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Hurricane Mitch: Impacts of Bioturbating Crustaceans in Shrimp Ponds and Adjacent Estuaries of Coastal Nicaragua

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Background

Unusually high storm precipitation, accompanied by wind-exacerbated destruction of vegetation and downslope slumping of topsoils, produced extremely high sediment loads in flood waters of drainage basins feeding the Gulf of Fonseca and adjacent coastlines of northwestern Nicaragua. In many areas, flood waters were reported to have destroyed existing mariculture pond levees and changed courses of natural distributaries. New accretion and redistribution of deltaic sediments filled some pond impoundments and in other cases buried productive vegetated natural substrates that formerly supported shrimp postlarvae and other estuarine-dependent invertebrates and fishes of commercial and ecological importance. In varied cases, these intertidal and shallow-subtidal varves entrapped or inundated richly organic plant debris (root and emergent foliage), which is ultimately subject to long-term hypoxic decomposition and subsequent leaching of highly reduced compounds.

Even where channels and ponds were reexcavated or impoundment levees rebuilt, reestablishment of prestorm redox and nutrient stratification in porewaters was expected to be long in coming. This process, modulated by newly recruited benthic infauna and anthropogenic manipulation of sediments, was foreseen to potentially bear on productivity of both mariculture ponds and natural habitats that support wild shrimp populations, the latter of which were long used as a source of some postlarval stock (PLs) used in local penaeid mariculture. Highly organic, postperturbation sediments potentially favor explosive growth in populations of pest infaunal macroinvertebrates that exploit anaerobic porewaters and dominate selected biotopes to the virtual exclusion of other species. Bioturbational and ventilatory effects of this infaunal assemblage are know to occur elsewhere, including other mariculture ponds, and to be highly detrimental to secondary production (Nates and Felder, 1998), at least until the atypically high organic loads of newly deposited sediments are largely exhausted. Such negative effects to penaeid survival and growth rates are believed to accrue through both depression of diel oxygen minima and elevation of reduce toxic compounds (ammonia and sulfides) in overlying waters (fig.1). In other settings, there are apparent benefits to plant and animal communities from the activities of these bioturbators (Felder, 2001).

This project was designed to evaluate the agents, processes and progress of decompositional effects in benthic and epibenthic environments of coastal Nicaragua, with special attention to dominant, process-modulating infaunal assemblages in penaeid shrimp culture ponds and adjacent intertidal and shallow subtidal habitats. Observations were designed to include population assessments of abundance and distribution in dominant, recently recruited burrowing assemblages as well as measurement of redox levels and nutrient efflux accruing to the overlying water column from their burrows in culture ponds and adjacent habitats. It was anticipated that, as in other tropical Central American shrimp culture ponds (Nates and others, 1997; Nates and Felder, 1998, 1999) such processes would be dominated by thalassinidean decapods. These bioturbators are known to undergo explosive population increases and become major pest problems in culture ponds containing high concentrations of benthic organic materials (Nates and Felder, 1999).

The project was undertaken in part to provide environmental insights and management tools for the penaeid shrimp mariculture industry. Potential for population explosion of pest species in culture ponds was to be evaluated, and ecologically compatible approaches to management of these populations to be proposed. As originally envisioned, this project would also provide data on densities of shrimp postlarvae in recovering natural estuaries, potentially for correlation with water quality data and changing conditions over time.

At the beginning of our initial visit to Nicaragua under this project (April 2000), meetings with Nicaraguan shrimp farming interests were held in Chinandega. Scientists from the National Oceanic and Atmospheric Administration (NOAA) also attended these meetings. Information from this meeting, along with our subsequent observations to regional shrimp farms, was used to tailor the originally proposed project design and emphases. Three observations, in particular, impacted the originally proposed project design: 1) It was apparent that pesticides were already widely in use to treat shrimp ponds, for intended complete eradication of burrowing organisms and other macroorganisms in ponds where they were thought to be potential vectors of "white spot" and other shrimp diseases; 2) there was, perhaps because of this treatment, no evidence that foreseen outbreaks of burrowing pest crustaceans had in fact occurred to date, at least in ponds on shrimp farms; 3) it was clear that our intended monitoring of wild penaeid PL stocks on periodic trips would be both logistically impractical and of limited interest to the regional commercial shrimp farmers (who were already in full transition to use of hatchery PLs and development of their own hatcheries).

Given these observations, our studies were broadened to encompass a more comprehensive examination of natural estuaries than originally envisioned, many adjacent to shrimp farming interests, to determine the presence or absence of potential pest crustacean populations and the level of threat (if any) that they posed to shrimp farming were it to be conducted without pesticide treatment.   Thus, our measurements of redox and nutrients in porewaters, effluent burrow waters, and surface waters were made primarily in these settings. Also, as the entire community of burrowing crustaceans was being apparently targeted by pesticide treatments on shrimp farms (all being perceived as putative vectors of shrimp disease), and downstream impacts of these treatments could impact adjacent coastal estuaries, our sampling program within and outside of shrimp ponds was expanded to more exhaustively sample and characterize this infaunal assemblage than originally envisioned. Work was tailored to include studies of measurable pesticide-sensitive lipid profiles in selected dominant infaunal genera to provide at least a minimum baseline for comparisons with ecological counterparts from regions not subjected to contaminant exposures. This was undertaken after the initial field trip revealed extensive regional use of the pesticide Neguvon® in aquacultural practice, and subsequent to the publication of a study indicating the value of lipid profiles in assessment of such contaminant effects (Nates and McKenney, 2000). Our project was also broadened to include genetic characterizations of dominant infaunal species, when it became apparent from morphological evidence that a potentially large part of this assemblage was comprised of possibly endemic species, previously unknown to science. This was deemed critically important, given the conservation implications that such discoveries have on future shrimp farm development and management in the region, including the advisability of continued pesticide use in coastal aquacultural settings.

Methodology

Field trips to Nicaragua were made by project investigators from the University of Louisiana at Lafayette during 10-16 April, 2000, 26 September - 1 October 2000, 13 - 17 August 2001, and 16 -22 November 2001. Sites were accessed by rental vehicles or vehicles provided by USAID; Sahlman shrimp farms provided boat access to sites on their properties. Sites were identified by proper names on available maps as well as by GPS coordinates (fig. 2), and those harboring significant infaunal thalassinidean populations were targeted for repeated survey. Dominant infaunal macrocrustacean biota (figs. 3A-F, 4A-F) was collected primarily with an extraction corer or "yabby pump" (figs. 5D,F); these collections were supplemented by hand-collecting of shallow burrowers and motile intertidal burrowers (figs. 5A-C). Prior to preservation of collected specimens in 95% ethyl alcohol, selected examples of burrowing thalassinideans, their common symbionts, and some other common crustacean species were photographed for archival of color in transparencies (Kodachrome 25). Exposures were made with a 35mm Nikon FE camera and 55mm Nikkor macrolens in the field, with subjects illuminated by mirrored sunlight and posed in water on a black felt background. Photographic vouchers were so labeled and also subsequently preserved in 95% ethyl alcohol for subsequent morphological examination and possible DNA sequencing.

Sampling protocols varied by site and date, depending on the site conditions at the time it was visited; unpredictable conditions on shrimp farms (often dry, recently treated with pesticides, or containing only precipitation) provided limited opportunities for collection and study of associated infauna or burrow effluent waters (figs. 6A,B). Natural estuaries of several drainages, ranging from large bay mouths to small tidal streams (figs. 5E,F and 6C), were thus the focus of most organismal and water sample collections. Where densely burrowed by thalassinideans (figs. 6D), population densities were estimated by 0.2-m² quadrat counts of burrow openings, samples of dominant burrowing animals were obtained, redox measurements were taken, and samples of burrow effluent waters, porewaters, and surface waters were drawn for later analysis in the laboratory (figs. 6E,F). Measurements of redox potential were made with a pH millivolt (mV) meter equipped with Pt-Cu and calomel reference probes (Cole-Parmer Digi-sense® Molel 5938-10). Field readings were corrected to those of a standard hydrogen electrode by adding +242mV to readings. Waters for nutrient analysis were drawn with a 60-ml disposable syringe equipped with an in-line discharge filter. Samples were transferred to sealed vials and frozen for return to the lab and subsequent analysis. Waters were analyzed for concentrations of phosphate, ammonium, nitrate/nitrite, and silicate on an Alphem® RFA/2 autoanalyzer following procedures of Strickland and Parsons (1972), except for ammonium which was determined in accord with Parsons and others (1984). Statistical and graphical analyses were conducted with Excel and Delta Graph software; significance was expressed as 95% CI.

Selected animal tissues and fecal pellets were analyzed for carbon and nitrogen content on a Leco® CHN Analyzer, Model 600. Analysis of lipids in selected animal tissues was undertaken in accord with all protocols of Nates and McKenney (2000) at the Gulf Breeze, Florida laboratory of the U. S. Environmental Protection Agency.

Taxonomic identifications were initially attempted for all dominant burrowing thalassinideans and associated alpheids using morphological characters and all available taxonomic literature. Morphologically problematic population samples of three putative genera (tables 1-3) were subsequently subjected to comparative DNA-based genetic analyses. Genomic DNA was isolated from skeletal muscle tissue using a phenol-chloroform extraction (Kocher and others, 1989). Selective amplification of a 599-basepair (bp) product (547 bp excluding primer regions) from the mitochondrial 16S rRNA gene was carried out by a polymerase-chain-reaction (PCR) (35-40 cycles: 1 min 94°C / 1 min 55°C / 2 min 72°C denaturing / annealing / and extension temperatures, respectively) using primers 16Sar (5'-CGCCTGTTTATCAAAAACAT-3'), 16SL2 (5'-TGCCTGTTTATCAAAAACAT-3), and 1472 (5'-AGATAGAAACCAACCTGG-3') (for references to primers see Schubart and others, 2000). PCR products were purified with Microcon-100® filters (Millipore Corp.) and sequenced with the ABI BigDye® Terminator Mix (PE Biosystems) on ABI Prism 310 and ABI 3100 Genetic Analyzers® (Applied Biosystems). Sequences were aligned manually with the multisequence-editing program ESEE (Cabot and Beckenbach, 1989). Sequence divergence was analyzed using Kimura 2-parameter distances and neighbor-joining (NJ) distance analysis (Saitou and Nei, 1987) with the program MEGA (Kumar and others, 1993). Significance levels were evaluated with the bootstrap method using 2000 replicates.

Results

No established populations of burrowing thalassinideans (figs. 3, 4A-C) were detected in commercial shrimp ponds during the entire term of study, despite extensive surveys of ponds and repeated attempts to extract animals with extraction corers (yabby pumps). Where successfully excavated, burrows in shrimp farms were on occasion found to harbor burrowing crabs assignable to *Uca* spp. (fig. 4F), *Goniopsis* sp. (fig. 5A) or *Panopeus* sp. (fig. 5B), usually in areas where empty ponds had accumulation water from recent rainfall (fig. 6B). Scattered burrows surrounded by characteristic fecal pellets of thalassinidean burrowers were, however, detected in drainage canals surrounding the Sahlman shrimp farm (site 4, fig. 2) on Isla Mangles Altas (though we were unsuccessful in extracting the animals), and extensive populations of thalassinideans were found immediately outside of shrimp ponds on Estero La Garita (site 22, fig. 2). Likewise, extensive populations, very actively reproducing, were found to densely populate shallow waters and shorelines of Estero de Las Peñitas (site 21, fig. 2), where regional shrimp farmers have established one hatchery and have a second major hatchery under construction. As for sites lacking shrimp farming operations, populations of burrowing thalassinideans were found in almost every estuary we examined, as well as along most open sandy shorelines of the Pacific Ocean. Particularly dense populations, with burrow openings exceeding 20-80/m², were found at Miramar, the aforementioned Estero de Las Peñitas and Estero La Garita, Paso de Caballos, the Poneloya estuary, Estero Aserradores, Estero Ciego, and Estero de Padre Ramos (sites 24, 22, 21, 20, 15, 14, 13, and 11, fig. 2). The densest of all populations encountered were in Estero Ciego, where densities of large burrows in some areas exceeded 140/m².

Dominant among these thalassinidean burrowers in Nicaraguan estuaries were large specimens (commonly exceeding 20 mm in carapace length) assignable to the genus *Lepidophthalmus,* these in turn resolving to three species differing in morphology of the ventral abdominal plating (fig. 3). Occurring as commensals in burrows with many of these animals were commensal alpheid shrimp assignable to, or closely related to, the genus *Leptalpheus* (fig. 4E). While burrow openings and ejecta mounds of *Lepidophthalmus* spp. typically dominated substrates in these settings (fig. 6D), two other thalassinideans (*Axianassa* sp. and *Upogebia* sp., figs. 4A, C) did co-occur with these dominants at much lower frequency. In sandy habitats of bay mouths, mouth bars, and open beaches, these thalassinidean species were usually replaced by either of two species assignable to *Callichirus* (fig. 4D), though usually at densities not exceeding 20/m². Also in the muddy sand substrates of the high salinity bay mouth of Estero de Padre Ramos (site 11, fig. 2) was a rarely occurring thalassinidean assignable to *Sergio* (fig. 4B). In upper intertidal extremes of such sandy habitats, crabs assignable to *Uca* (fig. 4F) and *Ocypode* (fig. 5C) were also common burrowers.

