UTILITY OF THE SOFT CORAL, *SINULARIA POLYDACTYLA*, AS A BIOMONITOR FOR POLYCHLORINATED BIPHENYLS (PCBS) IN TROPICAL MARINE WATERS: A PRELIMINARY ASSESSMENT

Paulina M. Welch
Peter J. Schupp
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WATER AND ENVIRONMENTAL RESEARCH INSTITUTE
OF THE WESTERN PACIFIC
UNIVERSITY OF GUAM

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by

Paulina M. Welch[a], Peter J. Schupp[b] & Gary R.W. Denton[a]

[a]Water and Environmental Research Institute of the Western Pacific
University of Guam, UOG Station, Mangilao, Guam 96923, USA

[b]Marine Laboratory,
University of Guam, UOG Station, Mangilao, Guam 96923, USA

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ABSTRACT

The objective of this research was to evaluate the soft coral *Sinularia polydactyla* as a biomonitor for polychlorinated biphenyls (PCBs). Potential influences on PCB accumulation were examined in sets of wild colonies. Uptake and depuration kinetics were measured by transplanting colonies and sampling over a 90 day period. All samples were lyophilized, extracted by accelerated solvent extraction, and analyzed by gas chromatography.

Spawning was discovered to impact PCB concentrations. Due to rapid physiological changes, within-colony differences were significant. Increases in lipid content in reproductively active portions of the colonies were not matched by increases in PCBs. During spawning, the new lipids were off-loaded while PCBs were not.

Post-spawn, within-colony differences abated. Gender and water column position were not significant factors. Most importantly, there was little variation among colonies. Analysis by lipid weight eliminated differences between age/size groups. Overall, the comparison studies revealed that *S. polydactyla* is well suited for biomonitoring outside of the spawning season.

The kinetics data provided strong evidence that Sinularian corals reflect ambient PCB levels. Uptake and depuration generally followed first-order kinetics. There was a degree of PCB metabolism and bioaccumulation in *S. polydactyla*. However, it was on par with what has been recorded in other aquatic species. Overall, it appears that *S. polydactyla* can be useful for PCB monitoring and Aroclor characterization. The use of *S. polydactyla* for at least basic PCB surveys or as part of a suite of monitoring organisms is warranted.

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Introduction

Pollution of land, air and water is sometimes an unintended consequence of industrial progress. When deleterious effects of pollution are recognized on human and animal health and on the quality of natural resources, measures are taken to eliminate the pollutant. An infamous group of industrial pollutants are polychlorinated biphenyls (PCBs). These compounds remain a major environmental concern even though they have been banned for three decades.

There are many approaches and tools for detecting and quantifying pollutants. A technique important in the regulation of PCBs is biomonitoring. PCB biomonitoring programs are well established in some parts of the world including mainland USA. However, biomonitoring programs are not well developed in subtropical and tropical regions of the world where PCB contamination is a growing concern. The purpose of this investigation was to evaluate a potential solution to the lack of a PCB biomonitoring tool appropriate for tropical coastal zones.

PCB Commercial History

Polychlorinated biphenyls (PCBs) are a group of synthetic organic chemicals widely used in industry until the late 1970's and early 1980's. Countries commercially producing PCBs included the United States, Germany, Italy, France, Japan and the former USSR (Connell and Miller 1984). The production of PCBs in the U.S. began in 1929 and ended in 1977. Termination resulted from the discovery that the chemicals were linked to adverse ecological and human health effects. The issuance of formal manufacturing and importing limitations by the USEPA followed in 1979. Finally, production was banned entirely in 1983 and restrictions on the use and disposal of existing PCB-containing equipment were set (USDHHS 1998). The estimated total production of PCBs from 1929 to 1980 is over 1.25 billion pounds in the U.S. alone, and 2.4 billion pounds worldwide (Erickson 1997).

Under the brand name Aroclor, the Monsanto Chemical Company of Illinois manufactured 99% of the technical PCBs used in the United States. The success of PCBs in industrial applications was largely due to their chemical inertness making them desirable for use as coolants, lubricants and solvents in a diversity of products including transformers, capacitors, refrigerators, and other electrical equipment, plastics, paints, and adhesives. Twelve formulations were offered over the years known as Aroclor 1221, 1232, 1242, 1248, 1254, 1260, 1262, 1268, and 1016 (USDHHS 1998).

Basic Structure of PCBs

Commercial formulations like Aroclor were mixes of related biphenyl compounds. The basic structure of the chlorinated biphenyl group is the biphenyl molecule shown in Figure 1.1. It is possible for 1-10 chlorine atoms to bond with the biphenyl at positions 2-6` resulting in 209 possible combinations called congeners. Commonly, the congeners are referred to by their numbers 1-209 designated by the

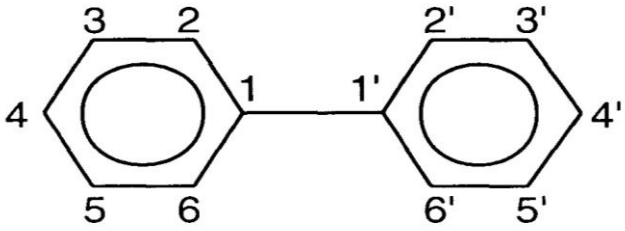

Figure 1: General polychlorinated biphenyl chemical structure. *Source:* Erickson 1997.

International Union of Pure and Applied Chemistry (IUPAC). Homologues are groups of congeners having the same degree of chlorination, or number of chlorines. The congeners within a homolog are isomers; they have the same chemical formula, but the chlorines are in different positions on the benzene rings. Positions 2, 2`, 6, and 6` are called ortho positions, 3, 3`, 5, and 5` are meta positions, and 4 and 4` are designated para positions (Erickson 1997).

Aroclors were not produced to have precise quantities of specific congeners. Desired qualities were obtained by varying the amount of chlorine added to the biphenyl parent chemical. Chlorine content by weight percent is indicated by the 3rd and 4th digits of the Aroclor number with the exception of 1016. Consequently, there were prevalent congeners in each mixture and some congeners never formed at all (USDHHS 1998).

Physical and Chemical Properties of PCBs

As a whole, PCBs are thermally and chemically stable. They are insoluble in water and soluble in nonpolar solvents and lipids. These characteristics increase with increased chlorination. Thus, the number of chlorine atoms per molecule is a determining factor in the degradation and bioaccumulation potential of a PCB congener. Certain configurations result in very environmentally persistent chemicals.

Smaller, less chlorinated congeners have relatively higher water solubility and are more diffuse in aqueous environments. They can partition easily into tissues, however, they are also generally readily metabolized and eliminated. In contrast, the highly chlorinated congeners are the least water soluble. The large nonpolar molecules bind strongly to organic matter in soil and sediment making them less available in the aqueous environment. Furthermore, their large size results in slow partitioning into tissues and inaccessibility to the active sites of cellular processes. It is the moderately chlorinated category of PCBs (5-7 chlorines) that is the most environmentally significant. These PCBs can partition into living organisms and resist metabolism (McFarland and Clarke 1989). This subgroup represents half of the 209 congener configurations and appear in high proportions in Aroclor formulations and environmental samples.

PCBs are further categorized by the configuration of their benzene rings which rotate freely around the bond connecting them. Planar, or coplanar, molecules are those in which the two rings are on the same plane. The other extreme, where the rings are at 90° with each other, is called nonplanar (USDHHS 1998). Due to steric hindrance, planarity is determined by chlorination at the ortho positions. Non-ortho or mono-ortho chlorinated biphenyls are generally

coplanar (Connell and Miller 1984; Erickson 1997). Twenty of the 209 congeners are considered to be coplanar.

Toxicity of PCBs

Adverse toxicological effects of PCBs have been documented in a wide spectrum of ecosystems and species (Connell and Miller 1984; Erickson 1997). Coplanarity is associated with high toxicological potential. Coplanar PCBs bare structural resemblance to dioxins and furans (McFarland and Clarke 1989; Erickson 1997). Foremost in this regard are PCB 77 (3,3',4,4'-tetrachlorobiphenyl), PCB 126 (3,3',4,4',5-pentachlorobiphenyl), and PCB 169 (3,3',4,4',5, 5'-hexachlorobiphenyl) which closely resemble the highly toxic TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) and dibenzofuran (2,3,4,7,8-pentachlorodibenzofuran).

According to McFarland and Clarke (1989), 36 of the 209 PCBs are environmentally threatening. The 36 congeners rate highly on potential toxicity, occurrence in environmental matrices, and relative abundance in biological samples. The first 16 in this group are primarily congeners with 5-7 chlorines and the worst players are coplanar.

PCBs, particularly those that are coplanar, have been shown to induce the P-450 enzyme system, which in turn produces intermediate products that are more toxic than the parent compound. Microsomal cytochrome P-450 enzyme systems catalyze oxidative biotransformation of aromatic ring compounds. Usually, this results in increased water solubility of these molecules. However, steric hindrance prevents the next step of detoxification and increases the stability and toxicity of the molecule (McFarland and Clarke 1989).

Global Transport of PCBs

The escape of PCBs into the environment was unforeseen and created widespread contamination that was not realized until the mid 1960s. They have since been discovered in all quarters of the globe and are now considered to be ubiquitous contaminants (Connell and Miller 1984; Erickson 1997). PCBs continually partition between all environmental compartments at rates determined by their physical and chemical properties.

In aquatic matrices, PCBs are predominantly associated with sediments because of their low water solubilities. However, the vapor pressure of most Aroclor formulations is such that a substantial fraction of the global PCB burden exists in the atmosphere in the vapor phrase. This is in large part responsible for the global dispersion of PCBs. Increased chlorination increases adsorption to particulates, and transfers PCBs to terrestrial and aquatic matrices by wet and dry deposition. Warmer temperatures increase volatilization and perpetuate dispersal (USDHHS 1998).

PCBs are soluble in and have a high affinity for biological lipids. This means that PCBs accumulate in the fatty tissues of living organisms and become concentrated (biomagnified) at higher trophic levels. This raises concern for the toxicological effects of PCBs on living organisms. However, with only 1% of the global PCB load associated with biota, this matrix does not contribute greatly to the dispersion and degradation of the global PCB burden.

PCB degradation occurs primarily in the atmosphere. Photochemical degradation occurs with half-life values ranging from 4-84 days for mono- and pentachlorobiphenyls respectively. In contrast, PCB half-lives in soil and sediment range from months to years due to binding with organic matter (USDHHS 1998).

As PCBs are dispersed globally and temporally, relative congener proportions change. Due to relative variations between congener half-lives, water solubility, affinity for soil, partitioning rates into biological lipids and susceptibility to degradation, congeners present in commercial formulations are not necessarily present in environmental samples. In fact, the similarity of a PCB profile of an environmental sample to an Aroclor profile is an indication of the proximity and age of the source. The further away and longer in time from the discharge, the more the relative congener proportions will be changed due to their physical properties.

Global Distribution of PCBs

Even though PCBs are not naturally found in the environment, and mass production stopped three decades ago, they continue to exist world-wide in soils, surface waters, indoor and outdoor air (USDHHS 1998) and the tissues and fluids of all living organisms (Connell and Miller 1984; Erickson 1997). The industrial use of PCBs in the USA, Japan, and Europe resulted in a belt of PCB contamination in the middle latitudes of the northern hemisphere. As PCBs were condemned in these regions, transformers and capacitors containing these chemicals were imported by developing countries in the subtropics and tropics. Consequently, PCBs contaminated tropical air and water and expanded the "PCB belt" southward (Tanabe 1991; Iwata *et al*. 1994).

An estimated 65% of PCBs ever produced is still in use or exists in landfills and 33% persists and cycles in the environment (Kimbrough 1995; Phillips and Rainbow 1998). PCBs have remained on USEPA's list of priority pollutants of concern for decades. The EPA's National Priorities List of U.S. hazardous waste sites has identified 432 PCB contaminated locations, one of which is in Guam.

Developing countries present an increasing risk of contributing to PCB pollution. Corroding machinery and inadequate containment at disposal facilities leak PCBs into the environment in many developing countries' industrialized cities (Connell and Miller 1984; Phillips 1995). In the Asia-Pacific region, including Japan, Russia, Hong Kong, India, China, and the Philippines, PCB levels in river and estuarine waters are relatively high by world standards. The Asia-Pacific Mussel Watch Program, which monitors these waters, reports that old transformers and capacitors containing PCBs are still being used throughout the region (Monirith *et al.* 2003). When improperly discarded these items of equipment currently rank among the more important contributing point sources to the global PCB contamination problem (Iwata *et al*. 1994).

The discharge of PCBs into the environment in tropical regions is of special concern to global pollution due to heightened dispersion potential. The sustained high temperatures and rainfall of the tropics expedite the dissipation and global redistribution of the semi-volatile, persistent PCBs (Tanabe 1991; Tanabe *et al.* 1994; Iwata *et al*. 1994). Currently, environmental PCB concentrations in Asian countries positively correlate with per-capita gross national product

(GNP). Environmental levels of these chemicals may thus be expected to increase further in developing countries with high economic growth rates (Monirith *et al*. 2003).

Routes of PCB Exposure

Today, the major sources of PCBs are poorly maintained hazardous waste sites, illegal dumping, burning of organic waste, the continued use of some appliances manufactured before 1979, and environmental cycling (Erickson 1997). The two most common routes of PCB exposure for the general public are inhalation of contaminated air and consumption of contaminated food (USDHHS 1998).

Due to their lipophilic quality, PCBs are passively absorbed across cell membranes in the lung alveoli and gastrointestinal tract (Albro and Fishbein 1972; Matthews and Anderson 1975). They are dispersed throughout body tissues and rapidly attain dynamic equilibrium with source concentrations (USDHHS 1998). Some of the lower chlorinated congeners are metabolized and excreted within a few days to weeks while the more highly chlorinated members are more persistent and preferentially accumulate in adipose tissue, particularly central fat stores (Matthews and Dedrick 1984).

The consumption of contaminated seafood by humans is an obvious health hazard. With PCB contaminated seafood, the problem is exacerbated by biomagnification (LeBlanc 1995; Oliver and Niimi 1988). Ingestion of PCB contaminated seafood is a major concern in many areas, including the South Pacific, where diets are predominantly fish-based (Carpenter 1998).

Benefits of Biomonitoring

Environmental monitoring was defined by Chapman *et al.* (1987) as repetitive measuring of an environmental parameter for the purpose of determining trends in that parameter. In aquatic environments this process is usually accomplished by analyzing water, sediments and/or biota for the contaminant(s) of interest and may have other objectives besides simply trend monitoring. For this reason, Phillips and Rainbow (1998) expanded upon the above definition to include the study of contaminant sources, transport pathways and sinks, the determination of baselines, the investigation of impacts of developments, the screening of effluents, and the study of environmental quality cause-and-effect relationships.

Biotic components used for monitoring purposes are referred to as biological monitors, or biomonitors. Phillips and Rainbow (1998) described aquatic biomonitors as organisms that accumulate contaminants in their tissues to levels reflective of ambient available concentrations and can be analyzed to identify the distribution, and abundance of biologically available fraction of these contaminants in the organism's immediate surroundings.

Biomonitoring has won favor over direct water and sediment analysis for several reasons. The concentrations of contaminants in open waters range in extremely small numbers (part per trillion to part per million). Thus, direct analysis requires sophisticated equipment, a high level of expertise, and it is time consuming, expensive, and susceptible to both analyte loss and contamination (Phillips and Rainbow 1998). Furthermore, direct analysis provides only a "snapshot" of contaminant levels which can markedly fluctuate due to intermittent discharges, tides, currents, weather, and interaction with biota among other factors (Phillips 1980).

Sediment analysis quantifies contaminants that are adhered to sediment, not contaminants that are interacting with biota living in the aqueous environment. Therefore, sediment analysis may not provide an accurate representation of which contaminants are being accumulated by aquatic organisms.

Certainly Semi Permeable Membrane Devices (SPMDs) are valuable tools for monitoring contaminants in areas where no biomonitors exist, but they do not negate the value of biomonitors. Differences in the accumulation of organic contaminants in SPMDs and mussels have been observed and actually elucidate the dissolved, colloidal, and particulate distribution of the contaminants. However, if insight into the introduction of organic pollutants into the food web is sought, biomonitoring is a more generous tool (Boehm *et al*. 2005; Richardson *et al*. 2005). Thorough evaluation of a species as a biomonitor can culminate in a tool that is not only advantageous in that it provides information on the biological availability of contaminants, but is also far less expensive and considerably more convenient than SPMD purchase and deployment.

Selection of a Biomonitor Species

The only essential quality of a biomonitor is that it must exhibit little to no metabolic regulation of the contaminant(s) of interest, i.e., levels accumulated should be dependent upon and therefore reflective of levels in the organism's immediate surroundings. Non-essential, but otherwise highly desirable prerequisites, first listed by Butler et al. (1971) and later added to by Phillips (1977), are summarized below:

- Exhibits high concentration factor of the pollutant without lethal consequences
- Sedentary, therefore representative of the area
- Abundant at study site
- Sufficiently long-lived to satisfy desired monitoring period
- Size provides adequate tissue for analysis
- Easy to identify and collect
- Able to withstand laboratory conditions
- Tolerates brackish water
- Amenable to translocation to desired study sites if not already present

Evaluation of Potential Biomonitor Species for PCB Assessment

It is now well accepted that contaminant bioaccumulation is potentially influenced by both intrinsic and extrinsic factors. Intrinsic factors influencing PCB accumulation include the organism's lipid content, size, age, reproductive stage, and tissue type. These factors can be overcome by deploying or sampling organisms that fit a range of criteria or normalizing the data (Phillips and Rainbow 1998). Intrinsic factors that cannot be controlled include metabolism and active uptake. Extrinsic, or environmental factors, can generally be circumvented and include ambient water temperature, salinity, and position in the water column. A thorough evaluation has to identify and describe how PCB accumulation by a species is influenced by intrinsic and extrinsic factors prior to its use as a biomonitor. Failure to do so may result in misleading or useless data (Phillips 1980).

Finally, the organism's rates of uptake and depuration of the contaminant of interest need to be ascertained. If the monitoring program is to produce a continuous record of pollutant availability, then sampling frequency has to be adjusted with respect to the contaminant's half-life in the

organism (Phillips and Rainbow 1998). PCBs are mixtures of individual congeners, each of which may behave differently in an organism. This is overcome by focusing monitoring programs on a limited group of PCBs that are of greatest concern. Equipped with kinetic rates for the congeners of interest allows sampling frequency to be optimized for the group. Furthermore, kinetic data makes it possible to generate quantitative analyses of aqueous contaminant concentrations in the organism's immediate surroundings. Without kinetic data, only qualitative assessments are possible.

Need for More Biomonitor Species

The establishment of a new biomonitor species adds versatility to biomonitoring programs. Creating scope in environments where traditional species do not exist expands the spatial and temporal scales of monitoring programs. In environments where more than one biomonitor species exists, it imparts opportunities to integrate ecosystem response and tweeze out overall trends. An approach using several species covers more ground, and increases accuracy by smoothing out idiosyncratic responses of a particular species. It presents a more complete environmental footprint of baselines and responses to pollutant point sources (Read *et al*. 2005). Ideally, a total picture is obtained by using a suite of organisms of diverse habitats, trophic levels and/or feeding strategies, which can provide a measure of contaminant levels in various environmental compartments (Rainbow and Phillips 1998).

Existing Research

A great deal of research exists establishing and employing biomonitors. However, most of the published research addresses heavy metals. Relatively little research has been conducted to expand the base of PCB biomonitors even though PCB biomonitoring is an important part of federal regulatory programs.

Bivalve mollusks are used in what is possibly the most extensive biomonitoring effort to date, the US Mussel Watch Program. The green-lipped mussel, *Perna viridis*, has been used heavily in subtropical areas, particularly the Indo-Pacific, and is potentially suitable for tropical environments (Kannan *et al.* 1989; Monirith *et al.* 2003). Marine sponges were studied, but indicated some levels of PCB metabolism (Perez *et al.* 2003). Damselfish larvae (*Abudefduf sordidus*) were studied to establish their PCB indicator potential in the Central Pacific Ocean via immunohistochemical methods (Kerr Lobel and Davis 2002).

Scattered PCB data from the monitoring of finfish are available. Skipjack tuna (*Katsuwonus pelamis*) has been used for off-shore and open ocean studies (Ueno *et al*. 2005). The drawback of finfish is their mobility. Depending on their range, a particular finfish may not accurately reflect the contamination of the water in which it was captured. It may also not be immediately available. Furthermore, with the use of mobile species spatial and temporal effects may be confounded (Phillips and Rainbow 1998).

Marine mammals are also common subjects of PCB investigations. The facts that cetaceans feed at the top of the food chain, have high bioaccumulative potential, demonstrate limited ability to enzymatically degrade PCBs, and pass large portions of their PCB load to the next generation through lactation make these animals the most affected victims of worldwide PCB pollution (Tanabe *et al.* 1994). However, cetaceans make poor biomonitors because of their mobility,

position in the food chain, and difficulty of sampling. Furthermore, sampling is generally limited to the incidental washed up carcass. They are good indicators of general global conditions but not useful for tracking specific sources.

The most neglected areas of research on PCB contamination of inland and coastal waters are subtropical and tropical regions (Phillips and Rainbow 1998). Overall, relationships between ambient and tissue PCB levels have not been evaluated in tropical species. There is an urgent need to fill this gap because of the rapidly growing industrialization and population of tropical regions. The region's reliance on seafood as a source of protein increases the concern about PCB contamination (Phillips 1989).

Previous researchers conducted a sweeping survey of a broad range of aquatic organisms in polluted areas on Guam. PCBs and heavy metals were measured. The research highlighted the potential of Sinularian corals for biomonitoring of PCBs. The study showed that the tissue concentrations of PCBs in soft corals reflected the high ambient levels of known PCB hot spots on Guam. The soft corals demonstrated a high accumulation capacity for PCBs and reflected homologue proportions that resembled commercial Aroclor mixtures (Denton *et al.* 1999; Denton *et al*. 2006). This indicates that Sinularian corals are potentially valuable monitoring tools and a thorough evaluation of their suitability is justified.

Characteristics of *Sinularia polydactyla*

Sinularia polydactyla (Class Anthozoa, subclass octocorallia (or Alcyonnaria), Order Alcyonacea, Family Alcyoniidae, Genus Sinularia, Species polydactyla) is a reef building species. They are the major reef builders on some reefs (Cornish and DiDonato 2004). *Sinularia polydactyla* grow in massive colonies. Older colonies can measure several meters in diameter (Fabricius and Alderslade 2001). Communities can consist of hundreds of individual colonies (Fabricius and Alderslade 2001). Colonies of *S. polydactyla* are shown in Plate I.

Each *S. polydactyla* colony is a unit of monomorphic polyps married by a fleshy mass, imbued with calcium carbonate sclerites (Gawel 1977; Wood 1989; Fabricius and Alderslade 2001). The colony has a bare trunk and a branching and lobed, polyp-bearing capitulum. The capitulum branches into primary lobes and finger-like secondary and sometimes tertiary lobes. The retractile polyps cover the entire capitulum, but are most dense in the tertiary lobes (Gawel 1977; Wood 1989). The lobes of *S. polydactyla* are long and finger-like of a leather texture, and therefore fall under the informal description "Finger coral" or "Leather coral" (Borneman 1999).

Sinularia polydactyla attach to hard, consolidated substratum on the sea floor or other structure as larvae after sexual reproduction, or as a clone colony after asexual propagation (Fabricius and Alderslade 2001). Sexual reproduction is accomplished by spawning which occurs on the fourth and fifth nights after the full moons in three consecutive months; March through May or April through June (Alino and Coll 1989; Slattery *et al*. 1999).

Sinularia polydactyla have slow recruitment and growth rates (Fabricius 1995). Population size is maintained predominantly by asexual reproduction via colony fission, fragmentation, or budding (Fabricius 1995). As a result, they are able to produce large areas of genetically identical colonies, or monospecific carpets (Fabricius and Alderslade 2001). Their communities

are stable, rather than ephemeral, and survive by maintaining low mortality rates and living to a great age (Fabricius 1995). Furthermore, *S. polydactyla* have few natural predators (Wylie and Paul 1989).

Sinularia polydactyla are suspension feeders. They filter particulate organic matter such as phytoplankton, microzooplankton, and bacterioplankton under 20 micrometers. They are able to remove large quantities of suspended particulate matter from the water. Capture by nematocysts is limited to small, weakly swimming zooplankton (Wood 1989; Borneman 1999; Fabricius and Alderslade 2001). The amount of organic matter in tropical waters is often low, therefore *S. polydactyla* host a symbiotic algae, zooxanthellea (group Dinoflagellata), in their gastrodermal cells that supplement the corals' diet by photosynthesis (Fabricius and Alderslade 2001).

Sinularia polydactyla are robust in terms of environmental conditions. They are found at depths from 3 to 40 meters, tolerate a range of temperatures and turbidity (including strong currents), freshwater input, and even occasional exposure to air during low tides (Borneman 1999; Fabricius and Alderslade 2001).

Soft Coral Distribution

Soft corals are major components of coral reefs in the tropical Indian Ocean, Red Sea and Indo-Pacific (Benayahu and Loya 1981; Wood 1989; Dai 1990; Fabricius 1997; Riegl and Piller 1999; Cornish and DiDonato 2004). The soft coral genus *Sinularia* is one of the major contributors to the animal cover of shallow coral reef habitats throughout the Indo-Pacific (Benayahu *et al*. 2002; Paulay *et al*. 2003). Their phenotypic plasticity (Gawel 1977; Borneman 1999) allows them to inhabit reefs from Africa, throughout the Red Sea, the Indian Ocean, and the Central-West Pacific to Hawaii. Areas where *S. polydactyla* has been recorded include but are not limited to: Madagascar, Indian Ocean Islands, Indonesia, Great Barrier Reef, New Caledonia, Polynesia, Taiwan, Vietnam, Ceylon and the Phillipine, Marshall, and Palau islands (Gawel 1977; Fabricius and Alderslade 2001).

On Guam, *Sinularia* occupy such diverse habitats as the windward barrier reef channel, leeward barrier reef channel, lagoon shelf, lagoon patch reef, and fringing reef flat (Gawel 1977). In some areas, *Sinularia* species have been recorded to constitute up to 95% of the living animal cover (Wylie and Paul 1989). Two species of *Sinularia* are predominant on Guam: *S. polydactyla* and *S. maxima*. *Sinularia polydactyla* has been reported as the dominant soft coral species at the following locations: Piti Bomb Hole, Shark's Hole, Cocos Lagoon, Apra Harbor, Facpi Island, Asan, Tepungan Channel, Mamaon Channel, Agana Boat Basin (Gawel 1977; Benayahu 1997; Denton *et al*. 2006). Other locations include: Dadi Beach, Luminao, and Agat (pers. observtn.).

Biomonitor Potential of *Sinularia* spp.

The information that research provides thus far about Sinularian corals shows that they satisfy the prerequisites for PCB biomonitoring candidacy. First, they have demonstrated accumulation of PCBs reflective of ambient levels. Second, they are sedentary, ample in size, abundant, and long lived. Third, they are easily identified and sampled. And fourth, Sinularian corals are robust, tolerate brackish water, and withstand translocation. Scoring high on the prerequisites, what remains to establish *Sinularia polydactyla* as a PCB biomonitor is a thorough evaluation of intrinsic factors that may affect PCB uptake and depuration.

Scope of Research
The recognized model for PCB kinetics studies for biomonitors in the field is the research conducted by Tanabe *et al*. (1987) with the green-lipped mussel, *Perna viridis*. Mussels were transplanted from an uncontaminated site in Hong Kong harbor to a heavily contaminated site nearby. Following 17 days of exposure, the mussels were back-transplanted to their origin for a 32 day depuration period. Sampling was carried out throughout the uptake and depuration periods to measure congener and homologue concentrations. Conducting the studies in the field not only generated congener and homologue kinetics rates and profiles, but also tested the suitability of the study organism for biomonitoring PCBs (Tanabe *et al*. 1987).

