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ACCUMULATION OF CURRENT-USE AND ORGANOCHLORINE PESTICIDES IN CRAB EMBRYOS FROM NORTHERN CALIFORNIA, USA

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Abstract—Invertebrates have long been used as resident sentinels for assessing ecosystem health and productivity. The shore crabs, *Hemigrapsus oregonensis* and *Pachygrapsus crassipes*, are abundant in estuaries and beaches throughout northern California, USA and have been used as indicators of habitat conditions in several salt marshes. The overall objectives of the present study were to conduct a lab-based study to test the accumulation of current-use pesticides, validate the analytical method and to analyze field-collected crabs for a suite of 74 current-use and legacy pesticides. A simple laboratory uptake study was designed to determine if embryos could bioconcentrate the herbicide molinate over a 7-d period. At the end of the experiment, embryos were removed from the crabs and analyzed by gas chromatography/mass spectrometry. Although relatively hydrophilic (log K_{OW} of 2.9), molinate did accumulate with an estimated bioconcentration factor (log BCF) of approximately 2.5. Following method validation, embryos were collected from two different Northern California salt marshes and analyzed. In field-collected embryos 18 current-use and eight organochlorine pesticides were detected including synthetic pyrethroids and organophosphate insecticides, as well as DDT and its degradates. Lipid-normalized concentrations of the pesticides detected in the field-collected crab embryos ranged from 0.1 to 4 ppm. Pesticide concentrations and profiles in crab embryos were site specific and could be correlated to differences in land-use practices. These preliminary results indicate that embryos are an effective sink for organic contaminants in the environment and have the potential to be good indicators of ecosystem health, especially when contaminant body burden analyses are paired with reproductive impairment assays. Environ. Toxicol. Chem. 2010;29:2593–2599. © 2010 SETAC

Keywords—Current-use pesticides Persistent organochlorine pollutants Crab embryos Accumulation

INTRODUCTION

A wide variety of pesticides are currently applied in agricultural and urban areas throughout the United States. Many different types of pesticides are applied concurrently and are transported off-site dissolved in water and bound to suspended sediments. The exposure of aquatic organisms to these currently applied pesticides and the resulting effects are not well understood.

The accumulation of hydrophobic organic chemicals by invertebrates has proven useful in environmental monitoring for polychlorinated biphenyls (PCBs) [1], organochlorine pesticides (OCPs) [2–4], polychlorinated benzo-*p*-dioxins and furans (PCDD/F) [1,5] and organophosphate insecticides (OPs) [3,6,7]. Many of these chemicals are known to cause sublethal effects in aquatic organisms, including reproductive impairment and decreases in hatching success [8–10]. Furthermore, early life stages are typically more sensitive to toxicants than adults and are commonly used in toxicity testing [11] and for overall ecosystem health assessments [12].

Invertebrates have long been used as resident sentinels for assessing ecosystem health and productivity [3,13,14]. Shore crabs are often chosen as appropriate bioindicators because they tend to be abundant in estuaries and beaches both in terms of numbers and biomass [15]. Sediment-dwelling crabs also tend to have small home ranges and are relatively immobile compared to other organisms such as fish. Two shore crabs, *Hemigrapsus oregonensis* and *Pachygrapsus crassipes* are abundant throughout northern California, USA and have been used as indicators of habitat conditions in several salt marshes [16]. These two species are hardy and abundant, even at contaminated sites.

The overall objective of the present study was to determine if crab embryos are effective bioindicators of pesticide contamination, particularly current-use pesticides. The specific objectives were to conduct a simple laboratory uptake study to determine if current-use pesticides concentrate in crab embryos, and measure pesticides in field-collected crab embryos, from two sites in Northern California with different types of land use.

MATERIALS AND METHODS

Study areas and sample collection

Ovigerous female crabs were collected from two sites in northern California in the summers of 2005 and 2006. *Hemigrapsus oregonensis* (*H. oregonensis*) and *Pachygrapsus crassipes* (*P. crassipes*) were collected from Bodega Bay (2005 and 2006) and from Stege Marsh (2006), California, USA. Bodega Bay is a shallow rocky inlet of the Pacific Ocean located northwest of San Francisco (CA, USA). Crabs were collected from a rocky intertidal area located directly downstream of a suburban golf course community. Stege Marsh is a U.S. Environmental Protection Agency (U.S. EPA) Superfund site located in San Francisco Bay within the city of Richmond (CA, USA). This salt marsh is surrounded by residential and industrial property and is impacted by a complex suite of urban and industrial contaminants. The marsh has had a long and complex history of contamination including heavy metals (Pb, As, Zn, and Cd) and organic contaminants (PCBs and polycyclic aromatic hydrocarbons [PAHs]). More recently, areas of the marsh

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have been contaminated with a suite of current-use pesticides including S-ethyl dipropylthiocarbamate (EPTC), cycolate, molinate, and prometryn, from a former agrochemical manufacturing plant [17]. Depending on species, crabs were collected from either the vegetated mid-intertidal (*P. crassipes*) or the lower rocky intertidal zone (*H. oregonensis*).

