for high body concentration of the metal with no apparent ill effects. The Ag was taken up in connective tissue and the lysosomes of the nephridia and gut then released into the urine. This ability to concentrate and sequester toxic heavy metals, rendering them inactive, would explain how many polychaetes collected from polluted sites may have much higher concentrations of metals in their bodies than would be expected based on the results of simple bioassay tests.

While total metal content of the sediments may not be indicative of their effects on the benthos the metal content of polychaetes has been utilized with variable results as an indicator of heavy metal burden of the sediments. Early work by Luoma & Bryan (1982) reported that *N. (H.) diversicolor*, *Perinereis cultrifera* (Family Nereididae) and *Nephtys hombergi*, exhibited Cu concentrations directly related those in the sediment. Later, however, Bryan & Gibbs (1987) found that the ampharetid *Melinna palmata* and the cirratulid *Tharyx marioni* seemed to be able to regulate, at least partially, their Cu content making these less likely to serve as indicators for this metal. *H. filiformis* and the nereid *Perinereis albuhitensis* were high in abundance at sites close to a sewage outfall on the west coast of Korea and were indicators of pollution but they also contained high levels of Cu, Pb and Cd indicating heavy metal pollution as well as organic enrichment. Working at sites in France and the United Kingdom, Berthet *et al.* (2003) found that the bioaccumulation of Cd and Cu (but not Zn) increased with metal content of the sediments in *N. (H.) diversicolor*. Conversely, Poirier *et al.* (2006) found no correlation between metal concentrations (Ag, Cd, Cu, Pb and Zn) in *N. (H.) diversicolor* and the sediments from which they were collected on the nearby French coast of the English Channel.

Many of the studies cited by Bryan & Langston (1992) in their review of heavy

metal pollution of the sediments in the United Kingdom, as well as later studies such as Berthet *et al.* (2003) and Poirier *et al.* (2006), use the nereid polychaete *N. (H.) diversicolor* as an indicator species. In Japan, Sun & Zhou (2007) used the taxonomically related species *Neanthes (Hediste) japonica* in their study of Cd and Cu (and hydrocarbons) uptake. These species are ideal candidates as indicator species as they are commonly taken in benthic samples and are relatively large animals but, as noted above, results of studies of heavy metal uptake in *N. (H.) diversicolor* have been variable. Saiz-Salinas & Francés-Zubillaga (1997), in fact, reported that *N. (H.) diversicolor* in 'Ría de Bilbao' (Spain) showed a wide range of concentrations of many metals with little correlation with the metal content of the sediments. They believed that the metal concentrations in *N. (H.) diversicolor* could better be explained by differences in diet and that this species should not be used as a biomonitor of sediment heavy metal levels. This confusion may be the result of geographical variation between populations or perhaps the existence of sibling species. When Sato & Nakashima (2003) examined syntypes of *H. japonica* (= *N. (H.) japonica*) they found two previously unrecognized new species. Species of *Neanthes*, as well as any other potential indicator species, should be well identified and any potential differences between geographic subpopulations fully described before use.

In tropical regions the species which has often used as an indicator species for heavy metal concentrations is *E. complanata*. This species is considered to be circumtropical, it is usually abundant and it is large and easily collected in intertidal and shallow waters. Reish *et al.* (1989), Marcano *et al.* (1996, 1997), Nusetti *et al.* (1998) and Vázquez-Núñez *et al.* (2007) have all conducted laboratory studies of uptake and its effects on this species using Cd, Cu, Hg and Zn. With regards to the use of *E. complanata* as an indicator of heavy metal concentrations a field situation, Méndez & Páez-Osuna (1998) monitored levels of Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn in *E. complanata* collected

from Mazatlán Bay in the Gulf of California (Mexico) and found high levels of Cu and Pb in the specimens taken from sediments high in those heavy metals. There were no significant correlations of metal concentrations between polychaete specimens and their sediments for the other six metals. Interestingly, these authors noted that there was a relationship between the levels of Cd, Ni, Pb and Zn and body size in *E. complanata*. They found lower concentrations of these metals in larger individuals than in smaller individuals. Given the ease of collection and the wide geographic range of this species it may be a valuable indicator species in tropical and semi-tropical regions where a temperate species such as *N. (H.) diversicolor* would not be expected to occur. Again care must be taken to ensure that this widespread species is not actually a species complex and physiological differences, such as differences in heavy metal uptake with size, are fully understood.