The Nicaragua species of *Lepidophthalmus* resolve genetically into three distinct clades, two of which represent known "ventrally plated" species, and one of which is new to science (table 1, fig. 7). All three occur sympatrically, though any one or two may dominate populations at a given sample site. Given the broad distribution of *Lepidophthalmus bocourti* among these, it was chosen as the estuarine subject for use in baseline characterizations of carbon, nitrogen, and fatty acid composition, while thalassinideans of the genus *Callichirus* were chosen as comparable subjects inhabiting surf and sandy shoreline habitats (tables 4 and 5).

The Nicaraguan specimens of the thalassinidean genus *Axianassa* may also represent an undescribed species (table 2, fig. 8), though close relationship to the poorly known and rarely collected Panamanian species *A. canalis* is suspected (but material of that species was not available for sequence comparisons at this writing). Without question, Nicaraguan materials of the commensal alpheoid shrimp associated with the aforementioned thalassinideans are new to science. While there appear to be among these no less than four species, all apparently new to science (table 3, fig. 9), one is closely related to *Leptalpheus mexicanus* (type materials and sequences remain to be compared with known materials of the latter species). These Nicaraguan materials resolve into clades representing an undescribed member of *Leptalpheus* in addition to apparently undescribed members of no less than three other undescribed genera.

Where densely concentrated populations of thalassinideans, dominated by *Lepidophthalmus* spp. and associates, occur in estuaries, there is clear indication that they mobilize reduced burrow waters into the water column (fig. 10). Redox potentials for these burrow waters are intermediate between those of shallow and deep pore waters but are clearly well below those of the overlying water column. While redox potentials less than 100 mv were commonly encountered in the muddy sands inhabited by these populations, no severely reducing pore waters (extremely low positive or negative redox potentials) were found in such densely colonized settings. While values ranged widely among samples and estuaries, there was weak evidence of elevated phosphate and nitrogen concentration in thalassinidean burrow water (fig. 11). No strongly elevated levels of ammonia were found in thalassinidean burrow effluents or adjacent pore waters during the entire course of study (figs. 11 and 12). Nutrient of shoreline surf waters outside of the estuary, the source waters of tidal flooding, were among the lowest of those measured, being lower only in rain waters accumulated in drained shrimp ponds (fig. 13). During September 2000, field work proceeded during heavy rainfall from a developing tropical storm, providing an opportunity to sample muddy floodwater draining both

Estero Ciego and Isla Mangles Altas (near Sahlman farm), and yielding very elevated measures of surface water nutrient loads (fig. 13).

Discussion and Management Recommendations

The issue of potential "pest" crustaceans:

Thalassinidean crustaceans do not, on the basis of present observations, represent an immediate or substantive threat to shrimp farming operations in Nicaragua. At this juncture it is not possible to determine whether these burrowers have ever densely populated or in any measure impacted shrimp farms there is at this juncture not possible to determine, largely because widespread use of pesticides (especially Neguvon® = trichlorfon) has been used extensively to eradicate marine organisms in ponds prior to stocking. Regardless, it is evident that resident populations of *Lepidophthalmus* spp. do occur in Nicaraguan estuaries and could readily be a source of larvae entering shrimp ponds, as they have in the southern Caribbean (Nates and Felder, 1998).

Biology of the Nicaraguan species remains poorly known, and one of the three is completely new to science. However, from our preliminary observations, it appears that all of these Pacific species may be somewhat limited in the potential to disrupt shrimp farming operations in Nicaragua, both because of habitat characteristics in the local setting and because of differences in biology. In terms of habitat characteristics, ponds on the shrimp farms visited were devoid of heavy benthic organic accumulations generally exploited by *Lepidophthalmus* spp., offering little in the way of raw materials for the "vertical conveyer" of organics and microbes generally believed to sustain these burrowers (see Bourgeois and Felder, 2001; Felder, 2001). Furthermore, many Nicaraguan shrimp ponds contained a combination of hard-packed sandy clay with a variable component of coarse, highly angular, poorly worn sand of recent volcanic origin; these are not typical substrates for most members of the genus (Felder and Rodrigues, 1993), and were not characteristic of the estuarine habitats in Nicaragua where *Lepidophthalmus* flourished. Also, the life history of at least two of the Nicaraguan species (*L. bocourti* and *L. eiseni*) appears to be less abbreviated and perhaps more complicated in terms of food dependencies than is true in western Atlantic species. None of our several attempts to lab-rear larvae of these species successfully produced a decapodid (first burrowing stage), even though this is rapidly achieved with minimal feeding in western Atlantic species (Felder and others, 1986; Nates and others, 1997). This implies possible constraints on within-pond population growth in Nicaraguan species of *Lepidophthalmus* subsequent to initial colonization from natural habitats. If the life cycle for these species cannot be completed within the contained waters and burrows of a shrimp farm, there may be little potential for accumulation of detrimental population densities. Furthermore, moderate to low population densities of *Lepidophthalmus* may actually have favorable effects on penaeid shrimp production, and detrimental effects are documented only where members of this genus have exploited pond substrates heavily loaded with decomposing organic materials and mobilized large volumes of reduced nutrients from pore waters in to the water column (Nates and Felder, 1998). Clearly, we did not see evidence of highly reduced environments or elevated levels of burrow effluent ammonia in the estuarine populations of Nicaragua. Thus, conceptual modeling of roles played by these thalassinideans in shrimp ponds does not imply that the impacts are detrimental (fig. 14).

The issue of endemic estuarine biota:

Perhaps the most significant finding of our studies is not the lack of potential threat to shrimp farmers from infaunal burrowers, but rather the remarkable levels of diversity, novelty, and genetic uniqueness (endemism) seen among the burrowing species sampled. While it is not uncommon to discover undescribed species in tropical waters of Mesoamerica, it is remarkable to find them at such levels of abundance and in settings where they potentially dominate ecological processes. It is of particular significance to discover that several among the few forms to date subjected to DNA sequence analyses may be extremely restricted in distribution, perhaps occurring in only a few Central American Pacific estuaries and playing key ecological roles where they occur. Given the potential for future shrimp farm development in these areas, the unknown range limits for the crustacean infaunal species we have found there, limited knowledge of their ecological significance, and the history of pesticide use in tropical aquacultural operations, it is urgent that efforts be initiated for both estuarine conservation and thorough characterization of the overall estuarine faunal assemblages in this region. With the lack of major geographical barriers along this region of the Pacific, narrowly endemized populations are not generally expected here but could be accounted for by broad disjunctures in the occurrence of persistently habitable estuarine waters. Some models also suggest that these endemics could have originated as relicts of glacial refugia (Felder, 2001).

To date we have investigated genetic affinities of only three of the more abundant burrowing macrocrustacean genera in Nicaragua, this being undertaken only after we realized from morphological studies that materials we had collected would not yield to classical identification literature and techniques. As the genetic procedures are costly, and were not an anticipated cost at the outset of this project, we had to limit which taxocenes were submitted to this level of analysis. Whether the remainder of our infaunal crustacean specimen holdings from coastal Nicaragua (*Callichirus*, *Panopeus*, *Eurytium*, *Pinnixa*, *Uca*, *etc.*) also reflect previously unappreciated levels of endemism must await additional funding for the required analyses. In any case, these and other ancillary collections of macrocrustaceans from our four trips have been archived for eventual study that will contribute to understanding of the diversity and endemism in the regional biota. It is urgent that such studies be carried to completion.

The general issue of pesticide use:

Our report of pesticide use in tropical penaeid shrimp aquaculture is not a new one, though in previous reports the pesticide applications have been Sevin® (carbaryl) and the applications were specifically for control of thalassinidean populations (Nates and Felder, 1998). In Nicaragua, the applications to penaeid shrimp pond waters (where acknowledged) instead usually consisted of Neguvon® (trichlorfon) and were putatively being made for eradication of all potential disease vectors, with the objective of eliminating perceived sources of "white spot" and other shrimp diseases. With minimal baseline information, all aquatic fauna introduced to ponds was seen as a potential source of disease, and thus all was to be eliminated. Presumably because of these applications (at least in part), we found little evidence of benthic fauna in recently drained shrimp culture ponds. Exceptions were found where rainwaters accumulated in fallow ponds and fiddler crabs (*Uca*) or a few other species managed to become established.

The fate and effects of these Neguvon® applications appear to require additional study. More must be learned about the fate of this pesticide and its degradation products in the penaeid shrimp product itself, and at least some studies are already underway on this subject in Nicaraguan government labs. A second issue involves environmental fates and pathways, including those which might ultimately impact natural estuaries downstream of commercial shrimp farms, with potential risk to systems that may harbor endemic estuarine fauna. To this end, we have undertaken baseline analyses of *Lepidophthalmus* and *Callichirus* fatty acid composition, as these parameters are reportedly impacted over time by pesticide exposures (Nates and McKenney, 2000). Periodic reevaluation of these measures should be undertaken. However, direct monitoring of degradation pathways and products is also needed, both in shrimp ponds and estuarine drainages, especially if wholesale pesticide use is to continue in the tropical shrimp farming industry. Meanwhile, fully contained recirculating approaches to shrimp farming provide an encouraging alternative, in that pesticide use would tend to be contained and in that the perceived need for it should be diminished. Without routine replacement of pond waters from surrounding habitats, other introductions of local fauna and the potential release of treatment agents is minimized. This approach is presently being adopted in several of the farms that we visited and should be encouraged.

The specific issue of Neguvon® (= trichlorfon) use:

Bays, estuaries, and mangroves in the American tropics are important nursery and fishing grounds but may also harbor a unique regional fauna that we have only begun to appreciate in terms of diversity and regional adaptation. Yet, these habitats are potentially major entry points of pesticides into the marine environment (Brecken-Folse and others, 1994), especially when these chemicals are applied to penaeid shrimp ponds sharing the same drainages. Among these chemicals are the organophosphorus insecticides, which have replaced organochlorine ones, putatively because of their mopre rapid biodegradation (Orús and others, 1990). Trichlorfon [dimethyl (2, 2, 2-trichloro-1-hydroxyethyl phosphonate)], also known by the trade names Anthon, Bovinos, Briten, Chlorophos, Ciclosom, Dylox, Dipterex, Ditrifon, Dylox, Dyrex, Equino-Aid, Foschlor, Leivasom, Neguvon, Masoten, Trichlorophon, Trinex, Phoschlor, Proxol, Trichlorophene, Totalene, Tugon, and Vermicide Bayer 2349 (Hayes and Laws, 1990), is one of the most popular organophosphorus insecticides. This compound has generally been used among cultured aquatic species for controlling planktonic invertebrates and metazoan fish ectoparasites (Martínez and others, 1991; Ngoh and Cullison, 1996). Trichlorfon has also been used to eliminate unwanted planktonic crustaceans, which compete for food with fish fry in nursery ponds (Juarez and Rouse, 1983). It is relatively soluble (14%) and moderately toxic by ingestion and dermal absorption (Extoxnet, 2000). Organophosphorus compounds, including trichlorfon, in general produce a specific inhibition of acetylcholinesterase, which in some cases is accompanied by the inhibition of the neuro target esterase (NTE). The modification of the NTE activity at pH 8 is responsible for the apparition of the syndrome of delayed neurotoxicity induced by some organophosphorus compounds (Repetto and others, 1988). It is important to note that in normal use, trichlorfon is very quickly hydrolyzed to form dichlorvos (O, O-dimethyl 2,2-dichloro-vynil phosphate) which is much more toxic (Cox and others, 1989). The decomposition rate depends on the aeration rate, the pH, and the temperature (Samuelsen, 1987).

Trichlorfon is highly toxic to birds (avian oral toxicity is 40-47 mg/kg), and both cold and warm water fish (2-180 ppm) (Sievers and others, 1995). In addition, it has moderate to high acute toxicity toward certain beneficial or nontarget insects and aquatic invertebrates (Extoxnet, 2000). Previous works showed that trichlorfon treatment alters growth, cell composition, ultrastructure and physiological processes in algae (Marco and others, 1990; Orús and others, 1990; Martínez and others, 1991; Marco and Orús, 1992). Moreover, the bioavailability of chemicals may be altered by physicochemical factors (salinity and temperature) and therefore may affect toxicity. Brecken-Folse and others (1994) found that grass shrimp (*Palaemonetes* spp.) exposed to trichlorfon, exhibited greater mortality with increasing salinity and temperature. Similar effects were reported in the amphipod *Gammarus pseudolimnaeus* by Marking and others (1991). More recently, Ludwig (1993) reported that the application of trichlorfon to fertilized culture ponds stocked with 5-day-old stripped bass fry resulted in an initial reduction in the concentration of rotifers and longer-term alteration of zooplankton successional stages, including changes in concentrations of cladoceran and copepod crustaceans.