Crab embryos were removed from the female's carapace using solvent-rinsed stainless steel tweezers, placed in clean, solvent-rinsed glass jars, and frozen at −40°C prior to analysis. All *H. oregonensis* samples were a composite of approximately five different females due to the small size of the individual clutches. *Pachygrapsus crassipes* clutch size is significantly larger than that of *H. oregonensis*; therefore, each of these samples was from a single crab.

Laboratory uptake study

A simple 7-d uptake study was conducted to determine if crab embryos were able to bioconcentrate hydrophilic, current-use pesticides from the water. In a previous study, abnormalities in crab embryos were correlated with sediment concentrations of two current-use pesticides (EPTC and molinate) from Stege Marsh (Kuivila et al., unpublished data). Molinate was chosen as the test herbicide for the uptake experiments because it is slightly more hydrophobic than EPTC and could have the potential to accumulate in crab embryos. Gravid female *H. oregonensis* collected from Bodega Bay in 2005 were exposed to two different concentrations (5 and 15 ppb) of molinate. The stages of the embryos were determined using a light microscope, and only crabs with embryos in stages 1 to 8 were used to avoid hatching out during the exposure. Uptake experiments were conducted in triplicate for each concentration, as well as a control. Ten gravid crabs were placed in glass containers, 2 L of sea water (with and without molinate) was added, and the crabs were incubated at 23°C with a 12 h light:dark cycle. To keep the concentration of molinate constant in each treatment group and to remove fecal material, the water was changed daily, respiked, and a water sample was collected to measure the change in concentration over the one-week experiment. After one week, the gravid crabs were removed from the containers and the stages of the embryos were determined. The embryos were removed from the female using stainless steel tweezers and placed in clean glass jars, and frozen at −40°C prior to extraction and analysis. Embryos were analyzed for molinate, as well as a suite of 73 other pesticides.

Water samples, collected daily, were filtered and extracted onto Oasis HLB® (hydrophilic liphophilic balance; Waters) solid phase extraction cartridges. The cartridges were dried under CO_2, eluted with ethyl acetate, and analyzed by gas chromatography–mass spectrometry (GC-MS) [18].

Extraction and chemical analysis

Thawed embryo samples (~0.1–2.0 g) were homogenized with approximately 20 g of baked (450°C for 4 h) sodium sulfate (Na_2SO_4) using a ceramic mortar and pestle, packed into solvent-rinsed glass columns (400 mm/L × 10 mm inner diameter [i.d.]), and spiked with [^{13}C] trifluralin, and [$^2H_{10}$] chlorpyrifos as recovery surrogates. Samples were extracted cold with 20 ml of dichloromethane (DCM) at a rate of 1 to 2 drops/s. Extracts were reduced to 0.75 ml using a Turbo Vap II (Zymark) operating at 25°C with high purity (>99.99 %) N_2. Ten percent by volume of each raw extract was allowed to evaporate to a constant weight in a fume hood for gravimetric lipid determination to the nearest 0.001 g using a microbalance.

A majority of the lipid was removed from the sample extracts using a 300 × 21.2 mm Phenogel (10 μm, 100 A; Phenomenex) gel permeation chromatography (GPC) column with DCM: MeOH (98:2 v/v) as the carrier solvent with a flow rate of approximately 4 ml/min. Following GPC clean-up, the samples were recombined, reduced to 0.75 ml, and exchanged to hexane.

Sample extracts were subjected to further clean-up using silica gel (100 mesh chromatographic grade; Thermo-Fisher Scientific) previously washed with DCM. A 200-ml glass column (400 mm/L × 10 mm i.d.), dry packed with 5 g of 9.1% (v/w) water deactivated silica gel previously activated at 130°C overnight, was then rinsed with 50 ml hexane. The more nonpolar pesticides (OCPs, OPs, and pyrethroids) were eluted with 50 ml of DCM, while the more polar pesticides were eluted with 10 ml of 5% acetone in DCM. Both fractions were combined, reduced to 0.75 ml, exchanged to ethyl acetate, and further reduced to 0.2 ml for instrumental analysis. Prior to analysis, 40 μl of a deuterated internal standard containing [$^2H_{10}$] acenaphthene, [$^2H_{10}$] phenanthrene, and [$^2H_{10}$] pyrene was added to each extract.