Differences in organic matter in the sediments, diet, and in regulatory capabilities of species all may act to complicate the use of polychaetes as indicators for metal pollution *in situ*. Further problems include the sometimes uncertain species identifications and the variance in physiological characteristics of species from different geographical regions. Finally, elevated levels of heavy metals often co-occur, (ex. if Cu levels are high it is often the case that Zn levels will also be high) and the effects of heavy metals on polychaetes are known to often be synergistic (Bryan & Langston 1992, Reish & Gerlinger 1997).

Another approach to establish ecologically relevant indicators of exposure to heavy metals is the identification of biomarkers, biochemical responses to the oxidative effects of these pollutants. An early application of this analysis to polychaetes was by Marcano *et al.* (1997) who assessed the levels of lysozyme activity in response to injection of bacteria in *E. complanata*. Lysozyme activity usually increases to protect against such a bacterial challenge but such an increase did not occur when the worms were exposed to elevated levels of copper. The presence of copper had an immunosuppressive

effect and perhaps a lysozymal assay could be used to assess exposure of an indicator species such as *E. complanata* to industrial wastes in a field situation.

Further workers have identified other biomarkers produced by polychaetes in response to oxidative pollutants such as heavy metals. Nusetti *et al.* (2001) also exposed *E. complanata* to elevated copper concentrations and found a resultant increase in melandialdehyde, an end product of saturated fatty acid breakdown due to oxidation. Additionally, they found the activity of the antioxidant enzyme glutathione reductase, which acts as a free radical scavenger in the detoxification of contaminants, was reduced in response to elevated copper. Geracitano *et al.* (2003) exposed the nereid *L. acuta* to elevated levels of copper and found that the enzyme catalase, which is thought to be produced in response to oxidative damage in the cell, increased in activity. Finally, Rhee *et al.* (2007) cloned the gene for the antioxidant enzyme glutathione S-transferase in *Neanthes succinea* (Family Nereididae), and found the expression of this gene increased significantly in response to elevated copper concentrations. Using specimens of this Nereid taken from an industrially polluted lake they found an increase in the expression of this gene as well as elevated levels of the cellular oxidative byproduct metallothionein proteins. These cellular indicators of oxidative damage and the suppression of the activity of antioxidant enzymes such as glutathione reductase and glutathione S-transferase could be used as biomarkers for oxidative stress resulting from heavy metal pollutants such as copper in polychaetes.

While there have been shown to be significant differences in biomarkers in polychaetes exposed to individual heavy metals in the lab there are numerous other environmental factors which may also result in oxidative stress. Abele-Oeschger & Oeschger (1995) demonstrated that in various life stages of the phyllodocid *Phyllodoce mucosa* there were differences in glutathione reductase activity as well as that of another antioxidant enzyme, superoxide-dismutase apparently due

to differences in exposure to solar radiation. Other environmental causes of oxidative stress may include hypoxia, temperature, H₂S levels, organic hydrocarbons and pesticides (Abele *et al.* 1998, Scaps & Borot 2000, Bocchetti *et al.* 2004, Ait Alla *et al.* 2006, Durou *et al.* 2007). The use of biomarkers in polychaete indicator species as indicators of elevated heavy metal contaminants in the field is thus far ambiguous and should be viewed as a general indicator of oxidative stress.

POLYCHAETES AS INDICATORS OF ORGANIC CONTAMINANTS

Organic contaminants include polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and pesticides. These toxic hydrocarbons enter coastal waters from many sources including automobile exhausts, oil spills, urban storm runoff, and agricultural runoff. Once in the water these contaminants are similar to heavy metals in that they adhere to suspended particulates and settle out in coastal sediments, often at high concentrations. These pollutants can be toxic (as well as carcinogenic and mutagenic) to both the benthos as well as the fish that feed upon them. Once again, total sediment load of these pollutants does not tell the entire story as the release of these materials is dependent on such things as sediment particulate size and organic content, for example. Polychaete indicator species have, therefore, been used to determine the bioavailability of these pollutants and identify ecological risks.