Although trichlorfon has been useful in aquaculture practices, the uncontrolled and indiscriminate use of it in shrimp aquaculture may mean, on the one hand, the destruction of a valuable ecosystem and, on the other, the rejection of the products on the market due to the presence of nonpermitted residues. Further research is needed to evaluate possible interactions between trichlorfon and pond muds, environmental degradation rates, biomagnification effects, and sublethal effects of the pesticide on growth, reproduction, and physiological health of crustacean populations.

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Figure Legends:

Figure 1. Conceptual model of potential effects on dissolved oxygen levels in penaeid shrimp ponds densely infested with thalassinideans.

Figure 2. Sites for samples of water and burrowing crustaceans, coastal Nicaragua, April 2000 - November 2001. Numbers correspond to locality names, general setting descriptions, GPS coordinates (n/a = not available), and sampling dates as follow: **1**.--UCA experimental shrimp farm and pump station, muddy volcanic sand Puerto Morazán, on Estero Real (12°50.666'N, 87°10.086'W), 14 April 2000; **2.**--Mainland dock for Sahlman shrimp farm, mangrove lined channel on Estero Cervantes (12°52.137'N, 87°21.021'W), 13 April 2000, 28 September 2000; **3.**--Sahlman shrimp farm, Isla Mangles Altas, on Estero Marotita, turbid estuarine channel (12°56.53'N, 87°19.60'W), 28 September 2000; **4.**-- Sahlman shrimp farm, Isla Mangles Altas, in and near Pond 30, clayey muds with volcanic sand (12°58.364'N, 87°18.977'W), 13 April 2000, 28 September 2000; **5.**--Fenicpesca, alias Potosí, near mouth of small urban-littered stream (13° 00.342'N, 87°30.032'W), 13 April 2000, 19 November 2001; **6**.--La Encajonada de los Cocos, supratidal and mouth of small stream, small rocks and mud (13°0.918'N, 87°30.667'W), 19 November 2001; **7.**--Santa Julia, poorly indurated rocky shore (GPS n/a), 19 November 2001; **8.**--El Rosario, coarse volcanic sand beach (13°02.822'N, 87°35.544'W), 19 November 2001; **9.**--Santa Elena, muddy sand estuarine shores and outer sandy beach (12°50.239'N, 87°35.194'W), 19 November 2001; **10.**--Las Enramadas, rocky shore with sand veneers (12°50.102'N, 87°34.973'W), 18 November

2001; **11.**--Estero de Padre Ramos, sandy shores of estuary mouth (12°45.18'N, 87°28.42'W); 14 April 2000, 28 September 2000, 15 August 2001; **12.**--Estero Nagualapa, rocky shore (12°40.805'N, 87°23.460'W); 20 November 2001; **13.**--Estero Ciego, muddy sand flats at ephemeral estuary mouth (12°39.468'N, 87°22.534'W), 15 April 2000, 29 September 2000, 15 August 2001, 20 November 2001; **14.**--Estero Aserradores, muddy sand with areas of exposed rock (12°37.352'N, 87°20.540'W), 29 September 2000, 15 August 2001, 20 November 2001; **15.**--Estero Paso de Cabellos, muddy sand, backbeach ponds and scrub mangroves (12°31.556'N, 87°12.507'W), 11, 12 April 2000, 30 September 2000, 15,16 August 2001; **16.**--Isla de Coriento, S. of bridge over estuary, rocks and mud along mangrove lined shore of hypersaline estuary (12°31.051'N, 87°12.028'W), 11 April 2000; **17.**--"La Cruz" shrimp farm ponds and very muddy, mangrove lined pump intake canal (GPS n/a), 12 April 2000; **18.**--"La Majeda" shrimp farm, empty ponds (12°26.209'N, 87°04.747'W), 12 April 2000; **19.**--"San Francisco" shrimp farm, empty ponds (12°30.422'N, 87°08.243'W), 12 April 2000; **20.**--Poneloya estuary, sandy backbeach ponds and muddy sand estuary mouth with some mangroves (GPS n/a), 27 September 2000, 14 August 2001, 21 November 2001; **21.**--Estero de las Peñitas near shrimp hatchery, estuarine shores with salt flats and clayey mud flats among mangroves (12°21.66'N, 87°01.25'W), 27 September 2000, 14 August 2001, 21 November 2001; **22.**--Estero La Garita near shrimp farm, muddy sand near mangroves (12°16.257N, 86°52.648'W), 17, 20 November 2001; **23.**--Puerto Sandino near power plant, sand flat in mouth of embayment, ranging from detrital substrates and salt flats near mangroves (12°10.519'N, 86°45.784'W), 17 November 2001; **24.**--Miramar, sandy beach (12°10.308'N, 86°45.801'W), 17 November 2001.

Figure 3. Dominant burrowing crustaceans collected from estuaries of coastal Nicaragua, April 2000 through November 2001, lateral and ventral views: (A, B) *Lepidophthalmus* sp. (undescribed); (C, D) *Lepidophthalmus bocourti*; (E, F) *Lepidophthalmus eiseni*.

Figure 4. Common burrowing crustaceans collected from estuaries and coastlines of Nicaragua, April 2000 through November 2001: (A) *Axianassa* sp.; (B) *Sergio* sp. (undescribed); (C) *Upogebia* sp.; (D) *Callichirus* sp.; (E) "*Leptalpheus*" sp. (undescribed, possible new genus); (F) *Uca* sp.

Figure 5. Common shallow burrowing crabs collected and typical estuarine collecting sites, coastal Nicaragua, April 2000 through November 2001: (A) *Goniopsis* sp.; (B) *Panopeus* sp.; (C) *Ocypode* sp.; (D) Use of "yabby pump" to sample infauna of mudflat near mangrove-lined mouth of Estero Ciego; (E) Estero de Padre Ramos, sandy shores near mouth of large estuary; (F) ephemeral opening of Estero Ciego to Pacific Ocean.

Figure 6. Typical penaeid shrimp ponds, estuarine collecting sites, and sampled habitats, coastal Nicaragua, April 2000 through November 2001: (A) Levee dividing dry penaeid shrimp culture ponds of UCA experimental farm on Estero Real; (B) water control gate and burrows of *Uca* sp. around accumulated rain waters, unfilled penaeid shrimp pond, UCA experimental farm on Estero Real; (C) Tidally exposed mudflat, densely burrowed by *Lepidophthalmus* spp., in Estero Ciego; (D) Typical slightly submerged burrows of

Lepidophthalmus spp., some harboring commensal alpheids (*Leptalpheus* sp. and yet to be named close relatives referred to as *"Leptalpheus"* spp.), in Estero Ciego; (E) Sampling of surface waters for subsequent nutrient analyses, Estero Ciego; (F) Measurement of redox potential in effluent waters from burrows of *Lepidophthalmus* spp., Estero Ciego.

Figure 7. Putative relationships of Nicaraguan species of *Lepidophthalmus* and extralimital congenerics based on sequencing of 16s mtDNA, portrayed as tree produced by neighbor-joining analysis of 16 sequences. *Axianassa canalis* was included as an external group. Numbers represent confidence values (Kimura-two parameter distances, 2000 bootstraps). Scale represents genetic distance. ECN, Estero Ciego, Nicaragua; PCN, Paso Caballos, Nicaragua; LPN, Las Peñitas, Nicaragua; MEX, México; MIS, Mississippi, USA; FLA, Florida, USA.

Figure 8. Putative relationships of Nicaraguan species of *Axianassa* and extralimital congenerics based on sequencing of 16s mtDNA, portrayed as tree produced by neighbor-joining analysis of 9 sequences. *Lepidophthalmus bocourti* was included as an external group. Numbers represent confidence values (Kimura-two parameter distances, 2000 bootstraps). Scale represents genetic distance. TEX, Texas, USA; FLA, Florida, USA; BRA Sao Paulo, Brazil; COL, Colombia; EAN, Estero Aserradores, Nicaragua; PON, Poneloya, Nicaragua.

Figure 9. Putative relationships of Nicaraguan species of commensal, burrow-dwelling alpheoid shrimp, including *Leptalpheus*, putative members of that genus (*“Leptalpheus"* spp.*)*, and extralimital congenerics, based on sequencing of 16s mtDNA and portrayed as tree produced by neighbor-joining analysis of 20 sequences. *Alpheus heterochaelis* and *A. viridari* were included as external groups. Numbers represent confidence values (Kimura-two parameter distances, 2000 bootstraps). Scale represents genetic distance. NCA, North Carolina, USA; MIS, Mississippi, USA; FLA, Florida, USA; BRA, Sao Paulo, Brazil; EAN, Estero Aserradores, Nicaragua; LPN, Las Peñitas, Nicaragua; PON, Poneloya, Nicaragua; PCN, Paso Caballos, Nicaragua.

Figure 10. Typical REDOX levels (mV, corrected to calomel electrode values) in effluent waters from thalassinidean burrows, adjacent surface waters, and varied depths of adjacent pore waters, from Estero Ciego, coastal Nicaragua, April 2000.

Figure 11. Concentrations of phosphate, ammonia, nitrates/nitrites, and silicates in interstitial porewaters of substrates, effluent waters from thalassinidean burrows, and adjacent surface waters in coastal estuaries densely populated by *Lepidophthalmus* spp. and their associates, coastal Nicaragua, April 2000 through September 2001.

Figure 12. Concentrations of phosphate, ammonia, nitrates/nitrites, and silicates in interstitial porewaters of substrates, effluent waters from thalassinidean burrows, and adjacent surface waters in coastal estuaries densely populated by *Lepidophthalmus* spp. and their associates, coastal Nicaragua, November 2001.

Figure 13. Atypical measures for concentrations of phosphate, ammonia, nitrates/nitrites, and silicates in coastal Nicaraguan waters. LOW EXTREMES were found in rain precipitation accumulated in drained shrimp ponds (Sahlman Farm) and in coastal surf waters immediately outside Estero Ciego. ESTUARINE FLOOD surface waters in both these locations were highly turbid which is believed to have augmented nutrient loads during September 2000.

Figure 14. Conceptual model suggesting effects of burrowing thalassinidean fauna on nutrient and carbon cycles in estuaries and potentially in shrimp ponds of coastal Nicaragua.

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Executive Summary

USGS Activity B7

Hurricane Mitch: Impacts of Bioturbating Crustaceans in Shrimp Ponds and Adjacent Estuaries of Coastal Nicaragua

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Populations of large burrowing crustaceans, sometimes known as mud shrimp or ghost shrimp, are known pests in commercial penaeid shrimp farms of tropical Central America. These animals have had tremendous negative impacts on commercial shrimp ponds in Colombia, especially where pond bottoms were richly organic. As a consequence of Hurricane Mitch, runoff and downslope slumping of soils in Nicaragua potentially buried large quantities of organic materials, in the process also destroying many shrimp farm levees. This raised the potential for infestation by populations of burrowing crustaceans as farm operations were reestablished, and prompted study of the invading populations and the phenomena by which these populations impact shrimp farms and natural estuaries.

Field research in Nicaragua centered on coastlines of the northwestern peninsula, especially in estuaries where shrimp farms have located, both on the Gulf of Fonseca and the Pacific Ocean. Twenty four sites were visited, some being repeatedly sampled, during four field trips between April 2000 and November 2001. No problem infestations of the burrowing shrimp were found on any of the Nicaraguan shrimp farms visited, though this was perhaps due to widespread use of pesticides in these farm operations. Extensive natural populations of the burrowing shrimp were found in the estuaries themselves, establishing that source populations are present in the region. However, while being close relatives of species reported to cause problems on shrimp farms in Colombia, these are unique species that appear to differ in biology and level of threat to the shrimp farming industry. They do not seem to inhabit extremely low oxygen muds, and thus do not show evidence of extensively mobilizing organic degradation products produced in such environments into the water column. They also do not appear to pose a high potential for rapid accumulations in the Nicaraguan shrimp farms, owing to characteristics of their larval development and sediment characteristics of the Nicaraguan ponds. As low level populations of these burrowing shrimp appear to have beneficial effects on at least some shrimp farm operations elsewhere, there is little present concern that they will cause negative impacts in Nicaragua. There is certainly no present obvious justification for treating ponds with pesticides to control them, although pesticide treatment is in extensive use for other perceived benefits by shrimp farmers. Most commonly, these pesticides are applied to kill all wild marine macrofauna that enters ponds before they are stocked with the commercial penaeid shrimp, as the wild marine fish and invertebrates are thought to be potential sources of shrimp diseases or to compete with the shrimp for food.

Remarkable in this study was the finding that many of the burrowing crustacean species were in fact previously unknown, undescribed forms, new to science. This was firmly established by genetic sequencing of DNA for several of the more common groups. Even some of the most dominant estuarine forms are totally new discoveries, and may be narrowly restricted in distribution to Nicaraguan and/or adjacent estuaries. This heightens concern that environmental quality be conserved in future management and development of the Nicaraguan shrimp farm industry, lest these remarkable populations be lost before their ecological roles in natural estuaries of the regions are fully appreciated. The use of pesticides in shrimp farm operations, especially, should be re-examined, and alternative management approaches taken if at all possible.