Sample extracts (1 μl injection volume) were analyzed on a Varian Saturn 2000 gas chromatograph (GC)–ion trap mass spectrometer (MS) and on an Agilent 6890 GC coupled to a micro electron capture detector (μECD). Analyte separation on the GC-MS was achieved using a 30 m × 0.25 mm i.d., 0.25 μm DB-5 ms fused silica column (Agilent Technologies) with helium as the carrier gas. The temperature of the splitless injector was held constant at 275°C. The GC oven temperature program was 80°C (hold 0.5 min), increase to 120°C at 10°C/min, increase to 200°C at 3°C/min (hold 5 min), followed by a third increase to 219°C at 3°C/min, and a final increase to 300°C at 10°C/min (hold 10 min). The transfer line and ion trap temperatures were 280°C and 220°C, respectively. The MS was operated in electron ionization mode with an emission current of 15 μA and no offset when run in full scan mode, and an emission current of 45 μA with a multiplier offset of 300 volts when using SIS (selective ion storage) mode. Data was collected in full scan and SIS modes.

Organochlorine pesticides were analyzed by GC-μECD with GC-MS confirmation. Analyte separation on the GC-μECD was achieved using a 30 m × 0.25 mm i.d., 0.25 μm DB-XLB fused silica capillary column (Agilent Technologies) with helium as the carrier gas. The flow was constant at 1.5 ml/min with an average linear velocity of 35 cm/s. Nitrogen was used as the makeup gas with a total flow of 64 ml/min. The temperature of the splitless injector and the detector were 250°C and 330°C, respectively. The GC temperature program was 75°C (hold 0.5 min) and increase to 300°C at 10°C/min (hold 10 min).

Quality control

All sample glassware was hand washed, baked at 450°C for 4 h, and finally rinsed with acetone and hexane prior to use. All solvents and other reagents used were ACS grade or better (Thermo Fisher Scientific). Pesticide reference materials were purchased from Chem Service, Riedel-de Haën, Supelco, and Ultra Scientific, or were donated by the U.S. EPA National Pesticide Repository. Purities ranged from 95 to 99%. Internal standards ([$^2H_{10}$] acenaphthene, [$^2H_{10}$] phenanthrene, and [$^2H_{10}$] pyrene) and surrogates ([$^2H_{10}$] chlorpyrifos [chlorpyrifos d_{10}] and [^{13}C] trifluralin) were purchased from Cambridge Isotope Labs. Neat pesticides were dissolved in acetone or methanol for an initial concentration of 1 mg/ml.

A performance-based quality assurance and quality control program, which included the parallel analysis of procedural blanks, matrix spikes, and replicates, was implemented to ensure high quality data. The GC-MS and GC-μECD were calibrated using standards that spanned the linear range of instrument response (0.025 to 5.0 ng/μl and 0.1 to 100 pg/μl, respectively). Instrument response was monitored every six to eight samples with mid-level check standards. Procedural blanks consisting of 20 to 30 g of baked Na_2SO_4 did not contain detectable levels of pesticides. Mean (± standard deviation) recoveries of chlorpyrifos d_{10} and [^{13}C] trifluralin added prior to sample extraction as recovery surrogates were 95 ± 19% and 102 ± 15%, respectively. Final method recoveries in spiked crab embryos ranged from 75 to 120%.

Limits of detection for all pesticides measured were calculated as the amount of analyte in the spiked sample that produced a signal greater than three times the background signal, and was calculated for both the GC-MS and GC-μECD (Table 1).

RESULTS AND DISCUSSION

Invertebrates are considered effective indicators of ecosystem health from a contaminant perspective due to their ability to accumulate contaminants and their relatively small home range. Persistent organic pollutants, particularly OCPs, have been of interest over the past few decades because they are hydrophobic in nature, tend to biomagnify, and have impacts on higher trophic level species. However, current-use pesticides (CUPs) are typically less hydrophobic than their predecessors, with the exception of certain pyrethroid insecticides, and only limited data is available as to their accumulation in aquatic organisms. The purpose of the present study was to determine if crab embryos could be effective indicators of environmental CUP contamination. Results indicate that several different classes of pesticides were detected, including thiocarbamate herbicides, pyrethroids, and organophosphate insecticides (all heretofore referred to as CUPs), and several OCPs.

