DEPARTMENT OF THE INTERIOR
U.S. FISH AND WILDLIFE SERVICE
REGION # 1

FY08 ENVIRONMENTAL CONTAMINANTS PROGRAM
ON-REFUGE INVESTIGATIONS SUB-ACTIVITY

PI - Contamination and Potential Impacts to Monk Seals
Project ID:1N51

by

Lee Ann Woodward, PhD
Resource Contaminants Specialist

for

Barry Stieglitz
Project Leader
Hawaiian and Pacific NWRC
June 14, 2008

Congressional District # HI02

II. INTRODUCTION

II.A. Background and Justification

The Hawaiian monk seals are distributed primarily in the Northwestern Hawaiian Islands. The population of this stock was approximately 1,300 animals in 1998. While the diet of the seals is highly variable (e.g., cephalopod, crustacean and teleost), they forage in relatively localized areas in association with a geographical subpopulation (Goodman-Lowe1998; Parrish et al. 2000). Migration of individuals among subpopulations is uncommon (Johnson and Kridler, 1983). The largest subpopulation of the monk seals is found at French Frigate Shoals. This group has been in decline since 1989 (Forney et al. 1999).

There are six main Hawaiian monk seal breeding colonies (French Frigate Shoals, Kure Atoll, Laysan Island, Lisianski Island, Pearl and Hermes Reef and Midway Atoll) in the Northwestern Hawaiian Islands (NWHI) (Gilmartin and Eberhardt 1995; Ragen and Lavigne 1999; Carretta et al., 2005, Antonelis et al. in press). The islands and atolls that comprise these colonies extend approximately 2,000 km northwest of the main Hawaiian Islands (*e.g.*, Kauai, Oahu, Maui) (Marine Mammal Commission 2002). A small number of monk seals have also been observed on the main Hawaiian Islands and recent surveys in 2000 and 2001 indicate that the number of animals on these islands appear to be increasing (Baker *et al*. 2004). Most animals show a high degree of fidelity to their birth colony even though land observations show that some individuals move between atolls or islands (Ragen and Lavigne 1999, Antonelis *et a*l. in press).

French Frigate Shoals (FFS) is located 700 miles Northwest of the main Hawaiian Islands, North Pacific Ocean. Tern and East Islands are the two largest islands at French Frigate Shoals. In 1942, the United States Navy (Navy) constructed aircraft refueling facilities at French Frigate Shoals. To accommodate the runway, the size of Tern Island was doubled by constructing a double-edged steel sheet sea wall. The island was filled with dredged, crushed coral and debris (Amerson 1971). Concurrently the United States Coast Guard (Coast Guard) operated a Long Range Navigation station on East Island from 1942-1952 and subsequently on Tern Island from 1952-1978 after the Navy's departure in 1946 (Amerson 1971). Control of French Frigate Shoals was returned to the United States Fish and Wildlife Service (Service) in 1979.