Table 1. Specimens of *Lepidophthalmus* spp. from Nicaragua and other areas compared by 16s mtDNA sequence analysis, analyzed phylogenetically in figure 7.

	Locality	Collection Date
Lepidophthalmus bocourti (LPN)	Las Peñitas, Nicaragua	August 14, 2001
Lepidophthalmus bocourti (PCN)	Paso Caballos, Nicaragua	September 30, 2000
Lepidophthalmus bocourti (PCN)	Paso Caballos, Nicaragua	August 15, 2001
Lepidophthalmus nov. sp. (LPN)	Las Peñitas, Nicaragua	August 14, 2001
Lepidophthalmus nov. sp. (PCN)	Paso Caballos, Nicaragua	August 16, 2001
Lepidophthalmus nov. sp. (PCN)	Paso Caballos, Nicaragua	August 16, 2001
Lepidophthalmus nov. sp. (LPN)	Las Peñitas, Nicaragua	September 27, 2000
Lepidophthalmus nov. sp. (LPN)	Las Peñitas, Nicaragua	August 14, 2001
Lepidophthalmus nov. sp. (LPN)	Las Peñitas, Nicaragua	September 27, 2000
Lepidophthalmus manningi (MEX)	Tabasco, México	February 14, 2001
Lepidophthalmus louisianensis (MEX)	Tamaulipas, México	April 21, 1998
Lepidophthalmus louisianensis (MIS)	Mississippi, USA	August 3, 1999
Lepidophthalmus louisianensis (FLA)	Florida, USA	August 9, 1999
Lepidophthalmus eiseni (ECN)	Estero Ciego, Nicaragua	April 15, 2001
Lepidophthalmus eiseni (ECN)	Estero Ciego, Nicaragua	April 15, 2001
Lepidophthalmus eiseni (MEX)	Nayarit, México	June 30, 2000

Table 2. Specimens of *Axianassa* spp. from Nicaragua and other areas compared by 16s mtDNA sequence analysis, analyzed phylogenetically in figure 8.

	Locality	Collection Date
Axianassa canalis? (EAN)	Estero Aserradores, Nicaragua	September 29, 2000
Axianassa canalis? (PON)	Poneloya, Nicaragua	August, 2001
Axianassa australis (BRA)	São Paulo, Brazil	October 9, 2001
Axianassa australis (BRA)	São Paulo, Brazil	October 9, 2001
Axianassa australis (TEX)	Texas, USA	July 2, 1998
Axianassa australis (TEX)	Texas, USA	February 29, 2000
Axianassa australis (COL)	Río Sinú, Colombia	March 6, 1997
Axianassa australis (FLA)	Florida, USA	July 29, 1999
Axianassa australis (FLA)	Florida, USA	July 29, 1999

Table 3. Specimens of burrow-dwelling alpheoid shrimp from Nicaragua and other areas compared by 16s mtDNA sequence analysis, analyzed phylogenetically in figure 9.

	Locality	Collection Date
“Leptalpheus” axianassae (BRA)	São Paulo, Brazil	October 9, 2001
“Leptalpheus” axianassae (BRA)	Florida, USA	July 27, 1999
Leptalpheus forceps (NCA)	North Carolina, USA	August 23, 2000
Leptalpheus forceps (FLA)	Florida, USA	August 9, 1999
Leptalpheus forceps (MIS)	Mississippi, USA	March 19, 1997
Fenneralpheus chacei (FLA)	Florida, USA	March 25, 1997
“Leptalpheus” nov. sp. A (PCN)	Paso Caballos, Nicaragua	September 30, 2000
“Leptalpheus” nov. sp. A (PCN)	Paso Caballos, Nicaragua	August 15, 2001
“Leptalpheus” nov. sp. A (LPN)	Las Peñitas, Nicaragua	August 14, 2001
“Leptalpheus” nov. sp. A (LPN)	Las Peñitas, Nicaragua	August 14, 2001
“Leptalpheus” nov. sp. A (PCN)	Paso Caballos, Nicaragua	April 12, 2000
“Leptalpheus” nov. sp. B (EAN)	Estero Aserradores	September 29, 2000
“Leptalpheus” nov. sp. B (PON)	Poneloya, Nicaragua	August 14, 2001
“Leptalpheus” nov. sp. C (LPN)	Las Peñitas, Nicaragua	August 14, 2001
“Leptalpheus” nov. sp. C (LPN)	Las Peñitas, Nicaragua	August 14, 2001
“Leptalpheus” nov. sp. C (LPN)	Las Peñitas, Nicaragua	August 14, 2001
“Leptalpheus” nov. sp. D (LPN)	Las Peñitas, Nicaragua	September 27, 2000
“Leptalpheus” nov. sp. D (PCN)	Paso Caballos, Nicaragua	August 16, 2001
“Leptalpheus” nov. sp. D (PCN)	Paso Caballos, Nicaragua	August 16, 2001
Fenneralpheus nov. sp. E (FLA)	Florida, USA	
Alpheus heterochaelis	Florida, USA	
Alpheus viridari	Texas, USA	

Table 4. Carbon and nitrogen content in hepatopancreas tissues of the thalassinideans *Lepidophthalmus bocourti* and *Callichirus* sp. and in fecal pellets of *L. bocourti*, coastal Nicaragua, April 2000; number of replicate analysis (n), mean ± CI (95%).

	Carbon (n=5)	Nitrogen (n=5)
L. bocourti	544.9 ± 130.9	40.3 ± 15.6
Callichirus sp.	451.1 ± 291.7	44.2 ± 4.4
Fecal pellets	161.8 ± 58.6	33.6 ± 13.5

Table 5. Fatty acid composition (% total fatty acids) in *Lepidophthalmus bocourti* and *Callichirus* sp.; n = number replicate analyses; n' = number of individuals analysed; nd = not detected; values are means ± 95% CI.

Fatty acids	Common name	*L. bocourti* (n=5; n'=5)	*Callichirus* sp. (n=5; n'=5)
10:00	capric	0.51 ± 0.18	0.52 ± 0.21
12:00	lauric	0.18 ± 0.04	0.41 ± 0.11
13:00	tridecanoic	0.15 ± 0.02	0.11 ± 0.02
14:00	myristic	22.51 ± 3.05	21.98 ± 5.65
15:00	pentadecanoic	4.50 ± 0.26	1.57 ± 0.20
16:00	palmitic	41.26 ± 1.89	41.21 ± 1.86
18:00	stearic	3.77 ± 0.70	6.93 ± 3.16
23:00	tricosanoic	0.17 ± 0.38	0.08 0.04
Total saturated		73.05	72.81
14:1w5	myristoleic	4.99 ± 0.67	4.89 ± 1.24
15:1w5	pentadecenoic	1.14 ± 0.06	0.43 ± 0.03
16:1w6	palmitoleic	9.76 ± 0.47	9.79 ± 0.41
17:1w7	heptadecenoic	0.25 ± 0.05	0.28 ± 0.11
18:1w9c	oleic	3.45 ± 0.42	3.33 ± 0.79
18:1w9t	elaidic	0.97 ± 0.19	1.77 ± 0.80
20:1w9	eicosenoic	0.06 ± 0.03	0.09 ± 0.05
24:1w9	nervonic	0.16 ± 0.07	0.08 ± 0.04
Total monounsaturates		20.78	20.66
18:3w6	gamma-linolenic	1.05 ± 0.09	0.26 ± 0.10
18:4w3	octadecatetraenoic	1.03 ± 0.10	0.30 ± 0.10
18:2w6c	linoleic	0.85 ± 0.09	0.86 ± 0.10
18:3w3	linolenic	0.23 ± 0.03	0.27 ± 0.03
18:2w6t	linolelaidic	0.74 ± 0.09	0.81 ± 0.06
20:4w6	arachidonic	nd	nd
20:5w3	eicosapentaenoic	0.28 ± 0.03	0.24 ± 0.08
22:6w3	docosahexaenoic	nd	0.10 ± 0.07
22:2w6	docosadienoic	0.01 ± 0.01	0.04 ± 0.02
Total polyunsaturates		4.19	2.88

Table 6. Selected publications since 1990 on bioturbation impacts of thalassinidean crustaceans. Where used below, the abbreviation *"C."* indicates species that remain treated under genus *Callianassa* sensu lato, despite recent restriction of that genus which excludes these species; the abbreviation "sh" is used to indicate shallow subtidal waters near in embayments or estuaries, or near beaches.

Location	Habitat	Focus	Authors	Taxa
Japan	intertidal	competition/snails	Tamaki (1994)	*Nihonotrypaea*
Japan	intertidal	competition/isopods	Tamaki and Suzukawa (1991)	*Nihonotrypaea*
Japan	intertidal	competition/eels	Tamaki and others (1992)	*Nihonotrypaea*
Australia	intertidal	community interactions	Dittman (1996)	*Trypaea*
Australia	intertidal	associated bivalves/redox	Kerr and Corfield (1998)	*Trypaea*
Australia	intertidal sand	bioturbation, carbon, microbes	Bird and others (2000)	*Biffarius*
New Zealand	intertidal sand	bioturbation effects	Berkenbusch and Rowden (1999)	*"C." filholi*
New Zealand	intertidal sand	effects on communities	Berkenbusch and Rowden (2000)	*"C." filholi*
South Africa	intertidal mudflat	hydrodynamics	Allanson and others (1992)	*Upogebia*
South Africa	intertidal	hydrocarbon accumulation	Jackson and Bidleman (1990)	*"C." kraussi*
South Africa	intertidal-sh subtidal	detrital food webs	Schlacher and Wooldridge (1996)	*"C. kraussi*
Red Sea	sh subtidal	organic cycling	Vaugelas and Buscail (1990)	*"C." laurae*
Medit	subtidal	sediment oxygen flux	Ziebis and others (1996a)	*"C." truncata*
Medit	subtidal	biogeochemistry	Ziebis and others (1996b)	*"C." truncata*

UK-N Sea	subtidal	sulfide adapt, metabolic	Johns and others (1997)	*Calocaris*, *Jaxea*, *Callianassa*
UK-N Sea	subtidal	activity, bioturbation	Stamhuis and others (1996, 1997)	*Callianassa*
UK-N Sea	subtidal	hydrodynamics, redox	Forester and Graf (1992, 1995)	*Callianassa*
UK-N Sea	subtidal	bioturbation	Hughes and Atkinson (1997)	*Callianassa*, *Jaxea*, *Calocaris*, *Upogebia*
UK-N Sea	subtidal	bioturbation	Rowden and Jones (1993, 1994, 1995), Rowden and others (1997, 1998) Nickell and others (1995)	*Callianassa*
N Am/Atl	intertidal	sediment modification	Over (1990)	*Callichirus*
N Am/Atl	sh subtidal, sand and rubble	burrows and impacts	Dworschak and Ott (1993)	*Glypturus*, *Axiopsis*, *Neocallichirus*, *Corallianassa*
N Am/Atl	intertidal-s. subtidal	effects on communities	Felder and Griffis (1994)	*Lepidophthalmus*, *Callichirus* 2 spp.
N Am/Atl	intertidal-s. subtidal	effects on associated biota	Felder (2001)	*Lepidophthalmus, Axianassa*, *Callichirus*
N Am/Atl	intertidal-sh subtidal	sulfide adapt, metabol	Bourgeois and Felder (2001)	*Callichirus*, *Lepidophthalmus*
S Am/Atl	sh subtidal	cycling, productivity	Murphy and Kremer (1992)	*Eucalliax*, *Neocallichirus*
S Am/Atl	intertidal-sh subtidal	cycling, redox in systems	Nates and Felder (1998)	*Lepidophthalmus*
N Am/Pac	intertidal	bioturbation and control	Dumbauld and others (1996, 1997)	*Neotrypaea*, *Upogebia*
N Am/Pac	intertidal	bioturbation	Myrick and Flessa (1996)	*Neotrypaea*

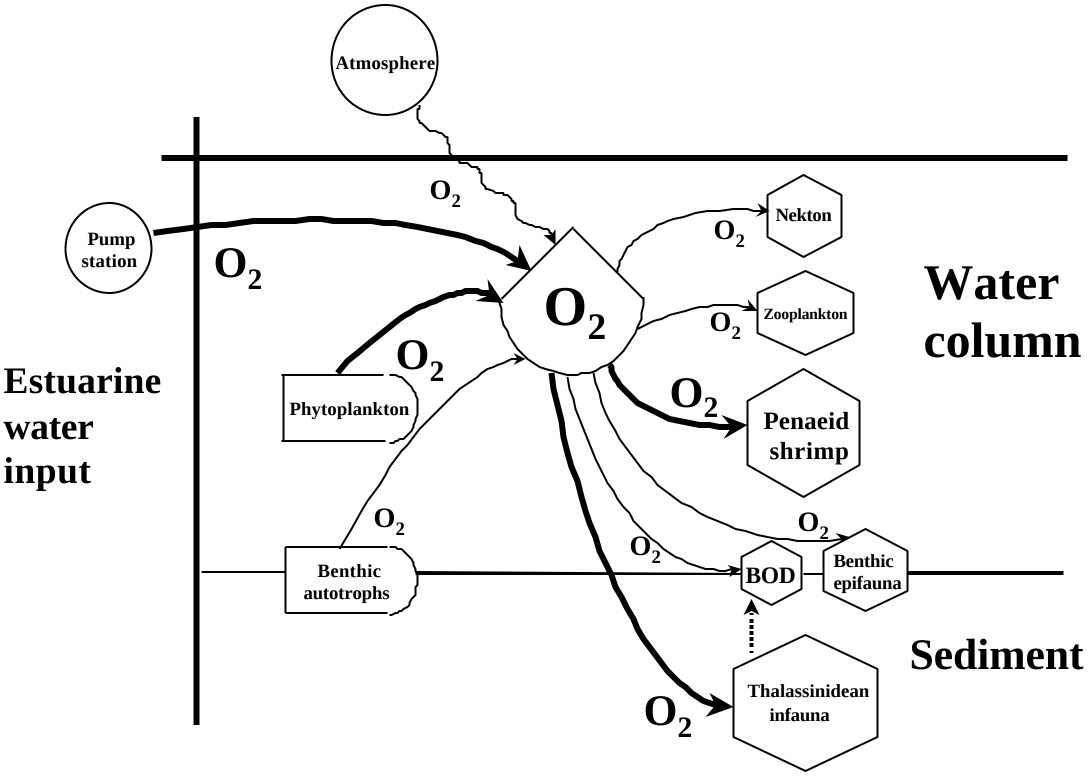

Figure 1. Conceptual model of potential effects on dissolved oxygen levels in penaeid shrimp ponds densely infested with thalassinideans.