Reish & Gerlinger (1997) reviewed laboratory toxicity tests conducted on polychaetes exposed to the water soluble portion of oil and found that extracted from refined oil was more toxic exposure to than that from crude oil. Similar results were seen when the effects of the water soluble fraction upon the reproduction of *O. diadema* and *C. serratus* were tested (Carr & Reish 1977). Based upon LC50 values, *N. arenaceodentata*, *O. diadema* and *Ophryotrocha puerilis*, were the most sensitive to either type of oil derivative while

C. capitata capitata, the cirratulid *Cirriformia spirobranchia* and *C. serratus* were comparatively less sensitive. More recently, Grant & Briggs (2002) conducted toxicity tests using the arenicolid *Arenicola marina* to analyze the effects of drill cuttings taken from adjacent to a North Sea oil platform. They found that sediments taken from 100m away from the oil platform had no effect on survival but even a 10% dilution of these sediments prevented feeding activity. It was also noted in this study that accompanying heavy metal concentrations in these drill cutting sediments were much too low to explain these toxic effects.

The uptake of organic hydrocarbons from the sediments and their accumulation by polychaetes capable of living in polluted regions has been often studied using deposit feeding species such as members of the Arenicolidae. Lyes (1979) found that *A. marina* took up very little labeled naphthalene, a water soluble component of crude oil, from contaminated sediments. Apparently much of the naphthalene remained bound to the sediments as it passed through the gut of the worm and any that was taken up by the worm was quickly released. This reduced uptake is in agreement with earlier work by Gordon *et al.* (1978) who found little evidence of uptake of PAHs in the same species collected from an area directly impacted by an oil spill in Canada. Weston *et al.* (2004) demonstrated that the initial uptake of benzo(a)pyrene (BaP) by *Abarenicola pacifica* (Family Arenicolidae) was due to absorption of dissolved BaP across the body wall but after several days of exposure the main source of uptake was from ingested material. Weston (1990) had previously shown, using [³H]benzo(a)pyrene, that *A. pacifica* was able to break down this material in the tissues and thus was capable of some level of regulation of tissue load. Surprisingly Weston found that the greatest bioaccumulation of BaP was in the worms exposed to the low organic, low BaP level sediments. The greatest correlation was not with levels of BaP in the surrounding water or sediments but with the feeding rate of the worms. Timmermann & Andersen (2003) conducted similar studies of the uptake of the

aromatic hydrocarbon pyrene by *A. marina* and also found highest bioaccumulation in the sediments with the lowest levels of organics and pyrene. The worms in the coarser, low organic sediments had the highest rates of feeding and these feeding rates correlated with body burden of pyrene. Worms in the high organic sediments with high total pyrene content had a rich food supply of organic material and/or its associated bacteria so they did not need to ingest as much material containing high levels of pyrene. What these results indicate is that subsurface deposit feeders such as these members of the Arenicolidae accumulate aromatic hydrocarbons not in relation to water or sediment load but in relation to ingestion rates making them poor candidates as indicator species of sediment loads.

C. capitata has also been explored for use as an indicator species for polycyclic aromatic hydrocarbon enrichment since it is capable of living in such polluted sediments. Linke-Gamenick *et al.* 2000, however, worked with three sibling species of *Capitella* and found that high sediment levels of fluoranthene reduced juvenile survival and inhibited reproduction in the intertidal species but not in the species from polluted sediments and from a hydrothermal vent. *Capitella* sp. 1 is one of the few species that can live in sediments contaminated with PAH and other organic pollutants (Forbes *et al.* 1994). Forbes *et al.* (1996) exposed this species to fluoranthene and found that it quickly took up the pollutant in a concentration dependent manner but body levels were undetectable after three days of exposure. This species is able to convert fluoranthene to more hydrophilic substances which are less toxic allowing its existence in heavily polluted sites (Forbes *et al.* 2001) but making it a poor candidate as an indicator species for hydrocarbon pollution.