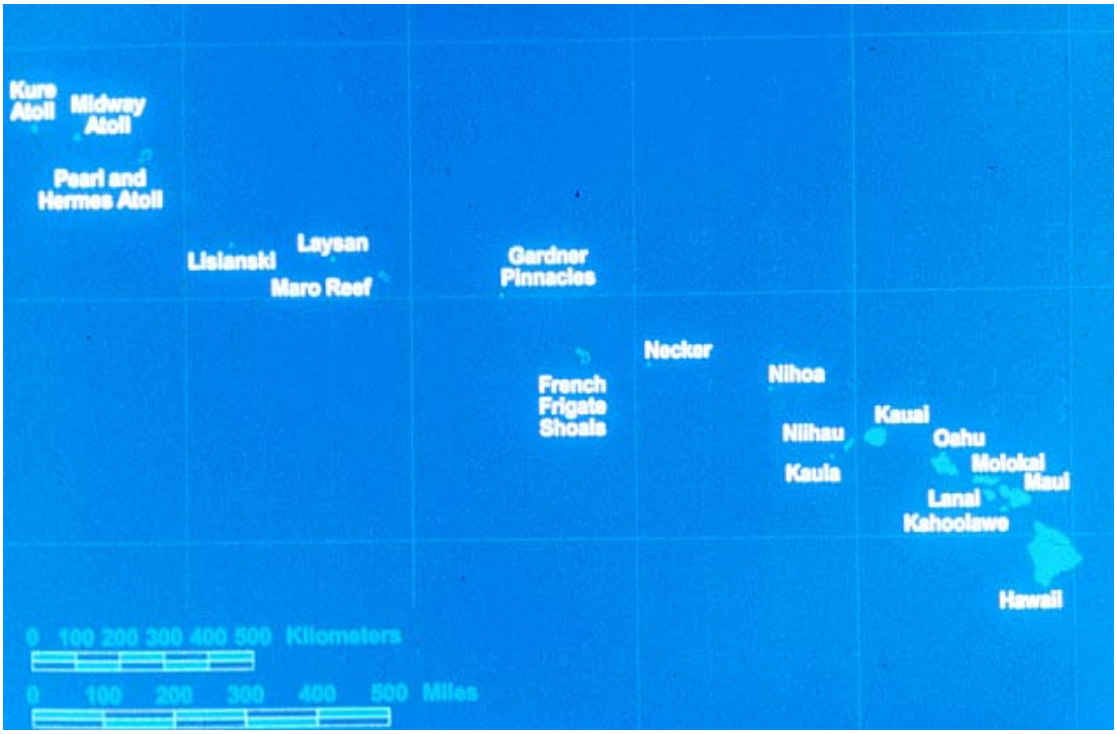

Figure 1. Map of the Hawaiian Islands including the Northwest Hawaiian Islands. Hawaiian Monk Seals were sampled at French Frigate Shoals, Kure Atoll, Laysan Island, Lisianski Island, Pearl and Hermes Reef and Midway Atoll.

Polychlorinated biphenyls (PCBs) were found in various marine species collected from French Frigate Shoals (Miao et al., 2000a & b and 2001). PCBs and chlorinated pesticides, collectively referred to as organochlorines (OCs), are lipophilic and bioaccumulative. Twelve of the 209 possible PCB congeners, are termed "dioxin-like" (Van den Berg *et al.* 1998), and generally considered the most toxic PCBs because they exert similar toxic effects as 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) (Safe 1987; 1994). Two important metabolites of the insecticide dichlorodiphenyltrichloroethane (DDT) are dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD) (USEPA 1999). Although open uses and the manufacture of PCBs in the U.S. were ceased in 1977 (Beeton *et al.* 1979) and use of DDT was banned in the U.S. in 1972 (Ahmed 1991; Dunlap 1998), they continue to be used in other parts of the world, and are found in environmental samples from all over the world (Barrie *et al.* 1992; Muir *et al.* 1999; Becker *et al.* 2000).

PCBs and DDTs have a variety of biological effects on various species of animals. In mammals, levels of PCBs greater than 4,000 ng/g in the liver are associated with lethality, levels greater than 10,000 ng/g in the fat show reproductive impairment (Kamrin and Ringer 1996). In pinnipeds, levels greater than 30,000 ng/g in the blubber and 3,000 ng/g in the liver are significantly associated with premature parturition (Kamrin and Ringer 1996). A study on harbor seals (*Phoca vitulina*) indicated that PCBs could cause immunosuppression (de Swart et

al. 1996). In addition, Brouwer and colleagues (1989) demonstrated that the harbor seals fed contaminated fish that contained high levels of PCBs and DDTs (average 1.5 mg and 0.4 mg per day, respectively) showed a reduction of both plasma retinol and thyroxine levels compared to seals that were fed fish that had low levels of these compounds (average 0.22 mg and 0.13 mg per day, respectively). Takei and Leong (1981) detected high levels of PCBs and p,p'-DDE in various tissues from a single captive adult male Hawaiian monk seal which died without known cause.