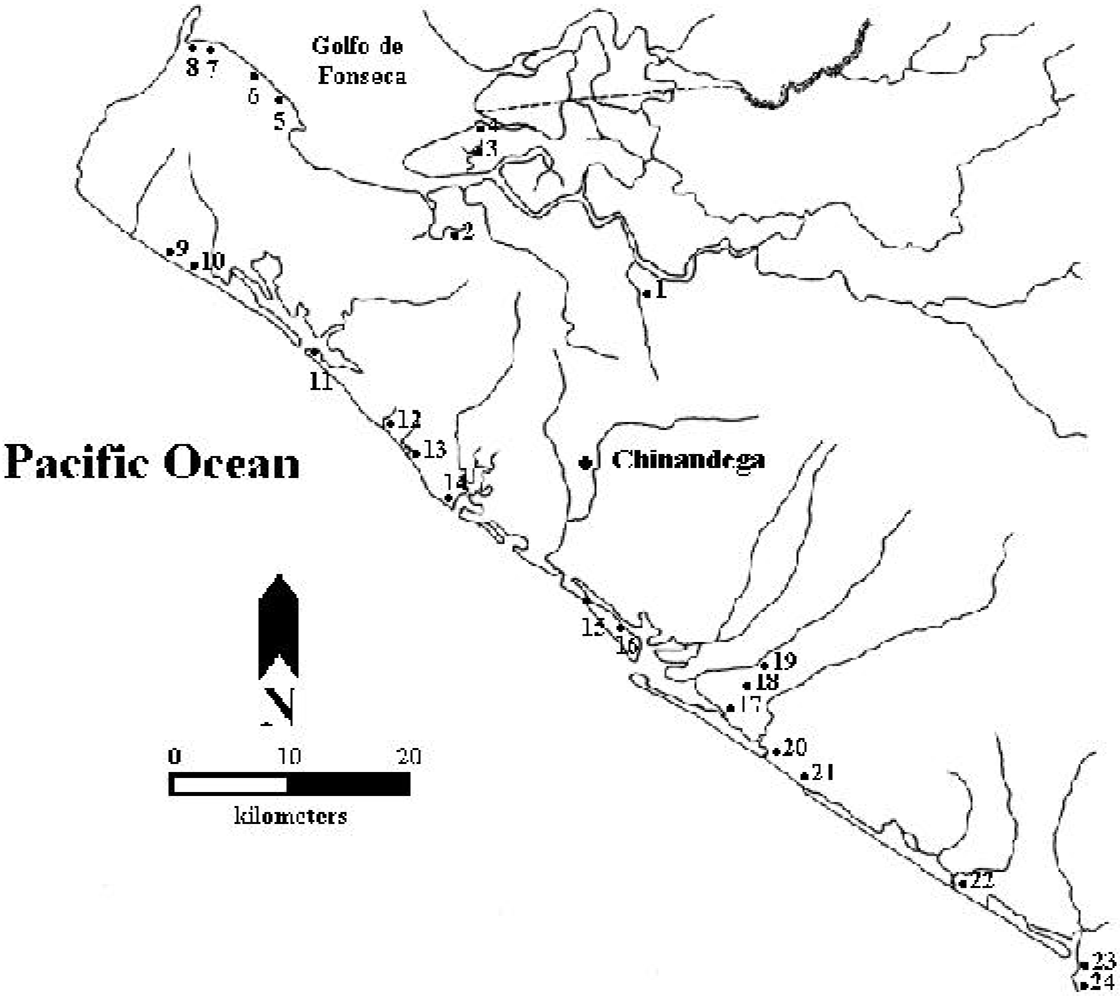

Figure 2. Sites for samples of water and burrowing crustaceans, coastal Nicaragua, April 2000 - November 2001. Numbers correspond to locality names, general setting descriptions, GPS coordinates (n/a = not available), and sampling dates as follow: **1**.--UCA experimental shrimp farm and pump station, muddy volcanic sand Puerto Morazán, on Estero Real (12°50.666'N, 87°10.086'W), 14 April 2000; **2.**--Mainland dock for Sahlman shrimp farm, mangrove lined channel on Estero Cervantes (12°52,137'N, 87°21.021'W), 13 April 2000, 28 September 2000; **3.**--Sahlman shrimp farm, Isla Mangles Altas, on Estero Marotita, turbid estuarine channel (12°56.53'N, 87°19.60'W), 28 September 2000; **4.**-- Sahlman shrimp farm, Isla Mangles Altas, in and near Pond 30, clayey muds with volcanic sand (12°58.364'N, 87°18.977'W), 13 April

2000, 28 September 2000; **5.**--Fenicpesca, alias Potosí, near mouth of small urban-littered stream (13° 00.342'N, 87°30.032'W), 13 April 2000, 19 November 2001; **6**.--La Encajonada de los Cocos, supratidal and mouth of small stream, small rocks and mud (13°0.918'N, 87°30.667'W), 19 November 2001; **7.**--Santa Julia, poorly indurated rocky shore (GPS n/a), 19 November 2001; **8.**--El Rosario, coarse volcanic sand beach (13° 02.822'N, 87°35.544'W), 19 November 2001; **9.**--Santa Elena, muddy sand estuarine shores and outer sandy beach (12°50.239'N, 87°35.194'W), 19 November 2001; **10.**--Las Enramadas, rocky shore with sand veneers (12°50.102'N, 87°34.973'W), 18 November 2001; **11.**--Estero de Padre Ramos, sandy shores of estuary mouth (12°45.18'N, 87°28.42'W); 14 April 2000, 28 September 2000, 15 August 2001; **12.**--Estero Nagualapa, rocky shore (12°40.805'N, 87°23.460'W); 20 November 2001; **13.**--Estero Ciego, muddy sand flats at ephemeral estuary mouth (12° 39.468'N, 87°22.534'W), 15 April 2000, 29 September 2000, 15 August 2001, 20 November 2001; **14.**--Estero Aserradores, muddy sand with areas of exposed rock (12°37.352'N, 87°20.540'W), 29 September 2000, 15 August 2001, 20 November 2001; **15.**--Estero Paso de Cabellos, muddy sand, backbeach ponds and scrub mangroves (12°31.556'N, 87°12.507'W), 11, 12 April 2000, 30 September 2000, 15,16 August 2001; **16.**--Isla de Coriento, S. of bridge over estuary, rocks, and mud along mangrove lined shore of hypersaline estuary (12°31.051'N, 87°12.028'W) 11 April 2000; **17.**--"La Cruz" shrimp farm ponds and very muddy, mangrove lined pump intake canal (GPS n/a), 12 April 2000; **18.**--"La Majeda" shrimp farm, empty ponds (12°26.209'N 87°04.747'W), 12 April 2000; **19.**--"San Francisco" shrimp farm, empty ponds (12°30.422'N, 87°08.243'W), 12 April 2000; **20.**-- Poneloya estuary, sandy backbeach ponds and muddy sand estuary mouth with some mangroves (GPS n/a), 27 September 2000, 14 August 2001, 21 November 2001; **21.**--Estero de las Peñitas near shrimp hatchery, estuarine shores with salt flats and clayey mud flats among mangroves (12°21.66'N, 87°01.25'W), 27 September 2000, 14 August 2001, 21 November 2001; **22.**--Estero La Garita near shrimp farm, muddy sand near mangroves (12°16.257N, 86°52.648'W), 17, 20 November 2001; **23.**--Puerto Sandino near power plant, sand flat in mouth of embayment, ranging from detrital substrates and salt flats near mangroves (12°10.519'N, 86°45.784'W), 17 November 2001; **24.**--Miramar, sandy beach (12°10.308'N, 86°45.801'W), 17 November 2001.

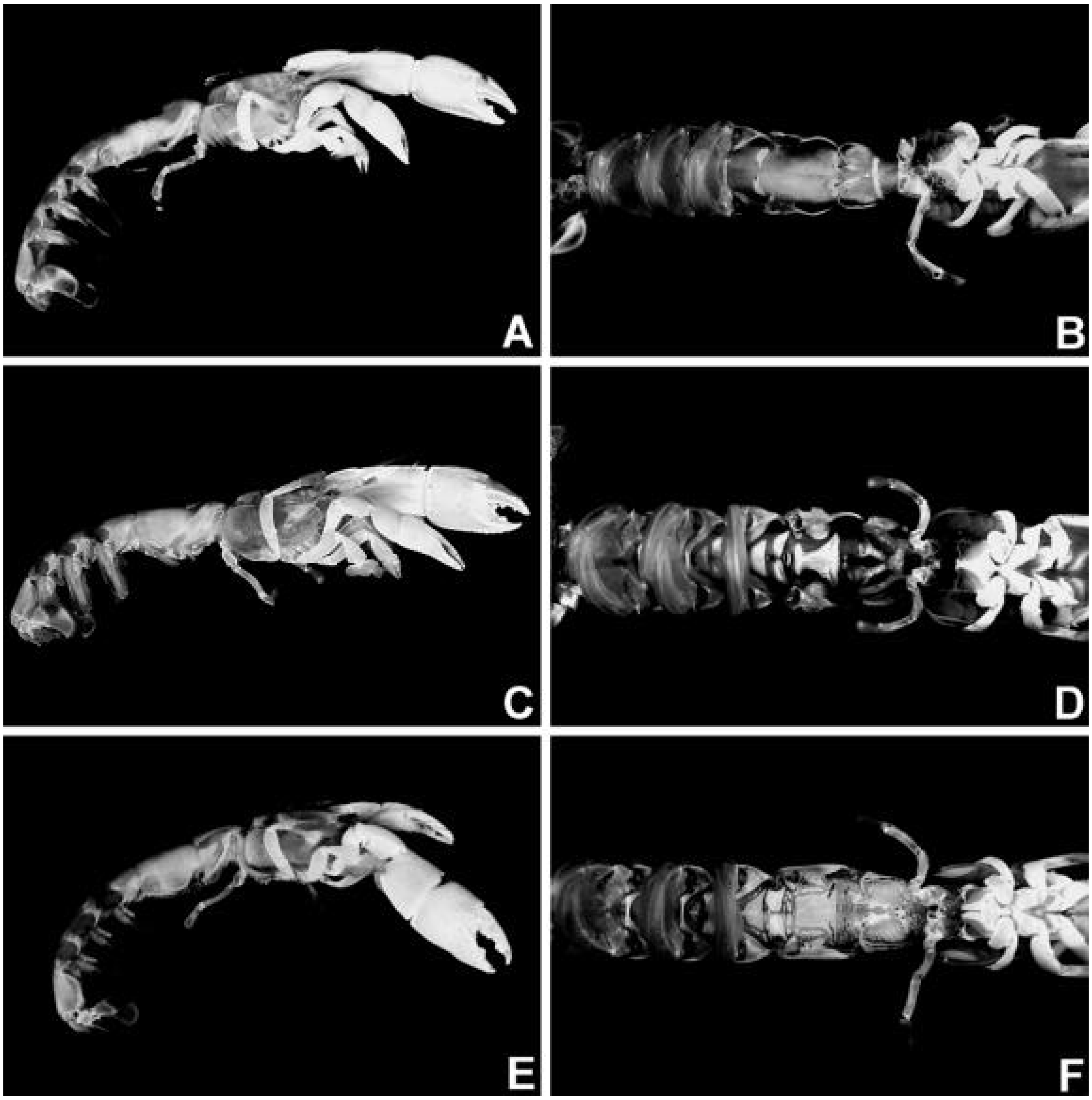

Figure 3. Dominant burrowing crustaceans collected from estuaries of coastal Nicaragua, April 2000 through November 2001, lateral and ventral views: A, B. *Lepidophthalmus* nov. sp.; C, D. *Lepidophthalmus bocourti*; E, F. *Lepidophthalmus eiseni*.