Laboratory kinetics studies are impractical. They tend to produce conflicting results due to the difficulties with dissolving and maintaining PCBs in solution. There are also a host of variables arising from incongruence with natural routes of uptake. This research followed the tested methods of Tanabe and co-investigators, but with *S. polydactyla*. Additionally, comparative studies were conducted to determine the effects of age, gender, season and water column position on tissue concentrations. The nature and scope of this study was intended to thoroughly examine *S. polydactyla* as a PCB monitor as defined by Phillips and Rainbow (1998).

Objectives of this Study
The overriding objective of this research was to evaluate the soft coral species *Sinularia polydactyla* as a biomonitor for polychlorinated biphenyls. Guidelines have been established by prior researchers which ensure that factors are examined which could influence contaminant accumulation. Disregarding such factors leads to nonsensical monitoring results, or worse yet; misleading information. Thus, each sub-objective of this study examined a factor that potentially influences the accumulation of PCBs in *S. polydactyla*. With knowledge of such factors, a biomonitoring program can be designed that produces reliable and accurate environmental monitoring. This research evaluates *S. polydactyla* as a biomonitor by examining the following factors:

- Variation within organisms: variation in PCB concentrations within a colony
- Variation between organisms: variation in PCB concentrations in different individuals at the same site
- Seasonal variation: PCB concentrations in colonies pre-spawn vs. post-spawn
- Gender: PCB concentrations in female colonies vs. male colonies
- Age: PCB concentrations in adult colonies vs. juvenile colonies
- Water column position: PCBs in colonies on the ocean floor vs. elevated colonies
- Rates of PCB uptake and depuration
  Amenability to transplantation

Potential Benefit of this Study
Soft corals can be reliable biomonitors of PCB pollution. Used appropriately, they will serve as convenient and inexpensive biomonitoring tools for sub-tropical and tropical aquatic environments. SPMDs are currently used by the EPA and other regulatory agencies to measure PCBs. Aside from being expensive, their handling requirements pose logistical and technical challenges for use on Guam, and even more so for more remote islands. Soft corals provide a more accessible and less resource intensive option for monitoring.

Materials and Methods

Experimental Site Descriptions

The studies described herein were conducted on *Sinularia polydactyla* representatives from two shallow reef sites along the western shoreline of central Guam (Fig. 2.1). Site selection was based on background PCB levels and *S. polydactyla* abundance. General information relating to each experimental site is given below.

Dadi Beach Reef

Dadi Beach (13°24`N, 144°39`E) is located on the SE side of the Orote Peninsula, a US naval reservation area juxtaposed to Apra Harbor. It lies approximately 2 km SE of the Orote Landfill, a disused military dumpsite and suspected source of PCBs (NAVFAC 2006). Marine fish from this part of the island contain relatively high levels of PCBs and a seafood advisory remains in effect between Orote Point and Rizal Beach, an 8 km stretch of coastline that encompasses the Dadi Beach area (NAVFAC 2006). The shallow reef off of Dadi Beach is spotted with small carpets of *S. polydactyla.* The PCB concentrations in samples from these corals are relatively elevated, reflective of the higher ambient PCB concentrations at this general location.

Piti Bomb Holes

The Piti Bomb Holes (13°25`N, 144°55`E) are located approximately 5 km west of Agana, the main business center of Guam, and 3 km NE of Apra Harbor, the island's only commercial port. Although scarred by WWII bomb craters, the area supports a rich diversity of marine flora and fauna and has been a marine preserve since 1997. The dense carpets of *S. polydactyla* that exist here contained relatively low levels of PCBs and representatives were transplanted from this site to Dadi Beach during the PCB uptake study. The site also served as a PCB depuration site for *S. polydactyla* from the latter location.

Colony Selection

Representative colonies of *S. polydactyla* were identified using the procedures outlined in Appendix A. Only adult colonies (Plate I) were selected for the experimental work described below unless stated otherwise. They were confined to representatives living at depths of 1.5-4.0 m that were separated by distances of at least 9 m to ensure genetic variability. The chosen colonies ranged in size (height and width) from 30-45 cm in the relaxed state. All were attached to similar substrates located approximately 15 cm above the ocean floor, with the exception of those colonies on calcareous pedestals (Plate II). Female colonies were identified by visual inspection for eggs (always present) on the interior of a peeled "finger". Male colonies were identified as those of adult size with no eggs. Juvenile (non-reproductive) colonies were recognized by their smaller height and width (≤15 cm), and fewer branches (Hoover *et al*. 2007).

Sample Collection

Underwater fieldwork was conducted with snorkeling or SCUBA diving gear. Samples were collected by snipping off a secondary branch of three to five 2.5-3.0 cm lobes (relaxed length) with scissors from the top-central area of the colony unless otherwise specified. The samples were immediately placed in labeled, pre-cleaned glass jars and capped with Teflon caps while underwater. The jarred samples were transported to the WERI laboratory on ice where they were rinsed *in vitro* with deionized water prior to storage in their original jars at -20°C.

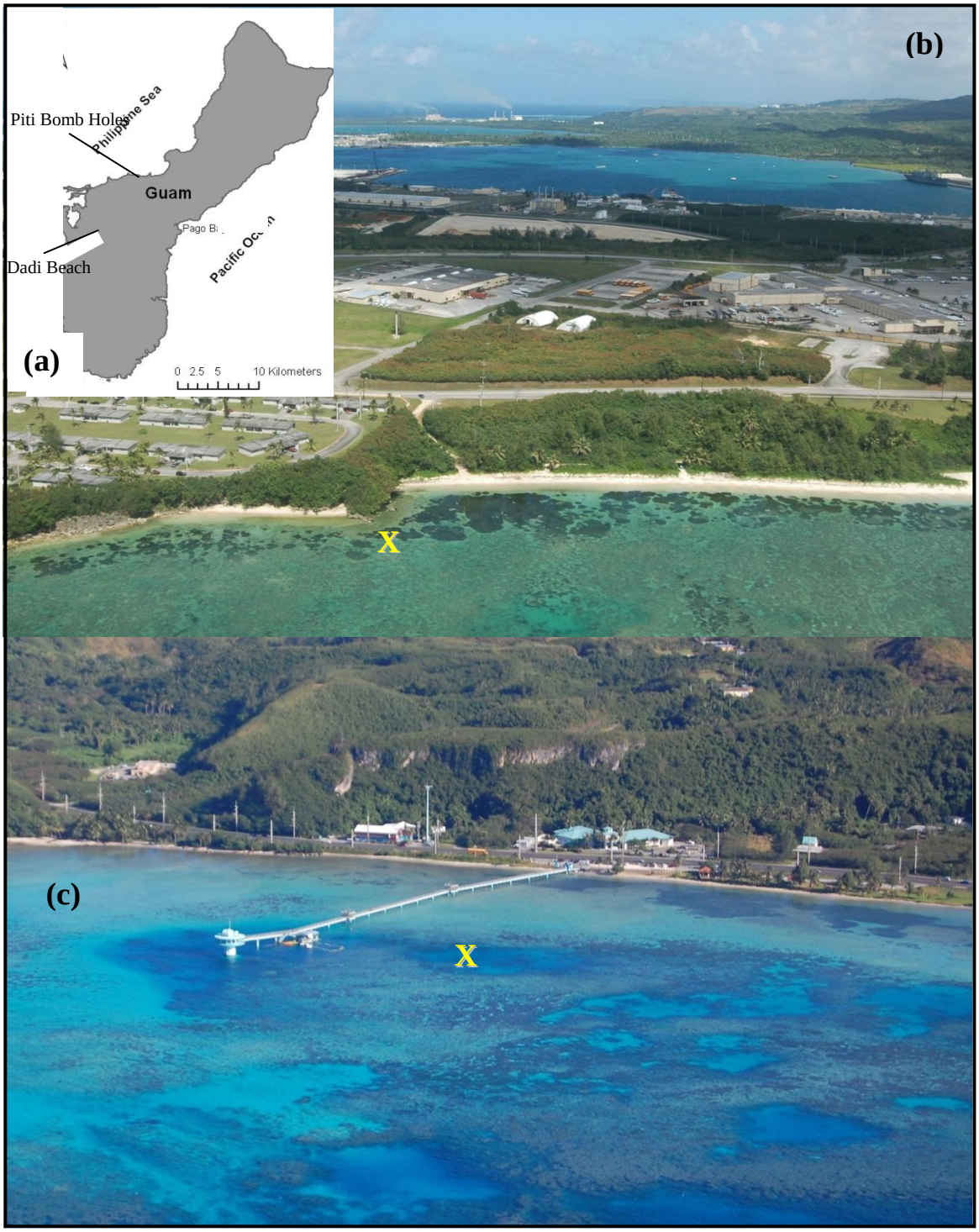

Figure 2: Map of Guam (insert a) showing location of study sites on the central western side of the island. Close-ups: PCB contaminated site, Dadi Beach (insert b) with Apra Harbor and power plants in background; PCB control site, Piti Bomb Holes (insert c), showing underwater observatory. 'X' denotes *S. polydactyla* removal and relocation points at each site during the uptake and depuration studies.

Experimental Design

Despite the recognized potential of *Sinularia* spp. for monitoring PCB contamination in tropical reef waters (Denton *et al*. 1999), fundamental information is lacking on the behavior of PCBs in these organisms in relation to variable parameters like growth, age, gender, reproductive development, spawning, and position in the water column. Likewise no data exists on PCB turnover rates in this group. Such information is vital in order to optimize sampling strategies for future monitoring purposes using *S. polydactyla*. The following experiments were therefore designed to fill in some of these essential data gaps.

PCB Variations Within and Between Colonies

Three female colonies at the Dadi site were sampled on May 10, 2007. Five samples were taken from the top-central region of each colony. Another five samples were taken from the bottom-peripheral areas of each colony.

PCB Variations Associated with Spawning

Ten female *S. polydactyla* colonies were tagged with colored zip ties at the Dadi site. One sample from each colony was taken on May 4, 2007; two days before the commencement of spawning. The same colonies were sampled again on May 10, 2007; three days after spawning.

PCB Variations Associated with Gender and Maturity

Ten female, 10 male, and 10 juvenile colonies were sampled on July 4, 2007. Two samples were taken for PCB analysis from each colony.

PCB Variations in Relation to Position in the Water Column

Two pairs of colonies were sampled on August 26, 2007; one female pair and one male pair. The pairs consisted of one colony located 2.5-5.0 cm above the sandy ocean floor and one colony attached to a calcareous pedestal approximately 30 cm above the ocean floor. Six samples were taken from the top-central regions of each colony. Another six samples were taken from the bottom-peripheral areas of each colony. The colonies were selected such that the top-central regions of the lower colonies corresponded in depth to the bottom-peripheral regions of the elevated colonies.

PCB Uptake and Depuration Kinetics

Six female colonies were selected for study at both sampling sites. The chosen colonies were adequately branched to allow multiple sampling events. They were also attached to substrate that could be easily broken loose with a hammer without damaging the colony. The colonies from Dadi were removed from their origin and transported in large coolers filled with seawater to Piti. Colonies from Piti were transported in the same manner to Dadi. The colonies were arranged equidistantly (4-5 ft apart) in a circle on the ocean floor. Three pieces of rebar were hammered into the ocean floor around each colony to wedge them in place. Zip ties were strapped across the substrata for added security. Each colony was tagged with colored zip ties. Three proximal native colonies at each site were also tagged as control specimens.

One sample was taken from each transplanted colony on days 0, 7, 15, 21, 44 and 90 (September 3-December 2, 2007). One sample was taken from each control colony on days 0, 21, and 90.

SPMD Deployment

Seawater concentrations of PCBs at each site were approximated using 15 cm *Semi Permeable Membrane Devices* (SPMDs) obtained from Environmental Sampling Technologies, Inc (EST). Sixty-four such SPMDs were assembled into 32 aluminum holders (two 2 SPMDs per holder). Twenty-eight SPMDs were deployed at Dadi and 32 at Piti. Two were used as field blanks at each site. The deployed SPMDs were attached by zip-ties to rocks or pipes approximately 15 cm off the ocean floor, evenly spaced (about 1-1.5 m apart), and each within 30 cm of the *S. polydactyla* colonies used during the kinetic studies (Plate III). The SPMDs were deployed on November 14, 2007 and retrieved 15 days later. As soon as the SPMDs were removed from the ocean, they were sealed in their original shipping cylinders, transported to the laboratory at WERI on ice and stored at -80°C. Later they were shipped back to EST on dry ice for dialysate removal and processing. Each SPMD had a mass of 0.885g, a total volume of 0.84 ml and a lipid volume of 0.167 ml.

Analytical Procedures

The analytical procedures described below were adapted from the following protocols: USEPA Methods 1668 Revision A, 3545A, 3620C, 3665A, 8082, and NOAA Technical Memorandum NOS ORCA 130. Appropriate quality control and quality assurance (QA/QC) protocols were adopted throughout to ensure the highest level of accuracy and precision. These protocols included method blanks, spiked blanks, matrix spikes, duplicates, surrogate and internal standards, and a marine mussel standard reference material (SRM 2977). All solvents used were pesticide grade and were checked for interfering contaminants by undergoing a 100-fold volume reduction. All analytical equipment was solvent-rinsed prior to use.

Tissue Preparation

The frozen *S. polydactyla* samples were lyophilized in a freeze dryer for 24 hours, in glass jars, loosely capped with aluminum foil. Once dried, they were ground to a fine powder with pestle and mortar, transferred into pre-cleaned glass vials with Teflon caps, and stored at -20°C.

Tissue Extraction

Samples were extracted by pressurized fluid extraction using a Dionex Accelerated Solvent Extractor 200 (ASE). Twenty-two milliliter extraction cells were prepared by inserting a cellulose filter, adding 5.5g of Florisil, a glass fiber filter and 0.5g of Hydromatrix (diatomaceous earth). This preparation achieved in-cell clean-up of the sample extracts. The Hydromatrix and Florisil used during this process were stored in a 100°C oven and kept in a desiccator during use.

Next, 0.2 ± 0.01 g of lyophilized, ground coral sample was mixed with 0.5g of Hydromatrix and transferred to the prepared extraction cells. Each sample was spiked with 25µl of 2 µl/ml (ppm) 4,4′-dibromooctafluorobiphenyl (DBOFB), a surrogate standard used to correct for PCB recoveries during the extraction process. The cells were topped-off with Hydromatrix and capped. Batches consisted of 11 samples, 1 method blank, and either a duplicate sample, matrix spike, or standard reference material (SRM 2977). The samples were extracted with high purity hexane under the following conditions:

Oven temperature: 125°C
Pressure: 1750 psi
Static time: 5 min (after 5 min pre-heat equilibration)

Flush volume: 70% of the cell volume
Nitrogen purge: 50 sec at 150 psi
Static Cycles: 2

All sample extracts were collected in calibrated collection vials and concentrated to ~0.4ml under a gentle stream of filtered compressed air in a 'Zymark TurboVap.' They were then spiked with 25µl of 2 ppm of the internal standard, pentachloronitrobenzene (PCNB), to correct for recoveries during the analytical stage. The extracts were adjusted to 0.5ml with high purity hexane using a Pasteur pipette and mixed by gently touching to a vortex mixer. Finally, the samples were transferred to 2-ml auto-sampler vials with 250µl glass inserts, capped and stored at 0°C. Analysis for PCBs typically followed within 72 hours.

Sulfuric Acid Clean-Up

Representative samples of *S. polydactyla* from each site were subjected to an additional clean-up procedure using sulfuric acid to remove any residual interfering co-extractants. During this procedure, the sample extract underwent an initial volume reduction to ~5 ml rather than ~0.4 ml. An approximately equal volume of concentrated sulfuric acid was then added to the extract and it was left to stand overnight. The following day the lighter hexane fraction was removed from the digest into a second calibrated collection vial and reduced to a final (PCNB spiked) volume of 0.5 ml by the method described above.

SPMD Dialysate Processing

EST returned the processed SPMD dialysates to WERI in 5-ml amber ampoules for PCB analysis. In preparation for analysis, the ampoules were cracked and the solutions contained therein quantitatively transferred to graduated glass vials with high purity hexane. Volume reduction, internal standard (PCNB) spiking and storage were accomplished in exactly the same way as described above for the tissue extractions.

High Resolution Gas Chromatographic Analysis

PCB analyses were performed with a 'Varian 3800' gas chromatograph equipped with an electron capture detector, a 60m x 0.25mm i.d. fused silica MDN-5S, polymethyl-5% phenylsiloxane (0.25µm film thickness) capillary column, and a Varian 8400 autosampler. Helium was the carrier gas and was set at a constant column flow rate of 1.0 ml/min. Nitrogen was the make-up gas. The injector (type 1177) and detector temperatures were held constant at 280°C and 320°C respectively. During injection, a split ratio of 100:1 was maintained for 1 minute. The column temperature was held at 50°C for the first minute of each run and then ramped up to 150°C at 30°/min. Immediately upon reaching 150°C, a second ramping up to 280°C at 2.5°C/min occurred and was held for 10 minutes. A final ramping up to 315°C at 20°C/min with a holding time of 5 minutes gave a total run time of 73 minutes.

PCB Calibration and Quantification

PCB detection and quantification was accomplished using 'Saturn Workstation' software. The analysis program was calibrated using an 'AccuStandard®' PCB calibration solution consisting of 20 PCBs chosen on the basis of their toxicity or prevalence in the environment. The 20 PCBs are listed in Appendix B along with potentially co-eluting congeners. Calibration curves were established on the basis of 6 concentrations of diluted standard solution: 5, 10, 20, 50, 100 and

200 ng/ml (ppb). Calibration solutions also included the surrogate (DBOFB) and internal (PCNB) standards at 100 ppb throughout. Calibration curves for PCBs 44, 77, and 187 are illustrated in Plate IV. Complete chromatographic separation was achieved for the 22 calibrated components (see Plate V).

Peaks were identified as target analytes if their retention times were offset by no more than ±0.03 minutes compared with the calibration standard. Congener quantification was based on peak area rather than peak height. Method detection limits (MDL) for individual chlorobiphenyls in the standard mix ranged from 0.572-3.687 ng/g on a tissue dry weight basis.

Accuracy determinations based on spiked samples and the standard reference material (SRM 2977) are shown in Table 1 and were considered reasonable. Precision estimates based on duplicate sample analysis averaged 23% of relative difference. The complete analytical data obtained for MDL determinations, accuracy and precision are presented in Appendix C.

Congener concentrations in the tissue extracts were calculated on both a dry weight and lipid weight basis (ng/g) after corrections for surrogate and internal standard recoveries. Lipid weight determination procedures for *S. polydactyla* tissues are described in Appendix D. Total PCB concentrations in each sample were calculated by summing the individual congener data ($\sum_{20}$PCB). All congener values below MDL were entered as zero for these computations.

Table 1: PCB Recovery from SRM 2977, Spiked Blank and Spiked Coral

PCB	Certified Value (ng/g dry wt.)	Study Mean ± 95% C.L. (ng/g dry wt.)	Spike Added (ng)	Spiked Blank Recovery (ng)	Spiked Coral Recovery (ng)
8	2.1 ± 0.15	-	100	103.99	114.74
18	2.65 ± 0.30	11.22 ± 1.69	100	108.56	103.36
28	5.37 ± 0.44	9.22 ± 6.28	100	109.73	103.68
52	8.37 ± 0.54	2.53 ± 1.56	100	88.9	78.11
44	3.25 ± 0.63	3.76 ± 0.76	100	105.49	84.06
66	3.64 ± 0.32	13.96 ± 4.73	100	111.88	79.22
101	11.2 ± 1.2	13.85 ± 3.69	100	108.95	94.21
77	none	3.55 ± 1.27	100	110.81	77.32
118	10.5 ± 1.0	10.85 ± 1.62	100	116.37	94.94
153	14.1 ± 1.0	12.38 ± 1.56	100	116.98	81.45
105	3.76 ± 0.49	3.01 ± 0.41	100	120.53	86.78
138	16.6 ± 1.6	6.08 ± 0.73	100	92.68	62.25
126	none	0.59 ± 0.37	100	117.73	86.73
187	4.76 ± 0.38	5.04 ± 1.05	100	115.95	72.07
128	2.49 ± 0.28	1.79 ± 0.18	100	121.13	106.94
180	6.79 ± 0.67	5.09 ± 0.86	100	112.53	69.42
170	2.95 ± 0.23	2.04 ± 0.38	100	114.54	86.21
195	none	-	100	105.88	76.36
206	none	-	100	102.64	59.57
209	none	-	100	-	69.78

Note: dashes indicate values below detection limits

Results

The experimental findings presented herein are based on analytical and statistical summaries for total PCBs (Σ_{20}PCBs), the nonplanar tetrachlorobiphenyl, PCB 44, the highly toxic coplanar tetrachlorobiphenyl, PCB 77, and the nonplanar heptachlorobiphenyl, PCB 187. All three of these congeners showed minimal interference with co-extractants (Appendix E) and occurred at sufficiently high levels at the contaminated site to warrant detailed examination. They also provided broad representation with respect to chlorine content, structural arrangement and potential toxicity of the group as a whole.

Evaluation of Intrinsic and Extrinsic Variables

A suite of experiments was conducted to determine the influence of intrinsic and extrinsic variables on PCB concentrations in *Sinularia polydactyla.* The complete analytical data sets for all detectable congeners in all experiments are tabulated in Appendix F.

PCB Variations Within and Between Colonies

The results of this investigation are graphically summarized in Figs. 3-5. The data bar and whiskers for each category are representative of the geometric mean (n=5) and 95% confidence limits respectively. Differences between groups were determined by Two-Way ANOVA and the Holm-Sidak Multiple Comparison Procedure at a significance level of 0.05. The statistical significance of each comparison is summarized in Table 3.

Table 2: Statistical Significance of Within and Between Colony Variations

Test	Dry/Lipid Wt. Basis	PCB 44	PCB 77	PCB 187	Total	Lipid %
Between Colonies	Dry	no	no	yes	no	n/a
	Lipid	no	no	yes	no	no
Within Colonies	Dry	yes	yes	no	yes	n/a
	Lipid	yes	yes	yes	yes	yes

Note: no/yes answers whether a statistically significant difference exists; n/a = not applicable

The results generally indicate that PCB concentrations and lipid weights vary significantly between tissues collected from the upper and lower portions of the same female colony, but not from the same portion of different female colonies from the same location. These findings held regardless of whether the PCB concentrations were expressed on a dry weight or lipid weight basis, although in the former instance the upper colony portions generally contained the higher levels of PCBs (Fig. 3) while the opposite occurred in the latter (Fig. 4). The PCB distribution discrepancy within colonies thus appears to be inversely related to the lipid content (Fig. 5), which is most unusual.

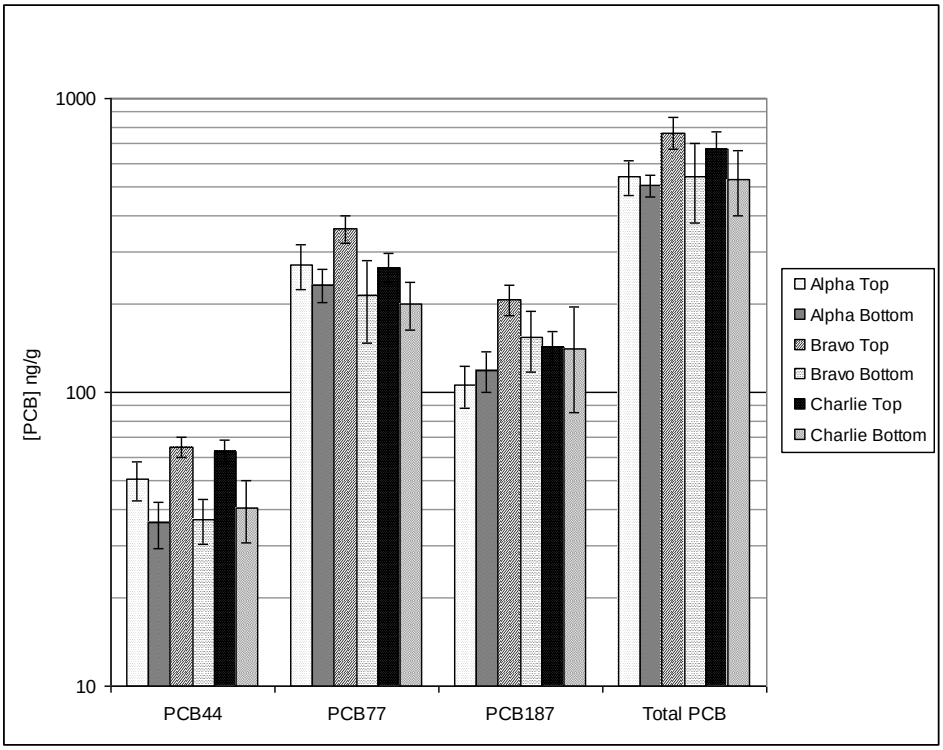

Figure 3: PCB variation within and between colonies (dry wt.).

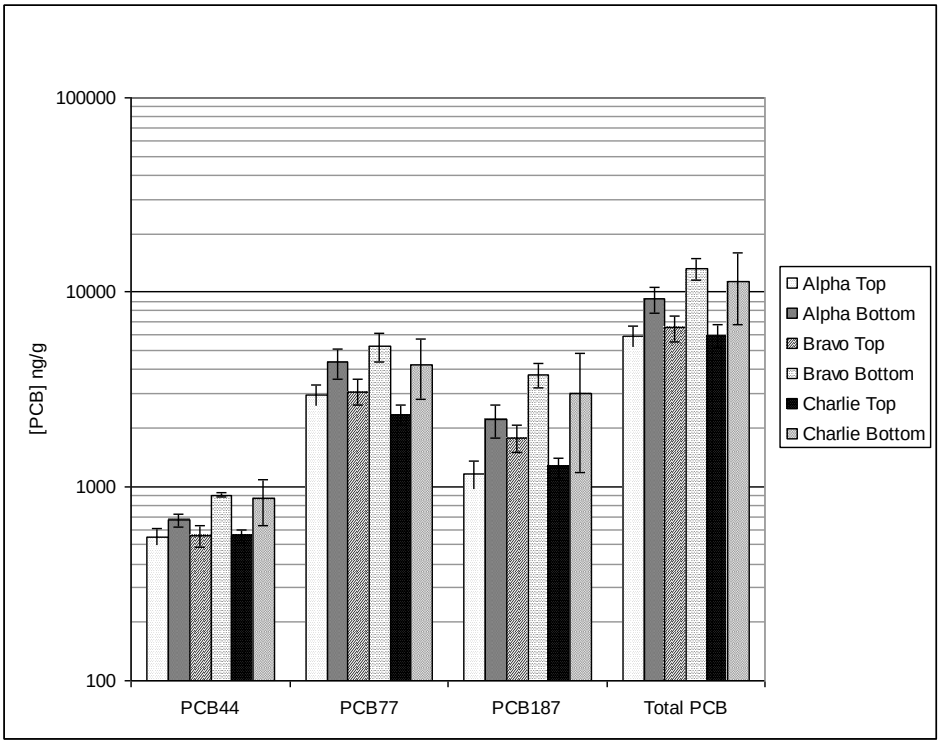

Figure 4: PCB variation within and between colonies (lipid wt.).