II.B. Scientific Objective(s)

The first objective of this study was to measure PCBs and DDTs in the Hawaiian monk seal population at French Frigate Shoals, North Pacific Ocean. Correlations between OC levels and age, sex, morphometric data were examined in monk seals.

The second objective of this study was to measure and compare PCBs and DDTs in the Hawaiian monk seal population from the six main Hawaiian monk seal breeding colonies (French Frigate Shoals, Kure Atoll, Laysan Island, Lisianski Island, Pearl and Hermes Reef and Midway Atoll) in the Northwestern Hawaiian Islands.

II.C. Management Action(s)

The results from this study have been used in Ecological Risk Assessments for Tern Island, French Frigate Shoals. This resulted in the Coast Guard removal of 1,600 cubic yards of PCB contaminated material from landfill costing approximately $2.6 Million. Unfortunately the Coast Guard cleanup site characterization and project scope greatly underestimated extent of contamination. USEPA has directed the Coast Guard to finish the remediation which would remove the remaining contamination (estimated cost $0.9 Million). To date the Coast Guard has not complied.

These data will be used as a baseline for all future monitoring of contaminant conditions in the NWHI.

III. METHODS

Personnel from the NOAA National Marine Fisheries Service's Pacific Islands Fisheries Science Center collected whole blood and biopsy blubber samples from free-ranging Hawaiian monk seals at four Northwestern Hawaiian Islands reproductive colonies (Laysan Island, Midway Atoll, Pearl and Hermes Reef and French Frigate Shoals) from 1998 –2002. Whole blood and a subset of biopsy blubber samples of Hawaiian monk seals were analyzed for selected OCs by a high-performance liquid chromatography/photodiode array (HPLC/PDA) method (Krahn *et al*. 1994) and for lipid classes and percent lipid by thin-layer chromatography with flame ionization detection (TLC/FID) (Krahn *et al.* 2001; Ylitalo *et al.* 2005).

IV. RESULTS

One hundred seventy five PCBs were measured in tissue samples from all the Hawaiian monk seals examined. Blubber ΣPCB levels ranged from 98 – 25,000 ng/g, lipid weight (lw) and blood ΣPCB levels from 50 – 35,000 ng/g, lw (Table 1). Virtually all (95%) of the blubber samples and most (> 50%) of the blood samples contained p,p'-DDE. Levels (lipid weight) of this DDT metabolite ranged from 50 – 5,500 ng/g in blubber and 6 – 5,500 ng/g in blood. The dioxin-like congeners PCBs 77, 126, 157, 169 and 189 were not detected in any of the samples. HCB was detected in five blubber samples, two from FFS, two from Laysan, and one from Pearl and Hermes Reef. Levels ranged from 6.1 –10 ng/g, lw in blubber but HCB was not detected in any blood samples. Blubber percent lipid ranged from 9.7 – 71%, composed primarily of triglycerides. The percent lipid in blood ranged from 0.015 – 0.58% and contained sterol/wax esters, cholesterol, triglycerides and phospholipids. OC levels in blubber were lower in adult females than in adult males or juveniles. Average levels of ΣPCBs, ΣPCB TEQs and p,p'-DDE (based on lipid weight) in blubber of adult females were 1.8 to 8 times lower than those of adult males and 1.8 – 3.6 times lower than levels in juveniles at Laysan and at Midway. There were no consistent differences in average concentration of OCs in blood however between juvenile and adult seals. For example, the average blood ΣPCB level was higher in adult females than juveniles and adult males at FFS but there were no differences in mean ΣPCBs among juveniles, adult males and adult females at Pearl and Hermes Reef.