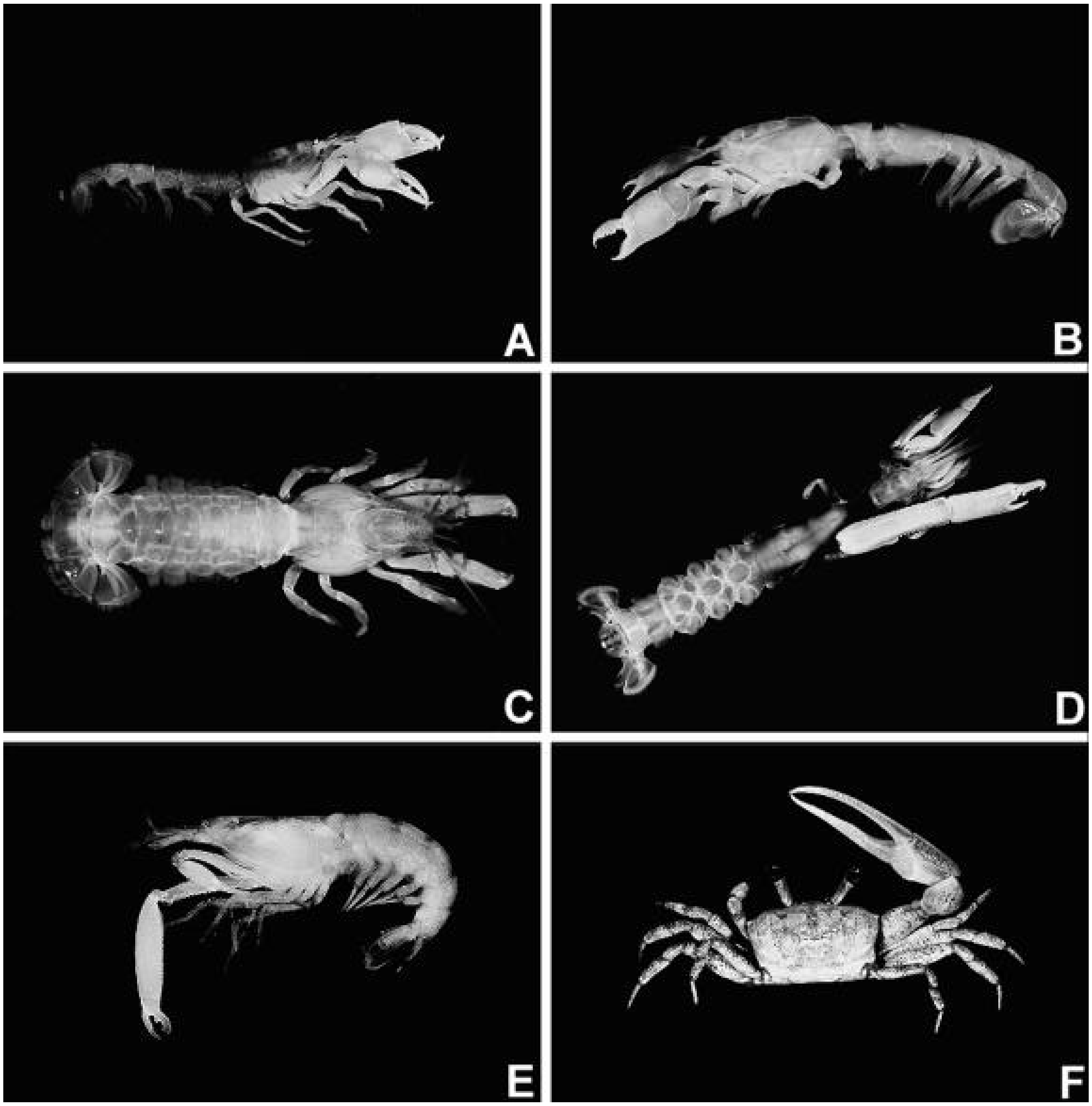

Figure 4. Common burrowing crustaceans collected from estuaries and coastlines of Nicaragua, April 2000 through November 2001: A. *Axianassa* nov. sp.; B. *Sergio* nov. sp.; C. *Upogebia* sp.; D. *Callichirus* sp.; E. "*Leptalpheus*" nov. sp.; F. *Uca* sp.

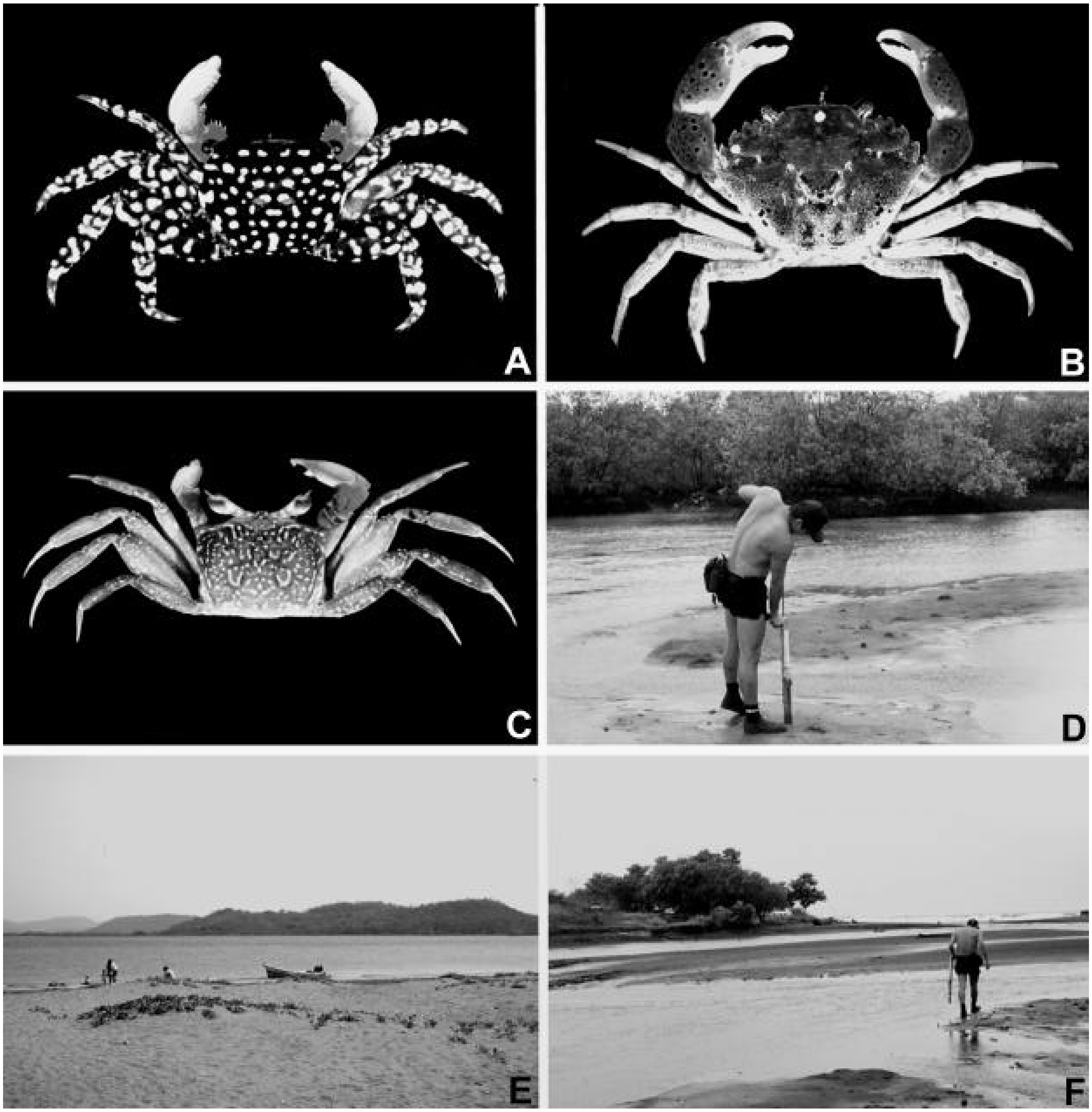

Figure 5. Common shallow burrowing crabs collected and typical estuarine collecting sites, coastal Nicaragua, April 2000 through November 2001: A. *Goniopsis* sp.; B. *Panopeus* sp.; C. *Ocypode* sp.; D. Use of "yabby pump" to sample infauna of mudflat near mangrove-lined mouth of Estero Ciego; E. Estero de Padre Ramos, sandy shores near mouth of large estuary; F. ephemeral opening of Estero Ciego to Pacific Ocean.

Figure 6. Penaeid shrimp culture ponds, estuarine collecting sites, and sampled habitats, coastal Nicaragua, April 2000 through November 2001: A. Dry culture ponds of UCA experimental farm, Estero Real; B. Control gate and burrows of *Uca* sp. near rain water, unfilled penaeid shrimp pond, UCA experimental farm, Estero Real; C. Tidally exposed mudflat, densely burrowed by *Lepidophthalmus* spp., Estero Ciego; D. Submerged burrows of *Lepidophthalmus* spp., some harboring commensal alpheids ("*Leptalpheus*" sp.), in Estero Ciego; E. Sampling of surface waters for subsequent nutrient analyses, Estero Ciego; F. Measurement of redox potential in effluent waters from burrows of *Lepidophthalmus* spp., Estero Ciego.

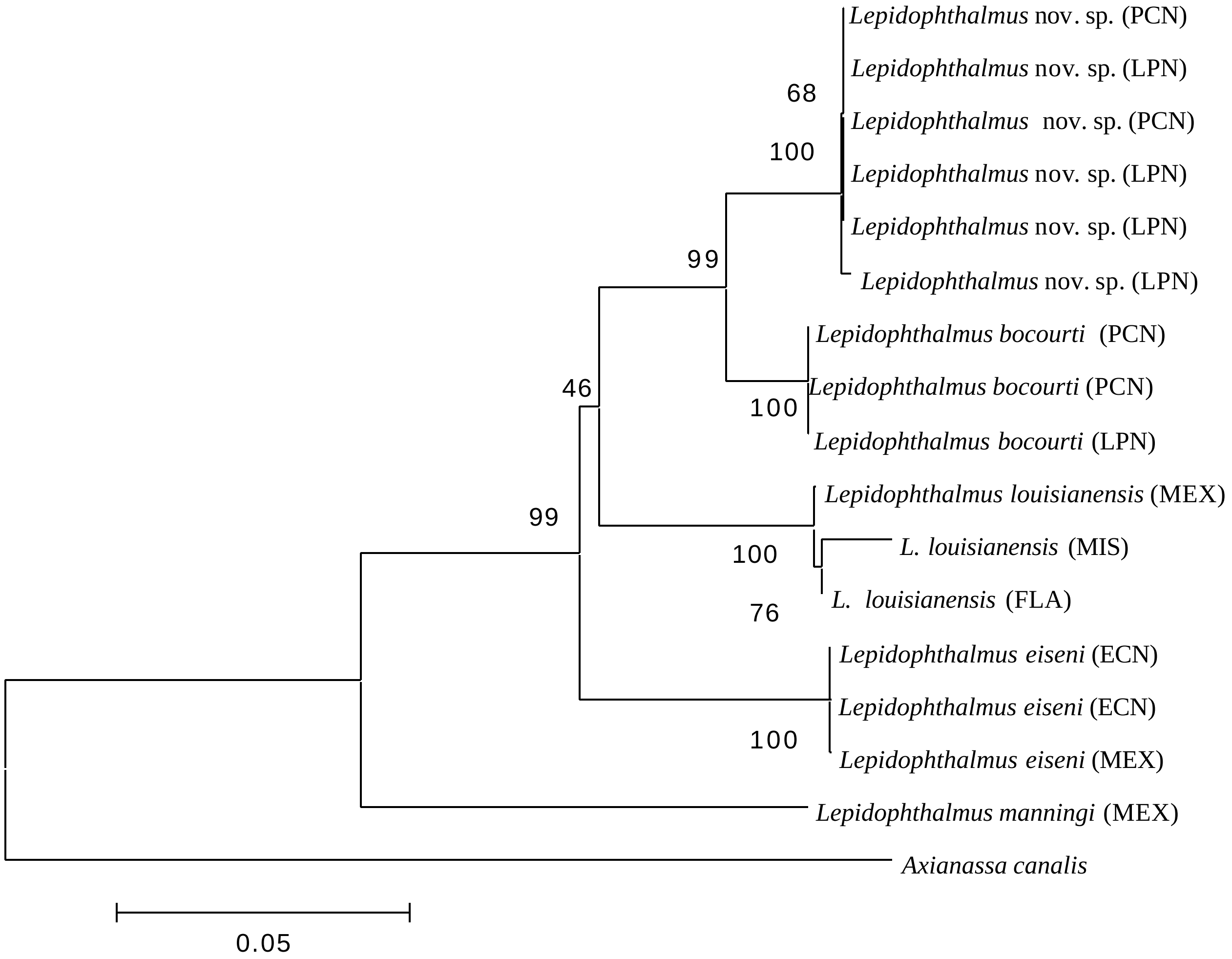

Figure 7. Putative relationships of Nicaraguan species of *Lepidophthalmus* and extralimital congenerics based on sequencing of 16s mtDNA, portrayed as tree produced by neighbor-joining analysis of 16 sequences. *Axianassa canalis* was included as an external group. Numbers represent confidence values (Kimura-two parameter distances, 2000 bootstraps). Scale represents genetic distance. ECN, Estero Ciego, Nicaragua; PCN, Paso Caballos, Nicaragua; LPN, Las Peñitas, Nicaragua; for others and all collection dates see table 1.