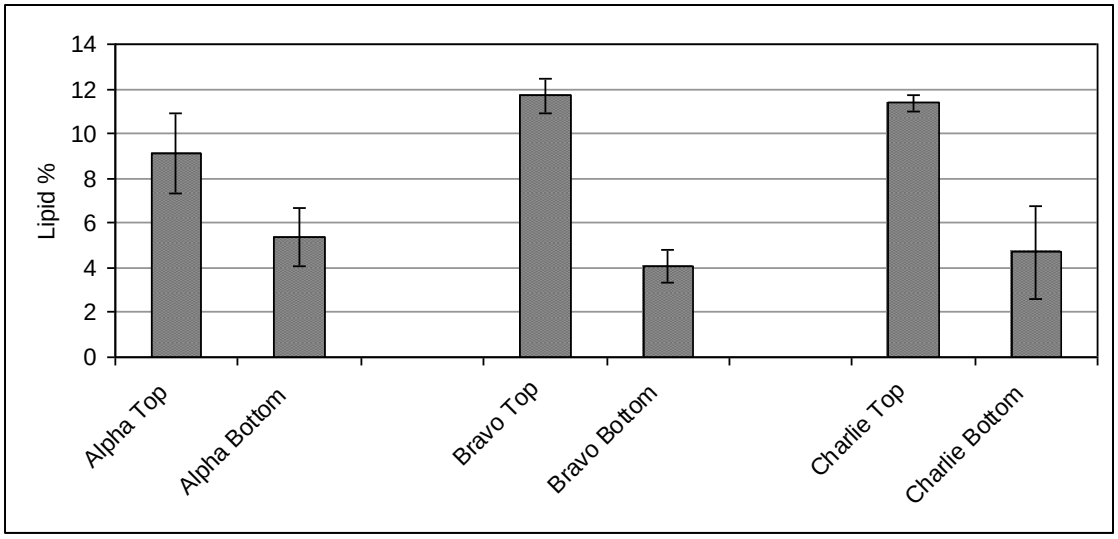

Figure 5: Lipid content variation within and between colonies.

PCB Variations Associated with Spawning

The findings of this part of the study are summarized in Fig. 6. PCB differences between the pre-spawn and post-spawned data sets (n=10 for each) were examined by paired t-test and only found to be significant ($P<0.05$) when calculated on a lipid weight basis. Under these circumstances, all PCB congeners (except PCB 187) and Σ_{20}PCBs were significantly higher in spent colonies despite lipid levels being significantly lower in this state.

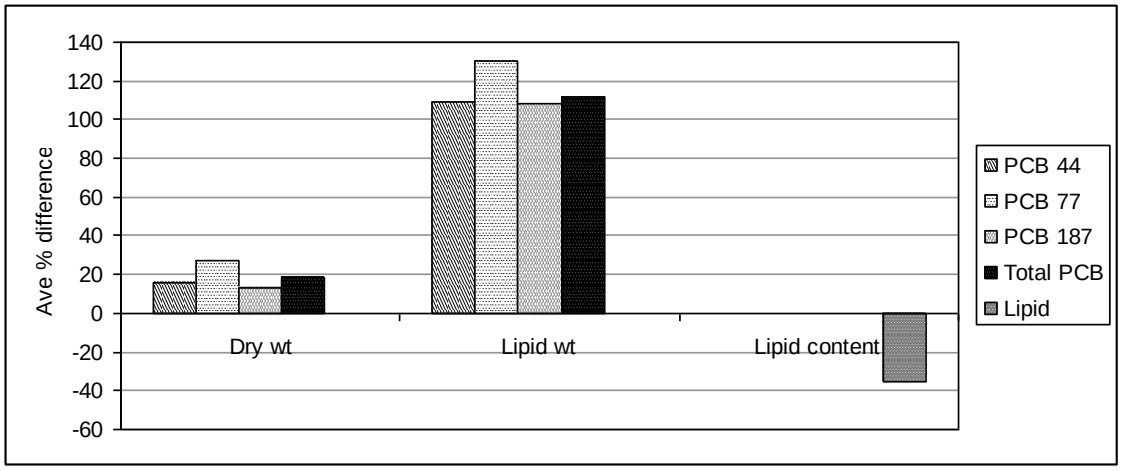

Figure 6: PCB variations associated with spawning relative to lipid content.

PCB Variations Associated with Gender and Maturity

The findings of this part of the study are presented in Figs. 7-9. Each bar graph shows the geometric mean (n=20) and 95% confidence limits for each category. Differences between groups were determined by One-Way ANOVA and the Holm-Sidak Multiple Comparison

Procedure at a significance level of 0.05. The statistical significance of each comparison is summarized in Table 3 and indicates that gender did not influence PCB concentrations on a dry weight or lipid weight basis (P>0.05). On the other hand, age/size was a significant factor (P<0.05) in dry weight calculations (with the exception of PCB 77) with adult/large colonies having higher dry weight based PCB levels than juvenile/small colonies.

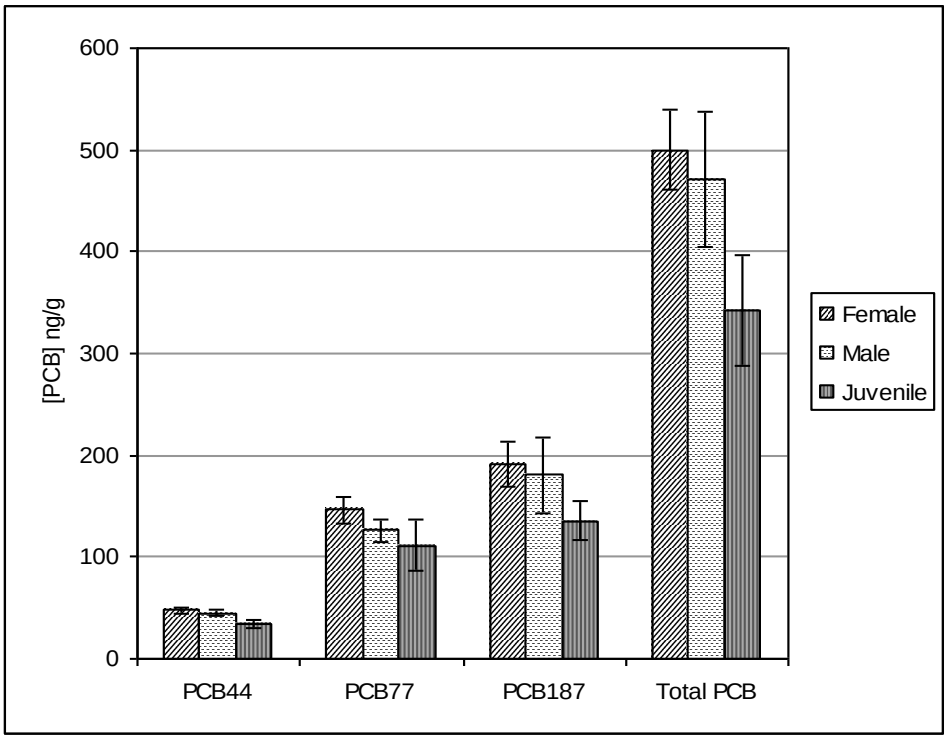

Figure 7: Influence of gender and maturity on PCB concentrations (dry wt.).

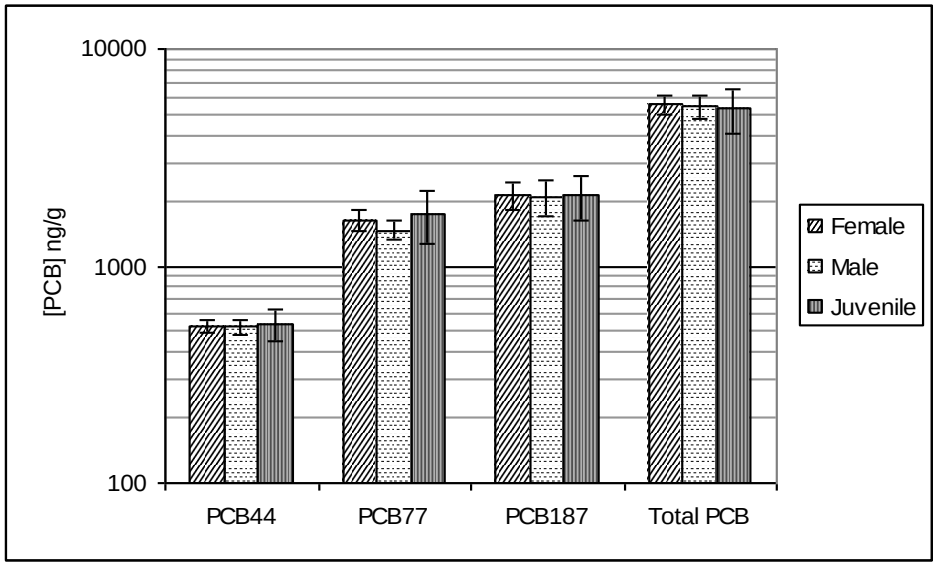

Figure 8: Influence of gender and maturity on PCB concentrations (lipid wt.).

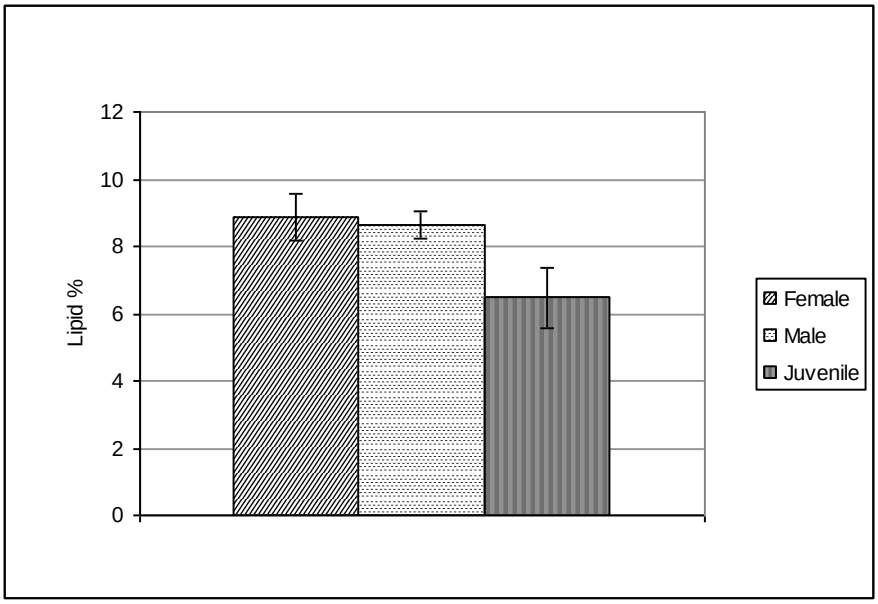

Figure 9. Influence of gender and maturity on lipid content.

Table 3: Statistical Significance of Gender and Maturity

Test	Dry/Lipid Wt. Basis	PCB 44	PCB 77	PCB 187	Total	Lipid %
Gender	Dry	no	no	no	no	n/a
	Lipid	no	no	no	no	no
Age/Size	Dry	yes	no	yes	yes	n/a
	Lipid	no	no	no	no	yes

Note: no/yes answers whether a statistically significant difference exists; n/a = not applicable

PCB Variations in Relation to Position in the Water Column

The results of this part of the study are presented graphically in Figs. 10-12. Each chart bar represents the geometric mean of six samples. Water column position and within colony differences in PCB levels were examined by Two-Way ANOVA and proved to be insignificant ($P>0.05$). The effect of gender on PCB levels was analyzed with One-Way ANOVA and the Holm-Sidak Multiple Comparison Procedure and found to be significant on both a dry and lipid weight basis. Lipid levels were found to be significantly ($P<0.05$) higher in female rather than male colonies by Two-Way ANOVA and also tended to be higher in upper rather than lower colony samples of both sexes. The statistical significance of each comparison is conveniently summarized in Table 4.

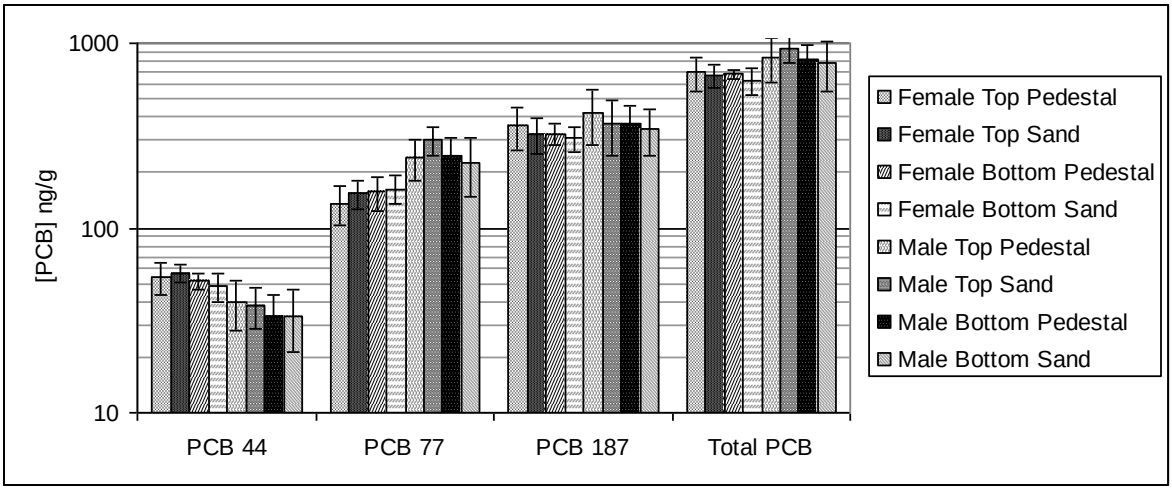

Figure 10: Influence of water column position, gender and within and between colony variation on PCB concentrations (dry wt.).

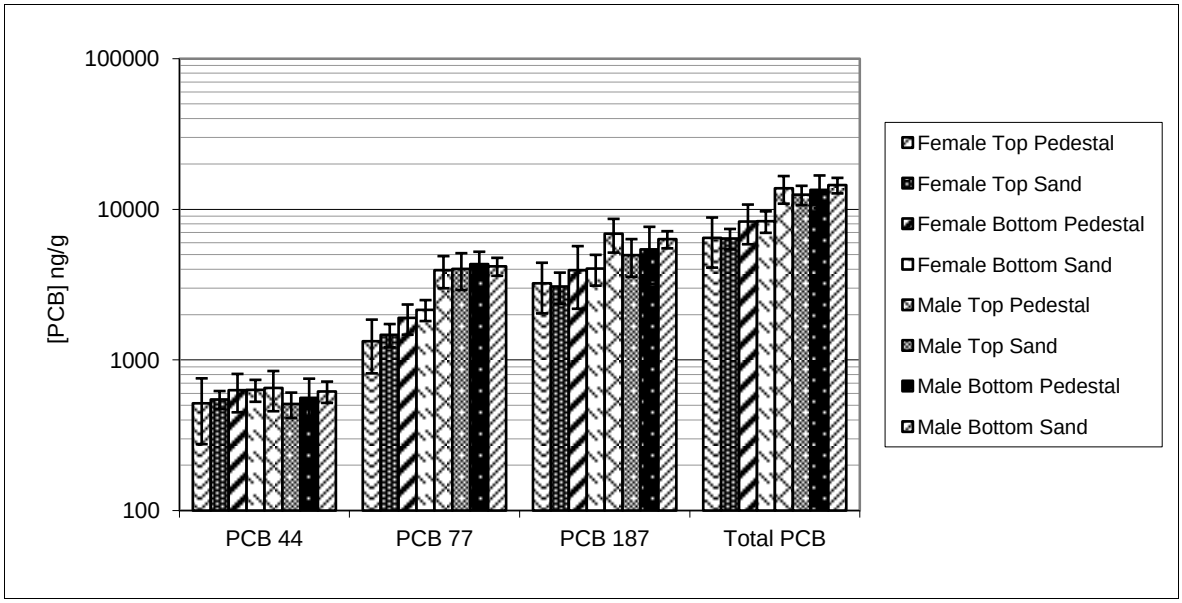

Figure 11: Influence of water column position, gender and within and between colony variation on PCB concentrations (lipid wt.).

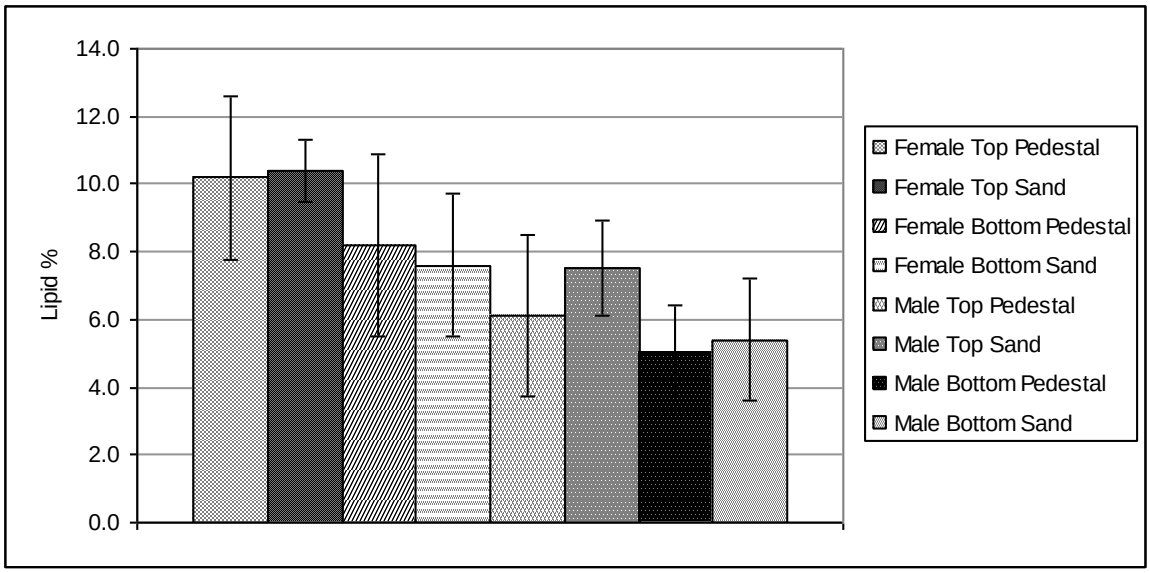

Figure 12: Influence of water column position, gender and within and between colony variations on lipid content.

Table 4: Statistical Significance due to Water Column Position, Gender and Within and Between Colony Variations

Test	Treatment	Dry or Lipid Wt. Basis	PCB 44	PCB 77	PCB 187	Total	Lipid %
Water Column	Pedestal vs. Sand (Female)	Dry	no	no	no	no	n/a
		Lipid	no	no	no	no	no
	Pedestal vs. Sand (Male)	Dry	no	no	no	no	n/a
		Lipid	no	no	no	no	no
Within Colonies	Top vs. Bottom (Female)	Dry	no	no	no	no	n/a
		Lipid	no	yes	no	yes	no
	Top vs. Bottom (Male)	Dry	no	no	no	no	n/a
		Lipid	no	no	no	no	no
Between Colonies	Female v. Male	Dry	yes	yes	no	yes	n/a
		Lipid	no	yes	yes	yes	yes

Note: no/yes answers whether a statistically significant difference exists; n/a = not applicable

Kinetics Studies

Kinetics determinations were conducted to examine the relationship between PCB concentrations in the ambient water and PCB concentrations in *S. polydactyla* tissue. Such information provides a better understanding of how to appropriately use a biomonitor and how to interpret the information it provides.

PCB Uptake and Depuration

Graphical representations of the PCB uptake and depuration data from the *S. polydactyla* transplant studies are shown in Fig. 13 and Fig. 14 for dry weight and lipid based calculations respectively. Plots are geometric means (n=6) with 95% confidence limits. Values obtained from resident specimens (controls) taken over the same time frame from each experimental site are also shown. A complete compendium of the raw data sets for all congeners analyzed is shown in Appendix G. Lipid content estimates of *S. polydactyla* during the uptake and depuration experiments are shown in Fig. 15.

PCB uptake and loss in *S. polydactyla* was assumed to follow simple first-order kinetics with the organism behaving as a single compartment (Neely 1979). This particular model requires that rates of uptake and loss are proportional to PCB levels in the surrounding water column and in the corals respectively, and in its simplest form may be written as:

Rate of PCB accumulation = Rate of PCB uptake - Rate of PCB loss

The rate of change in PCB levels in *S. polydactyla* can, therefore, be expressed as:

$$dC/dt = k_u C_w - k_d C \qquad (1)$$

where: C = PCB concentrations in *S. polydactyla* (ppb = ng/g)
C_w = PCB concentrations in the water (ppb = ng/ml)
k_u = is the kinetic rate constant for PCB uptake ($time^{-1}$)
k_d = is the kinetic rate constant for PCB loss ($time^{-1}$)
t = time

The integrated form of Equation 1 is:

$$C = (k_u/k_d)C_w[1-e^{-kdt}]+C_{(start)}e^{-kdt} \qquad (2)$$

Where: $C_{(start)}$ = the PCB concentration at the start of that particular time interval.

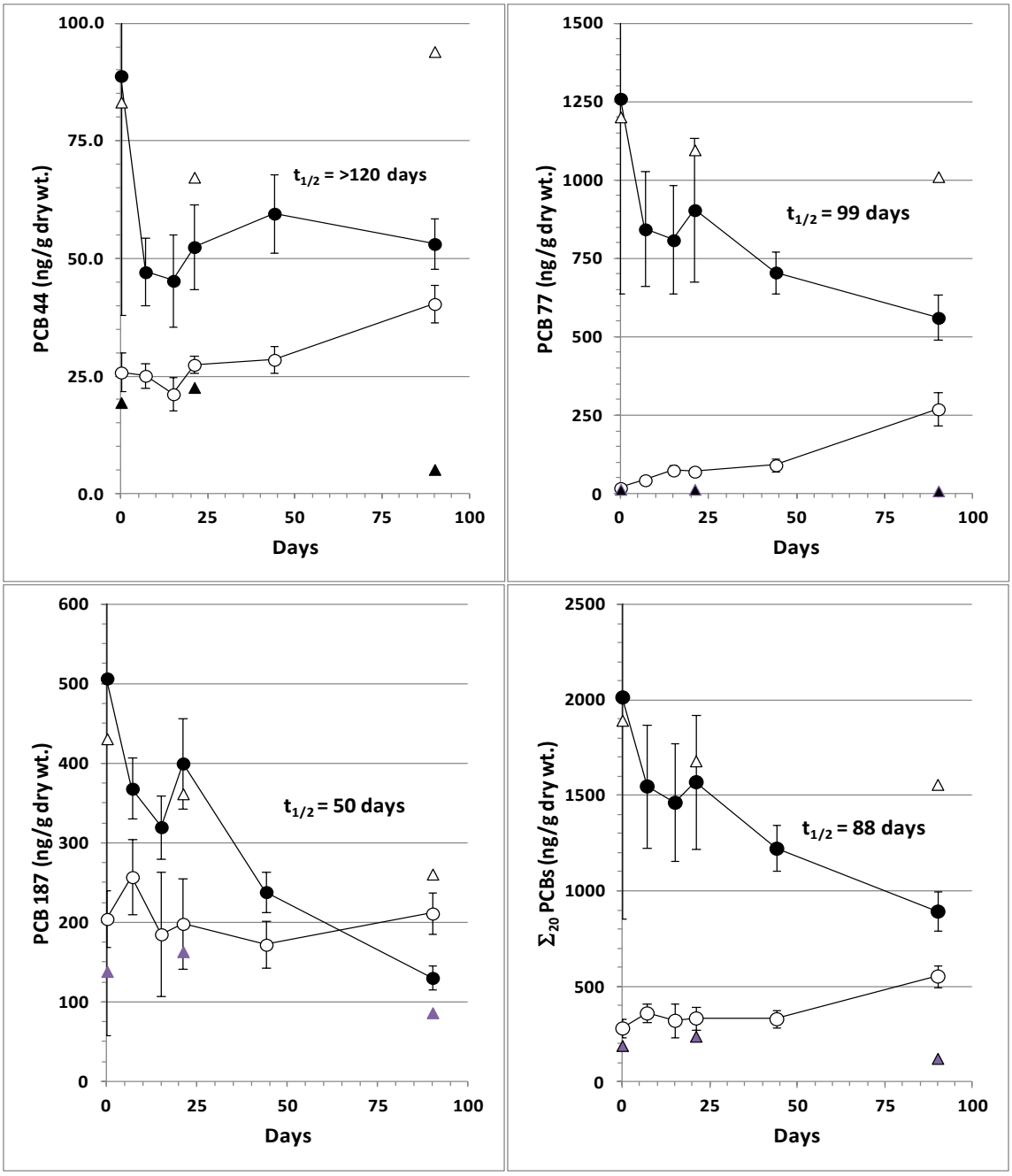

Figure 13: Dry weight based PCB levels in *S. polydactyla* colonies transplanted between Piti Bomb Holes (relatively clean) and Dadi Beach Reef (relatively contaminated). Open circles [○] = Piti Bomb Holes colonies transplanted to Dadi Beach Reef; closed circles [●] = Dadi Beach Reef colonies transplanted to Piti Bomb Holes; open triangles [△] = Dadi Beach Reef resident colonies; filled triangles [▲] = Piti Bomb Holes resident colonies. Plots are geometric means; error bars are 95% confidence intervals (shown for transplants only). PCB half-lives ($t_{1/2}$) are calculated based on a single compartment, first-order kinetics, bioconcentration model (see text for further details).

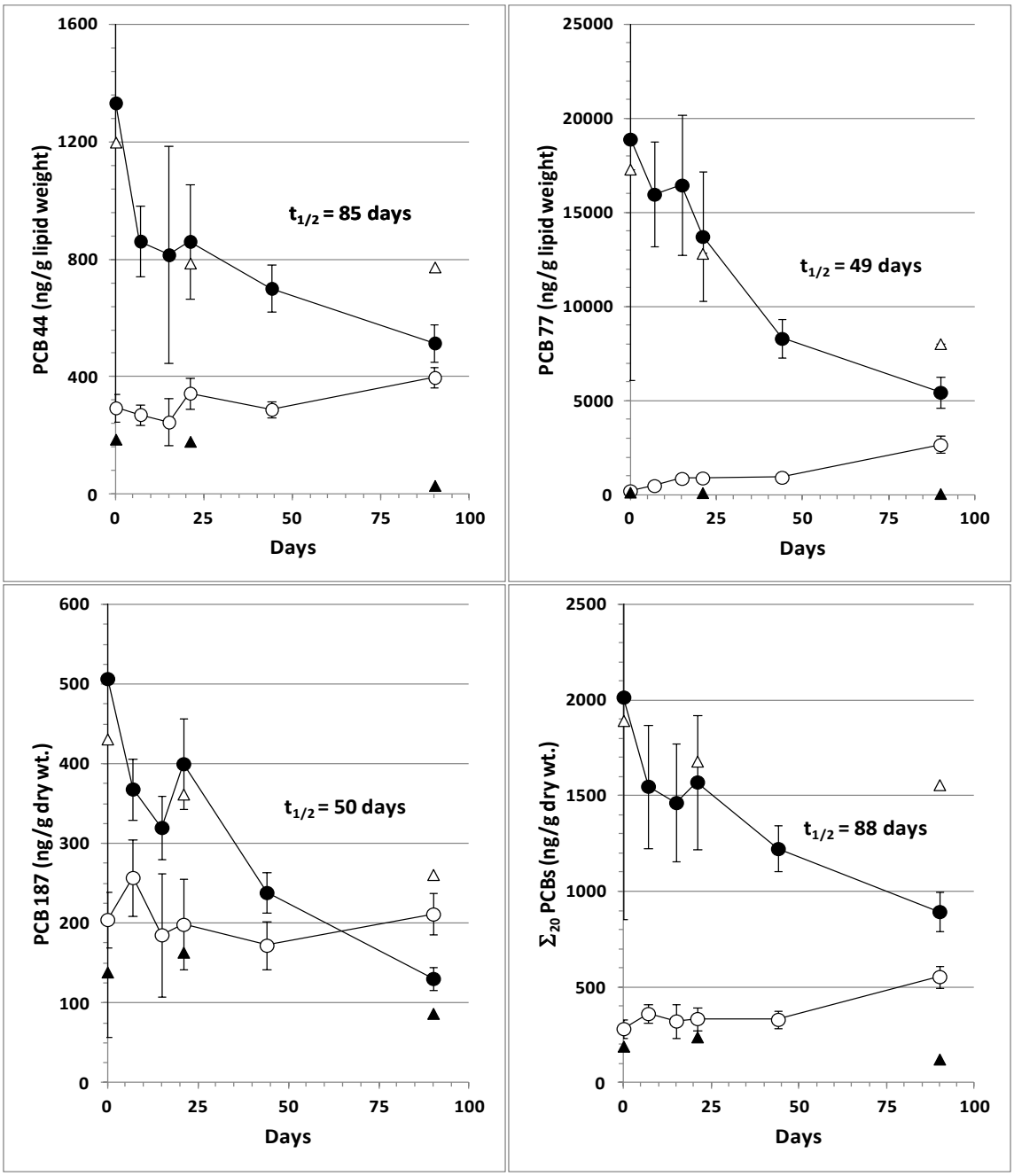

Figure 14: Lipid weight based PCB levels in *S. polydactyla* colonies transplanted between Piti Bomb Holes (relatively clean) and Dadi Beach Reef (relatively contaminated). Open circles [○] = Piti Bomb Holes colonies transplanted to Dadi Beach Reef; closed circles [●] = Dadi Beach Reef colonies transplanted to Piti Bomb Holes; open triangles [△] = Dadi Beach Reef resident colonies; filled triangles [▲] = Piti Bomb Holes resident colonies. All plots are geometric means; error bars are 95% confidence intervals (shown for transplants only). PCB half-lives ($t_{1/2}$) were calculated based on a single compartment, first-order kinetics, bioconcentration model (see text for further details).