Concentrations of OCs measured in matched blood and blubber pairs of 63 Hawaiian monk seals were significantly correlated. Percent lipid values were only weakly correlated for matched pairs of blood and blubber samples. Moreover, some of the larger, more lipophilic OCs (*e.g.*, 170/194, 180) that were measured in most of the biopsy blubber samples (> 75%) were detected in < 20% of the corresponding whole blood samples. Blubber ΣPCB and p,p'-DDE levels increased with age throughout life in males. They also increased with age in females but only until seals were sexually mature and then decreased. The exception in females was the oldest one (13 years old) which had higher levels of ΣPCBs and p,p'-DDE in blubber than did younger adult females.

Concentrations of OCs were more variable with age in blood than in blubber in males and females. The average ΣPCB and ΣPCB TEQ levels in blubber were higher in seals at Midway than at other colonies, after accounting for seal age. Although PCBs 138 and 153 were the largest contributors to blubber ΣPCBs in seals from all breeding colonies, more PCB congeners were measured in seals at Midway than in seals at the other colonies. Average blubber p,p'-DDE levels (data not shown), based on wet and lipid weights, were more similar among seals from different colonies than were average concentrations of PCBs or PCB TEQs. Mean DDE (based on lipid weight) was only significantly greater for juveniles at Midway compared with those at Laysan and FFS ($p < 0.0001$). Adult females from Laysan had a significantly higher ($p = 212$ 0.0035) mean lipid concentration than adult females from Midway Atoll. OC concentrations in

blood did not differ among juveniles, adult males and adult females throughout the NWHI. Though the average ΣPCB level (based on lipid weight) was higher in juveniles at Midway than at FFS, the average PCB TEQ levels were higher in adult males at FFS versus Pearl and Hermes Reef . PCB 101 and PCB 153, as well as other PCBs (e.g., PCBs 28, 52, 66, 95, 110) were the predominant congeners measured in whole blood samples of Hawaiian monk seals throughout the NWHI. Seals at Midway had a wider suite of PCB congeners in blood than seals at the other colonies. Average blood levels of p,p'-DDE did not vary significantly ($p > 0.05$) among colonies. Average blood percent lipid of seals from Pearl and Hermes Reef was higher than in seals from all other colonies. Those differences were significant for juveniles ($p = 0.0067$) and adult males ($p < 0.0001$) but not for adult females ($p > 0.05$).

Summary

Concentrations of ∑PCBs, ∑PCB TEQs and p,p'-DDE (both wet weight and lipid weight) in matched blood and blubber samples from 63 Hawaiian monk seals were significantly correlated ($p < 0.05$). Reproductive status (*i.e.*, juvenile, adult male, reproductive female) appeared to influence the concentrations of OCs measured in blubber of Hawaiian monk seals, with reproductive females having decreased blubber concentrations compared to adult males or sexually immature monk seals at each site. In general, the blubber concentrations of ∑PCBs and p,p'-DDE increased with age in both male and females up to sexual maturity (> 4 years of age). In animals older than age four years, OC levels increased with increasing age in males whereas contaminant concentrations appeared to decrease with increasing age in females, presumably due to transfer of these compounds from mother to pup during gestation and lactation. However, in monk seal blood, no consistent differences in blood OC levels were found based on sex or reproductive status. Mean levels of PCBs measured in blubber of juvenile, adult male and reproductive female monk seals were elevated in animals from Midway Atoll compared to seals from the other breeding sites. The average blubber p,p'-DDE levels, however, were not significantly different among monk seals from the four colonies. Additionally, there were no clear differences in mean OC levels of whole blood among Hawaiian monk seal colonies.

Although the concentrations of PCBs and PCB TEQs measured blubber and blood of a small number of monk seals were above threshold levels associated with immune dysfunction (Table 3, Figures 2 & 3) in various species of mammals, the findings of the current study suggest that levels of these contaminants in monk seals are not elevated compared to other populations of pinnipeds from the eastern North Pacific.