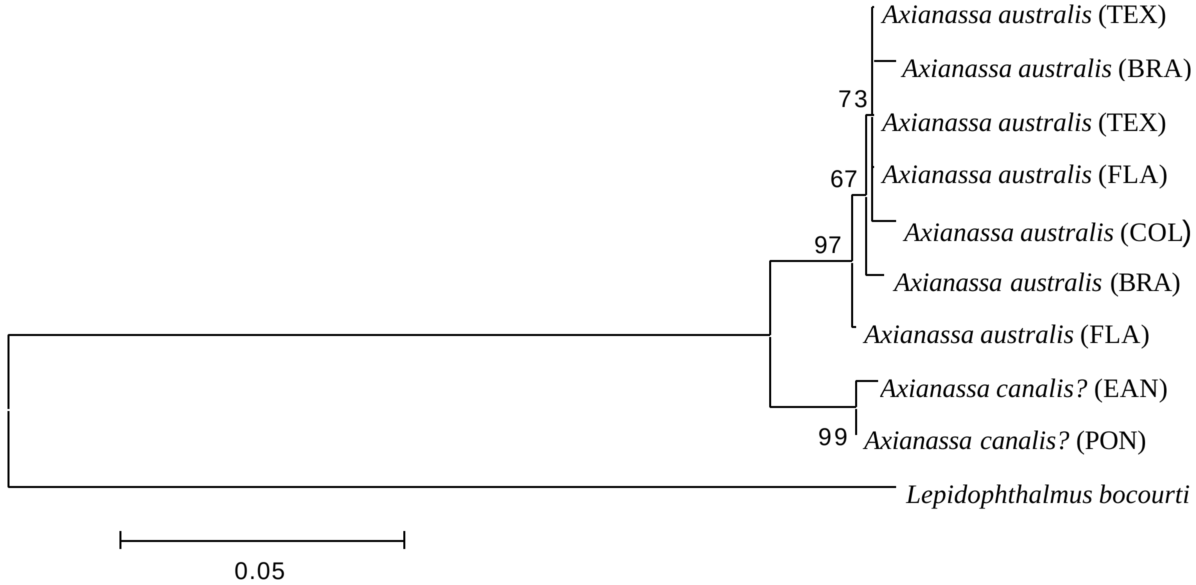

Figure 8. Putative relationships of Nicaraguan species of *Axianassa* and extralimital congenerics based on sequencing of 16s mtDNA, portrayed as tree produced by neighbor-joining analysis of 9 sequences. *Lepidophthalmus bocourti* was included as an external group. Numbers represent confidence values (Kimura-two parameter distances, 2000 bootstraps). Scale represents genetic distance. EAN, Estero Aserradores, Nicaragua; PON, Poneloya, Nicaragua; for others and all collection dates see table 2.

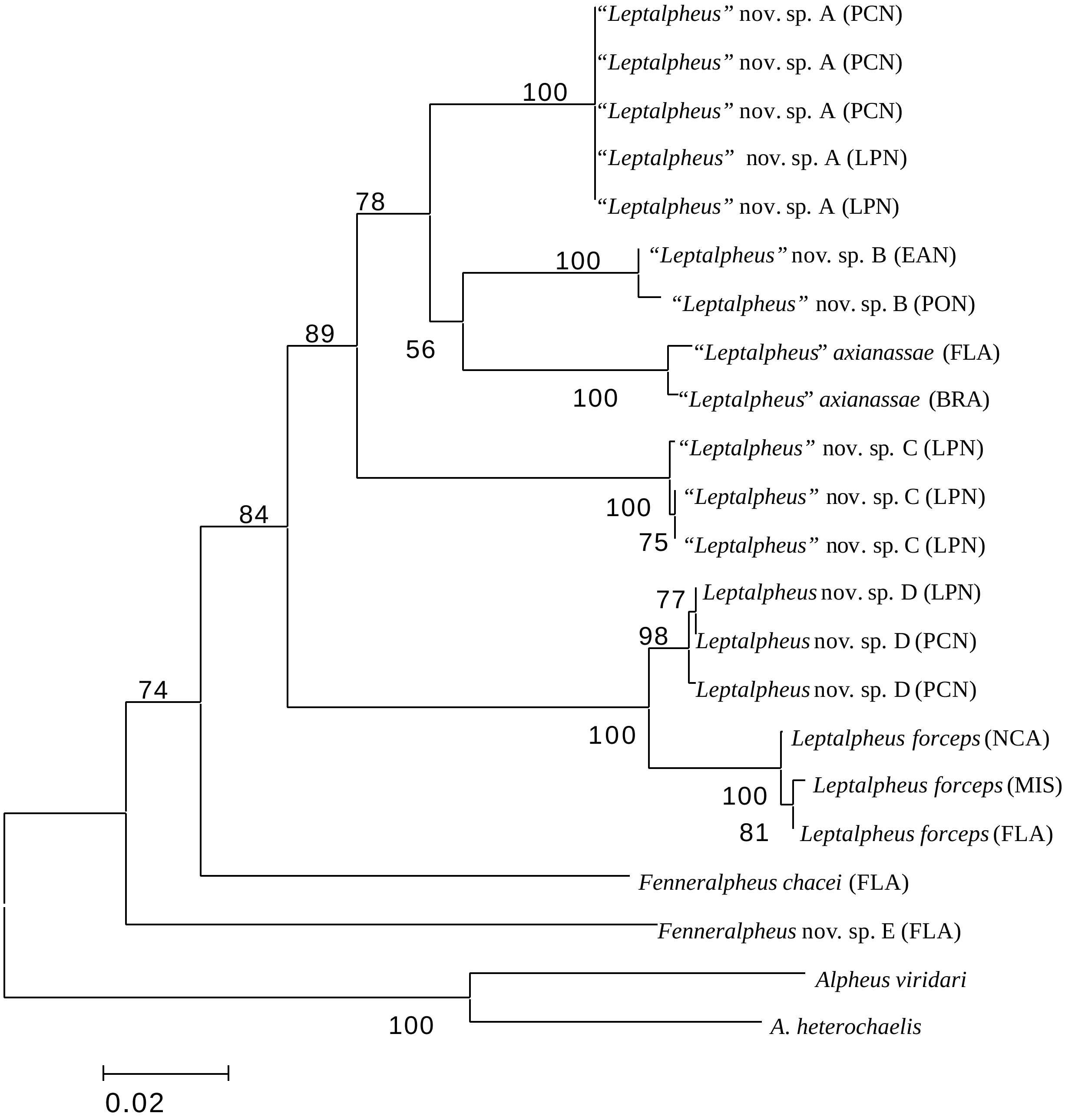

Figure 9. Putative relationships of Nicaraguan species of commensal, burrow-dwelling alpheoid shrimp, including *Leptalpheus*, putative congenerics ("*Leptalpheus")*, and relatives (*Fenneralpheus*), based on 16s mtDNA portrayed as neighbor-joining analysis of 20 sequences. *Alpheus heterochelis* and *A. viridari* were included as external groups. Numbers represent confidence values (Kimura-two parameter distances, 2000 bootstraps). Scale represents genetic distance. EAN, Estero Aserradores, Nicaragua; LPN, Las Peñitas, Nicaragua; PON, Poneloya, Nicaragua; PCN, Paso Caballos, Nicaragua; for others and all collection dates see table 3.

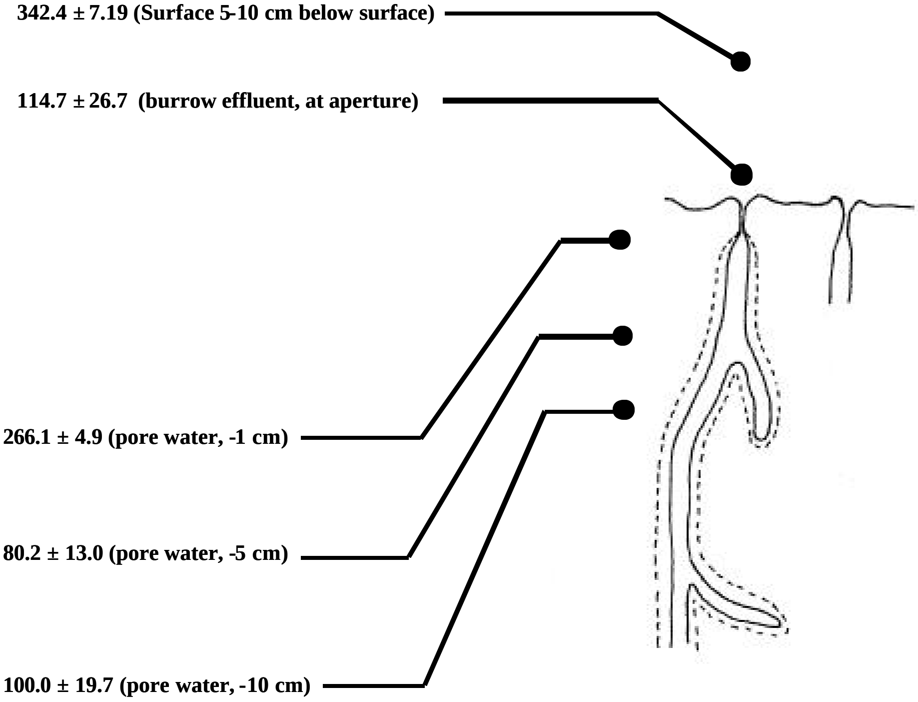

Figure 10. Typical REDOX levels (mV, corrected to calomel electrode values) in effluent waters from thalassinidean burrows, adjacent surface waters, and varied depths of adjacent pore waters, from Estero Ciego, coastal Nicaragua, April 2000.

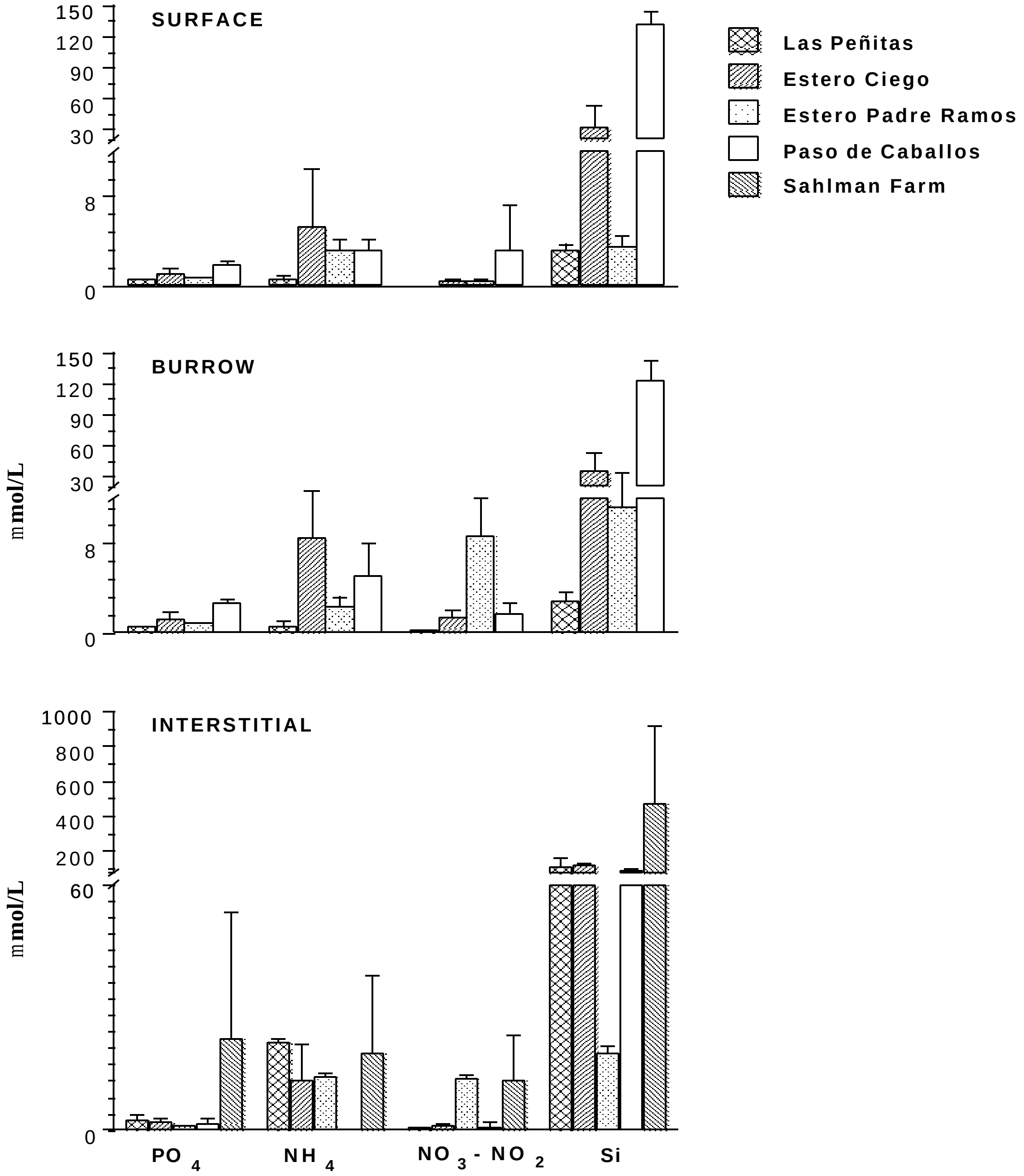

Figure 11. Concentrations of phosphate, ammonia, nitrates/nitrites, and silicates in interstitial porewaters, effluent waters from thalassinidean burrows, and adjacent surface waters in coastal estuaries densely populated by *Lepidophthalmus* spp. and their associates and from interstitial porewaters of a shrimp farm in coastal Nicaragua, April 2000 - September 2001.