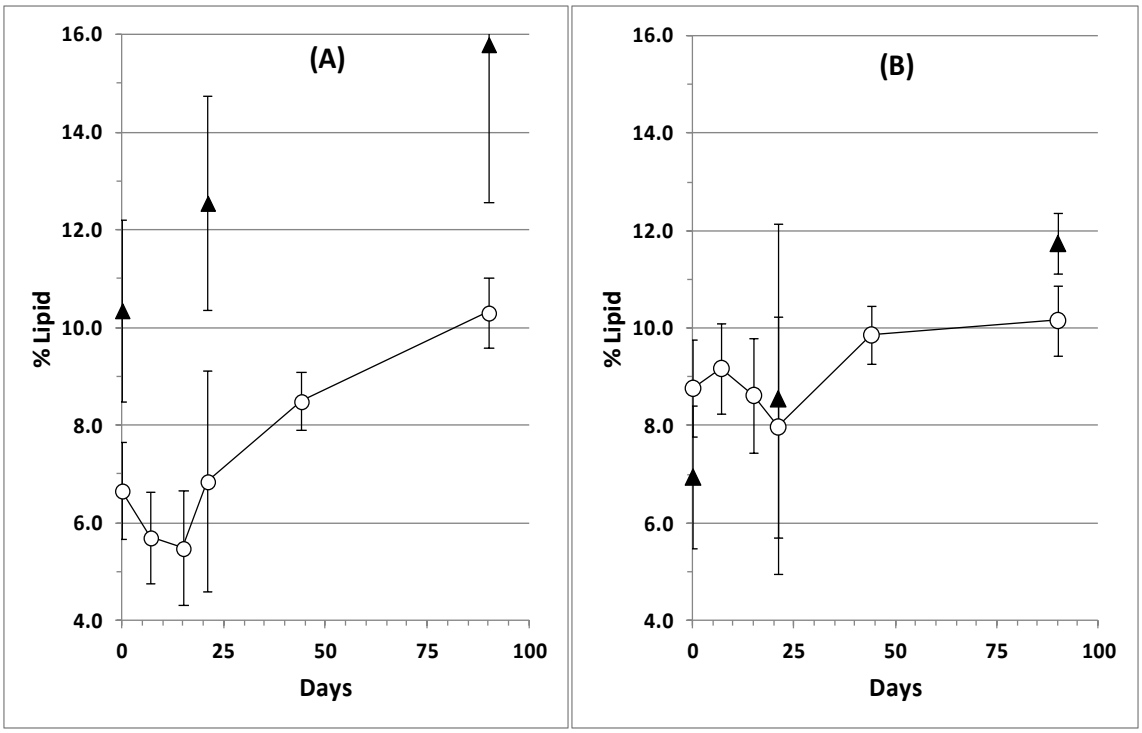

Figure 15: Changes in lipid content of *S. polydactyla* colonies transplanted (open circles [○]) between Piti Bomb Holes (relatively clean) and Dadi Beach Reef (relatively contaminated) over the 90 day PCB uptake (Graph A) and depuration (Graph B) period. The lipid content of resident colonies at each site over the same time frame is also shown (filled triangles [▲]). All plots are geometric means; error bars are 95% confidence intervals.

During the present study, loss rate constants (k_d) of interest were equivalent to the slopes of regression lines fitted to the depuration data plotted against time on natural log-linear graph paper (Spacie and Hamelink 1982). The regression equations, R squared values (r^2) and linear correlation coefficients (r) obtained for each congener by this means are summarized in Table 5 and are generally supportive of a first-order process.

Once values for k_d were determined the biological half-life ($t_{1/2}$) of each congener was calculated from Equation 3 as follows:

$$t_{1/2} = \ln 2/k_d = 0.693/k_d \qquad (3)$$

Equation 4 estimates values of k_u by rearrangement of Equation 1 above as follows:

$$k_u = k_d(C_{(end)} - C_{(start)}[e^{-kdt}])/C_w(1-e^{-kdt}) \qquad (4)$$

where $C_{(end)}$ = the PCB concentration at the end of that uptake time interval
C_w = the SPMD derived seawater PCB concentrations (see below)

Kinetic rate constants and congener half-lives are displayed in Table 6: The overall uptake rate constant shown is the average of the uptake rates constants calculated for all sampling time intervals.

Table 5: Regression Statistics of Natural Log Transformed Depuration Data vs. Time

PCB	Dry Wt.			Lipid Wt.		
	Equation	r^2	***r***	***Equation***	r^2	***r***
44	-0.0014x + 4.063	0.037	0.193	-0.0082x +6.939	0.808	0.899
77	-0.0070x + 6.912	0.768	0.876	-0.0142x + 9.813	0.962	0.981
187	-0.0138x + 6.109	0.94	0.969	-0.0203x + 8.942	0.985	0.985

Table 6: Kinetic Rate Constants and Congener Half-Lives on Dry and Lipid Weight Basis

PCB	Dry Wt.			Lipid Wt.		
	k_u	k_d	$t_{1/2}$	k_u	k_d	$t_{1/2}$
44	57852	0.0014	495	1477623	0.0082	85
77	24097	0.0070	99	323914	0.0142	49
187	38056	0.0138	50	673215	0.0203	34

SPMD Ambient Water Concentration Determinations

Ambient water PCB concentrations were calculated per directions of Environmental Sampling Technologies, the SPMD manufacturer, utilizing the USGS Excel program titled "SPMD Water Concentration Estimator" version 4.1[1]. The program assumes an ambient water temperature of 10°C to calculate PCB concentrations. The actual ambient water temperatures at the experimental sites were near 26°C, therefore, the C_w values calculated herein are slightly overestimated. Performance reference compounds (PRCs) were not used, thus, the effects of flow were not accounted for in the present research.

PCB concentrations in SPMDs and derived seawater PCB concentrations are listed in Table 7 for the congeners of interest and Σ_{20}PCBs. The complete SPMD analytical data sets for all PCBs examined during this study are listed in Appendix H.

[1] available at: www.cerc.usgs.gov/Branches.aspx?BranchId=8

Table 7: PCBs Accumulated in Semi-Permeable Membrane Devices and Derived C_w

PCB	SPMD Mean (ng/SPMD)		C_w (pg/L) Mean and (Range)	
	Piti	Dadi	Piti	Dadi
8	8.5	13.1	162.8 (113.6-251.1)	251.3 (95.5-458.1)
18	7.7	60	115.8 (69.9-169.2)	895.9 (502.5-1131.5)
28	2.8	-	14.0 (n/d-14.0)	n/c
52	-	0.5	n/c	4.9 (n/d-4.9)
44	0.5	0.8	1.8 (1.7-1.9)	3.2 (0.8-5.2)
66	-	-	n/c	n/c
101	-	-	n/c	n/c
77	0.1	5.3	1.4 (0.5-2.1)	122.2 (29.1-194.3)
118	0.1	-	1.7 (0.1-5.5)	n/c
153	0.2	0.1	4.1 (2.4-6.6)	2.4 (1.8-3.4)
105	0.03	-	0.5 (n/d-0.5)	n/c
138	19.2	0.9	266.5 (n/d-266.5)	12.9 (0.5-19.5)
126	4.7	1.8	141.8 (130.5-153.0)	54.5 (12.4-76.0)
187	0.3	4	5.8 (2.9-8.5)	75.4 (24.7-133.4)
128	0.1	-	1.9 (n/d-1.9)	n/c
180	3.7	0.02	94.1 (n/d-94.1)	0.5 (n/d-0.5)
170	-	-	n/c	n/c
195	-	-	n/c	n/c
206	0.1	0.1	4.9 (3.5-7.1)	5.5 (3.9-7.6)
209	-	0.1	n/c	*

* = C_w calculation not available; n/c = not calculable; n/d = not detected; dashes indicate values below detection limits

Discussion

The following discussion explains the question that was set forth by each investigation, then explores interpretations of the results. Patterns, relationships, and correlations are elucidated. Exceptions and unsettled questions are pointed out. Theoretical implications are stated where appropriate.

PCB Verification with Sulfuric Acid

The clean-up method employed in this research does not remove certain pesticides which may coelute and interfere with the desired analytes. Concentrated sulfuric acid digests pesticides, yet leaves PCBs intact. Thus, H_2SO_4 cleanup verified PCB contribution of the quantitative GC result. Three congeners, 44, 77, and 187, were selected as the foci of the statistical analyses throughout this research based on the evidence that these were in fact PCBs and occurred at sufficiently high levels at the contaminated site. It should be noted here that $\sum_{20}$PCB data reflects concentrations of PCBs and possibly portions of coeluting, non-PCB compounds like pesticides,

as well as co-eluting PCBs. Therefore, $\sum_{20}$PCB data is included for consideration, but will not be given much weight in drawing conclusions.

The three bona fide PCBs available for this study make a good representation of the PCB chemical group. PCB 44 and 77 are both tetrachlorobiphenyls; nonplanar and coplanar respectively. PCB 187 is a heptachlorobiphenyl. The diversity of the three PCBs helps make this first study of soft coral kinetics more fruitful. However, further research is warranted to determine if uptake and depuration rates for other significant PCBs show similar patterns in *S. polydactyla*.

Evaluation of Intrinsic and Extrinsic Variables

PCB Variations Within and Between Colonies

The reliability and efficiency of a biomonitor is in large part determined by the variability of analyte concentrations in the organisms. This is an inverse relationship where low variability makes a biomonitor more reliable and more efficient (Phillips and Rainbow 1998). With low variability, changes in analyte concentrations can be detected more readily and with fewer samples. A biomonitor may still be valuable if variability exists but follows fixed, predictable patterns. Appropriate sampling procedures and schedules can maneuver within the constraints of recognizable patterns to eliminate variables in monitoring data. Seemingly random variability between and within organisms cannot be controlled and produces nonsensical and misleading data. Thus, the first step in evaluating a biomonitor is to determine the typical breadth of variability in analyte concentrations within and between organisms.

The comparison of PCB concentrations in *S. polydactyla* colonies revealed that there was little variation between colonies. One case of significant difference in PCB 187 occurred among the sampled colonies. However, it was noted that colony "A" was smaller than colonies "B" and "C" which may have influenced the result (factors of age/size are addressed later). Overall, the results of this study indicate that the differences between one colony and another are sufficiently low to produce viable monitoring data.

Significant patterns arose in the comparison of PCB concentrations within *S. polydactyla* colonies. The two groups in the comparison were the "Tops", samples taken from the top central areas of the colonies, and the "Bottoms", samples taken from the lower, peripheral areas of the colonies. Calculated on a dry weight basis, "Tops" had higher PCB concentrations (differences for PCB 44 and PCB 77 were significant; differences for PCB 187 were non-significant but might have been with a larger sample size). These differences were not surprising as tissue PCB concentrations calculated on dry weight are known to be misrepresentative due to the lipophilicity of PCBs. It is well established that variations in PCB concentrations are often normalized by adjusting the data relative to the lipid content of the tissue (Phillips and Rainbow 1998). Lipid analysis showed that "Tops" had higher lipid levels than "Bottoms". Thus, it was expected that the differences seen on a dry weight basis would be eliminated when the data was calculated on a lipid weight basis.

Analysis of the data calculated on lipid weight proved to be surprising and emphasized the importance of carrying out an evaluation of a biomonitor such as the present study. On a lipid-

weight basis, the "Bottoms" samples had higher PCB concentrations than the "Tops" (significantly higher in all cases). It is worth noting once more that the "Tops" contained greater proportions of lipid than the "Bottoms". Clearly, there is a factor effecting the distribution of PCBs and lipids in *S. polydactyla*. This is the type of factor that must be identified and controlled in a monitoring protocol.

The factor producing the confounding data may be related to anatomical or chemical differences between the "Tops" and "Bottoms". The samples for this comparison were collected in the middle of spawning season when lipid content is fluxing. Seasonal changes are addressed in the next section. Alternatively, the outcome might be a factor of chemical saturation. Soft corals depend on chemical defenses for protection. The secondary metabolites that provide protection in *S. polydactyla* are both polar and non-polar. They are most highly concentrated in the tips and new growth of the coral (Van Alstyne *et al*. 1994). The high concentrations of chemicals in the "Tops" might have a halting effect on the passive transport of PCBs into the tissue. The lower levels of secondary metabolites in lower and older portions of the corals might be a reason for the lipids in those areas to be more available to PCBs.

PCB Variations Associated with Spawning

Seasonal variation is an important factor to examine, particularly in organisms that spawn regularly like soft corals. It is crucial to understand when a monitoring species undergoes spawning, and what changes occur at that time. Otherwise, monitoring data may falsely indicate a rise or fall in ambient PCB concentrations. The results might be in fact reflecting changes in the anatomical composition of the organism or events in the reproductive process. Spawning involves a rapid build-up of lipid, followed by off-loading of lipid with the release of gametes. Previous to this research, there was no knowledge of how this lipid flux influences the accumulation of PCBs in soft corals.

The comparison of pre-spawn and post-spawn samples in this study agrees with the notion that seasonal reproduction alters analytical data. Again the results were non-intuitive. There were no significant differences in PCB concentrations between female colonies pre-spawn and post-spawn when calculated on a dry weight basis. On the other hand, data calculated on a lipid weight basis showed differences pre-spawn and post-spawn. Surprisingly, the post-spawn PCB concentrations were higher (PCB 44 and 77 significantly; 187 non-significantly) despite lipid levels being lower. . Based on other research, one would expect steady proportions of PCBs and lipids. Evidently, there are unique events occurring during soft coral spawning that disturb PCB-lipid equilibrium.

The soft coral spawning season involves a rapid gain in lipid content, particularly in the secondary and tertiary lobes. It appears from "Within and Between" comparisons in this study that the new lipid is either shielded physically or chemically from PCB accumulation, or it is simply gained and off-loaded faster than PCBs are equilibrated. In the latter case, PCB and lipid levels are out of equilibrium due to the rapid change in lipid content rather than ambient PCB concentrations. Either way, during the spawning process, PCB-lipid proportions seem meaningless.

The insignificant changes in PCB dry weight concentrations pre- and post-spawn indicate that PCBs are not off-loaded during the lipid off-loading process. Much of the lipid flux might be associated with the eggs that are released during spawning. This provides evidence that *S. polydactyla* is not purged of PCBs by releasing gametes. The absence of PCBs in the eggs could be due to the fast rate of lipid gain followed by the expulsion of the eggs which would preclude equilibrium with PCBs. The eggs are also known to have high levels of defensive metabolites, which might prevent the passive transport of PCBs into the eggs (Slattery *et al*. 1998).

PCB Variations Associated with Gender and Maturity

Gender did not have a significant effect on data calculated on either a dry or lipid weight basis. It is important to note that sampling for this comparison was conducted in July, one month after the final spawning of the year. As with other organisms, age/size was a significant factor in dry weight calculations (with the exception of PCB 77). Adult/large colonies had higher dry weight based PCB levels than juvenile/small colonies. In this case, calculating the data on a lipid weight basis had the typical effect of eliminating differences between the two age/size groups. Accordingly, lipid content was significantly different, with adult/large colonies having more. This set of results is more typical relative to generalizations made from other biomonitor evaluations. The regularity of the lipid-normalized data across age and gender supports the hypothesis that outside of the spawning season, *S. polydactyla* reflects ambient PCB levels.

PCB Variations in Relation to Position in the Water Column

The stimulus to examine the effect of water column position was not only a recommendation of the literature, but also to settle the question of whether the differences observed within colonies could be accounted for by this factor. *Sinularia polydactyla* is a reef-building soft coral (Schumacher 1997; Fabricious and Alderslade 2001). The calcareous structure built by the coral in effect becomes a pedestal upon which the living coral resides. Consequently, the coral with a pedestal is elevated in the water column above corals residing on the ocean floor. This phenomenon provided an opportunity to examine whether the differences observed between "Tops" and "Bottoms" (discussed in "Within/Between Variation" section) were symptomatic of differences in PCB levels in the water column or differences within the organism itself.

The paired colonies were selected such that the "Tops" of the lower "Sand" colony were at the same level in the water column as the "Bottoms" of the elevated "Pedestal" colony. In essence, any variation between these groups would be caused by a factor other than stratified PCB distribution in the water column. Furthermore, a lack of variation between the "Bottoms" of the lower colony and the "Bottoms" of the elevated colony would nullify the theory that "Bottoms" were accumulating PCBs from the sediments on which they rested. Statistical analysis revealed that the factor of water column position was not significant in any case.

Interestingly, within colony differences were not as pronounced as in the previous examination (discussed in the "Within and Between" section). It is important to consider that these samples were collected in August when spawning was not occurring. The samples collected for the first "Within Colony" variation study were collected in May, which was the middle of the spawning season. The absence of the spawning variable resulted in statistically insignificant differences between "Tops" and "Bottoms". This discovery supports the biomonitoring capacity of *S. polydactyla* outside of the spawning season.

Finally, gender was re-examined in this data set and found to be a significant factor in both dry and lipid weight based PCB data. The samples for the first examination of gender were collected in the beginning of July. The samples for the second comparison were collected at the end of August. It is not readily apparent why PCB differences between gender were observed in this data set, especially when "Within Colony" differences were not present. The sampling notes reveal that the male and female colonies were located roughly 100 yards apart and were found at different depths (3-4ft: males, 8-10ft: females). The simplest explanation would be that the colonies were too far apart and were exposed to different ambient PCB concentrations.

It is notable that in the first gender comparison, lipid content did not vary between males and females. In the second gender comparison, lipid content was significantly higher in females. The higher female lipid levels might suggest an upset in lipid-PCB equilibrium levels if the lipids were newly deposited. However, new lipid deposition would be expected to create within-colony differences between "Tops" and "Bottoms". A simpler explanation is that the difference in lipid content was a reflection of greater depth and colder ambient water temperatures rather than a gender-specific event.

Finding male sand-pedestal and female sand-pedestal pairs in the same location proved to be too limiting. Therefore, the distance between the male pairs and the female pairs may have been too great to assume that the male and female groups were exposed to the same ambient environment. Under such faulty site selections, gender would not be the singular variable in this comparison and hence could not be claimed as the cause of the observed differences.

Kinetics Studies

PCB Uptake and Depuration

One of the goals of the kinetics study was to investigate whether the uptake and depuration of PCBs in *S. polydactyla* follows first-order kinetics. Plotted on a natural-log scale, the depuration data were linear. This strongly suggests that depuration was following first-order kinetics. The correlation coefficients were 0.8 or better for all the depuration data sets except PCB 44 dry-weight. All of the correlation coefficients were higher for data calculated on lipid weight than dry weight. This is a signal that PCB accumulation in *S. polydactyla* is associated with the lipid content of the soft coral.

The data for the uptake of PCBs in *S. polydactyla* plotted linearly as well. Control data indicated that the ambient concentrations of the PCBs dropped rapidly during the time of the experiment. In the cases of PCBs 44 and 77, the ambient concentrations remained high enough that the PCBs were still being accumulated. Unfortunately, PCB 187 concentrations were too low and the PCB was depurated from the experimental specimens. For PCBs 44 and 77, the correlation coefficients were better for data calculated on dry-weight than lipid weight, which contrasts the depuration data.

Lipid content of all the experimental and control colonies increased during the kinetics study. If lipid was being laid down very rapidly, it could be hypothesized that the PCBs weren't depurating; they were being diluted. However, if that were the case, then uptake would not have

been observed for PCBs 44 and 77. The data clearly show that there was movement of PCBs 44 and 77 into the corals even though lipid content was increasing and ambient PCB concentrations were dropping. The increase in lipid content could potentially explain why the uptake data was less linear. It seems again that PCB uptake does not keep pace with lipid deposition in the soft corals.

Lipid increase was an uncontrolled variable during the experiment. Fortunately, lipid increase was nearly equal at both experimental sites. We can assume that the effect of lipid increase was the same on both study groups, cancelling out. If the lipid increase promoted sequestration, or diluted PCB concentrations, it would have the same effect on both sets of results. Therefore, the fact that PCB concentrations decreased linearly at the depuration sites and increased linearly at the uptake sites indicates that the PCB concentrations in the corals were reflecting ambient conditions.

It is noteworthy that the controls at both the Dadi Beach site and the Piti Bomb Holes site were depurating. Another uncontrolled variable was changing ambient PCB concentration. Nonetheless, ambient PCB 44 and 77 concentrations were greater at the Dadi Beach site than at Piti Bomb Holes over the same period.

The results of this kinetics study provide strong evidence that corals are suitable for monitoring PCBs and give a general idea for monitoring timetables. The linearity of the graphs and high correlation coefficients, even with small sample sizes, indicates that *S. polydactyla* reflects changes in ambient PCB levels on practical time scales. The half-lives of the PCB congeners 44, 77, and 187 were 85, 49 and 34 days respectively when calculated on a lipid weight. A reasonable sampling frequency to establish a concentration trend would therefore be every couple of months. At least one more kinetics study with greater sample sizes is called for to define the kinetic rate constants with greater confidence and for more congeners.

Preferential PCB Accumulation

The purpose of setting out SPMDs at the sampling sites was to measure the concentrations of PCBs in the ambient water (C_w) using an established monitoring device. These values were necessary for calculating kinetic rate constants, but the data also provides insight into metabolic activities in *S. polydactyla*. SPMDs overcome variability issues in biomonitors associated with metabolism. Therefore, comparing the profile of PCBs accumulated by the SPMDs and the profiles from *S. polydactyla* can give an indication if any metabolism or bioaccumulation is occurring in the corals.

The comparison (Appendix I) revealed that at both the Dadi Beach site and the Piti Bomb Holes site, the SPMDs had higher proportions of PCB congeners 8 and 18; SPMDs and corals were about equal in proportions of PCB 126; and the corals had higher proportions of PCBs 52, 44, 153 and 77 and much higher proportions of congener 187

Biological degradation of PCBs has been documented in organisms when the congeners have fewer than 4 chlorines (Matthews and Anderson 1975; Matthews and Dedrick 1984; Erickson 1997). PCBs 8 and 18 have two and three chlorines respectively. Thus, it is possible that the higher proportions of these congeners in the SPMDs are a reflection of their metabolism by the

corals. The corals at both sites showed proportionately higher concentrations of PCB 187. There are 7 chlorines in PCB 187. Higher chlorinated PCBs are more lipophillic than lesser chlorinated congeners (Connell and Miller 1984; Erickson 1997). PCB 77 was also preferentially accumulated, especially at Dadi. This is a coplanar PCB, and its bioaccumulation is supported by other research as well (Tanabe *et al*. 1987).

Previous kinetics studies have revealed patterns in PCB accumulation similar to the ones discovered in this work. A study with green-lipped mussels showed higher proportions of coplanar PCBs relative to total PCBs. Coplanar PCBs were shown to take longer to equilibrate into lipid tissue, and much longer to depurate than their nonplanar counterparts (Kannan *et al*. 1989). This dynamic would result in the enhanced bioaccumulation of coplanar PCBs in chronic exposure conditions. The suspected source of PCBs at Dadi Beach is the Orote Landfill which has been in operation since 1944. The high proportions of congeners 77 and 187 may be reflective of chronic exposure of the resident corals.

A kinetics study with American oysters also showed slower depuration of planar PCBs compared to nonplanar PCBs of the same chlorination level. Additionally, lower molecular weight PCBs were accumulated faster than higher molecular weight PCBs (Gardinali *et al*. 2004).

A study with the sponge, *Spongia officinalis*, demonstrated substantial preferential accumulation of PCBs having 7 and 8 chlorine atoms. The least chlorinated PCBs, with 3 and 4 chlorines, appeared to be undergoing some form of metabolism (Perez *et al*. 2003). It is a point of interest that 4 similar yet different aquatic species displayed parallel patterns in PCB accumulation. It is plausible that the biological organisms and PCBs are interacting in ways that are universal to many organisms.

It is important to determine how ambient PCB concentrations are reflected in the living organisms used for monitoring. From this initial study, it seems that there is a degree of PCB metabolism and bioaccumulation in *S. polydactyla*. However, this is occurring at the low end and high end of the chlorination scale and not throughout the midrange. Overall, it appears that *S. polydactya* can be useful for PCB monitoring, and will also offer valuable information about the fate of PCBs in aquatic organisms.

PCB Profiling

PCB profiles offer information about the sources of PCBs. Each Aroclor is chlorinated to a certain degree. Different congeners will be absent, present, or predominant in each Aroclor profile. The profiles detected in *S. polydactyla* from the Dadi site offer clues to which Aroclors may have contaminated the region. The detection of PCB 105 indicates Aroclor 1242 as a source because this is the only Aroclor containing this congener (USDHHS 1998).

Aroclor 1242 would also be the source of PCB 44. Aroclor 1016 is the only other Aroclor with PCB 44, but 1016 is not a likely candidate based on the profile as a whole. The high levels of PCB 187 indicate the presence of Aroclor 1254 or 1260. Aroclors 1242, 1254 and 1260 are also sources of PCBs 77 and 126. Even at this stage of evaluation, it seems reasonable to use *S. polydactyla* for fast and efficient information on the presence or absence of particular Aroclors in a water body.

Conclusions and Recommendations

The present study has produced a generous buffet of information about the dynamics of PCBs in *Sinularia polydactyla.* There is still information that needs to be brought to the table before the soft coral can be used as a biomonitor without reservation. An over-arching issue is the number of PCBs studied. The limitations of this study only allowed 3 PCBs to be analyzed statistically. Optimally, the comparisons and kinetics studies would be recreated to research a larger suite of PCBs. The following is a summary of the conclusions that can be drawn from this work and recommendations for information that needs to be pursued.