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VI. BUDGET

EXPENDITURES	Year 1 FY 2000	Year 2 FY 2001	All Years
Field Operations			
Cooperators: University of Hawaii National Marine Fisheries Service (NMFS)			
Hormone Analysis (UH student)	$ 20,000	$ 0	**$ 0**
Chemical Analysis (Non-PACF) NMFS Montlake Lab (UH student)	$ 20,000	$ 0	**$ 20,000**
Travel	$ 4,000	$ 0	**$ 4,000**
Supplies/equipment	$ 10,000	$ 0	**$ 10,000**
U-Hawaii Subtotal	$ 54,000	$ 0	**$ 54,000**
U-Hawaii Overhead (8%)	$ 4,320	$ 0	**$ 4,320**
U-Hawaii Total	$ 58,320	$ 0	**$ 58,320**
Personnel - Data Analysis	$ 3,000	$ 10,000	**$ 13,000**
Personnel - Report Writing	$ 2,000	$ 10,000	**$ 12,000**
Operational Subtotal	$ 63,320	$ 20,000	**$ 83,320**
Refuge Overhead (8.7%)	$ 5,509	$ 1,740	**$ 7,249**
PACF Funding			
Analytical Subtotal	$ 0	$ 0	**$ 0**
TOTAL FUNDING	**$ 68,829**	**$ 21,740**	**$ 90,569**

Table 1: Mean (± SE) ages, percent lipid and ΣPCBs, ΣPCB TEQs and p,p'-DDE levels
measured in blubber of juvenile and adult Hawaiian monk seals at four breeding colonies in the Northwestern Hawaiian Islands.

	Age (years)	Percent lipid	wet weight ∑PCBs ng/g	wet weight ∑PCB TEQs pg/g	wet weight *p,p′*-DDE ng/g	lipid weight ∑PCBs ng/g	lipid weight ∑PCB TEQs pg/g	lipid weight *p,p′*-DDE ng/g
French Frigate Shoals								
juvenile (n = 28)	0.78 ± 0.048	44 ± 3.2	340 ± 80	7.5 ± 2.6	250 ± 59	980 ± 220	21 ± 6.4	700 ± 160
adult male (n = 7)	12 ± 0.96	38 ± 5.4	480 ± 120	9.1 ± 4.0	160 ± 38	1,400 ± 280	25 ± 9.1	440 ± 78
		p = 0.4171	*p* = 0.0754	*p* = 0.4643	*p* = 0.9619	*p* = 0.0812	*p* = 0.3829	*p* = 0.7798
Laysan Island								
juvenile (n = 3)	1 ± 0.0	47 ± 1.5	400 ± 120	4.9 ± 1.6	260 ± 89	850 ± 240	11 ± 3.3	560 ± 190
adult male (n = 9)	15 ± 1.4	46 ± 4.3	400 ± 100	4.5 ± 1.1	280 ± 94	910 ± 230	9.8 ± 2.1	590 ± 170
reproductive female (n = 2)	12 ± 4.2	57 ± 7.5	290 ± 210	2.9 ± 2.3	190 ± 140	480 ± 300	4.7 ± 3.5	300 ± 210
		p = 0.5404	*p* = 0.6617	*p* = 0.3887	*p* = 0.8192	*p* = 0.4520	*p* = 0.2076	*p* = 0.8074
Midway Atoll								
juvenile (n = 13)	1.6 ± 0.29	42 ± 3.4	1,300 ± 200	18 ± 3.1	410 ± 95	3,200 ± 510	43 ± 6.4	930 ± 190
adult male (n = 5)	9.8 ± 1.5	27 ± 4.3	2,500 ± 1,300	18 ± 4.8	410 ± 110	8,800 ± 4,000	69 ± 13	1,500 ± 240
reproductive female (n = 6)	9.5 ± 1.1	27 ± 2.6	300 ± 75	3.2 ± 0.80	76 ± 25	1,100 ± 270	12 ± 2.5	270 ± 77
		***p* = 0.0087**	***p* = 0.0009**	***p* = 0.0003**	***p* = 0.0030**	***p* = 0.0005**	***p* = 0.0002**	***p* = 0.0018**
Pearl and Hermes Reef								
adult male (n = 6)	7.8 ± 1.8	40 ± 5.2	1,100 ± 630	13 ± 7.0	570 ± 390	2,400 ± 1,300	28 ± 15	1,200 ± 840

Significant differences (one-way ANOVA, $\alpha = 0.05$) in concentrations of OCs based on reproductive status for each breeding colony are shown in bold.