Rust *et al.* (2004) exposed six species of polychaetes to high BaP levels and found that *N. succinea* and *N. virens* seemed to be able to convert this PAH to less toxic metabolites most effectively while the maldivian *Clymenella torquata* was least effective leading them to warn against the use of *N. virens* as an indicator

species for PAH contamination. Jørgensen *et al.* (2005) found that *N. virens* had a great ability to regulate its body content of pyrene, another PAH. Kane Driscoll & McElroy (1996) exposed *N. (H.) diversicolor*, the spionid *Scolecopides viridis* and the orbiniid *Leitoscoloplos fragilis* to BaP and found that the first two species were capable of rapidly metabolizing this hydrocarbon while it was metabolized much more slowly by *L. fragilis*. Tissue accumulation of this hydrocarbon was highest in *L. fragilis* making it the better choice of these three species as an indicator of PAH levels.

Another species shown to be very tolerant of PAHs is the spionid *Streblospio benedicti*. Chandler *et al.* (1997) exposed these worms to high levels of PAH in the lab and found elevated levels of the pollutant in their tissues with no apparent effects on mortality. There were some indications of a reduction in larval settlement/metamorphosis success but these were not significant. This species is a frequent dominant in hydrocarbon-contaminated sediments and its ability to concentrate this type of pollutant makes it a potential indicator species for hydrocarbon contamination.

While *C. capitata* has been identified as a dominant in an oil spill region (Sanders *et al.* 1980) there have been few species identified from field studies as possible indicators of chronic organic pollution. When Lenihan *et al.* (1995) transplanted sediments to areas near McMurdo Station in Antarctica polluted mainly by petroleum hydrocarbons they found *Ophryotrocha claperedii* (Family Dorvilleidae) to be numerically dominant at the most polluted sites. *O. claperedii* was also abundant at intermediately polluted sites but was accompanied by species of *Gyptis* and *Tharyx*. Most of the other polychaetes transplanted to the hydrocarbon polluted sites from clean areas died.

Venturini & Tommasi (2004) worked with polychaete assemblages in two bays in north-east Brazil and found that the stations with high organic, high PAH sediments were dominated by carnivorous species while those of sandy sediments with low PAH levels were dominated by subsurface deposit feeders. Ingole *et*

al. (2006) found that the most abundant polychaete in the area surrounding the *MV River Princess*, an oil transport ship containing oil that had been grounded for three years off Goa, India, was the carnivore *Pisionideus indica*. In both of these studies the carnivorous species in these high PAH stations are also regarded as indicative of high organic content so they may not necessarily be acting as indicators of PAH contamination.

Intertidally, Ditzel Faraco & Lana (2003) dumped diesel fuel directly on mangrove sediments in southeast Brazil they found no significant difference between the fauna in these plots and in nearby control plots after recovery. In fact a much greater effect was seen if the sediments were allowed to dry out before treatment than if diesel oil was poured directly on them. Dutrieux *et al.* 1989 found that in chronically oil polluted Indonesian mangroves populations of a Nereididae, *Nereis* sp., showed a decrease in average size along a pollution gradient. Additionally, the capitellid *Puliella* sp. was resistant to all but the highest levels of oil and decreased in size with increasing pollution perhaps making these species good candidates as indicator species based on average body size.

While some species of polychaetes are often found in oil polluted sediments it is difficult to characterize any as indicator species of elevated PAH conditions. In field situations the species present in regions high in PAHs are usually species which have been recognized as organic enrichment species. Many species (*ie. A. pacifica, Capitella* sp.) are very capable of regulating body levels of these contaminants and body burden is more closely related to feeding effort than contaminant levels of the environment.

Pesticides applied to the terrestrial environment often end up in coastal waters. Additionally, pesticides are sometimes applied directly to intertidal or shallow waters in order to reduce the effects of various pests to financially important aquacultural species such as oysters and fish. Those pesticides which break down slowly in the environment such as organochlorides are especially worrisome

as they may build up as they pass through the food chain resulting in biomagnification in the higher trophic levels. Little is known about the accumulation of these pesticides in the benthos and their effects on benthic communities and the use of polychaetes as indicator species for pesticide pollution is rare.