Laboratory uptake study

A one-week laboratory exposure study was conducted using field-collected crabs from Bodega Bay (Table 2) to determine if molinate would bioconcentrate in crab embryos. Molinate, a thiocarbamate herbicide, was used because it was previously detected at relatively high concentrations (8.9 to 61.1 μg/kg dry weight) in Stege Marsh sediments and was correlated with abnormalities in crab embryos (data not shown). The total percent lipid in the composite samples depended on the stage of each embryo in the sample and ranged from 1.4 to 5.1%

Table 1. Type of use, chemical class, Log K_{OW}, and limits of detection (μg/g lipid wt) for all compounds analyzed in both species of crab embryos (*Hemigrapsus oregonensis* and *Pachygrapsus crassipes*) in Stege Marsh and Bodega Bay (CA, USA)[a]

Compound	Type of Use	Chemical class	Log K_{OW}	LOD (μg/g lipid wt)	Compound	Type of use	Chemical class	Log K_{OW}	LOD (μg/g lipid wt)
Ethalfluralin	H	Aniline	5.1	0.04	Malathion	I	Organophosphate	2.9	0.04
Pendamethalin	H	Aniline	5.2	0.04	Methidathion	I	Organophosphate	2.4	0.04
Trifluralin	H	Aniline	5.3	0.04	Methylparathion	I	Organophosphate	3.4	0.04
Alachlor	H	Chloroacetanilide	2.9	0.04	Phosmet	I	Organophosphate	2.8	0.04
Metolachlor	H	Chloroacetanilide	3.5	0.04	Allethrin	I	Pyrethroid	5.5	0.04
Cyproconazole	F	Conazole	2.9	0.1	Bifenthrin	I	Pyrethroid	7.3	0.02
Metconazole	F	Conazole	3.9	0.1	Cyfluthrin	I	Pyrethroid	5.6	0.04
Myclobutanil	F	Conazole	2.9	0.04	λ-Cyhalothrin	I	Pyrethroid	6.8	0.02
Propiconazole	F	Conazole	3.7	0.1	Cypermethrin	I	Pyrethroid	6.6	0.04
Tebuconazole	F	Conazole	3.7	0.1	Deltamethrin	I	Pyrethroid	4.6	0.04
Tetraconazole	F	Conazole	3.6	0.1	Esfevalerate	I	Pyrethroid	6.2	0.02
α − Chlordane	Leg	Organochlorine	5.9	0.01	Fenpropathrin	I	Pyrethroid	6.0	0.02
Aldrin	Leg	Organochlorine	5.7	0.01	τ-Fluvalinate	I	Pyrethroid	7.0	0.04
p,p′-DDD	D	Organochlorine	6.0	0.01	Permethrin	I	Pyrethroid	6.1	0.02
p,p′-DDE	D	Organochlorine	6.2	0.01	Resmethrin	I	Pyrethroid	5.4	0.04
p,p′-DDT	Leg	Organochlorine	5.9	0.01	Sumithrin	I	Pyrethroid	6.0	0.04
Dieldrin	Leg	Organochlorine	6.2	0.01	Tetramethrin	I	Pyrethroid	4.6	0.04
Endosulfan I	I	Organochlorine	4.7	0.01	Azoxystrobin	F	Strobilurin	2.5	0.04
Endosulfan II	I	Organochlorine	4.8	0.01	Pyraclostrobin	F	Strobilurin	4.2	0.1
Endosulfan sulfate	D	Organochlorine	NA	0.01	Trifloxystrobin	F	Strobilurin	4.5	0.04
Endrin	Leg	Organochlorine	4.7	0.01	Butylate	H	Thiocarbamate	4.1	0.04
α-HCH	Leg	Organochlorine	3.8	0.01	Cycloate	H	Thiocarbamate	2.8	0.04
β-HCH	Leg	Organochlorine	3.9	0.01	EPTC	H	Thiocarbamate	3.2	0.04
δ-HCH	Leg	Organochlorine	3.6	0.01	Molinate	H	Thiocarbamate	2.9	0.04
γ-HCH	Leg	Organochlorine	3.7	0.01	Pebulate	H	Thiocarbamate	3.8	0.04
Heptachlor	Leg	Organochlorine	5.2	0.01	Thiobencarb	H	Thiocarbamate	3.4	0.04
Heptachlor epoxide	D	Organochlorine	4.4	0.01	Chlorothalonil	F	Misc	2.9	0.04
Hexachlorobenzene	Leg	Organochlorine	5.9	0.01	DCPA	H	Misc	4.8	0.02
Isodrin	Leg	Organochlorine	4.7	0.01	Fipronil	I	Misc	3.8	0.02
Methoxychlor	Leg	Organochlorine	5.0	0.02	Fipronil desulfinyl	D	Misc	NA	0.02
cis-Nonachlor	Leg	Organochlorine	5.4	0.01	Fipronil sulfide	D	Misc	NA	0.02
trans-Nonachlor	Leg	Organochlorine	5.4	0.01	Fipronil sulfone	D	Misc	NA	0.02
Oxychlordane	Leg	Organochlorine	NA	0.01	Iprodione	F	Misc	3.1	0.04
Pentachloronitrobenzene	F	Organochlorine	5.5	0.02	Methoprene	I	Misc	5.2	0.04
Pentachloroanisole	D	Organochlorine	NA	0.02	Napropamide	H	Misc	3.4	0.04
Chlorpyrifos	I	Organophosphate	5.1	0.04	Oxyfluorfen	H	Misc	4.5	0.04
Diazinon	I	Organophosphate	3.8	0.04	Piperonyl butoxide	S	Misc	4.8	0.04