The study of variation "within and between" colonies produced confounding results. In short, during the spawning season, there were more lipids in the "Tops", but more PCBs in the "Bottoms". Young lobes, from which the "Tops" were sampled, are active growth sites. The short exposure of the new growth and rapid lipid accumulation might, therefore, be reasons for the low PCB levels in this region of the colonies. It certainly appears from the present data that lipid loading and off-loading out-paces that of PCBs.

Alternatively, high levels of relatively polar metabolites in the actively growing tips may inhibit or at least slow the passive diffusion of PCBs into these tissues from the surrounding water. On the other hand, the source of the high lipid content might be the large number of eggs within the tips. The lipids in the eggs might not be drawing in PCBs because of protective mechanisms or impermeability of the egg membranes. Outside of the spawning season, there were no significant differences between "Tops" and "Bottoms."

The seasonal variation study with pre- and post-spawn female colonies produced patterns congruent with the "within and between" study. The pre-spawn females had higher lipid concentrations than post-spawn. However, the lipid-based PCB concentrations were significantly higher post spawn. Dry-weight based PCB concentrations were not significantly different between the two groups. It appears, then, that lipid content fluxes rapidly during the reproductive season, but PCB load does not. The spawning season of *S. polydactyla* occurs from March through June. It appears that biomonitoring with *S. polydactyla* is viable if timed to avoid the spawning season.

The two "within and between" colony comparisons, and the seasonal comparison are laden with information. We can reasonably deduce that PCBs are not off-loaded during spawning. We've also learned that the rapid build-up of lipid during spawning does not result in a proportionate accumulation of PCBs. Regardless of the mechanism preventing uniform PCB distribution, it is clear that lipid based data derived during the spawning season is unreliable. Monitoring during the spawning season must be avoided when using *S. polydactyla.* Outside of the spawning season, the coral has more uniform PCB-lipid proportions. A critical study to conduct next would be to trace lipid content in *S. polydactyla* for at least one full year to check for other periods of rapid lipid accumulation of off-loading.

The data for the comparison of gender and age/size play by textbook rules. Gender did not have an effect on PCB concentrations. Higher dry weight PCB concentrations in larger/older colonies were balanced out by calculating the concentrations by lipid weight. This data set, collected in

July, showed that *S. polydactyla* could potentially behave very nicely as a biomonitor during non-spawning months. This study, timed right after the conclusion of spawning, supports the hypothesis that the physiological events of spawning are to blame for interfering with PCB-lipid proportions rather than the basic structure and function of the organism.

Around Guam, as in other locations, female colonies are predominant. Unfortunately, then, it would not be practical to circumvent spawning variables by sampling only male colonies. Therefore, it is necessary to pin down the changes that occur in female colonies throughout the year. This study was limited to conducting each comparison individually and only once or twice. A more thorough study ought to conduct comparisons of gender, age/size, and within and between colonies simultaneously and multiple times in the span of a year.

The comparison of PCB concentrations accumulated in different positions of the water column revealed no significant differences. We can state with some degree of confidence that water column position, or contact with the substrata, will not significantly affect monitoring data in an environment where PCBs are water-borne. If the source of PCBs was contaminated sediment, the outcome may be different. No study was made of soft corals that are intermittently exposed during low tide. Portions of a colony bathed in contaminants collected on the surface of the water could potentially behave differently as well.

The linearity of the kinetics graphs and high correlation coefficients, even with small sample sizes, indicates that *S. polydactyla* adequately reflects changes in ambient PCB levels. A question that immediately jumps out is why are the depuration coefficients much better than the uptake coefficients, especially since all corals underwent synchronous lipid increases? As mentioned earlier, it is possible that the lipid was accumulated faster than the PCBs could proportionately accumulate and this lag resulted in lower correlation coefficients. It is suspected that the lipid increase was due to dropping temperatures, considering the study ran from September through December during which sea temperature drops 1-2°C on average.

Regardless, the lipid-based PCB concentrations produced better correlation coefficients than dry-weight analysis. Even with the changing lipid volume, it appears that the soft corals were accumulating PCBs reflective of ambient levels. It would be appropriate to conduct kinetics studies with larger sample sizes, and of course target a larger suite of PCBs in order to accurately calculate total ambient PCB concentrations from *S. polydactyla* samples. Additionally, SPMDs should be deployed and collected in staggered increments to match the sampling design for the coral. The current research was constrained to one set of SPMDs deployed only for 15 of the 90 days of the kinetics study.

Conducting a broader and more extensive kinetics study is also necessary because there appears to be a degree of PCB metabolism and bioaccumulation occurring in *S. polydactyla*. The observed metabolism of lower chlorinated PCBs and preferential accumulation of higher chlorinated and planar PCBs matches observations made in other aquatic species. In the cases of the PCBs studied, the phenomenon is neither dramatic nor unique to soft corals. Nonetheless, kinetics studies with the simultaneous use of SPMDs will illustrate whether a particular PCB of interest is reflective of ambient concentrations.

The information provided in this research supports the use of *S. polydactyla* for at least basic PCB surveys or as part of a suite of monitoring organisms. The uptake and depuration rates appear to be short enough to detect fresh PCB sources, yet long enough to make trend monitoring feasible. *Sinularia polydactyla* can certainly assist in identifying the presence or absence of particular Aroclors in an area. Additionally, this study provides a plethora of data from contaminated and uncontaminated sites across 8 months which can be used as benchmarks for quick comparisons.

PCB monitoring via *S. polydactyla* also presents many technical advantages. The most unique attribute is the capacity for repeated sampling of the same organism. This eliminates the effects of genetic differences. Genetic variability is a major unknown to eliminate and makes potential soft coral biomonitoring very exciting. Ample tissue is also available for numerous sampling events.

Furthermore, *S. polydactyla* are immobile. The corals can be found where expected when they are needed, significantly simplifying sampling tasks. This also makes *S. polydactyla* excellent for mapping contamination as they are reflecting time-averaged concentrations at a specific location. Another attribute of *S. polydactyla* is its abundance and tolerance of transplantation. This broadens potential applications and increases the success rates of research.

In summing, then, Sinularian corals can be valuable monitoring tools for tropical areas, just like mussels are for temperate zones. Their widespread occurrence alone in the tropics and sub-tropics makes their use appealing. The presence of soft corals in waters that are suspected of PCB contamination provides an immediate sampling device. After investigating the issues addressed in this chapter, *S. polydactyla* will be a thoroughly evaluated biomonitoring species and it is expected to become a popular environmental tool in tropical waters.

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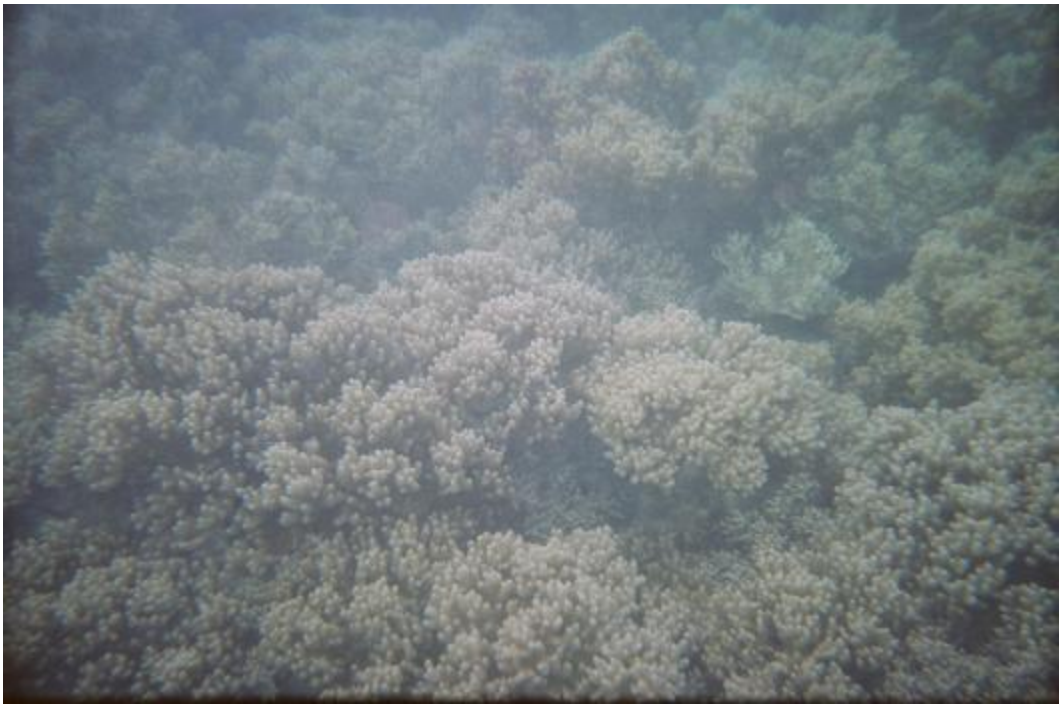
Plate I

Colonies of *Sinularia polydactyla*



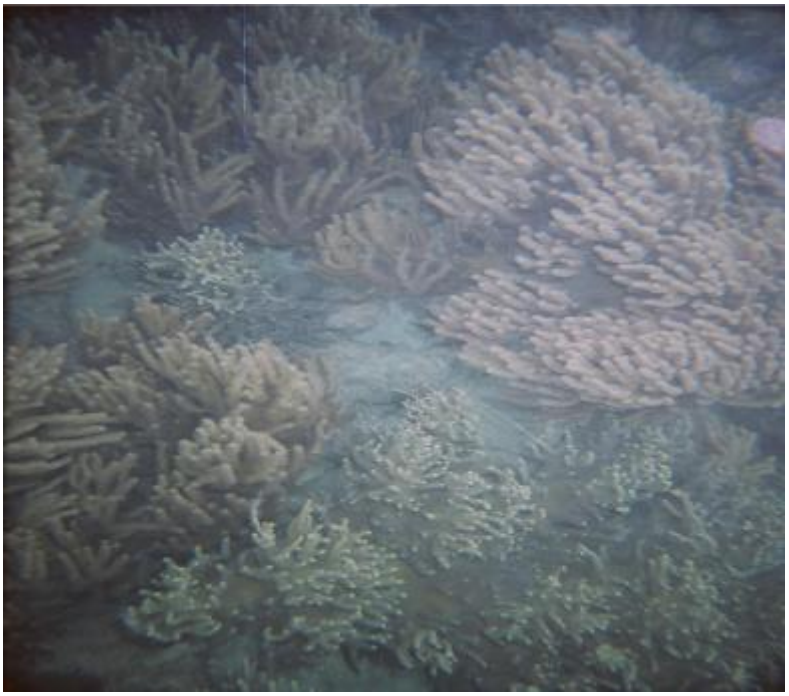
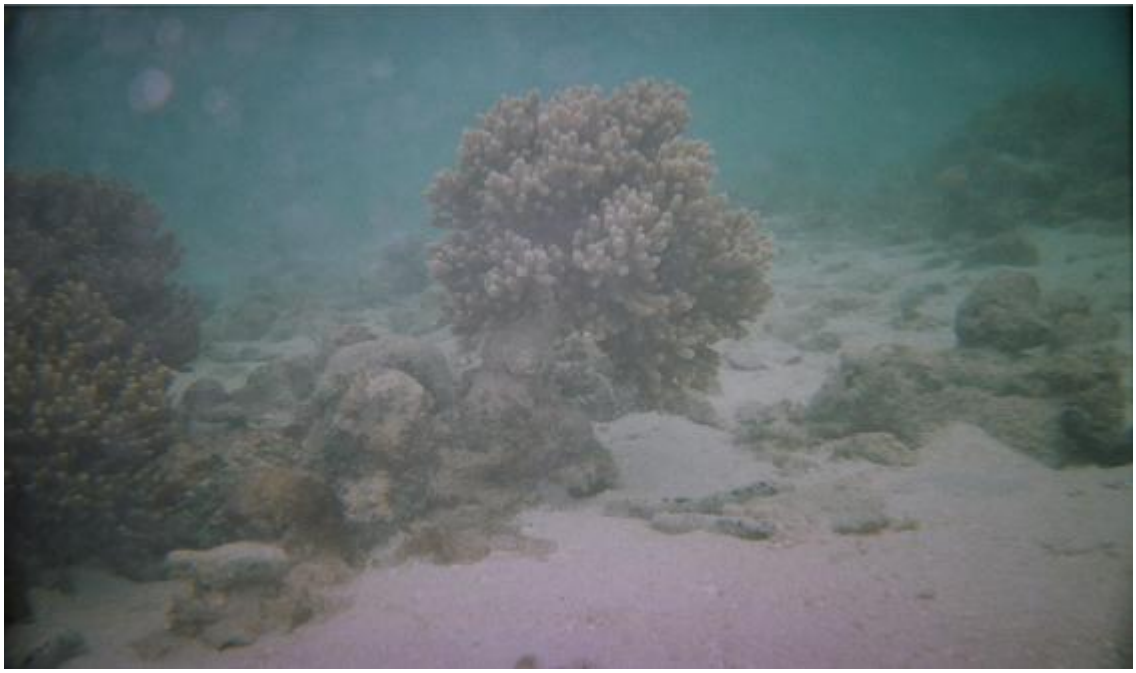


Plate II

***Sinularia polydactyla* on Pedestals**



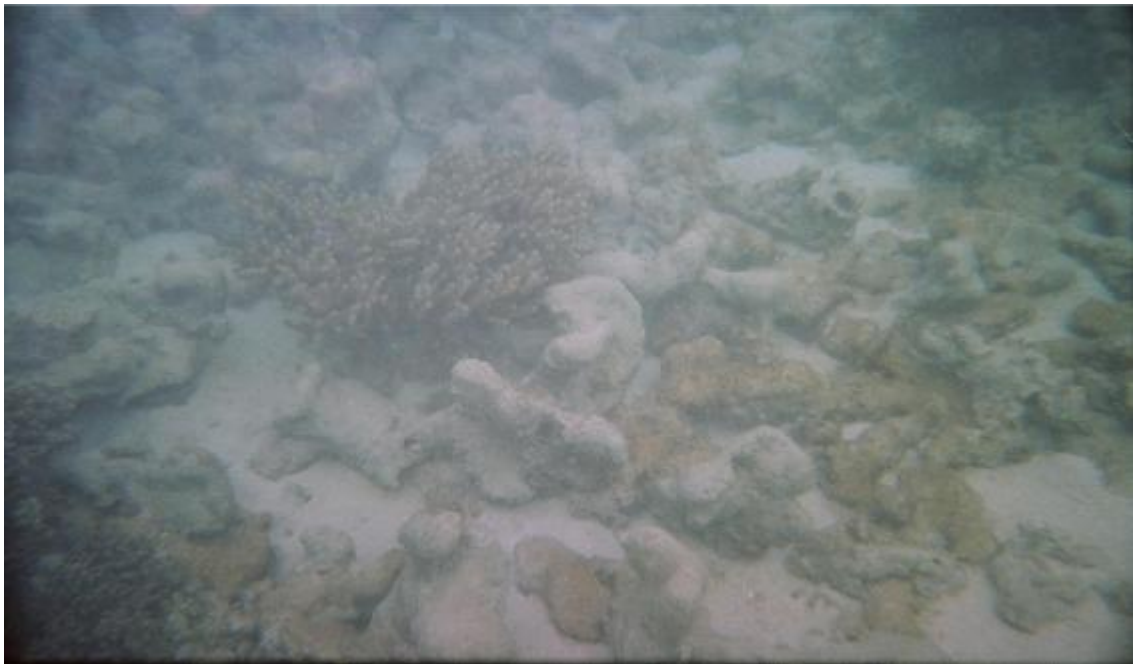
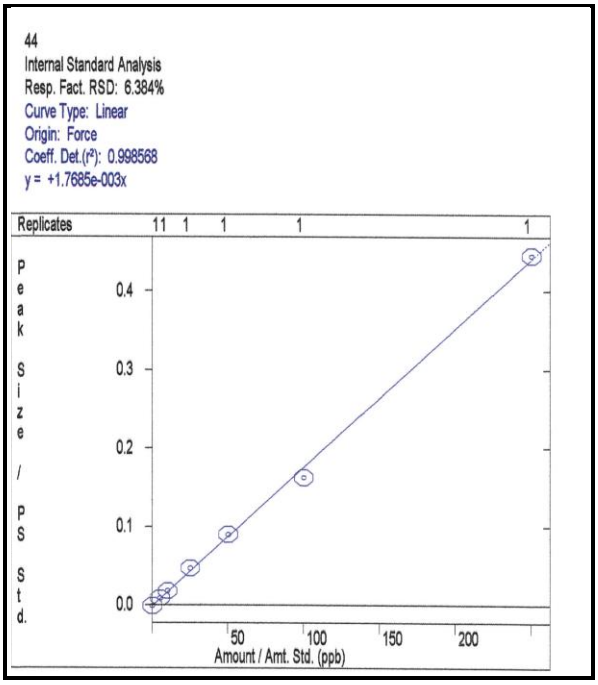


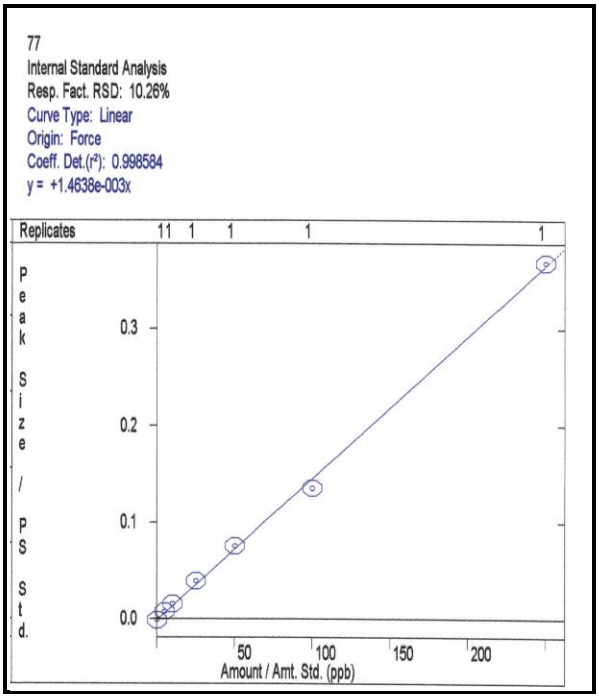
Plate III

Placement of SPMDs Next to *Sinularia polydactyla* Colony

Plate IV

Internal Standard Calibration Curves: PCBs 44, 77, and 187





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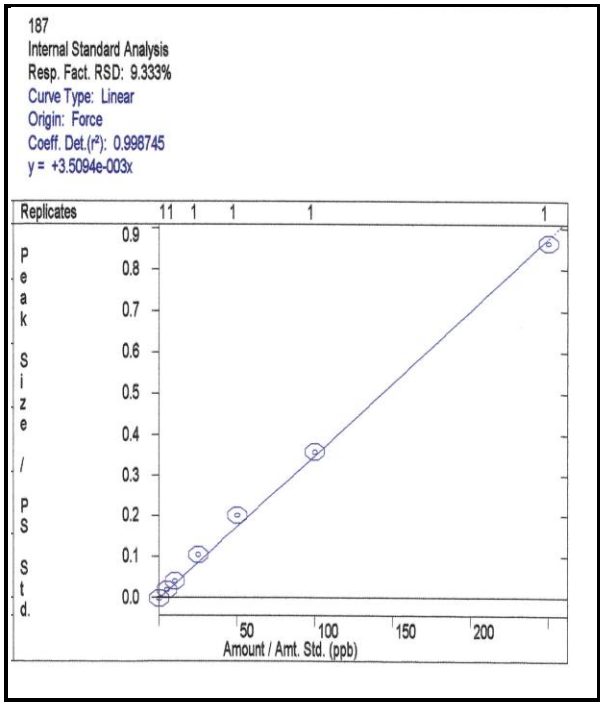
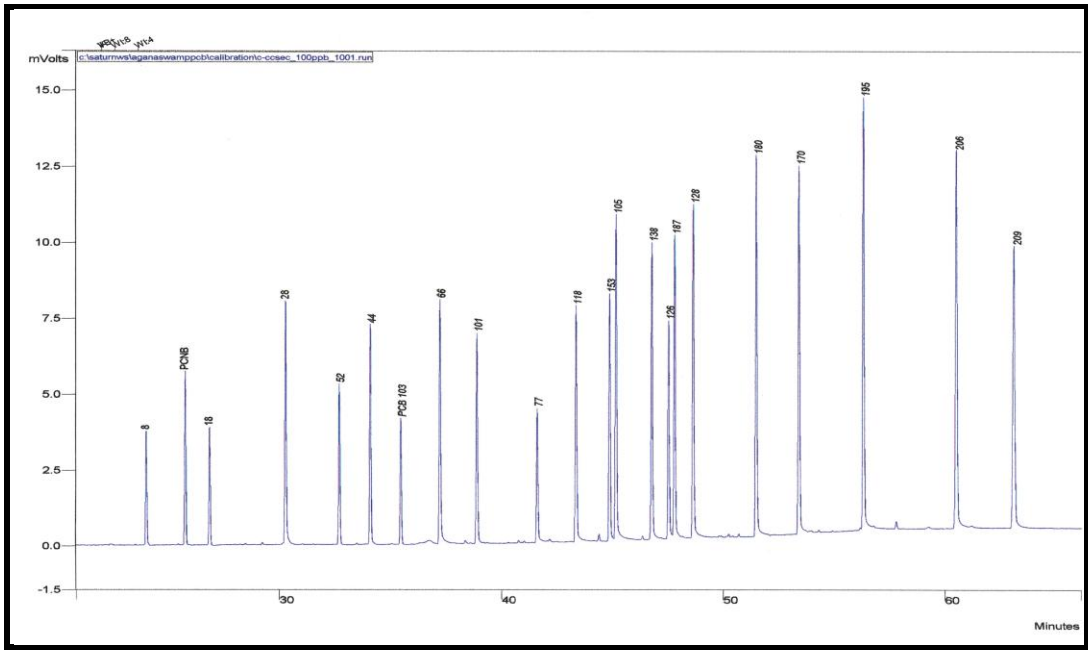


Plate V

Complete Chromatographic Separation of PCB Calibration Standard



Appendix A

Field and Laboratory Procedures for Identification of *Sinularia* Species

Field Identification

Field identification of *Sinularia polydactyla* is based on appearance and texture. *S. polydactyla* is a brown to green "finger" or "leather" coral. It contracts and becomes compacted, rigid and rough when molested. A single lobe or "finger" of the colony is peeled apart by hand to determine texture. Due to the lattice structure of its sclerites, *S. polydactyla* tear roughly. This distinguishes *S. polydactyla* from its common relative, *Sinularia maxima*, which has a parallel sclerite structure and tears easily (Janes and Wah 2005; personal communication with Dr. Marc Slattery 2007). A plume of brown mucus (zooxanthellae) is also released from *S. polydactyla* when torn. The sclerite structure may also be tested by pinching off the tip of a lobe. *S. polydactyla* is very resistant to being plucked, whereas *S. maxima* "pops" off easily.

Laboratory Identification

Remove a 2-3 cm lobe from several representative colonies with scissors, place in clean, seawater filled container and transport on ice to the laboratory. Rinse each sample was with deionized water. Remove a sliver of tissue from the tip of each lobe with a scalpel and place on a glass microscope slide with a drop of bleach. Allow the tissues to digest in the bleach for 5-10 minutes. Rinse with deionized water before examining under a light microscope.

The surface sclerites of *S. polydactyla* are club-like with stubby projections and terminal warts. The clubs have rough, slightly crooked shafts and pointed ends. Some of the terminal warts have distinct "Y" shapes projections. The surface sclerites of *S. maxima* are leaf-like (leptoclatus); the leaves projecting at angles of 90° from one other. The *S. maxima* sclerites are approximately half the size of those belonging to *S. polydactyla*, and the shafts are smoother with blunt ends (Janes and Wah 2005; personal communication with Dr. Marc Slattery 2007).

Appendix B

PCB Congeners in Calibration Standard and Co-eluting PCBs

Calibration Standard PCB	Structural Arrangement	Co-Eluting PCB	Structural Arrangement
8	2,4'	5	2,3
18	2, 2', 5	15	4, 4'
28	2, 4, 4'	31	2, 4', 5
52	2, 2', 5, 5'	43	2, 2', 3, 5
44	2, 2', 3, 5'	none	
66	2, 3' 4, 4'	80	3, 3', 5, 5'
101	2, 2', 4, 5, 5'	79	3, 3', 4, 5'
77	3, 3', 4, 4'	154	2, 2', 4, 4', 5, 6
118	2, 3', 4, 4', 5	106	2, 3, 3', 4, 5
153	2, 2', 4, 4', 5, 5'	none	
105	2, 3, 3', 4, 4'	none	
138	2, 2', 3, 4, 4', 5'	158	2, 3, 3', 4, 4', 6
126	3, 3', 4, 4', 5	129	2, 2', 3, 3', 4, 5'
187	2, 2', 3, 4', 5, 5', 6	159	2, 3, 3', 4, 5, 5'
128	2, 2', 3, 3', 4, 4'	none	
180	2, 2', 3, 4, 4', 5, 5'	none	
170	2, 2', 3, 3', 4, 4', 5	none	
195	2, 2', 3, 3', 4, 4', 5, 6	none	
206	2, 2', 3, 3', 4, 4', 5, 5', 6	none	
209	2, 2', 3, 3', 4, 4', 5, 5', 6, 6'	none	

Appendix C

QA/QC Data 1: MDL (ng/g dry wt.)

PCB	Replicates							Std Dev	MDL	LCL	UCL
	1	2	3	4	5	6	7				
8	6.199	3.871	3.120	3.356	3.302	3.338	3.423	1.082	3.400	2.176	7.480
28	3.953	3.234	2.966	2.487	3.021	2.707	2.946	0.466	1.464	0.937	3.222
52	2.337	2.215	2.429	2.367	2.633	2.230	2.677	0.182	0.572	0.366	1.258
44	4.273	4.130	3.964	4.447	4.546	3.982	4.128	0.223	0.701	0.449	1.543
66	5.967	5.895	5.197	6.182	6.646	5.753	5.764	0.442	1.390	0.889	3.058
101	4.353	4.447	4.032	4.641	4.796	4.267	4.524	0.251	0.789	0.505	1.736
77	5.474	4.157	3.812	4.457	4.874	5.104	5.548	0.660	2.075	1.328	4.564
118	3.877	4.362	3.862	4.750	5.060	4.251	4.310	0.435	1.368	0.876	3.010
153	3.648	4.378	3.462	4.767	5.126	4.270	4.363	0.583	1.831	1.172	4.029
105	3.726	4.246	3.070	4.479	4.801	4.247	4.390	0.571	1.793	1.148	3.945
138	3.405	3.776	2.520	3.057	4.473	3.200	4.285	0.694	2.180	1.396	4.797
126	3.340	4.182	3.329	4.760	4.886	4.032	4.034	0.611	1.920	1.229	4.223
187	3.565	4.770	3.071	5.439	5.703	4.562	4.644	0.943	2.963	1.896	6.519
128	3.877	4.978	3.323	5.656	6.334	4.823	4.972	1.013	3.185	2.038	7.007
180	3.660	5.101	2.971	5.378	6.138	4.933	5.191	1.082	3.400	2.176	7.480
170	3.242	4.991	2.854	5.506	6.025	4.831	5.057	1.165	3.662	2.344	8.057
195	2.765	4.665	2.601	5.180	5.671	4.511	4.727	1.173	3.687	2.360	8.112
206	2.117	4.179	2.717	4.717	5.206	3.900	4.133	1.085	3.411	2.183	7.505
209	1.858	3.726	2.744	4.391	4.853	3.581	3.811	1.001	3.147	2.014	6.924
$\sum_{20}$PCB	71.64	81.30	62.04	86.02	94.09	78.52	82.93	10.31	32.41	20.74	71.30

MD = method detection limit; LCL = lower control limit; UCL = upper control limit

QA/QC Data 2: Recovery from SRM 2977 (ng/g dry wt.)