Reish & Gerlinger (1997) reviewed the relatively few studies of the effects of pesticides on polychaetes up to that time. They listed LC50 values which had been established for DDT and endosulfan on *N. arenaceodentata* (Pesch & Hoffman 1983, Reish *et al.* 1985); endosulfan and chlordane for *N. virens* (McLeese *et al.* 1982); and carbaryl and parathion-ethyl for the arenicolid *Arenicola cristata* (Conti 1987). Some species have shown a resistance to certain pesticides. For example, *N. virens* was not adversely affected by endrin, dieldrin, or DDT at the levels tested by McLeese *et al.* (1982) and *N. arenaceodentata* was not affected by the insecticides bactimos and altosid at the levels tested by Reish *et al.* (1985). Reish *et al.* (1989) found that survival of *E. complanata* was not affected by the highest levels of DDT they tested but the ability of these worms to regenerate anterior or posterior ends was reduced.

While adults are capable of living in pesticide polluted sediments Hill & Nelson (1992) showed that the number of worms able to complete metamorphosis and settle were reduced in *Capitella* sp. 1 upon exposure to the pesticide lindane. Flemer *et al.* (1995) found that when the concentration of the pyrethroid fenvalerate was increased in settlement plots, the capitellid *Mediomastus ambiseta* actually increased in number and there was no negative effect on abundances at the concentrations used in their study. Hansen *et al.* (1999) reported a reduced growth rate and reproductive output of *C. capitata* sp. 1 to nonylphenol, a breakdown product of surfactants and an emulsifier of pesticides, and noticed a reduction in time to first reproduction. Murdoch *et al.* (1997) conducted a chronic response study of *N. arenaceodentata* to two organohalines and found that while survival and growth were not affected there was

a noticeable effect upon reproduction. Mendez (2005, 2006) has exposed *Capitella* sp. 1 and *Capitella* sp. B to the insecticide Teflubenzuron used to treat ectoparasitic infestations of salmon and saw no adult mortality but did note juvenile mortality, delayed metamorphosis, reduced feeding activity and reduced body size. Adult survival seems relatively resistant to pesticides and perhaps the nature of pesticide effects upon polychaete reproduction would provide a more sensitive assay in the use of polychaetes as indicator species.

There has been little testing of the field responses of benthic polychaetes to pesticides. Pridmore *et al.* (1992) applied the organochloride pesticide chlordane on an intertidal sandy beach in New Zealand and found that of the five most abundant polychaetes species present only the subsurface deposit feeding *H. filiformis* showed a significant decrease in abundance after two months. The surface feeding spionid *A. oxycephala* the deposit feeding orbinid *Orbinia papillosa* and the predators *Goniada emeriti* (Family Goniadidae) and *Sphaerosyllis semiverrucosa* (Family Syllidae) were unaffected by the chlordane. Dumbauld *et al.* (2001) applied the pesticide Carbaryl (SEVIN®), an organocarbamate used to eradicate burrowing shrimp, to oyster beds in Washington state and found essentially no effect on the polychaete populations, including the numerically dominant capitellid polychaete *Mediomastus californiensis*. Similarly, in Ernst *et al.* (2001) study of the effects of azamethiphos and cypermerthrin on the benthos adjacent to salmon pens, no polychaetes were reduced in abundance. Finally, Granberg *et al.* (2008) has exposed the spionid *Marenzelleria neglecta* to Baltic Sea sediments contaminated with PCBs and chlorinated pesticide residues for over 85 days and found no accumulation of these compounds in the animals. These field experiments have shown that polychaetes are relatively resistant to, and do not seem to accumulate pesticides, in field experiments and would be poor candidates as indicator species for these pollutants in field situations.

Scaps *et al.* (1997) has used a more precise approach to the detection of the effects of

pesticides on the polychaete *N. (H.) diversicolor*. In laboratory studies these authors noted an inhibitory effect by three organophosphate pesticides and carbaryl, a carbamate pesticide, on acetylcholinesterase activities. This inhibition of this enzyme was interpreted as a response to contaminant stress and exposure to such pollutants and was a sensitive test with significant inhibition exhibited at levels of 10⁻⁶M for the pesticides tested. This method could be a good candidate to use for testing toxicity of other organophosphate pesticide pollutants using *N. (H.) diversicolor* as the assay species. Ait Alla *et al.* (2006) has used *N. (H.) diversicolor* as their test organism to evaluate the use of four enzyme activity markers in polluted and unpolluted estuarine sites in Morocco. Pollutants often co-occur and this approach attempts to parse out the responses to different types of pollutants by the effects upon the different types of enzymes. They noted a high inhibition of acetylcholinesterase activity in the polluted population which they interpreted as most likely due to pesticide pollution. Care must be taken when using this method to assay for pesticide effects as Scaps & Borot (2000) have shown that salinity and, to a lesser extent, temperature may also affect acetylcholinesterase activity in *N. (H.) diversicolor*. As a further note of caution Hamza-Chaffai *et al.* (1998) also found that elevated levels of the heavy metal copper also can affect acetylcholinesterase in a bivalve. Despite these caveats these enzyme markers could act as a powerful means to monitor pesticide effects on the benthos using polychaetes such as *N. (H.) diversicolor*.