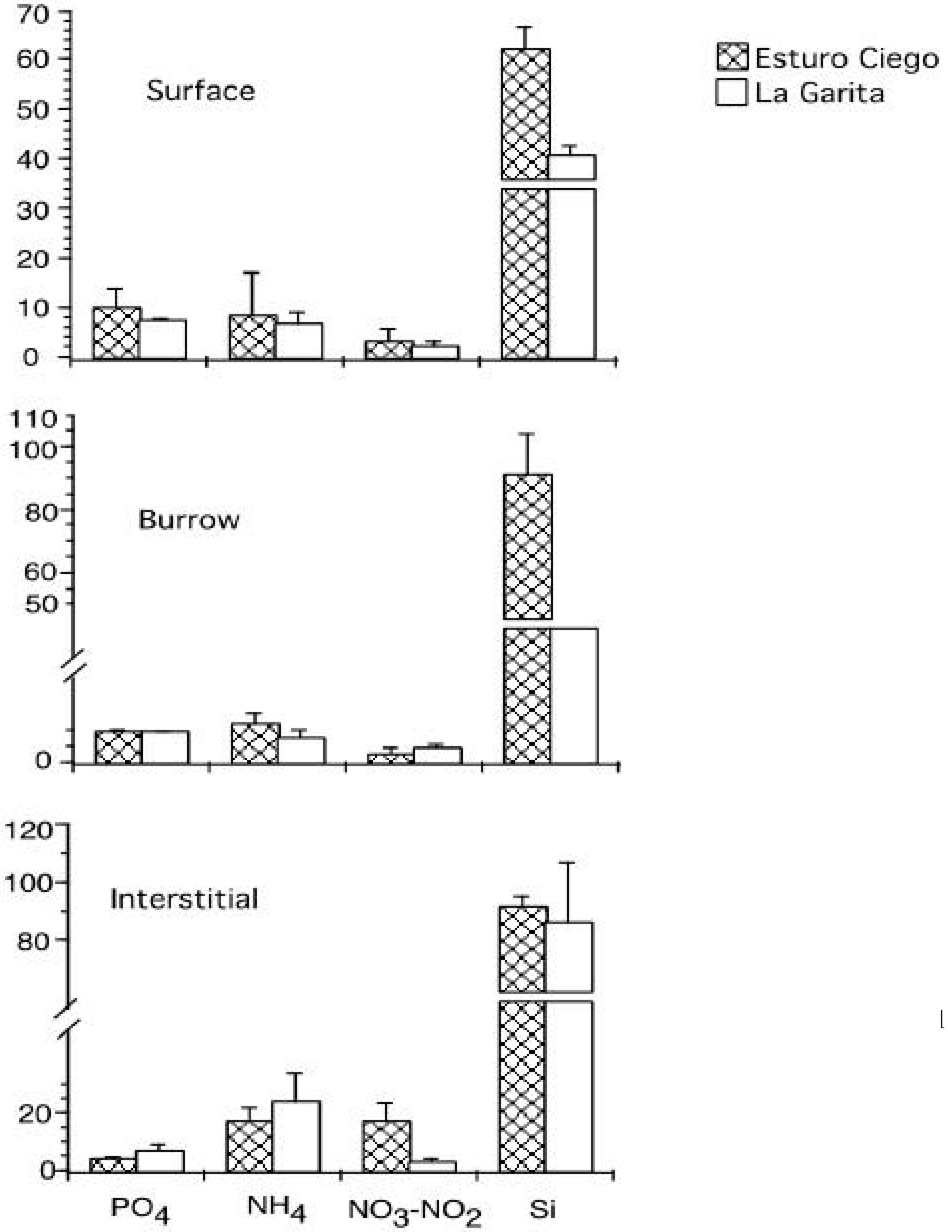

Figure 12. Concentrations of phosphate, ammonia, nitrates/nitrites, and silicates (µmol/L) in interstitial porewaters of substrates, effluent waters from thalassinidean burrows, and adjacent surface waters in coastal estuaries densely populated by *Lepidophthalmus* spp. and their associates, coastal Nicaragua, November 2001.

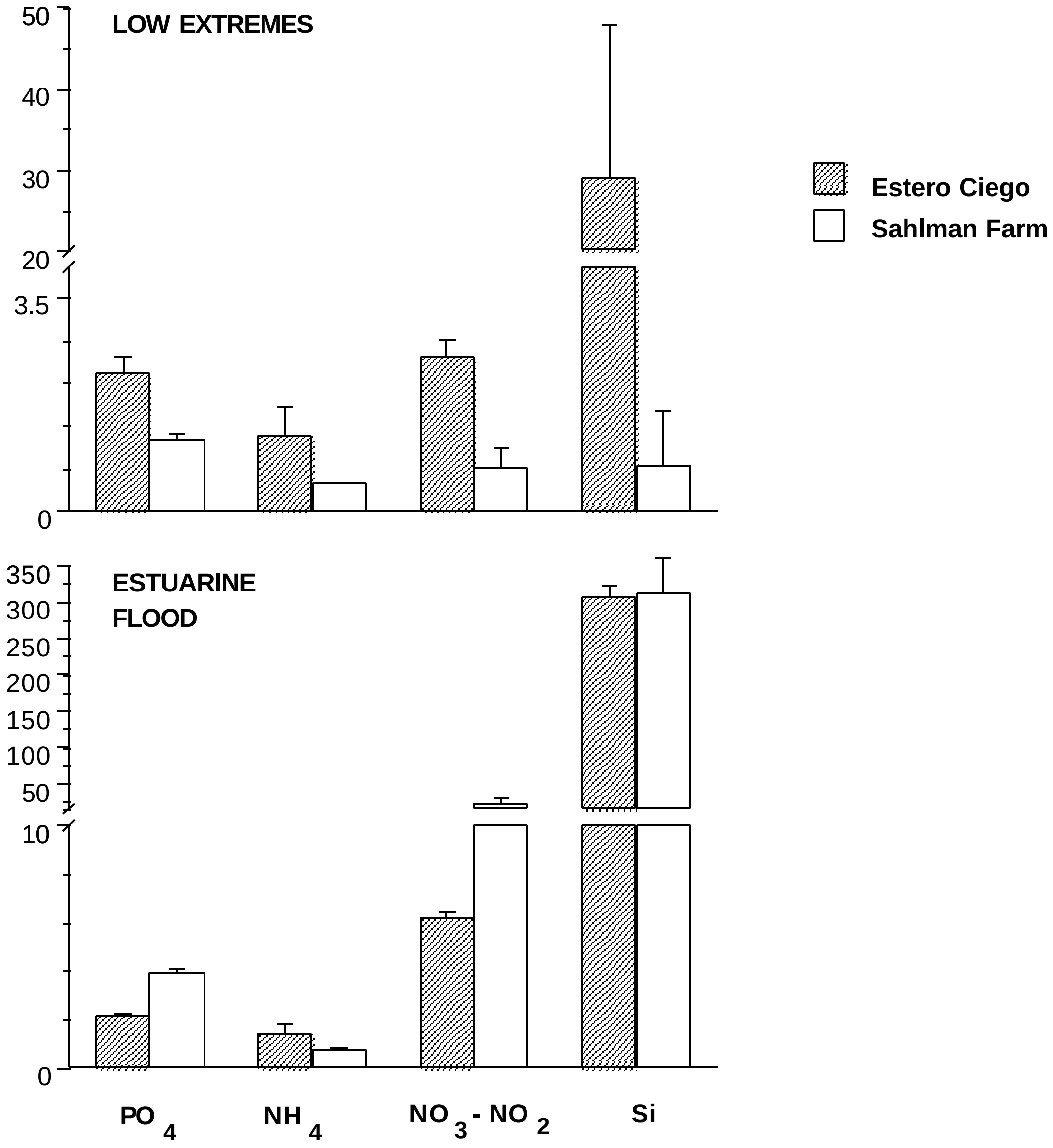

Figure 13. Atypical measures for concentrations of phosphate, ammonia, nitrates/nitrites, and silicates (µmol/L) in coastal Nicaraguan waters. LOW EXTREMES were found in rain precipitation accumulated in drained shrimp ponds (Sahlman Farm) and in coastal surf waters immediately outside Estero Ciego. ESTUARINE FLOOD surface waters in both these locations were highly turbid which is believed to have augmented nutrient loads during September 2000.

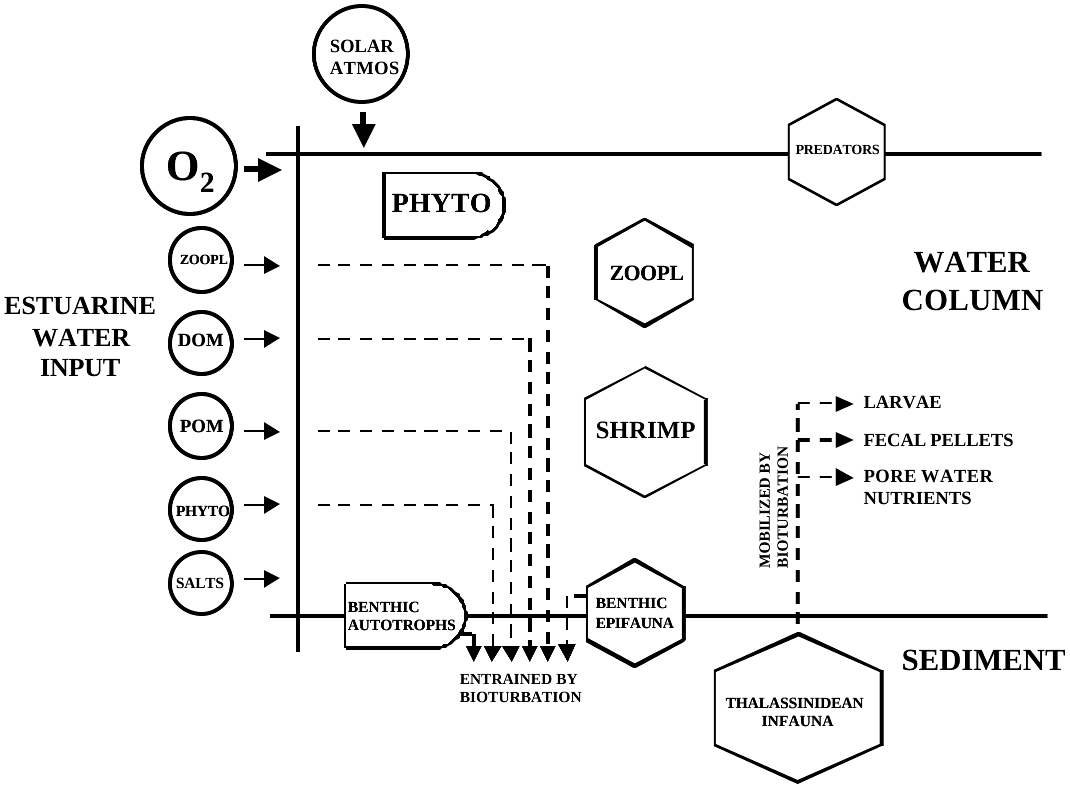

Figure 14. Conceptual model representing effects of organic entrainment and subsequent remobilization, both modulated by bioturbation of burrowing thalassinidean fauna, with resultant impacts on nutrient and carbon cycles in estuaries and potentially shrimp ponds of coastal Nicaragua.