	Date	June 7, 2007	June 7, 2007	July 3, 2007	July 26, 2007	August 6, 2007	October 15, 2007
PCB	**8**	—	—	3.566	—	—	—
	18	11.774	10.130	14.314	9.468	10.386	—
	28	18.124	15.826	—	3.094	5.043	4.003
	52	—	0.445	5.341	2.199	2.729	1.950
	44	4.995	4.600	3.479	2.715	2.725	4.030
	66	9.874	10.535	25.432	11.652	11.125	15.110
	101	10.344	12.066	21.635	11.464	10.270	17.313
	77	4.865	1.539	5.368	2.647	2.291	4.558
	118	10.582	10.365	14.161	9.735	8.251	11.979
	153	11.559	12.345	14.030	10.314	10.700	15.303
	105	2.932	2.824	3.805	2.403	2.668	3.408
	138	5.977	6.697	7.434	5.370	4.909	6.079
	126	0.863	0.681	—	—	—	0.223
	187	6.378	3.977	6.208	3.854	3.701	6.106
	128	1.776	1.916	2.078	1.461	1.627	1.872
	180	4.423	4.899	6.102	4.346	4.069	6.706
	170	1.997	1.931	—	1.880	1.620	2.769
	195	—	—	—	—	—	—
	206	—	—	—	—	—	—
	209	—	—	—	—	—	—
	$\sum_{20}$PCB	106.462	100.774	132.952	82.601	82.113	101.411

Dashes indicate values below detection limits.

QA/QC Data 3: Duplicate PCB Analyses (ng/g dry wt.)

	Source	F3 Post	F3 Post'	J3	J3'	M7	M7'	F10 rep	F10 rep'	DBY 9/3	DBY 9/3'
	Lipid %	12.2		9.0		8.6		10.9		6.5	
PCB	8	—	—	—	—	—	—	—	—	—	—
	18	7.54	8.39	5.44	5.23	7.78	6.99	3.22	4.77	—	—
	28	—	—	—	—	—	—	—	—	—	—
	52	76.7	68.4	53.7	45.7	79.8	95.2	53.7	45.8	3.46	3.35
	44	54.4	56.5	54.2	50.6	45.6	45.2	41.2	52.1	17.7	20.4
	66	—	—	—	—	—	—	—	—	—	—
	101	—	—	—	—	3.29	2.87	—	—	—	—
	77	326	373	191	169	115	119	84.5	116	13.0	14.0
	118	—	—	—	—	—	—	—	—	—	—
	153	36.0	44.8	—	—	22.4	21.8	20.3	28.0	—	—
	105	6.88	9.10	6.81	5.95	8.69	9.93	5.37	8.13	8.05	9.32
	138	—	—	—	—	—	—	—	—	—	—
	126	13.5	20.8	—	—	12.4	17.2	14.0	23.0	8.94	9.20
	187	146	189	217	177	237	249	137	199	170	167
	128	—	—	—	—	—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—	—	—
	∑20PCB	667	770	528	453	532	567	359	477	221	224

F=female; J=juvenile; M=male; D=Dadi site; B=blue; Y=yellow; G=green; S=sand; T=transplant; C=control; Dashes=values below detection limits.

QA/QC Data 3: Duplicate PCB Analyses (ng/g dry wt.) Cont'd

	Source	DOG 9/18	DOG 9/18'	DG 10/17	DG 10/17'	SFT2	SFT2'	A Top	A Top'	A Btm	A Btm'
	Lipid %	9.8		12.3		10.9		7.6		7.3	
PCB	**8**	—	—	—	—	—	—	—	11.2	9.47	10.04
	18	—	—	1.84	—	2.94	3.31	5.82	7.24	6.08	6.44
	28	—	—	—	—	—	—	—	—	—	—
	52	16.8	12.4	10.9	9.35	76.1	81.4	76.1	88.5	72.1	76.4
	44	11.4	37.8	34.3	32.4	49.9	53.0	40.8	54.2	41.4	43.9
	66	—	—	—	—	—	—	—	—	—	—
	101	29.2	—	—	—	—	—	—	—	—	—
	77	64.5	62.0	86.8	122	89.9	101	207	325	235	249
	118	—	—	—	—	—	—	—	—	—	—
	153	—	—	—	—	19.6	23.0	29.6	50.1	34.7	34.6
	105	5.54	20.2	25.6	13.1	7.73	8.48	—	5.20	4.73	5.01
	138	—	—	—	—	—	—	—	—	—	—
	126	5.20	19.8	18.9	—	17.3	18.4	—	—	3.15	—
	187	95.0	241	190	263	199	224	77.4	145	109	115
	128	—	—	—	—	—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—	—	—
	∑20PCB	228	393	368	440	463	513	437	686	515	540

F=female; J=juvenile; M=male; D=Dadi site; B=blue; Y=yellow; G=green; S=sand; T=transplant; C=control; Dashes=values below detection limits.

QA/QC Data 4: Duplicate Lipid Analysis (g)

Source	DO 10/17	DO 10/17'	PCB 9/3	PCB 9/3'	DG 10/17	DG 10/17'
dry wt	0.428	0.456	0.296	0.301	0.268	0.357
vial wt	21.408	21.555	21.477	21.514	21.476	21.501
vial +lipid wt	21.449	21.607	21.503	21.539	21.509	21.545
lipid wt	0.041	0.052	0.026	0.025	0.033	0.044
Lipid %	9.6	11.4	8.8	8.3	12.3	12.3

D=Dadi; O=Orange; G=green.

Appendix D

Lipid Extraction and Quantification Procedure Adopted for this Study

Lipid Extraction

Samples were hexane extracted in the Dionex Accelerated Solvent Extractor 200 (ASE). Extraction cells (22 ml) where charged with a cellulose filter, 0.5g of 'Hydromatrix' (diatomaceous earth), and 0.4 ± 0.1 g of lyophilized, ground coral sample (weighed out to the nearest 0.001 g) mixed with 1g of 'Hydromatrix,' and topped-off with 'Hydromatrix,'. The ASE was programmed as follows:

Oven temperature: 130°C
Pressure: 1750 psi
Static time: 5 min (after 5 min pre-heat equilibration)
Flush volume: 80% of the cell volume
Nitrogen purge: 50 sec at 150 psi
Static Cycles: 2

Lipid extracts were collected in pre-cleaned 40-ml collection vials weighed to the nearest 0.001 gram. Upon evaporation to dryness in the 'Zymark TurboVap,' the residual lipids were heated to 100°C for 24 h in an oven. After cooling to room temperature in a desiccator at room temperature, the vials of lipid were weighed to the nearest 0.001 gram. All necessary precautions were taken to keep the vials free of extraneous material that could affect their final weights, e.g., fingerprints, dust and dirt.

Final lipid weights were determined by subtracting the weight of the vials from the weight of the vial plus lipid. The percentage lipid in each sample was calculated as follows:

Final lipid weight/Sample dry weight x 100

Appendix E

PCB Peak Verification by Sulfuric Acid Clean-Up

Selected *Sinularia polydactyla* samples were subjected to an additional clean-up procedure using sulfuric acid to remove any co-extractants with similar chromatographic retention times as the PCB peaks of interest. During this procedure, the sample extract underwent an initial volume reduction to ~5 ml rather than ~0.4 ml. After adding an approximately equal volume of concentrated sulfuric acid and leaving overnight, the lighter hexane fraction was removed from the digest and reduced to a final volume of 0.5 ml as described earlier. PCB recoveries from *S. polydactyla* samples are summarized in the table below and precede the raw data sets.

1. PCB Verification and Congener Recoveries following Sulfuric Acid Clean-Up

PCB Congener	Congener Detects in Samples (n=19)		% Recovey after Acid Cleanup	
	Before Acid Cleanup	After Acid Cleanup	Range	Mean
8	7	6	0-97	66
52	18	16	0-338	63
44	18	15	47-105	75
66	1	1	-	99
77	19	19	86-189	115
118	1	0	-	0
153	10	2	0-127	23
105	18	16	0-144	98
126	12	1	-	46
187	19	19	87-187	118

PCBs 18, 28, 101, 138, 128, 180, 170, 195, 206, 209 consistently below detection in all samples.

2. PCB Verification with Sulfuric Acid Clean-Up (data as ng/g dry wt.)

	Date	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08
	Source	PB	PB*	PY	PY*	POG	POG*	DOG	DOG*	PG	PG*	PBY	PBY*	DCB	DCB*	DB	DB*	PCO	PCO*
PCB	8	20.6	11.6	16.7	11.6	15.1	8.82	—	—	10.5	—	10.5	10.3	15.9	10.4	—	—	—	—
	18	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	52	41.7	30.3	37.6	19.4	52.2	23.3	3.07	5.41	73.0	18.3	71.6	23.3	70.1	15.3	8.73	—	—	—
	44	58.3	43.3	54.8	31.4	58.5	45.3	38.2	31.8	50.9	38.3	57.8	27.9	89.9	76.7	39.2	—	5.72	4.81
	66	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	101	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	11.6
	77	624	726	562	586	641	623	251	319	530	490	641	756	906	1030	224	270	7.88	14.9
	118	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	10.7	—
	153	37.5	—	38.2	—	44.5	—	—	—	43.6	—	53.2	—	—	—	—	5.03	—	7.20
	105	7.48	8.47	6.51	—	5.68	8.18	14.2	13.9	5.72	4.05	5.99	7.42	6.41	7.90	12.0	12.0	4.14	—
	138	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	126	44.2	—	40.1	—	33.4	—	14.0	—	42.7	—	38.6	—	—	—	—	—	—	—
	187	140	188	108	133	118	208	164	225	131	120	162	211	241	272	239	261	63.0	61.5
	128	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8.44
	209	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$PCB	974	1008	864	781	968	917	484	596	888	671	1040	1035	1330	1413	523	548	91.4	104

*=extract sulfuric acid cleaned, P=Piti; D=Dadi; C=control; O=orange, Y=yellow, B=blue, G=green; dashes=values below detection limits.

2. PCB Verification with Sulfuric Acid Clean-Up (data as ng/g dry wt.) Cont'd

	Date	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	12/2/08	5/9/07	5/9/07	5/9/07	5/9/07
	Source	DBY	DBY*	DY	DY*	PCB	PCB*	PO	PO*	DCY	DCY*	DG	DG*	DCO	DCO*	DO	DO*	Psmpl	Psmpl*	Dsmpl	Dsmpl*
PCB	**8**	—	—	—	—	—	—	—	—	14.6	13.3	—	—	16.5	14.8	—	—	—	—	—	—
	18	—	—	—	—	—	—	—	—	—	—	—	—	—	15.2	—	—	—	—	—	—
	28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	52	6.99	3.27	12.1	3.01	4.19	2.37	42.0	26.5	91.4	22.3	7.30	4.68	109	38.3	5.05	—	11.4	38.4	75.6	44.8
	44	36.9	26.9	50.4	30.7	—	—	41.1	36.9	100.8	87.3	38.4	—	91.8	96.4	40.7	—	19.7	14.2	49.5	23.1
	66	—	—	—	—	—	4.08	—	—	—	—	—	—	—	—	—	—	10.2	10.1	—	—
	101	—	—	—	—	—	11.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	77	218	222	396	596	21	29	411	352	1165	1267	285	277	981	1038	282	248	39.5	47.1	374	398
	118	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	153	—	—	—	—	17.1	18.0	32.7	—	80.6	—	—	5.99	77.1	—	—	5.06	—	—	73.8	93.9
	105	11.8	14.5	13.9	15.5	26.7	21.2	4.16	5.73	5.32	6.91	15.0	17.0	8.74	8.63	13.1	10.8	—	—	14.9	16.2
	138	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	126	—	—	—	—	—	6.65	15.7	—	31.0	—	15.1	—	32.9	—	12.7	—	—	—	27.6	12.8
	187	214	200	186	259	200	174	132	139	205	281	238	299	360	452	240	211	139	163	347	409
	128	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$**PCB**	488	467	658	904	270	267	679	560	1694	1678	599	604	1677	1663	594	474	219	273	963	998

*=extract sulfuric acid cleaned, P=Piti; D=Dadi; C=control; O=orange, Y=yellow, B=blue, G=green; dashes=values below detection limits.

Appendix F

Intrinsic/Extrinsic Variables 1: PCB Variations Within and Between Colonies (data as ng/g dry wt.)

	Date	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07
	Source	A Top 1	A Top 2	A Top 3	A Top 4	A Top 5	A Top 5'	A Btm 1	A Btm 2	A Btm 3	A Btm 4	A Btm 5	A Btm 5'	B Top 1	B Top 2	B Top 3	B Top 4	B Top 5
PCB	**8**	13.6	—	12.1	—	—	11.2	—	—	—	8.22	9.47	10.0	8.07	—	—	13.3	10.8
	18	6.01	5.36	5.42	5.66	5.82	7.24	5.57	5.78	5.13	6.15	6.08	6.44	5.06	5.23	—	—	5.13
	28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	52	80.9	69.0	91.6	81.7	76.1	88.5	60.5	54.7	38.7	72.9	72.1	76.4	77.8	66.4	—	130	107
	44	55.1	43.9	62.7	52.5	40.8	54.2	32.9	35.4	27.0	45.9	41.4	43.9	66.3	57.0	65.6	73.3	63.7
	66	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	101	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	77	293	244	348	278	207	325	207	224	214	290	235	249	365	302	387	414	337
	118	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	153	—	35.0	—	40.7	29.6	50.1	35.1	34.8	37.9	—	34.7	34.6	34.3	27.1	—	—	45.1
	105	4.11	—	4.02	—	—	5.20	—	4.63	4.79	6.49	4.73	5.01	6.58	5.09	7.05	9.20	7.71
	138	—	—	—	—	—	—	18.4	—	—	—	—	—	—	—	—	—	31.9
	126	—	0.86	—	—	—	—	—	—	—	—	3.15	—	30.7	24.0	—	13.3	10.6
	187	125	103	125	107	77	145	111	112	109	157	109	115	230	161	209	233	212
	128	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$**PCB**	578	501	649	566	437	686	470	471	486	587	515	540	824	648	669	887	831

Dashes=values below detection limits.

Intrinsic/Extrinsic Variables 1: PCB Variations Within and Between Colonies (data as ng/g dry wt.) Cont'd

	Date	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07
	Source	B Btm 1	B Btm 2	B Btm 3	B Btm 4	B Btm 5	C Top 1	C Top 2	C Top 3	C Top 4	C Top 5	C Btm 1	C Btm 2	C Btm 3	C Btm 4	C Btm 5
PCB	**8**	—	—	9.14	11.2	—	11.7	13.6	11.2	16.0	11.9	—	8.17	11.5	—	—
	18	3.11	3.66	5.53	5.06	4.33	6.36	6.18	7.15	4.72	5.49	3.64	4.69	7.40	4.17	6.37
	28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	52	33.4	34.9	143	91.9	86.2	204	202	106	150	112	78.7	127	71.8	45.6	92.0
	44	30.0	30.9	46.3	43.5	35.5	69.0	67.3	58.7	66.3	54.5	39.5	51.2	49.0	23.8	45.7
	66	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	101	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	77	171	200	355	225	165	312	295	260	243	229	220	236	197	133	230
	118	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	153	—	—	—	29.1	20.9	—	29.7	—	—	30.7	37.3	37.4	22.3	23.4	35.8
	105	4.69	5.52	9.05	9.67	6.92	9.74	12.2	6.77	8.77	5.74	9.92	15.8	9.92	5.35	5.80
	138	—	23.1	45.3	26.7	16.3	—	—	—	—	—	—	—	—	—	23.5
	126	21.6	24.8	25.1	14.6	11.1	17.7	22.9	—	—	—	22.6	31.4	20.5	6.23	5.45
	187	133	151	227	155	120	161	157	135	154	111	204	232	109	90.3	118
	128	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$**PCB**	397	474	865	612	466	792	806	585	643	560	616	744	498	332	562

Dashes=values below detection limits.

Intrinsic/Extrinsic Variables 1: PCB Variations Within and Between Colonies (data as ng/g lipid wt.)

	Date	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07
	Source	A Top 1	A Top 2	A Top 3	A Top 4	A Top 5	A Top 5'	A Btm 1	A Btm 2	A Btm 3	A Btm 4	A Btm 5	A Btm 5'	B Top 1	B Top 2	B Top 3	B Top 4	B Top 5
	Lipid %	10.2	6.6	11.4	10.7	7.6	7.6	4.8	4.9	3.8	6.9	7.3	7.3	12.2	13.1	10.8	11.5	11.0
PCB	**8**	133	—	107	—	—	146	—	—	—	120	130	138	66.2	—	—	116	98.6
PCB	**18**	58.8	81.0	47.6	52.7	76.1	94.7	116	119	135	89.5	83.6	88.6	41.6	40.1	—	—	46.7
PCB	**28**	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PCB	**52**	792	1042	805	760	994	1157	1263	1128	1020	1061	993	1052	638	509	—	1131	970
PCB	**44**	539	663	551	488	534	709	687	729	712	668	570	604	544	436	607	636	579
PCB	**66**	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PCB	**101**	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PCB	**77**	2864	3686	3061	2591	2705	4243	4313	4619	5640	4226	3227	3419	2996	2313	3585	3598	3067
PCB	**118**	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PCB	**153**	—	528	—	379	387	654	732	717	998	—	477	476	281	207	—	—	410
PCB	**105**	40.2	—	35.3	—	—	68.0	—	95.4	126	94.5	65.0	68.9	54.0	39.0	65.3	79.9	70.1
PCB	**138**	—	—	—	—	—	—	384	—	—	—	—	—	—	—	—	—	290
PCB	**126**	—	13.0	—	—	—	—	—	—	—	—	43.4	—	252	184	—	115	96.5
PCB	**187**	1226	1553	1099	1000	1012	1893	2323	2300	2872	2291	1497	1586	1891	1235	1936	2027	1929
PCB	**128**	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PCB	**180**	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PCB	**170**	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PCB	**195**	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PCB	**206**	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PCB	**209**	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$**PCB**	5654	7566	5705	5272	5708	8965	9819	9706	11503	8549	7085	7431	6765	4963	6194	7703	7557

Dashes=values below detection limits.

Intrinsic/Extrinsic Variables 1: PCB Variations Within and Between Colonies (data as ng/g lipid wt.) Cont'd

	Date	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07
	Source	B Btm 1	B Btm 2	B Btm 3	B Btm 4	B Btm 5	C Top 1	C Top 2	C Top 3	C Top 4	C Top 5	C Btm 1	C Btm 2	C Btm 3	C Btm 4	C Btm 5
	Lipid %	3.2	3.6	5.2	4.9	3.9	11.5	11.4	10.8	12.0	11.2	3.3	4.6	8.0	2.6	7.1
	8	—	—	177	231	—	102	119	104	134	105	—	176	143	—	—
	18	97.3	103	107	104	110	55.3	54.1	66.5	39.4	48.8	109	101	92.3	163	89.4
	28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	52	1044	980	2763	1888	2192	1772	1771	989	1251	998	2365	2726	895	1778	1292
	44	939	867	894	894	902	600	590	546	555	484	1186	1103	611	929	642
	66	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	101	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	77	5334	5612	6854	4629	4195	2716	2585	2418	2035	2035	6620	5086	2455	5180	3232
	118	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PCB	**153**	—	—	—	597	531	—	260	250	—	273	1122	804	278	914	503
	105	146	155	175	199	176	84.7	107	63.0	73.3	51.0	298	339	124	209	81.5
	138		648	876	549	413	—	—	—	—	—	—	—	—	—	330
	126	675	695	485	301	282	154	201	155	—	—	680	676	256	243	76.6
	187	4167	4228	4389	3176	3057	1401	1375	1254	1289	985	6122	4993	1360	3523	1655
	128	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$**PCB**	12401	13289	16720	12567	11859	6886	7062	5847	5376	4980	18502	16003	6215	12939	7901

Dashes=values below detection limits.

Intrinsic/Extrinsic Variables 2: PCB Variations in Pre- and Post-Spawn Colonies (data as ng/g dry wt.)

	Date	5/4/07	5/4/07	5/4/07	5/4/07	5/4/07	5/4/07	5/4/07	5/4/07	5/4/07	5/4/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07
	Source	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F1*	F2*	F3*	F4*	F5*	F6*	F7*	F8*	F9*	F10*
PCB	**8**	—	—	—	—	9.61	—	9.79	—	—	—	—	—	—	—	—	—	contaminated	—	—	—
	18	8.54	3.88	7.62	3.91	10.5	4.46	7.59	4.94	3.04	4.05	8.36	5.13	7.54	4.80	8.50	7.68		5.47	4.72	4.60
	28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	—	—
	52	56.8	34.2	73.5	48.9	96.2	43.5	102	57.9	45.2	43.0	71.0	48.2	76.7	62.3	86.0	75.5		52.9	57.2	37.9
	44	63.3	27.4	53.6	30.0	72.8	34.2	70.2	38.0	29.9	31.5	62.8	35.3	54.4	44.1	65.7	53.8		39.2	36.1	31.4
	66	—	—	—	—	—	—	—	—	—	12.3	—	—	—	—	—	11.9		—	9.29	10.0
	101	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	—	—
	77	450	131	389	198	453	102	409	146	131	151	461	160	326	337	399	235		155	157	176
	118	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	—	—
	153	56.0	29.9	51.6	34.8	45.0	24.7	47.6	25.6	28.0	26.4	—	34.1	36.0	—	37.9	28.8		28.1	23.9	30.9
	105	7.67	4.62	8.82	4.12	7.68	5.14	8.35	4.81	—	4.17	8.52	6.17	6.88	6.00	7.24	5.75		5.42	—	—
	138	—	—	—	—	—	—	30.5	—	14.2	13.2	—	—	—	—	—	18.9		—	9.62	—
	126	10.5	—	14.1	7.18	18.3	—	16.7	7.42	9.9	11.0	15.7	6.07	13.5	20.1	18.1	10.8		9.66	9.58	7.93
	187	301	97.2	211	80.8	197	74.6	260	75.5	69.7	69.8	315	112	146	134	173	132		84.8	57.5	73.0
	128	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	—	—
	180	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	—	—
	170	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	—	—
	195	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	—	—
	206	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	—	—
	209	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	—	—
	$\sum_{20}$**PCB**	954	328	810	408	910	289	962	360	331	366	942	407	667	609	795	580		380	365	372

*=post-spawn samples; F=female; dashes=values below detection limits.

Intrinsic/Extrinsic Variables 2: PCB Variations in Pre- and Post-Spawn Colonies (data as ng/g lipid wt.)

	Date	5/4/07	5/4/07	5/4/07	5/4/07	5/4/07	5/4/07	5/4/07	5/4/07	5/4/07	5/4/07
	Source	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
	Lipid %	13.3	7.3	11.2	15.3	14.1	12.1	16.6	17.3	18.7	15.5
PCB	**8**	—	—	—	—	68.2	—	58.9	—	—	—
	18	64.4	53.4	67.9	25.5	74.2	37.0	45.6	28.6	16.2	26.0
	28	—	—	—	—	—	—	—	—	—	—
	52	428	472	655	319	682	361	616	335	241	277
	44	477	377	478	196	517	284	422	220	159	202
	66	—	—	—	—	—	—	—	—	—	79.1
	101	—	—	—	—	—	—	—	—	—	—
	77	3396	1801	3471	1290	3215	847	2458	843	700	971
	118	—	—	—	—	—	—	—	—	—	—
	153	422	412	460	227	319	205	286	148	149	170
	105	57.8	63.7	78.6	26.9	54.5	42.6	50.2	27.8	—	26.8
	138	—	—	—	—	—	—	183	—	75.7	84.9
	126	79.0	—	125	46.9	129	—	101	42.9	52.8	70.4
	187	2270	1339	1880	527	1399	619	1563	437	372	449
	128	—	—	—	—	—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$**PCB**	7195	4517	7215	2658	6459	2396	5784	2081	1766	2356

*=post-spawn samples; F=female; dashes=values below detection limits.

Intrinsic/Extrinsic Variables 2: PCB Variations in Pre- and Post-Spawn Colonies (data as ng/g lipid wt.) Cont'd

	Date	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07	5/10/07
	Source	F1*	F2*	F3*	F4*	F5*	F6*	F7*	F8*	F9*	F10*
	Lipid %	4.9	7.6	12.2	7.0	10.9	7.8	11.8	7.2	9.3	7.0
PCB	8	—	—	—	—	—	—	contaminated	—	—	—
	18	171	67.4	62.0	68.5	78.0	98.9		75.6	50.6	65.3
	28	—	—	—	—	—	—		—	—	—
	52	1456	634	630	889	789	973		731	613	538
	44	1288	464	447	629	603	693		542	386	446
	66	—	—	—	—	—	153		—	100	142
	101	—	—	—	—	—	—		—	—	—
	77	9441	2109	2680	4810	3661	3032		2135	1684	2497
	118	—	—	—	—	—	—		—	—	—
	153	—	448	296	—	348	371		388	256	439
	105	175	81.1	56.6	85.6	66.5	74.1		74.8	—	—
	138	—	—	—	—	—	244		—	103	—
	126	322	79.8	111	287	166	139		133	103	112
	187	6455	1466	1197	1915	1584	1697		1171	616	1035
	128	—	—	—	—	—	—		—	—	—
	180	—	—	—	—	—	—		—	—	—
	170	—	—	—	—	—	—		—	—	—
	195	—	—	—	—	—	—		—	—	—
	206	—	—	—	—	—	—		—	—	—
	209	—	—	—	—	—	—		—	—	—
	$\sum_{20}$PCB	19308	5349	5481	8684	7293	7474		5250	3911	5275

*=post-spawn samples; F=female; dashes=values below detection limits.

Intrinsic/Extrinsic Variables 3: PCB Variations Due to Colony Gender and Maturity (data as ng/g dry wt.)

	Date	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07
	Colony	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F1'	F2'	F3'	F4'	F5'	F6'	F7'	F8'	F9'	F10'
PCB	**8**	—	—	sample compromised	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	18	4.69	4.50		4.23	4.57	4.70	5.19	2.46	3.80	2.64	3.28	3.96	3.81	4.42	5.40	4.77	—	5.14	4.23	3.22
	28	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	52	60.6	69.4		—	75.6	80.6	65.6	44.8	94.2	82.1	46.6	59.0	61.8	63.6	68.0	63.2	48.4	64.3	69.0	53.7
	44	61.1	41.4		40.8	52.1	45.2	43.3	32.2	58.1	49.5	47.5	47.0	45.1	44.3	54.6	49.9	46.8	53.3	51.9	41.2
	66	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	101	51.6	—		—	—	—	—	—	—	—	4.85	—	—	—	—	—	—	—	—	—
	77	203	149		127	151	139	167	91.6	186	115	139	157	160	147	158	148	156	149	150	84.5
	118	—	—		—	—	—	—	0.96	—	—	—	—	—	—	—	—	—	—	—	—
	153	42.3	—		—	28.7	33.3	36.7	18.8	34.0	—	35.4	36.6	40.8	34.1	36.1	36.6	37.1	—	30.9	20.3
	105	8.03	5.60		—	5.97	5.69	5.42	4.01	6.25	6.23	6.81	5.96	5.62	5.58	8.14	6.97	5.84	8.23	6.38	5.37
	138	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	126	—	—		—	13.7	14.1	17.9	9.9	17.3	—	14.1	19.3	15.8	15.8	15.5	19.1	23.6	—	17.1	14.0
	187	239	272		148	126	182	220	132	199	183	146	266	209	150	158	202	217	261	208	137
	128	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	180	—	—		—	—	—	—	—	—	0.78	—	—	—	—	—	—	—	—	—	—
	170	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	209	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$**PCB**	670	542		320	458	504	561	337	599	439	444	595	541	465	504	530	535	542	538	359

F=female; dashes=values below detection limits.