Table 2: Mean (± SE) lipid content and concentrations of ΣPCBs, ΣPCB TEQs and p,p'-DDE in whole blood of juvenile and adult Hawaiian monk seals sampled at four breeding colonies in the Northwestern Hawaiian Islands.

			wet weight			**lipid weight**		
	Age (years)	**Percent lipid**	**∑PCBs** ng/g	**∑PCB TEQs** pg/g	***p,p'*-DDE** ng/g	**∑PCBs** ng/g	**∑PCB TEQs** pg/g	***p,p'*-DDE** ng/g
French Frigate Shoals								
juvenile (n = 64)	1.0 ± 0.15	0.13 ± 0.014	1.5 ± 0.25	0.017 ± 0.0044	0.24 ± 0.055	1,800 ± 360	22 ± 7.4	330 ± 97
adult male (n = 9)	11 ± 0.85	0.043 ± 0.0061	1.5 ± 0.39	0.026 ± 0.011	0.12 ± 0.045	4,100 ± 980	81 ± 30	270 ± 100
reproductive female (n = 6)	12 ± 1.1	0.094 ± 0.015	6.3 ± 2.2	0.019 ± 0.011	ND†	6,800 ± 1,900	17 ± 8.0	ND†
		***p* = 0.0133**	***p* = 0.0024**	***p* = 0.0162**	*p* = 0.6453	***p* = 0.0003**	***p* = 0.0008**	*p* = 0.2202
Laysan Island								
juvenile (n = 4)	1.0 ± 0.0	0.037 ± 0.0085	1.5 ± 0.53	0.018 ± 0.0066	ND†	4,900 ± 2,100	60 ± 28	ND†
adult male (n = 10)	15 ± 1.2	0.051 ± 0.010	3.5 ± 2.4	0.045 ± 0.037	ND†	6,400 ± 3,200	70 ± 55	ND†
reproductive female (n = 1)†	15	0.055	1.6	0.012	ND†	2,900	21	ND†
		p = 0.4678	*p* = 0.8807	*p* = 0.8106		*p* = 0.8242	*p* = 0.9793	
Midway Atoll								

juvenile (n = 17)	1.5 ± 0.23	0.086 ± 0.0080	3.3 ± 0.62	0.041 ± 0.0084	0.52 ± 0.13	3,900 ± 590	47 ± 6.4	610 ± 150
adult male (n = 6)	9.3 ± 0.86	0.096 ± 0.014	7.1 ± 2.6	0.045 ± 0.015	0.42 ± 0.19	6,300 ± 2,000	42 ± 10	400 ± 160
reproductive female (n = 13)	9.3 ± 1.3	0.10 ± 0.0087	3.4 ± 1.1	0.030 ± 0.0065	0.15 ± 0.058	3,400 ± 1,100	32 ± 7.9	120 ± 42
		p = 0.3624	p = 0.4422	p = 0.4618	p = 0.1233	p = 0.2227	p = 0.1521	**p = 0.0476**
Pearl and Hermes Reef								
juvenile (n = 4)	2.0 ± 0.47	0.29 ± 0.13	3.3 ± 0.31	0.018 ± 0.0076	0.14 ± 0.075	2,100 ± 780	15 ± 9.1	140 ± 82
adult male (n = 7)	7.4 ± 1.5	0.30 ± 0.055	3.9 ± 0.67	0.022 ± 0.0062	0.59 ± 0.46	1,800 ± 530	12 ± 4.9	290 ± 240
reproductive female (n = 4)	9.8 ± 2.1	0.26 ± 0.14	2.8 ± 0.35	0.025 ± 0.011	0.23 ± 0.15	1,900 ± 710	20 ± 7.7	160 ± 87
		p = 0.8645	p = 0.6291	p = 0.9977	p = 0.9413	p = 0.9633	p = 0.9531	p = 0.9474

Significant differences (one-way ANOVA, $\alpha = 0.05$) in OC concentrations based on reproductive status for each breeding colony are shown in bold.
[†] Not included in statistical analysis

ND = not detected in any of the samples analyzed.