CONCLUSIONS

The polychaetes have been utilized extensively in the assessment of the ecological state of benthic communities, especially the response of those communities to pollution. What is evident in this review is that the assessment of the effects of pollution upon the benthos is quite complex and there are no simple means for doing this. Polychaetes (as well as other taxa)

differ greatly in their responses to environmental pollutants. Populations in a polluted region may differ in their resistance to those pollutants from nearby populations of the same species in an unpolluted region. The idea that a single polychaete species, or groups of species, will always occur in an environmentally stressed, or environmentally benign, situation is incorrect as numerically dominant species may change temporally in any dynamic system. A further cause of difficulty is that most field studies have ignored the usual co-occurrence of pollutants and any synergistic effects of this mix of pollutants. Despite these problems, the use of polychaetes as indicator species has shown to be a useful tool in any assessment of the environmental quality of benthic communities.

There is a proven history for the use of polychaetes in the toxicological assessment of water quality. Polychaete species differ in their sensitivities to individual pollutants and often it is the early life stages (eggs, embryos, larvae) as well as the effects upon growth and reproductive output which provide the greatest sensitivity. The adsorption of many toxic substances onto the sediments and organic material, rendering them less available to benthic organisms, make application of laboratory toxicological studies to field situations problematic. An additional concern is ensuring that the test species is properly identified. The use of well established cultures of polychaete species can usually eliminate this concern.

The use of polychaetes as indicators of community diversity and, therefore, overall health of the benthic community has value but it is apparent that there are no cosmopolitan positive or negative indicator species which will identify a community as healthy or unhealthy. Each region is inhabited by many species capable of a rapid response to an environment impacted by conditions toxic to other species. Which of these species will respond to polluted conditions and become numerically dominant seems to vary with time. Even the polychaete species characteristic of an unpolluted region may vary temporally. Some have espoused the use of higher taxonomic levels

of polychaetes (families, orders) as indicators of the state of the benthos but these methods have shown limited success. Other methods such as an 'annelid pollution index' or the use of polychaete feeding guilds have also shown limited success. Polychaete species can be used as positive or negative indicators for the effects of pollutants on the benthic community but only within the confines of a specific sampling program. Those species recognized as indicators should be viewed as specific for the area studied and for the time period of the study.

Numerous species of polychaetes have been identified whose abundances could be used to indicate organic enrichment of an area. Sewage input is the greatest source of organic material to coastal waters although organic enrichment may occasionally occur naturally. It is often not the total amount of organic material deposited into a region but the amount relative to the ability of that region to break down that material. Too much organic input may lead to anoxic conditions in the sediments and this is what will affect the benthic community. Especially in the case of sewage input, the analysis of the effects of organic input is confounded by the associated input of many other types of pollutants (heavy metals, pesticides, and others). Organic enrichment at aquaculture facilities provides a useful means to assess the effects of organic enrichment without interference from other pollutants and many of these studies indicate that members of the *C. capitata* complex, the genus *Ophryotrocha* and polydorid spionids are the usual numerical dominants.

Much study has been done on the effects of heavy metals on polychaetes, including many ecotoxicological studies. The results of these laboratory studies often cannot be applied to the field situation as these metals are adsorbed onto the sediments segregating them from the benthic organisms. Many species of polychaetes are able to exist in sediments containing very high heavy metal loads. Some species only take up heavy metals by diffusion from the interstitial water which deposit feeders may take in greater amounts through ingestion during feeding. Many polychaetes have shown a

great capability to regulate the body burden of these metals thus reducing their utility as indicator organisms. Recent studies on the use of biomarkers in polychaetes in order to recognize biochemical responses to the oxidative stress of heavy metal pollutants has shown some promise but further work is needed to overcome the lack of specificity of these methods.