Intrinsic/Extrinsic Variables 3: PCB Variations Due to Colony Gender and Maturity (data as ng/g dry wt.) Cont'd

	Date	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07
	Colony	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M1'	M2'	M3'	M4'	M5'	M6'	M7'	M8'	M9'	M10'
PCB	**8**	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8.62	—	8.13	9.20	—	8.06
	18	5.82	7.07	6.73	6.42	—	6.51	7.78	5.39	5.55	4.56	4.18	3.23	4.83	5.49	11.3	5.39	5.50	—	—	6.62
	28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	52	79.1	72.7	65.7	58.1	103.1	87.7	79.8	111.1	96.4	87.1	81.2	83.4	73.5	59.9	113.4	73.6	78.1	92.3	63.2	55.3
	44	54.6	49.3	47.1	43.9	50.3	47.4	45.6	52.4	45.2	42.2	44.6	40.8	39.9	37.3	60.7	44.3	43.5	46.8	33.5	39.5
	66	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	101	5.69	48.1	—	39.8	—	—	3.29	—	2.55	—	2.21	1.07	2.99	—	—	—	—	26.4	—	1.81
	77	157	156	147	127	168	122	115	159	147	129	114	97.9	114	91.6	169	105	104	131	80.6	110
	118	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	15.7	—	—
	153	—	—	—	—	—	22.6	22.4	27.6	28.1	21.0	—	—	—	—	—	—	—	20.2	—	—
	105	10.4	8.86	9.84	7.64	11.3	8.26	8.69	10.6	10.4	9.41	6.72	6.98	6.46	5.07	10.94	8.55	7.47	8.72	6.34	7.82
	138	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	126	—	—	—	—	—	10.0	12.4	21.0	17.5	16.9	—	—	—	—	—	—	—	97.6	—	—
	187	195	274	193	155	331	133	237	285	273	153	111	146	131	86	287	125	168	225	131	124
	128	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$**PCB**	507	616	469	438	663	437	532	672	626	463	364	379	373	285	661	363	415	673	314	354

M=male; dashes=values below detection limits.

Intrinsic/Extrinsic Variables 3: PCB Variations Due to Colony Gender and Maturity (data as ng/g dry wt.) Cont'd

	Date	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07
	Colony	J1	J2	J3	J4	J5	J6	J7	J8	J9	J10	J1'	J2'	J3'	J4'	J5'	J6'	J7'	J8'	J9'	J10'
PCB	**8**	—	—	—	—	13.0	—	—	—	—	—	—	—	—	—	—	—	19.1	—	—	9.75
	18	4.24	4.33	5.44	3.26	3.10	—	3.74	3.44	3.30	4.17	4.97	5.06	4.05	3.60	4.92	2.87	6.02	2.01	2.88	4.72
	28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	52	49.6	44.3	53.7	54.0	24.5	33.3	72.2	49.3	42.6	59.8	36.0	41.4	27.6	36.1	29.6	25.8	36.5	29.6	33.8	38.8
	44	38.3	40.0	54.2	36.9	17.7	10.5	43.6	46.4	41.2	40.0	35.1	37.3	32.5	36.5	41.7	30.2	37.5	30.1	33.8	31.1
	66	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	101	—	—	—	—	10.1	—	—	—	—	—	—	—	—	—	27.5	2.22	4.00	—	—	—
	77	129	185	191	136	37.3	—	132	90.1	80.3	131	127	178	129	175	106	78.5	129	61.1	63.8	125
	118	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	153	—	—	—	23.8	—	—	28.2	20.6	16.4	23.8	—	—	—	—	—	19.5	—	—	—	—
	105	4.65	5.84	6.81	4.92	—	—	5.69	6.05	4.30	5.43	—	4.83	4.73	5.98	7.26	—	—	—	2.61	—
	138	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	126	—	—	—	15.4	—	—	25.5	12.3	9.40	21.1	—	—	7.44	—	—	—	—	—	—	—
	187	149	180	217	112	46	71.0	181	177	111	133	123	153	173	183	168	129	177	102	82.1	129
	128	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	18.1	—	—	—
	180	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$**PCB**	375	460	528	387	152	115	492	406	308	419	325	420	379	441	384	288	427	225	219	338

J=juvenile; dashes=values below detection limits.

Intrinsic/Extrinsic Variables 3: PCB Variations Due to Colony Gender and Maturity (data as ng/g lipid wt.)

	Date	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07
	Source	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F1'	F2'	F3'	F4'	F5'
	Lipid %	8.1	7.4		6.3	9.5	9.9	7.4	8.7	11.4	6.6	9.4	9.1	10.3	10.2	10.0
PCB	8	—	—		—	—	—	—	—	—	—	—	—	—	—	—
PCB	18	58.0	61.0		67.3	48.1	47.6	70.5	28.4	33.3	40.3	35.0	43.8	37.0	43.5	54.0
PCB	28	—	—		—	—	—	—	—	—	—	—	—	—	—	—
PCB	52	750	940		—	796	815	892	518	827	1252	497	652	601	627	680
PCB	44	756	562		649	549	458	588	371	510	755	507	519	439	436	546
PCB	66	—	—		—	—	—	—	—	—	—	—	—	—	—	—
PCB	101	639	—		—	—	—	—	—	—	—	51.7	—	—	—	—
PCB	77	2507	2019		2029	1595	1410	2273	1057	1633	1756	1485	1729	1552	1444	1581
PCB	118	—	—		—	—	—	—	11.1	—	—	—	—	—	—	—
PCB	153	524	—		—	302	337	499	217	298	—	377	404	396	336	361
PCB	105	99.3	76.0		—	62.9	57.6	73.7	46.3	54.9	95.0	72.7	65.8	54.6	54.9	81.4
PCB	138	—	—		—	—	—	—	—	—	—	—	—	—	—	—
PCB	126	—	—		—	144	143	243	114	152	—	150	213	153	156	155
PCB	187	2951	3689		2349	1327	1837	2984	1523	1746	2786	1559	2943	2027	1479	1577
PCB	128	—	—		—	—	—	—	—	—	—	—	—	—	—	—
PCB	180	—	—		—	—	—	—	—	—	11.9	—	—	—	—	—
PCB	170	—	—		—	—	—	—	—	—	—	—	—	—	—	—
PCB	195	—	—		—	—	—	—	—	—	—	—	—	—	—	—
PCB	206	—	—		—	—	—	—	—	—	—	—	—	—	—	—
PCB	209	—	—		—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$PCB	8283	7347		5095	4825	5105	7623	3887	5255	6696	4735	6569	5260	4575	5036

sample compromised

F=female; dashes=values below detection limits.

Intrinsic/Extrinsic Variables 3: PCB Variations Due to Colony Gender and Maturity (data as ng/g lipid wt.) Cont'd

	Date	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07
	Source	F6'	F7'	F8'	F9'	F10'	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
	Lipid %	8.6	8.3	10.7	11.1	10.9	8.3	9.2	7.6	8.6	8.8	9.3	8.6	10.7	8.8	9.3
	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	18	55.8	—	48.2	38.1	29.6	70.3	76.8	88.7	75.1	—	70.3	90.1	50.3	63.3	49.2
	28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	52	739	581	603	621	494	955	790	866	680	1171	948	924	1036	1101	940
	44	583	561	500	467	379	659	536	621	513	572	513	528	489	515	456
	66	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	101	—	—	—	—	—	68.7	—		466	—	—	38.1	—	29.1	—
	77	1727	1876	1399	1352	777	1896	1691	1941	1486	1904	1314	1336	1478	1681	1387
	118	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PCB	153	428	445	—	278	187	—	—	—	—	—	244	260	258	320	226
	105	81.4	70.1	77.2	57.4	49.3	126	96.2	130	89.4	128	89.2	101	98.9	118	102
	138	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	126	223	283	—	154	129	—	—	—	—	—	108	144	195	200	182
	187	2357	2600	2451	1875	1256	2349	2976	2540	1815	3755	1435	2742	2661	3115	1652
	128	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$PCB	6193	6416	5078	4842	3301	6124	6688	6188	5124	7530	4721	6162	6266	7143	4993

F=female; M=male; dashes=values below detection limits.

Intrinsic/Extrinsic Variables 3: PCB Variations Due to Colony Gender and Maturity (data as ng/g lipid wt.) Cont'd

	Date	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07
	Source	M1'	M2'	M3'	M4'	M5'	M6'	M7'	M8'	M9'	M10'	J1	J2	J3	J4	J5
	Lipid %	6.6	8.3	7.8	9.2	9.3	9.8	8.9	8.1	8.3	7.7	5.8	5.0	9.0	5.4	4.4
	8	—	—	—	—	92.8	—	91.6	114	—	105	—	—	—	—	298
	18	63.1	38.7	61.6	59.7	122.0	55.0	62.0	—	—	85.9	72.7	87.1	60.7	60.1	70.9
	28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	52	1226	1001	937	651	1220	752	880	1139	760	717	851	891	600	998	560
	44	673	490	510	405	653	452	490	578	402	511	656	805	605	681	405
	66	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	101	33.4	12.9	38.2	—	—	—	—	327	—	23.4	—	—	—	—	232
	77	1719	1175	1452	996	1820	1076	1170	1621	969	1427	2210	3729	2134	2519	854
	118	—	—	—	—	—	—	—	194	—	—	—	—	—	—	—
PCB	**153**	—	—	—	—	—	—	—	249	—	—	—	—	—	439	—
	105	101	83.7	82.4	55.1	118	87.3	84.2	108	76.3	101	79.8	117	76.0	91.0	—
	138	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	126	—	—	—	—	—	—	—	1205	—	—	—	—	—	284	—
	187	1681	1750	1674	936	3089	1280	1892	2774	1570	1613	2563	3630	2426	2078	1053
	128	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$**PCB**	5497	4551	4755	3103	7115	3703	4670	8309	3778	4584	6433	9259	5901	7151	3474

M=male; J=juvenile; dashes=values below detection limits.

Intrinsic/Extrinsic Variables 3: PCB Variations Due to Colony Gender and Maturity (data as ng/g lipid wt.) Cont'd

	Date	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07	7/4/07
	Source	J6	J7	J8	J9	J10	J1'	J2'	J3'	J4'	J5'	J6'	J7'	J8'	J9'	J10'
	Lipid %	8.2		6.3	8.7	6.9	6.0	7.2	6.2	7.6	4.8	6.9	4.5	8.2	10.0	6.8
	8	—		—	—	—	—	—	—	—	—	—	420	—	—	144
	18	—		54.7	37.8	60.1	83.5	70.2	65.0	47.4	103	41.7	132	24.4	28.9	69.7
	28	—		—	—	—	—	—	—	—	—	—	—	—	—	—
	52	406		783	488	863	606	575	444	475	622	375	804	360	339	573
	44	128		737	471	576	589	518	522	480	876	439	825	366	339	459
	66	—		—	—	—	—	—	—	—	—	—	—	—	—	—
	101	—		—	—	—	—	—	—	—	576	32.2	88.1	—	—	—
	77	—	sample compromised	1432	920	1891	2126	2478	2078	2308	2224	1141	2831	742	640	1851
	118	—		—	—	—	—	—	—	—	—	—	—	—	—	—
PCB	**153**	—		327	188	343	—	—	—	—	—	283	—	—	—	—
	105	—		96.0	49.2	78.2	—	67.1	76.1	78.7	152	—	—	—	26.1	—
	138	—		—	—	—	—	—	—	—	—	—	—	—	—	—
	126	—		196	108	304	—	—	120	—	—	—	—	—	—	—
	187	865		2819	1266	1921	2061	2122	2776	2408	3518	1876	3888	1242	823	1903
	128	—		—	—	—	—	—	—	—	—	—	398	—	—	—
	180	—		—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—		—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—		—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—		—	—	—	—	—	—	—	—	—	—	—	—	—
	209	—		—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$PCB	1400		6445	3528	6035	5465	5829	6081	5797	8072	4188	9386	2735	2197	5001

J=juvenile; dashes=values below detection limits.

Intrinsic/Extrinsic Variables 4: PCB Variations Due to Colony Water Column Depth and Gender (data as ng/g dry wt.)

	Source	PFB1	PFB2	PFB3	PFB4	PFB5	PFT1	PFT2	PFT3	PFT4	PFT5	SFB1	SFB2	SFB3	SFB4	SFB5
PCB	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	18	—	4.05	2.56	2.71	2.66	1.90	2.80	3.53	—	1.82	—	—	3.52	—	—
	28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	52	69.2	40.6	60.0	63.0	33.5	49.9	77.0	94.5	43.7	76.7	67.5	—	76.7	68.7	51.8
	44	46.9	57.9	56.4	54.7	43.5	42.5	61.3	69.2	61.2	41.7	46.8	36.1	56.6	50.7	39.5
	66	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	101	41.1	79.6	—	—	—	—	5.10	—	44.5	—	—	29.8	—	—	—
	77	146	223	149	155	123	156	138	184	139	84.7	165	115	183	177	140
	118	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	153	42.9	60.2	31.3	34.6	36.3	43.6	36.5	43.7	48.1	20.1	35.4	—	41.7	35.4	31.1
	105	8.85	9.83	8.09	9.88	9.50	8.37	9.67	10.8	12.2	7.57	9.73	8.84	10.5	10.2	8.47
	138	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	126	14.4	—	20.1	23.7	17.8	10.4	15.7	17.3	35.0	17.9	19.2	—	13.4	19.9	16.3
	187	324	260	301	373	373	380	334	412	514	221	298	263	311	307	260
	128	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$PCB	693	736	628	717	640	693	680	835	897	471	642	453	697	670	547

P=pedestal; S=sand; F=female; T=top; B=bottom; dashes =values below detection limits.

Intrinsic/Extrinsic Variables 4: PCB Variations Due to Colony Water Column Depth and Gender (data as ng/g dry wt.) Cont'd

	Source	SFB6	SFT1	SFT2	SFT3	SFT4	SFT5	SFT6	PMB1	PMB2	PMB3	PMB4	PMB5	PMT1	PMT2	PMT3
PCB	**8**	—	—	—	—	—	—	—	—	—	—	7.81	—	7.85	—	—
	18	3.55	1.90	3.31	—	3.07	3.74	—	2.26	2.84	2.46	4.74	1.57	3.71	—	—
	28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	52	56.7	62.6	81.4	89.6	73.5	76.0	58.4	49.6	59.6	66.2	80.3	38.2	—	90.7	—
	44	63.0	47.8	53.0	52.4	68.9	64.9	58.3	25.1	33.2	35.9	53.7	24.4	52.9	43.8	21.4
	66	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	101	7.31	—	—	—	4.76	—	6.14	—	—	—	7.48	40.5	71.2	—	27.4
	77	217	172	101	126	182	172	189	257	322	234	320	153	340	270	150
	118	3.26	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	153	60.9	35.8	23.0	30.7	—	32.4	54.2	—	51.5	52.0	65.6	43.9	—	59.7	—
	105	13.8	11.0	8.48	10.5	13.4	10.4	13.5	11.6	16.6	14.9	17.7	10.0	22.0	18.7	11.6
	138	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	126	—	26.1	18.4	18.4	—	19.2	—	17.5	34.9	31.5	40.1	13.2	—	34.7	—
	187	425	343	224	269	386	290	472	480	368	387	468	220	692	448	267
	128	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$PCB	850	701	513	597	732	669	852	843	889	824	1065	545	1189	966	477

P=pedestal; S=sand; F=female; M=male; T=top; B=bottom; dashes =values below detection limits.

Intrinsic/Extrinsic Variables 4: PCB Variations Due to Colony Water Column Depth and Gender (data as ng/g dry wt.) Cont'd

	Source	PMT4	PMT5	SMB1	SMB2	SMB3	SMB4	SMB5	SMT1	SMT2	SMT3	SMT4	SMT5	SMT6
PCB	**8**	—	—	—	—	—	—	—	—	—	8.82	—	—	—
	18	—	2.75	4.56	—	—	3.27	3.76	3.70	2.38	3.97	2.03	3.67	2.26
	28	—	—	—	—	—	—	—	—	—	—	—	—	—
	52	101	55.0	108	108	29.1	69.3	69.9	63.3	79.7	92.8	75.9	117	57.1
	44	55.0	36.3	50.7	47.9	17.0	27.0	38.2	41.8	33.8	53.7	31.6	51.7	24.4
	66	—	—	—	—	—	—	—	—	—	—	—	—	—
	101	45.6	47.7	—	—	26.8	—	—	63.3	—	5.42	35.4	3.69	63.4
	77	274	215	335	272	96.7	230	299	279	382	387	211	306	272
	118	—	3.46	3.63	—	—	—	—	—	—	—	—	—	—
	153	—	56.7	73.2	56.4	29.8	51.9	64.5	80.6	85.1	—	49.4	69.3	59.0
	105	21.2	14.1	20.2	26.2	7.66	14.0	14.1	16.6	13.1	20.8	15.6	21.0	10.7
	138	—	—	—	—	—	—	—	—	—	—	—	—	—
	126	—	20.1	34.6	49.0	10.2	25.2	24.0	23.5	11.3	—	28.4	39.2	6.90
	187	473	340	457	408	186	318	431	516	327	589	334	461	167
	128	—	—	—	—	—	—	—	—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$**PCB**	969	791	1087	968	404	738	944	1088	935	1161	783	1073	663

P=pedestal; S=sand; M=male; T=top; B=bottom; dashes =values below detection limits.

Intrinsic/Extrinsic Variables 4: PCB Variations Due to Colony Water Column Depth and Gender (data as ng/g lipid wt.) Cont'd

	Source	PFB1	PFB2	PFB3	PFB4	PFB5	PFT1	PFT2	PFT3	PFT4	PFT5	SFB1	SFB2	SFB3	SFB4	SFB5
	Lipid %	8.5	10.9	6.5	12.5	5.0	10.8	6.9	12.4		11.6	9.6	4.4	11.5	7.1	5.9
	8	—	—	—	—	—	—	—	—		—	—	—	—	—	—
	18	—	37.1	39.6	21.8	53.4	17.6	40.8	28.4		15.6	—	—	30.6	—	—
	28	—	—	—	—	—	—	—	—		—	—	—	—	—	—
	52	813	372	925	506	673	464	1122	760		659	702	—	668	965	873
	44	551	531	870	440	874	395	894	557		358	487	823	493	712	666
	66	—	—	—	—	—	—	—	—		—	—	—	—	—	—
	101	483	730	—	—	—	—	74.4	—		—	—	680	—	—	—
	77	1713	2045	2299	1244	2480	1451	2017	1479		728	1717	2631	1598	2491	2361
	118	—	—	—	—	—	—	—	—		—	—	—	—	—	—
PCB	**153**	504	551	483	278	729	405	532	351		173	368	—	363	498	525
	105	104	90.1	125	79.3	191	77.8	141	87.2		65.1	101	202	91.3	144	143
	138	—	—	—	—	—	—	—	—		—	—	—	—	—	—
	126	170	—	310	190	357	96.3	230	139		154	199	—	117	279	275
	187	3803	2386	4640	2995	7505	3533	4865	3312		1899	3100	5989	2711	4320	4387
	128	—	—	—	—	—	—	—	—		—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—		—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—		—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—		—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—		—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—		—	—	—	—	—	—
	$\sum_{20}$PCB	8141	6743	9692	5754	12863	6440	9916	6715		4053	6675	10325	6072	9408	9231

sample compromised

P=pedestal; S=sand; F=female; T=top; B=bottom; dashes =values below detection limits.

Intrinsic/Extrinsic Variables 4: PCB Variations Due to Colony Water Column Depth and Gender (data as ng/g lipid wt.) Cont'd

	Source	SFB6	SFT1	SFT2	SFT3	SFT4	SFT5	SFT6	PMB1	PMB2	PMB3	PMB4	PMB5	PMT1	PMT2	PMT3
	Lipid %	9.3	11.6	10.9	8.5	10.2	11.3	10.3	5.5	7.9	5.0	5.3	5.9	10.2	7.2	2.8
	8	—	—	—	—	—	—	—	—	—	—	148	—	76.7	—	—
	18	38.3	16.3	30.3	—	29.9	33.0	—	41.0	35.9	49.4	90.0	26.8	36.2	—	—
	28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	52	612	539	745	1050	717	670	568	900	755	1330	1526	650	—	1257	—
	44	680	412	485	614	673	572	568	456	421	722	1020	415	517	607	754
	66	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	101	78.9	—	—	—	46.5	—	59.8	—	—	—	142	691	696	—	962
	77	2341	1480	927	1481	1780	1515	1842	4671	4086	4695	6072	2612	3322	3741	5268
	118	35.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PCB	**153**	657	308	210	360	—	285	527	—	652	1045	1247	748	—	827	—
	105	149	94.8	77.6	123	131	91.9	131	211	210	299	337	171	215	259	409
	138	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	126	—	225	169	216	—	169	—	318	443	633	761	225	—	481	—
	187	4589	2957	2054	3150	3765	2560	4590	8715	4662	7784	8885	3749	6765	6211	9376
	128	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$PCB	9181	6032	4698	6994	7143	5896	8287	15311	11266	16556	20228	9288	11629	13383	16769

P=pedestal; S=sand; F=female; M=male; T=top; B=bottom; dashes =values below detection limits.

Intrinsic/Extrinsic Variables 4: PCB Variations Due to Colony Water Column Depth and Gender (data as ng/g lipid wt.) Cont'd

	Source	PMT4	PMT5	SMB1	SMB2	SMB3	SMB4	SMB5	SMT1	SMT2	SMT3	SMT4	SMT5	SMT6
	Lipid %	5.4	7.6	6.4	7.9	2.5	5.4	6.9	8.8	6.9	7.1	7.9	10.1	5.0
	8	—	—	—	—	—	—	—	—	—	124	—	—	—
	18	—	36.3	70.9	—	—	60.0	54.7	42.0	34.2	55.8	25.7	36.4	44.8
	28	—	—	—	—	—	—	—	—	—	—	—	—	—
	52	1886	725	1676	1375	1172	1274	1019	719	1147	1305	962	1162	1133
	44	1024	479	787	607	686	496	556	474	487	755	400	512	485
	66	—	—	—	—	—	—	—	—	—	—	—	—	—
	101	847	630	—	—	1077	—	—	719	—	76.2	449	36.6	1257
	77	5091	2835	5208	3451	3891	4221	4361	3171	5500	5445	2673	3030	5400
	118	—	45.6	56.4	—	—	—	—	—	—	—	—	—	—
PCB	**153**	—	748	1137	716	1201	954	939	916	1226	—	626	687	1171
	105	394	186	313	333	308	258	205	188	188	293	198	208	212
	138	—	—	—	—	—	—	—	—	—	—	—	—	—
	126	—	265	537	621	413	463	350	267	162	—	360	388	137
	187	8789	4490	7106	5174	7503	5850	6274	5858	4713	8280	4227	4576	3316
	128	—	—	—	—	—	—	—	—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$**PCB**	18031	10440	16891	12276	16252	13576	13760	12355	13457	16335	9920	10637	13156

P=pedestal; S=sand; M=male; T=top; B=bottom; dashes =values below detection limits.

Appendix G

Kinetics Studies 1: PCB Uptake in Colonies Transplanted from Piti Bomb Holes to Dadi Beach (data as ng/g dry wt.)

	Date	9/3/07	9/10/07	9/18/07	9/24/07	10/17/07	12/2/07	9/3/07	9/10/07	9/18/07	9/24/07	10/17/07	12/2/07	9/3/07	9/10/07	9/18/07	9/24/07
	Source	DTO	DTO	DTO	DTO	DTO	DTO	DTY	DTY	DTY	DTY	DTY	DTY	DTB	DTB	DTB	DTB
PCB	**8**	—	—	—	—	—	—	—	—	—	7.36	—	—	—	—	—	—
	18	—	—	—	—	2.38	—	4.93	—	—	—	3.64	—	2.94	—	—	—
	28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	52	6.54	11.0	11.2	12.8	6.33	5.05	7.96	9.75	18.1	9.76	5.48	12.1	7.13	18.7	14.3	8.52
	44	27.3	26.2	16.4	27.8	25.3	40.7	34.0	24.2	20.2	24.8	27.1	50.4	24.1	19.6	19.1	24.5
	66	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	101	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	77	15.7	39.7	70.1	67.9	54.7	282	34.0	42.8	79.6	76.3	124	396	13.6	55.1	93.8	69.7
	118	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	153	17.0	—	—	—	—	—	—	—	—	6.82	—	—	—	—	—	—
	105	17.0		6.20	10.38	14.4	13.1	10.32	7.90	7.20	8.25	11.3	13.9	12.9	14.0	7.52	9.30
	138	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	126	—	13.3	—	9.19	10.7	12.7	8.56	7.99	6.07	—	9.78	—	11.6	11.5	6.84	9.66
	187	193	259	130	290	149	240	259	183	124	114	131	186	187	232	142	189
	128	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$**PCB**	277	349	234	418	262	594	359	275	255	248	312	658	259	351	283	310

D=Dadi site; T=transplanted; O=orange; Y=yellow; B=blue; dashes indicate values below detection limits.

Kinetics Studies 1: PCB Uptake in Colonies Transplanted from Piti Bomb Holes to Dadi Beach (data as ng/g dry wt.) Cont'd

	Date	10/17/07	12/2/07	9/3/07	9/10/07	9/18/07	9/24/07	10/17/07	10/17/07	12/2/07	9/3/07	9/10/07	9/18/07	9/18/07	9/24/07	10/17/07	12/2/07
	Source	DTB	DTB	DTG	DTG	DTG	DTG	DTG	DTG'	DTG	DTOG	DTOG	DTOG	DTOG'	DTOG	DTOG	DTOG
PCB	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	18	—	—	4.26	—	—	—	1.84	—	—	3.80	—	—	—	—	2.36	—
	28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	52	3.77	8.73	7.59	11.9	21.0	10.9	10.9	9.35	7.30	5.79	11.6	16.8	12.4	12.6	7.90	3.07
	44	24.7	39.2	29.3	29.6	28.8	29.1	34.3	32.4	38.4	23.9	25.5	11.4	37.8	30.0	32.5	38.2
	66	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	101	—	—	—	—	—	—	—	—	—	—	—	29.2	—	—	—	—
	77	80.7	224	27.2	50.2	107	67.4	86.8	122	285	17.6	39.9	64.5	62.0	60.4	119	251
	118	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	153	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	105	10.9	12.0	10.8	14.2	16.0	15.4	25.6	13.1	15.0	9.10	10.0	5.54	20.2	12.9	14.7	14.2
	138	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	126	9.68	—	10.3	12.0	13.3	12.6	18.9	—	15.1	9.54	10.0	5.20	19.8	11.2	14.7	14.0
	187	164	239	269	307	334	205	190	263	238	173	244	95.0	241	163	213	164
	128	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$PCB	294	523	358	425	520	341	368	440	599	242	341	228	393	290	404	484

D=Dadi site; T=transplanted; O=orange; Y=yellow; B=blue; G=green; dashes indicate values below detection limits.