Age Class	PCB118 TEQ	HQ	PCB156 TEQ	HQ	PCB118 +156	HI
Adult Female nonparous	81.9	**1.82**	22.86	0.51	107.76	**2.39**
Juvenile Female	49.9	**1.11**	48.15	**1.07**	98.05	**2.18**
Subadult Female nonparous	7.4	0.16	37.14	0.82	44.54	0.99
Adult Female parous	7.3	0.16	27.96	0.62	35.26	0.78
Adult Male	164.1	**3.65**	52.09	**1.16**	216.19	**4.8**
Juvenile Male	49.5	**1.1**	35.04	0.78	84.54	**1.88**
Subadult Male	9.4	0.21	35.8	0.8	45.2	**1.004**

Table 3. Hazard Quotents (HQ) and Hazard Index (HI) for Hawaiian Monk Seals at Tern Island, French Frigate Shoals.

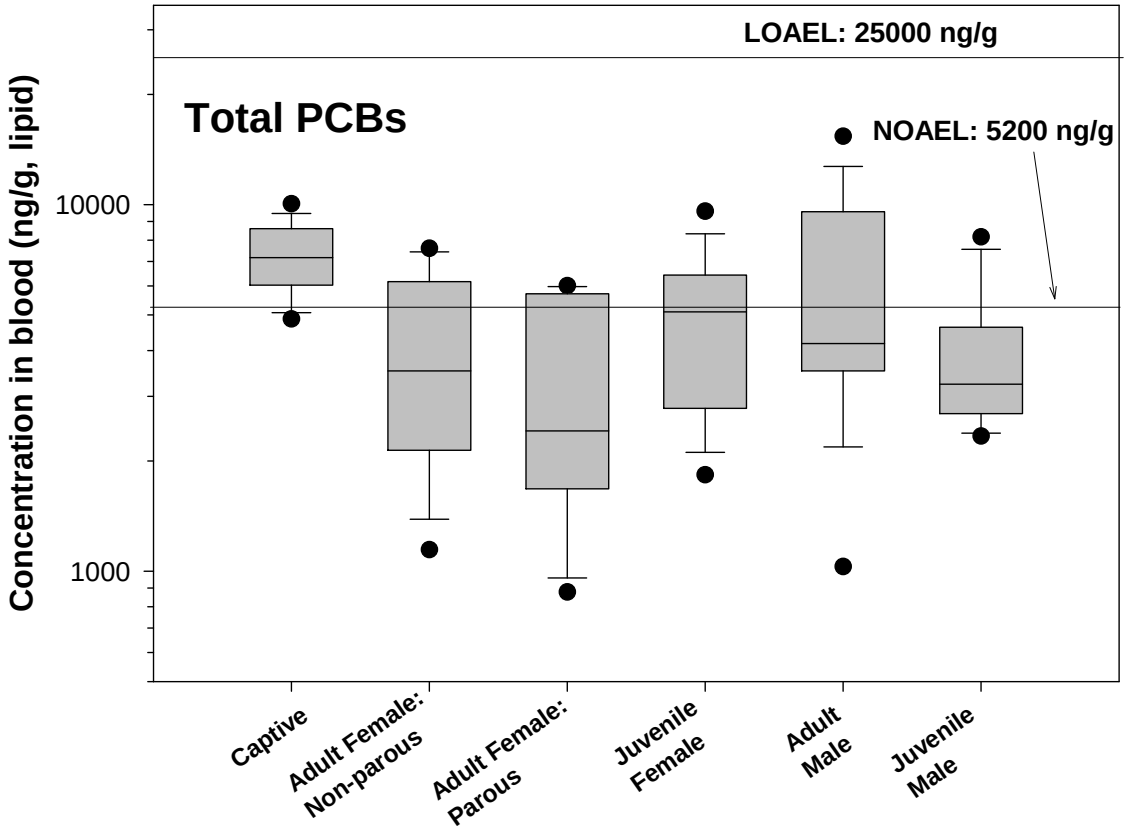

Figure ??. Comparison of concentrations of total PCBs (ng/g, lipid) to reproductive effect thresholds from Kamrin and Ringer (1996) and Kannan et al. (2000).