Many polychaete species have shown a relatively high ability to regulate organic contaminants such as polycyclic aromatic hydrocarbons (PAH) and pesticides. Life history characteristics such as reproductive output and body size (growth rate) may be more sensitive indicators of environmental degradation due to organic contaminants than adult survivorship. The inhibition of the activity of the enzyme acetylcholinesterase in polychaetes may prove useful as a response to pesticide input but this biomarker also may lack specific specificity.

Many species shown above to have been useful as indicator species for various types of environmental pollutant based stress occur in the Gulf of Nicoya, Costa Rica. Potential species of use include capitellids of the genera *Capitella* and *Mediomastus*, the nereid *N. succinea*, the amphinomid *E. complanata* and cirratulids of the genera *Chaetozone* and *Cautleriella*. As previously mentioned though, each area may have its own set of species which best act to indicate the health of the environment and further studies in the Gulf of Nicoya will help to recognize those species. Hopefully this review will stimulate the analysis of those areas of the Gulf which are being impacted by pollutants and lead to any necessary remediation.

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RESUMEN

Los poliquetos son usualmente el taxón más abundante en comunidades bentónicas y han sido muy utilizados como especies indicadoras de condiciones ambientales. Esta revisión encuentra que, mientras el uso de especies indicadoras para un contaminante en particular no es simple, los poliquetos pueden proveer un medio útil para evaluar los efectos de condiciones ambientales pobres. Los poliquetos pueden ser usados como indicadores sensibles de la calidad de agua, especialmente en términos de los efectos de contaminantes en las características del ciclo de vida. También pueden ser utilizados como indicadores generales de la diversidad de la comunidad pero aquellas especies indicadoras de baja diversidad pueden ser diferentes geográficamente y temporalmente. Mientras que las aguas servidas son generalmente una mezcla con mucho material orgánico y otros contaminantes como metales pesados y plaguicidas, las situaciones de alto contenido de material orgánico asociadas con acuicultura indican que miembros del complejo de especies *Capitella capitata* y del género de dorvilleidos *Ophryotrocha* son a menudo dominantes. Algunas especies de poliquetos son capaces de vivir en sedimentos con alto contenido de metales traza y la carga corporal de estos metales a menudo no refleja las concentraciones en los sedimentos debido a regulación por estas especies. Muchas especies parecen relativamente resistentes a contaminantes orgánicos y plaguicidas y los efectos de estos contaminantes en los ciclos de vida pueden proveer un método de evaluación más sensible. Estudios recientes han tenido algún éxito usando biomarcadores en poliquetos para indicar contaminación general por metales pesados o plaguicidas. Una lista, de especies de poliquetos conocidas por ocurrir en densidades apreciables en el Golfo de Nicoya, Costa Rica, y que a menudo han sido usadas como indicadores de contaminación, se incluye como taxa con potencial para monitoreo ambiental en este estuario tropical.

Palabras clave: Polychaeta, especies indicadoras, contaminación marina, diversidad, metales, plaguicidas, materia orgánica, estuario tropical, Costa Rica.

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APPENDIX 1

List of genera and species names mentioned in the text.