Kinetics Studies 1: PCB Uptake in Colonies Transplanted from Piti Bomb Holes to Dadi Beach (data as ng/g dry wt.) Cont'd

	Date	9/3/07	9/3/07	9/10/07	9/18/07	9/24/07	10/17/07	12/2/07	9/3/07	9/24/07	12/2/07	9/3/07	9/24/07	12/2/07	9/3/07	9/24/07	12/2/07
	Source	DTBY	DTBY'	DTBY	DTBY	DTBY	DTBY	DTBY	DCY	DCY	DCY	DCB	DCB	DCB	DCO	DCO	DCO
PCB	**8**	—	—	—	—	—	—	—	—	—	14.6	8.58	10.8	15.9	12.1	11.7	16.5
	18	—	—	—	—	—	2.58	—	3.61	—	—	4.17	—	—	4.72	4.37	—
	28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	52	3.46	3.35	9.40	7.23	14.4	10.6	6.99	107	88.3	91.4	118	109	70.1	132	84.3	109
	44	17.7	20.4	27.0	20.4	29.2	29.4	36.9	79.5	56.1	101	64.6	62.1	89.9	113	87.7	91.8
	66	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	101	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	77	13.0	14.0	38.6	50.3	89.6	87.4	218	1180	950	1165	867	914	906	1700	1523	981
	118	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	153	—	—	—	—	—	—	—	—	—	80.6	—	—	—	—	—	77.1
	105	8.05	9.32	11.1	7.53	8.11	15.8	11.8	10.7	9.60	5.32	11.5	11.0	6.41	13.2	10.6	8.74
	138	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	126	8.94	9.20	12.4	6.51	8.37	14.5	—	45.5	—	31.0	—	66.0	—	54.0	40.0	32.9
	187	170	167	352	319	292	171	214	416	311	205	375	372	241	514	410	360
	128	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$**PCB**	221	224	450	411	442	331	488	1842	1415	1694	1449	1545	1330	2542	2171	1677

D=Dadi site; T=transplanted; C= Dadi site resident control; O=orange; Y=yellow; B=blue; dashes indicate values below detection limits.

Kinetics Studies 1: PCB Uptake in Colonies Transplanted from Piti Bomb Holes to Dadi Beach (data as ng/g lipid wt.) Cont'd

	Date	9/3/07	9/10/07	9/18/07	9/24/07	10/17/07	12/2/07	9/3/07	9/10/07	9/18/07	9/24/07	10/17/07	12/2/07	9/3/07	9/10/07	9/18/07	9/24/07
	Source	DTO	DTO	DTO	DTO	DTO	DTO	DTY	DTY	DTY	DTY	DTY	DTY	DTB	DTB	DTB	DTB
	Lipid %	8.5		10.1	6.9	9.6	8.9	10.1	8.9	10.5	10.0	8.1	11.5	7.9	9.4	7.4	6.5
	8	—		—	—	—	—	—	—	—	73.4	—	—	—	—	—	—
	18	—		—	—	24.9	—	48.6	—	—	—	45.1	—	37.1	—	—	—
	28	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—
	52	76.9		111	185	66.1	56.7	78.6	110	172	97.3	68.0	105	89.7	199	192	132
	44	321		162	403	264	457	335	273	192	248	336	438	303	208	257	378
	66	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—
	101	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—
	77	184		694	984	571	3170	336	483	756	760	1539	3443	171	585	1262	1079
	118	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—
PCB	153	200		—	—	—	—	—	—	—	68.0	—	—	—	—	—	—
	105	199		61.3	150	150	147	102	89.2	68.4	82.2	141	121	163	149	101	144
	138	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—
	126	—		—	133	112	142	84.6	90.2	57.6	—	121	—	146	122	92.0	149
	187	2273		1284	4202	1551	2701	2555	2064	1174	1139	1621	1618	2356	2461	1907	2917
	128	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—
	180	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—
	209	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$PCB	3253		2313	6058	2739	6674	3540	3110	2420	2467	3871	5726	3266	3724	3812	4799

sample compromised

D=Dadi site; T=transplanted; O=orange; Y=yellow; B=blue; dashes indicate values below detection limits.

Kinetics Studies 1: PCB Uptake in Colonies Transplanted from Piti Bomb Holes to Dadi Beach (data as ng/g lipid wt.) Cont'd

	Date	10/17/07	12/2/07	9/3/07	9/10/07	9/18/07	9/24/07	10/17/07	10/17/07	12/2/07	9/3/07	9/10/07	9/18/07	9/18/07	9/24/07	10/17/07	12/2/07
	Source	DTB	DTB	DTG	DTG	DTG	DTG	DTG	DTG'	DTG	DTOG	DTOG	DTOG	DTOG'	DTOG	DTOG	DTOG
	Lipid %	9.6	10.7	15.3	9.7	11.7	7.1	12.3	12.3	9.5	6.7	9.0	9.8	9.8	10.3	10.0	11.0
PCB	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	18	—	—	27.8	—	—	—	14.9	—	—	56.7	—	—	—	—	23.7	—
	28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	52	39.3	81.9	49.7	123	179	154	88.2	75.8	77.2	86.4	129	171	126	122	79.4	27.9
	44	258	368	192	306	246	412	278	263	406	356	284	117	386	290	326	346
	66	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	101	—	—	—	—	—	—	—	—	—	—	—	298	—	—	—	—
	77	842	2104	178	519	916	953	705	991	3015	263	445	659	633	584	1200	2273
	118	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	153	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	105	114	113	70.8	147	137	218	208	106	159	136	112	56.6	207	124	148	128
	138	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	126	101	—	67.6	124	113	178	154	—	160	142	111	53.1	202	109	147	127
	187	1710	2244	1757	3173	2858	2899	1542	2130	2522	2578	2723	970	2465	1579	2137	1485
	128	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$PCB	3064	4910	2343	4392	4450	4814	2991	3566	6339	3619	3803	2325	4019	2808	4061	4388

D=Dadi site; T=transplanted; O=orange; B=blue; G=green; dashes indicate values below detection limits.

Kinetics Studies 1: PCB Uptake in Colonies Transplanted from Piti Bomb Holes to Dadi Beach (data as ng/g lipid wt.) Cont'd

	Date	9/3/07	9/3/07	9/10/07	9/18/07	9/24/07	10/17/07	12/2/07	9/3/07	9/24/07	12/2/07	9/3/07	9/24/07	12/2/07	9/3/07	9/24/07	12/2/07
	Source	DTBY	DTBY'	DTBY	DTBY	DTBY	DTBY	DTBY	DCY	DCY	DCY	DCB	DCB	DCB	DCO	DCO	DCO
	Lipid %	6.5	6.5	9.0	4.5	7.9	10.1	9.6	5.8	6.5		6.9	7.7	11.4	8.4	12.5	12.1
	8	—	—	—	—	—	—	—	—	—		124	141	139	144	93.4	137
	18	—	—	—	—	—	25.5	—	62.6	—		60.0	—	—	56.4	34.9	—
	28	—	—	—	—	—	—	—	—	—		—	—	—	—	—	—
	52	53.4	51.7	105	159	183	104	72.8	1853	1350		1694	1430	614	1574	674	901
	44	273	315	301	449	371	290	384	1377	858		931	812	788	1345	701	761
	66	—	—	—	—	—	—	—	—	—		—	—	—	—	—	—
	101	—	—	—	—	—	—	—	—	—		—	—	—	—	—	—
	77	201	215	431	1107	1141	863	2267	20450	14532		12487	11938	7935	20310	12182	8135
	118	—	—	—	—	—	—	—	—	—		—	—	—	—	—	—
PCB	153	—	—	—	—	—	—	—	—	—	sample compromised	—	—	—	—	—	639
	105	124	144	124	166	103	156	123	186	147		166	143	56.1	158	85.2	72.4
	138	—	—	—	—	—	—	—	—	—		—	—	—	—	—	—
	126	138	142	138	143	107	144	—	789	—		—	862	—	646	320	272
	187	2626	2582	3921	7008	3724	1685	2231	7208	4758		5402	4860	2113	6138	3280	2981
	128	—	—	—	—	—	—	—	—	—		—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—	—		—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—		—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—		—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—		—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—	—		—	—	—	—	—	—
	$\sum_{20}$PCB	3415	3450	5018	9032	5629	3267	5077	31925	21646		20864	20185	11645	30371	17370	13899

D=Dadi site; T=transplanted; C=Dadi site resident control; O=orange; Y=yellow; B=blue; dashes indicate values below detection limits.

Kinetics Studies 2: PCB Loss in Colonies Transplanted from Dadi Beach to Piti Bomb Holes (data as ng/g dry wt.)

	Date	9/3/07	9/10/07	9/18/07	9/24/07	10/17/07	12/2/07	9/3/07	9/10/07	9/18/07	9/24/07	10/17/07	12/2/07	9/3/07	9/10/07	9/18/07	9/24/07
	Source	PTO	PTO	PTO	PTO	PTO	PTO	PTY	PTY	PTY	PTY	PTY	PTY	PTB	PTB	PTB	PTB
PCB	**8**	—	—	—	—	—	—	—	—	—	—	—	16.7	10.6	—	—	9.52
	18	12.4	—	—	—	—	—	6.63	—	—	4.35	4.42	—	4.30	—	—	—
	28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	52	185	62.4	—	74.9	124	42.0	125	81.6	180	90.2	104	37.6	47.6	69.3	53.8	86.3
	44	224	38.2	42.6	48.4	73.1	41.1	107	53.7	68.2	66.6	70.7	54.8	49.2	47.2	46.5	59.3
	66	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	101	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	77	2858	574	532	562	619	411	1557	1060	1085	1238	802	562	709	866	730	852
	118	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	153	—	116	—	—	—	32.7	—	213	193	—	88.2	38.2	—	142	133	124
	105	44.7	8.70	9.50	10.2	15.1	4.16	13.7	10.9	17.8	10.5	9.11	6.51	8.32	10.8	10.3	12.6
	138	—	25.4	—	—	—	—	—	37.6	—	—	—	—	—	35.5	31.4	—
	126	—	26.6	30.6	52.3	—	15.7	61.1	59.7	106	—	56.5	40.1	—	39.4	24.1	51.6
	187	1762	348	351	352	263	132	501	412	403	393	225	108	249	335	286	340
	128	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	180	14.6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$PCB	5102	1200	966	1100	1095	679	2372	1929	2052	1803	1361	864	1078	1545	1315	1536

P=Piti site; T=transplanted; O=orange; Y=yellow; B=blue; dashes indicate values below detection limits.

Kinetics Studies 2: PCB Loss in Colonies Transplanted from Dadi Beach to Piti Bomb Holes (data as ng/g dry wt.) Cont'd

	Date	10/17/07	12/2/07	9/3/07	9/10/07	9/18/07	9/24/07	10/17/07	12/2/07	9/3/07	9/10/07	9/18/07	9/24/07	10/17/07	12/2/07	9/3/07	9/10/07
	Source	PTB	PTB	PTG	PTG	PTG	PTG	PTG	PTG	PTOG	PTOG	PTOG	PTOG	PTOG	PTOG	PTBY	PTBY
PCB	8	—	20.6	—	—	—	8.21	—	10.5	—	16.3	—	—	—	15.1	—	—
	18	3.53	—	3.45	—	—	—	2.45	—	5.47	—	—	—	2.82	—	3.05	—
	28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	52	71.5	41.7	102	—	94.4	91.2	110	73.0	106	73.0	64.7	124	100	52.2	118	93.7
	44	45.2	58.3	67.7	40.1	33.4	44.1	54.3	50.9	90.9	62.4	50.6	63.7	58.9	58.5	67.7	45.4
	66	—	—	—	—	—	—	—	—	—	13.1	—	—	—	—	—	—
	101	—	—	—	—	—	—	—	—	—	—	—	—	80.6	—	—	—
	77	651	624	887	669	776	828	637	530	1373	1180	792	1325	763	641	1043	869
	118	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	153	—	37.5	—	—	169	—	80.5	43.6	—	241	178	275	88.0	44.5	—	182
	105	8.39	7.48	8.91	11.7	8.40	11.3	7.53	5.72	12.8	13.8	9.55	13.1	8.18	5.68	12.6	9.58
	138	—	—	—	—	30.3	—	—	—	—	37.6	—	—	—	—	—	31.9
	126	46.6	44.2	—	64.7	33.7	68.6	49.0	42.7	58.7	54.9	34.7	65.8	46.8	33.4	—	41.2
	187	221	140	348	313	270	365	213	131	499	439	336	522	224	118	444	378
	128	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$PCB	1047	974	1418	1099	1415	1417	1154	888	2145	2134	1465	2389	1372	968	1688	1650

P=Piti site; T=transplanted; O=orange; Y=yellow; B=blue; G=green; dashes indicate values below detection limits.

Kinetics Studies 2: PCB Loss in Colonies Transplanted from Dadi Beach to Piti Bomb Holes (data as ng/g dry wt.) Cont'd

	Date	9/18/07	9/24/07	10/17/07	12/2/07	9/3/07	9/24/07	12/2/07	12/2/07	9/3/07	9/24/07	12/2/07	9/3/07	9/24/07	12/2/07
	Source	PTBY	PTBY	PTBY	PTBY	PCY	PCY	PCY	PCY	PCB	PCB	PCB	PCO	PCO	PCO
PCB	**8**	—	—	—	10.5	—	—	—	—	—	8.50	—	—	—	—
	18	—	—	2.62	—	—	—	—	—	2.47	—	—	—	—	—
	28	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	52	113	52.5	146	71.6	3.56	6.54	—	0.38	5.57	13.5	4.19	—	5.59	—
	44	38.0	39.1	60.0	57.8	15.1	16.4	5.33	4.24	31.4	35.2	—	15.5	20.1	5.72
	66	—	—	—	—	—	5.37	—	—	—	—	—	—	—	—
	101	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	77	1085	846	790	641	12.3	4.94	7.85	3.64	26.5	41.5	21.3	10.0	16.1	7.88
	118	—	—	—	—	—	—	—	17.0	—	—	—	—	15.2	10.7
	153	254	—	—	53.2	—	—	—	—	—	—	17.1	—	—	—
	105	10.9	10.3	10.0	5.99	4.46	6.68	4.71	3.73	22.6	20.0	26.7	3.60	6.84	4.14
	138	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	126	27.1	57.8	53.5	38.6	—	—	—	—	16.5	16.9	—	6.78	5.92	—
	187	293	456	295	162	120	122	51.1	52.9	238	359	200	93.8	100	63.0
	128	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	209	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$**PCB**	1821	1461	1357	1040	155	162	69.0	82.0	343	495	270	130	170	91.4

P=Piti site; T=transplanted; C=Piti resident controls; O=orange; Y=yellow; B=blue; dashes indicate values below detection limits.

Kinetics Studies 2: PCB Loss in Colonies Transplanted from Dadi Beach to Piti Bomb Holes (data as ng/g lipid wt.)

	Date	9/3/07	9/10/07	9/18/07	9/24/07	10/17/07	12/2/07	9/3/07	9/10/07	9/18/07	9/24/07	10/17/07	12/2/07	9/3/07	9/10/07	9/18/07	9/24/07
	Source	PTO	PTO	PTO	PTO	PTO	PTO	PTY	PTY	PTY	PTY	PTY	PTY	PTB	PTB	PTB	PTB
	Lpd %	5.3			4.8	9.0	10.2	7.6	5.3	6.5	11.2	9.0	11.7	5.5	4.6	3.5	5.9
	8	—			—	—	—	—	—	—	—	—	142	192	—	—	163
	18	234			—	—	—	87.4	—	—	38.7	48.9	—	77.7	—	—	—
	28	—			—	—	—	—	—	—	—	—	—	—	—	—	—
	52	3504			1572	1383	411	1643	1534	2773	803	1154	321	860	1499	1532	1474
	44	4243			1016	816	402	1413	1010	1048	593	783	468	888	1022	1324	1013
	66	—			—	—	—	—	—	—	—	—	—	—	—	—	—
	101	—			—	—	—	—	—	—	—	—	—	—	—	—	—
	77	54073	sample compromised	sample compromised	11797	6910	4029	20520	19936	16675	11017	8880	4803	12817	18732	20806	14564
	118	—			—	—	—	—	—	—	—	—	—	—	—	—	—
PCB	153	—			—	—	321	—	3997	2962	—	976	326	—	3065	3788	2122
	105	845			214	168	40.7	181	204	274	93.8	101	55.6	150	233	295	215
	138	—			—	—	—	—	707	—	—	—	—	—	768	895	—
	126	—			1098	—	154	805	1122	1627	—	626	343	—	852	686	881
	187	33337			7398	2933	1289	6604	7747	6196	3502	2495	922	4503	7253	8150	5812
	128	—			—	—	—	—	—	—	—	—	—	—	—	—	—
	180	276			—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—			—	—	—	—	—	—	—	—	—	—	—	—	—
	195	—			—	—	—	—	—	—	—	—	—	—	—	—	—
	206	—			—	—	—	—	—	—	—	—	—	—	—	—	—
	209	—			—	—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$PCB	96513			23094	12210	6647	31252	36258	31555	16047	15063	7381	19489	33423	37477	26245

P=Piti site; T=transplanted; O=orange; Y=yellow; B=blue; dashes indicate values below detection limits.

Kinetics Studies 2: PCB Loss in Colonies Transplanted from Dadi Beach to Piti Bomb Holes (data as ng/g lipid wt.) Cont'd

	Date	10/17/07	12/2/07	9/3/07	9/10/07	9/18/07	9/24/07	10/17/07	12/2/07	9/3/07	9/10/07	9/18/07	9/24/07	10/17/07	12/2/07	9/3/07
	Source	PTB	PTB	PTG	PTG	PTG	PTG	PTG	PTG	PTOG	PTOG	PTOG	PTOG	PTOG	PTOG	PTBY
	Lpd %	7.6	9.3	8.1	5.6	6.6		9.3	9.9	7.9	7.5		7.1	7.6	11.1	6.2
	8	—	221	—	—	—		—	107	—	217		—	—	136	—
	18	46.5	—	42.5	—	—		26.5	—	69.5	—		—	37.1	—	49.4
	28	—	—	—	—	—		—	—	—	—		—	—	—	—
	52	941	447	1257	—	1427		1191	739	1341	973		1755	1318	471	1916
	44	596	624	832	722	505		587	516	1153	832		902	773	528	1097
	66	—	—	—	—	—		—	—	—	175		—	—	—	—
	101	—	—	—	—	—		—	—	—	—		—	1058	—	—
	77	8570	6688	10908	12044	11723	sample compromised	6880	5365	17420	15729	sample compromised	18767	10020	5779	16889
	118	—	—	—	—	—		—	—	—	—		—	—	—	—
PCB	153	—	402	—	—	2548		870	441	—	3209		3900	1155	402	—
	105	110	80.2	110	211	127		81.4	57.9	162	184		186	107	51.2	204
	138	—	—	—	—	458		—	—	—	502		—	—	—	—
	126	613	474	—	1165	509		530	432	744	732		932	615	301	—
	187	2911	1498	4281	5638	4081		2305	1329	6326	5856		7398	2937	1062	7190
	128	—	—	—	—	—		—	—	—	—		—	—	—	—
	180	—	—	—	—	—		—	—	—	45.0		—	—	—	—
	170	—	—	—	—	—		—	—	—	—		—	—	—	—
	195	—	—	—	—	—		—	—	—	—		—	—	—	—
	206	—	—	—	—	—		—	—	—	—		—	—	—	—
	209	—	—	—	—	—		—	—	—	—		—	—	—	—
	$\sum_{20}$PCB	13788	10435	17430	19779	21378		12471	8986	27216	28455		33840	18021	8730	27346

P=Piti site; T=transplanted; O=orange; Y=yellow; B=blue; G=green; dashes indicate values below detection limits.

Kinetics Studies 2: PCB Loss in Colonies Transplanted from Dadi Beach to Piti Bomb Holes (data as ng/g lipid wt.) Cont'd

	Date	9/10/07	9/18/07	9/24/07	10/17/07	12/2/07	9/3/07	9/24/07	12/2/07	12/2/07	9/3/07	9/24/07	12/2/07	9/3/07	9/24/07	12/2/07
	Source	PTBY	PTBY	PTBY	PTBY	PTBY	PCY	PCY	PCY	PCY	PCB	PCB	PCB	PCO	PCO	PCO
	Lpd %	5.9	6.0		8.7	9.8	12.1	14.6	17.6	17.6	8.8	10.7	13.3	10.5	12.6	16.9
	8	—	—		—	107	—	—	—	—	—	79.0	—	—	—	—
	18	—	—		30.2	—	—	—	—	—	28.1	—	—	—	—	—
	28	—	—		—	—	—	—	—	—	—	—	—	—	—	—
	52	1584	1884		1682	728	29.4	44.8	—	—	63.4	126	31.6	—	44.3	—
	44	768	632		691	587	125	112	30.3	24.1	357	328	—	148	160	33.8
	66	—	—		—	—	—	36.8	—	—	—	—	—	—	—	—
	101	—	—		—	—	—	—	—	—	—	—	—	—	—	—
	77	14704	18057		9099	6517	102	33.8	44.6	—	301	386	160	95.5	127	46.5
	118	—	—		—	—	—	2.33	—	96.8	—	—	—	—	121	63.4
PCB	153	3071	4222		—	541	—	—	—	—	—	—	129	—	—	—
	105	162	181		116	60.9	36.9	45.7	26.8	—	258	186	201	34.3	54.3	24.5
	138	540	—		—	—	—	—	—	—	—	—	—	—	—	—
	126	697	451	sample compromised	615	392	—	—	—	—	188	157	—	64.7	47.0	—
	187	6392	4876		3391	1643	990	834	290	301	2713	3340	1510	895	794	372
	128	—	—		—	—	—	—	—	—	—	—	—	—	—	—
	180	—	—		—	—	—	—	—	—	—	—	—	—	—	—
	170	—	—		—	—	—	—	—	—	—	—	—	—	—	—
	195	—	—		—	—	—	—	—	—	—	—	—	—	—	—
	206	—	—		—	—	—	—	—	—	—	—	—	—	—	—
	209	—	—		—	—	—	—	—	—	—	—	—	—	—	—
	$\sum_{20}$PCB	27919	30302		15623	10576	1283	1110	392	422	3909	4602	2033	1237	1348	540

P=Piti site; T=transplanted; C=Piti resident controls; O=orange; Y=yellow; B=blue; dashes indicate values below detection limits.

Kinetic Studies 3: PCB Geometric Means in Colonies Transplanted from Piti Bomb Holes to Dadi Beach (data as ng/g)

Sampling Day		0	7	15	21	44	90
Time Interval (days)		0	7	8	6	23	46
Dry Wt PCB	44	25.5	25.1	22.8	27.5	28.4	40.4
	77	18.9	44.0	74.8	71.3	94.2	270
	187	205	257	197	199	177	212
Lipid Wt PCB	44	291	272	217	345	288	398
	77	215	489	873	894	955	2663
	187	2337	2800	1955	2489	1791	2084

Kinetic Studies 3: PCB Interval and Overall Uptake Rate Constants in Colonies Transplanted to Dadi Beach (data as ng/g)

		kd	ku1	ku2	ku3	ku4	ku5	Ku
Dry Wt PCB	44	0.0014	-5501.53	-81265.11	254552.20	24852.51	96624.34	57852.48
	77	0.0070	31162.49	34906.80	-523.78	12899.42	42042.29	24097.44
	187	0.0138	141616.00	-58564.02	39685.75	21613.21	45930.41	38056.27
Lipid Wt PCB	44	0.0082	-137931.20	-1527180.09	7378024.88	39480.48	1635719.93	1477622.80
	77	0.0142	361668.71	472153.53	131734.22	129225.31	524787.50	323913.85
	187	0.0203	1570646.47	-763391.29	1780484.05	166065.24	612270.18	673214.93

Appendix H

PCBs Accumulated by SPMDs (data as ng/SPMD)

	Dialysate ID	Hex bl	Dial bl	Piti field bl	Dadi field bl	Piti 1	Piti 2	Piti 3	Piti 4	Piti 5	Dadi 1	Dadi 2	Dadi 3	Dadi 4	Dadi 5	Piti ave	Dadi ave
PCB	**8**	–	0.15	–	–	6.48	5.91	–	8.43	13.06	4.97	19.51	23.84	5.90	11.16	8.47	13.08
	18	–	2.05	–	–	5.57	4.68	–	9.42	11.33	33.64	74.91	45.33	70.24	75.75	7.75	59.97
	28	–	–	–	–	–	–	2.76	–	–	–	–	–	–	–	2.76	–
	52	–	–	0.20	0.12	–	–	–	–	–	0.45	–	–	–	–	–	0.45
	44	–	0.44	–	–	–	–	–	0.45	0.49	0.21	1.36	0.44	1.08	1.13	0.47	0.84
	66	5.23	3.81	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	101	2.23	1.45	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	77	–	–	–	–	–	0.02	–	0.07	0.09	1.26	8.45	4.44	5.90	6.52	0.06	5.32
	118	–	0.06	0.07	–	0.01	0.03	0.39	0.05	–	–	–	–	–	–	0.12	–
	153	–	0.12	–	–	0.12	0.13	–	0.23	0.32	–	0.16	0.09	0.10	0.12	0.20	0.12
	105	–	0.39	–	–	–	–	–	–	0.03	–	–	–	–	–	0.03	–
	138	–	–	0.14	–	–	–	19.19	–	–	0.04	–	0.88	1.40	1.39	19.19	0.93
	126	–	–	0.19	–	–	–	–	4.31	5.05	–	2.51	0.41	2.13	2.15	4.68	1.80
	187	0.06	0.22	0.16	0.04	0.15	0.37	–	0.25	0.45	1.30	7.00	2.80	4.14	4.57	0.30	3.96
	128	–	–	0.17	–	–	–	–	0.12	–	–	–	–	–	–	0.12	
	180	0.05	0.06	0.15	–	–	–	3.67	–	–	–	–	–	0.02	–	3.67	0.02
	170	–	–	0.11	–	–	–	–	–	–	–	–	–	–	–	–	–
	195	–	–	0.10	–	–	–	–	–	–	–	–	–	–	–	–	–
	206	–	–	0.14	–	0.10	0.09	–	0.17	0.12	–	0.18	0.09	0.15	0.10	0.12	0.13
	209	–	–	0.10	–	–	–	–	–	–	–	0.08	0.06	–	0.05	–	0.06

Hex=hexane; Dial=dialysis; bl=blank; dashes=values below detection limits. Due to EST Inc. processing: Piti 1, 2, Dadi 1, 2, 3, 4 = 4 SPMDs each; Piti 3, 4, 5 = 8 SPMDs each; Dadi 5 = 10 SPMDs; field bl = 2 SPMDs each. Data displayed was calculated per SPMD accordingly. 2 Dadi SPMDs were lost to biofouling.

Appendix I

Sequestration of PCBs in *S. polydactyla* vs. SPMDs

	Source	Kinetics Controls	SPMDs	Kinetics Controls	SPMDs
	Site	Dadi ng/g	Dadi ng/spmd	Piti ng/g	Piti ng/spmd
	Date	9/3/2007-12/2/2007	11/14/07-11/29/07	9/3/2007-12/2/2007	11/14/07-11/29/07
PCB	**8**	12.88	13.08	8.50	8.47
	18	4.22	59.97	2.47	7.75
	28	—	—	—	2.76
	52	100.95	0.45	5.62	—
	44	82.79	0.84	16.57	0.47
	66	—	—	5.37	—
	101	—	—	—	—
	77	1131.88	5.32	15.19	0.06
	118	—	—	14.34	0.12
	153	78.83	0.12	17.11	0.20
	105	9.68	—	10.35	0.03
	138	—	0.93	—	19.19
	126	44.88	1.80	11.53	4.68
	187	355.99	3.96	139.99	0.30
	128	—	—	—	0.12
	180	—	0.02	—	3.67
	170	—	—	—	—
	195	—	—	—	—
	206	—	0.13	—	0.12
	209	—	0.06	—	—
	$\sum_{20}$**PCB**	1740.61	86.69	196.68	47.94

Dashes=values below detection limits. Data represent average result for group.