Figure 2. Taken from Coast Guard Ecological Risk Assessment for Tern Island, French Frigate Shoals (USCG 2001).

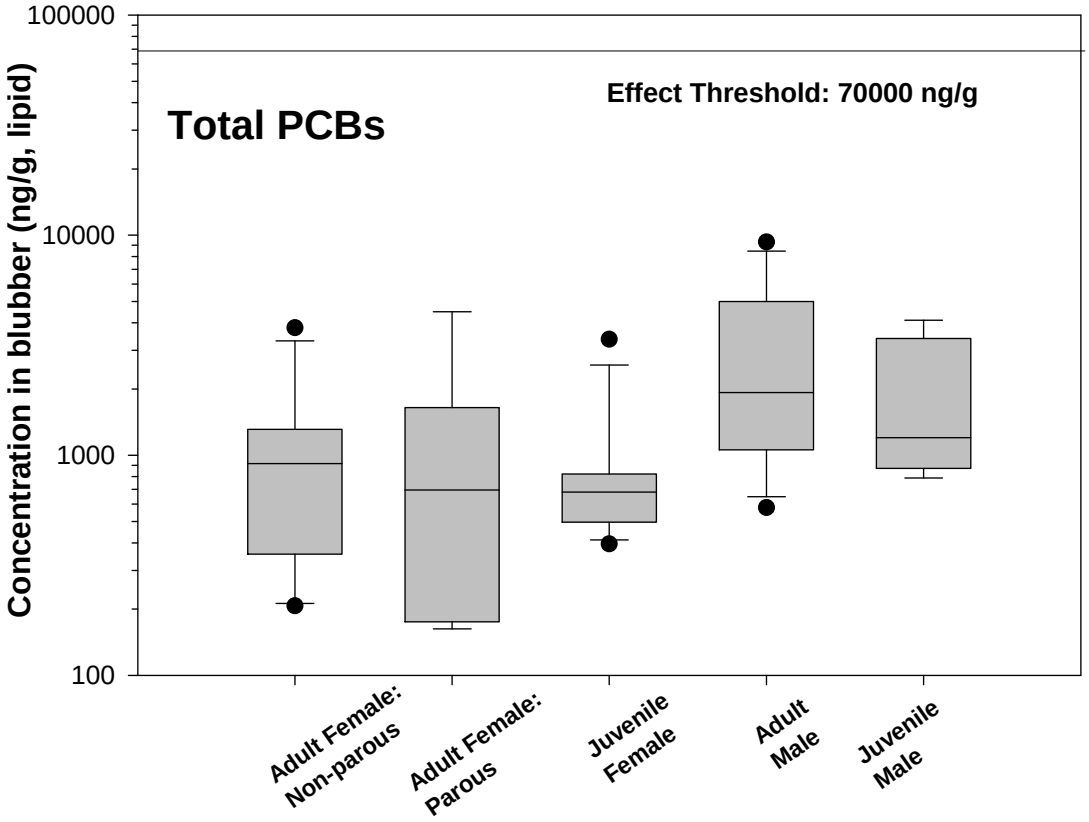

Figure ??. Comparison of concentrations of total PCBs (ng/g, lipid) in blubber to reproductive effect thresholds from Kamrin and Ringer (1996).

Figure 3. Taken from Coast Guard Ecological Risk Assessment for Tern Island, French Frigate Shoals (USCG 2001).