- Abarenicola pacifica* Healy and Wells, 1959
Aglaophamus uruguayi Hartman, 1953
Arenicola cristata Stimpson, 1856
Arenicola marina (Linnaeus, 1758)
Australonereis ehlersi (Augener, 1913)
Amphiglena mediterranea (Leydig, 1851)
Anabothrus (= *Sosane*) *gracilis* (Malmgren, 1866)
Aonides oxycephala (Sars, 1862)
Capitella capitata (Fabricius, 1780)
C. capitata capitata
Capitella capitata species complex
Capitella sp. I
Capitella sp. B
Caulleriella
Caulleriella sp.
Ceratocephale
Chaetozone setosa Malmgren, 1867
Chaetozone
Cirriformia luxuriosa (Moore, 1904)
Cirriformia spirobranchia (Moore, 1904)
Cirriformia tentaculata (Montagu, 1804)
Cirratulus cirratus (Müller, 1776)
Clymenella torquata Leidy, 1855
Ctenodrilus serratus (O. Schmidt, 1857)
Dinophilus gyrotiliatus O. Schmidt, 1857
Diopatra neapolitana delle Chiaje, 1841
Dorvillea (*Schistomeringos*) *annulata* Moore, 1906
Euchone sp.
Eurythoe complanata (Pallas, 1766)
Exogone verugera (Claparède, 1868)
Galeolaria caespitosa Savigny, 1818
Glycera alba (Müller, 1788)
Goniada emeriti Audouin and Milne Edwards, 1833
Gyptis
Halosydna johnsoni (Darboux, 1899)
Harmothoe imbricata (Linnaeus, 1776)
Heteromastus filiformis (Claparède, 1864)
Hydroides dianthus (Verrill, 1873)
Hydroides elegans (Haswell, 1883)
Laeonereis acuta Treadwell, 1923
Leitoscoloplos fragilis (Verrill, 1873)
Lumbrinereis spp.
Magelona papillicornis Müller, 1858
Malacocerus fuliginosus (Claparède, 1868)
Maldane sarsi Malmgren, 1865
Marenzelleria neglecta Sikorski and Bick, 2004
Mediomastus ambiseta (Hartman, 1947)
Mediomastus californiensis Hartman, 1944
Mediomastus fragilis Coquillett, 1902
Melinna palmata Grube, 1870
Myriochele oculata (Zachs, 1923)
Neanthes caudata (delle Chiaje, 1828)
Neanthes (*Hediste*) *diversicolor* (Müller, 1776)
Neanthes (*Hediste*) *japonica* (Izuka, 1908)
Neanthes arenaceodentata (Moore, 1903)
Nephtys australiensis Fauchald, 1965
Nephtys hombergi Savigny, 1818
Nereimyra punctata O.F. Müller, 1788
Nereis aibuhitensis (Grube, 1878)
Nereis virens Grube, 1851
Ophryotrocha adherens Paavo, Bailey-Brock and Åkesson, 2000
Ophryotrocha claperedii Studer, 1878
Ophryotrocha diadema Åkesson, 1976
Ophryotrocha hartmanni Huth, 1933
Ophryotrocha labronica La Greca and Bacci, 1963
Ophryotrocha puerilis Claparède and Metschnikow, 1869
Orbinia papillosa (Ehlers, 1907)
Owenia fusiformis delle Chiaje, 1844
Paramphinome
Paraprionospio pinnata (Ehlers, 1901)
Pectinaria koreni (Malmgren, 1866)
Perinereis albuhitensis Grube, 1878
Perinereis cultrifera (Grube, 1840)
Pholoe minuta (Fabricius, 1780)
Phyllodoce mucosa Oersted 1843
Pionosyllis heterocirrata (Hartmann-Schröder, 1959)
Pisionidens indica (Aiyar and Alikunhi, 1940)

<i>Platynereis dumerili</i> (Audouin and Milne Edwards, 1833)	<i>Sabella pavonina</i> Savigny, 1820
<i>Poecilochaetus serpens</i> Allen, 1904	<i>Scalibregma inflatum</i> Rathke, 1843
<i>Polydora ciliata</i> (Johnston, 1838)	<i>Scolecopsis squamata</i> (Müller, 1806)
<i>Polydora cornuta</i> Bosc, 1802	<i>Scolecopides viridis</i> Verrill, 1873
<i>Polydora ligni</i> (Webster, 1879)	<i>Sphaerosyllis semiverrucosa</i> Ehlers, 1913
<i>Polydora websteri</i> Hartman, 1943	<i>Streblospio benedicti</i> Webster, 1879
<i>Prionospio heterobranchia</i> Moore, 1907	<i>Streblospio gynobranchiata</i> Rice and Levin, 1998
<i>Prionospio perkinsi</i> Maciolek, 1985	<i>Syllis</i> spp.
<i>Prionospio</i> spp.	<i>Terebellides stroemi</i> Sars, 1835
<i>Protoarcia oerstedii</i> (Claparède, 1864)	<i>Tharynx marioni</i> (Saint-Joseph, 1894)
<i>Pseudopolydora paucibranchiata</i> (Okuda, 1937)	<i>Tharynx</i>
<i>Puliella</i> sp.	<i>Tharynx</i> sp.