[a] NA = not applicable; D = degradate; F = fungicide; H = herbicide; I = insecticide; Leg = organochlorine insecticide; HCH = hexachlorohexane; S = synergist; LOD = limits of detection; DDD = dichlorodiphenyldichloroethane; DDE = dichlorodiphenyldichloroethylene; DDT = dichlorodiphenyltrichloroethane; DCPA = dichloropropionanilide; EPTC = *S*-ethyl dipropylthiocarbamate.

Table 2. Site information, lipid weight (%) and concentration (μg/g lipid wt) of total current-use (CUP) and organochlorine (OCP) pesticides detected in crab embryos collected from Bodega Bay and Stege Marsh (CA, USA) in 2005 and 2006[a]

Sample location	Year collected	Lipid (%)	ΣCUP (μg/g lipid wt)	ΣOCP (μg/g lipid wt)
Bodega Bay (*Hemigrapsus oregonensis*)				
BML Dorms 1	2005	1.4	0.493	0.300
BML Dorms 2	2005	3.7	2.73	1.08
BML Dorms 3	2005	4.2	5.61	6.88
BML Dorms 4	2005	2.6	16.8	1.54
BML Dorms 5	2005	4.8	405	2.21
BML Dorms 6	2005	2.1	8.82	0.385
BML Dorms 7	2005	5.6	1.33	0.858
BML Dorms 8	2005	4.6	2.01	1.56
BML Dorms 9	2005	5.1	1.81	13.2
BML Dorms 10	2005	3.0	0.409	0.356
BML Dorms 11	2005	3.4	0.303	0.343
BML Dorms 12	2005	3.1	0.809	0.354
Bodega Bay (*Pachygrapsus crassipes*)				
North Bodega Harbor	2006	7.4	0.003	0.077
Doran Boat Launch 1	2006	2.3	0.170	0.132
Doran Boat Launch 2	2006	4.7	0.600	0.154
Stege Marsh (*Hemigrapsus oregonensis*)				
Bridge 1	2006	2.8	0.868	1.00
Bridge 2	2006	5.4	0.024	1.16
Bridge 3	2006	4.9	0.204	0.769
Bridge 4	2006	4.2	0.273	2.17
Bridge 5	2006	6.8	0.208	0.655
Stege Marsh (*Pachygrapsus crassipes*)				
Station C	2006	3.0	ND	1.34
Station D1	2006	4.8	0.334	0.977
Station D2	2006	8.6	0.013	0.424
Station D3	2006	8.1	ND	0.382
Station D4	2006	10.8	0.049	0.208
Station D5	2006	4.1	2.80	1.07
Station G	2006	7.1	0.005	0.251
Station M	2006	11.7	0.868	0.555

[a] ND = not detected; BML = Bodega Marine Laboratory.

(Table 2). Therefore, molinate concentrations in the embryos were lipid normalized to decrease variability in the pesticide concentrations between composite embryo samples. Molinate concentrations in the embryos from the high (15 ppb) treatment group were 5.1 ± 0.68 μg/g lipid weight, while concentrations from the low (5 ppb) treatment group were 1.0 ± 0.12 μg/g lipid weight. No molinate was detected in embryos from the control treatment group or in the seawater used in the experiments.

The tendency of chemicals to bioconcentrate in organisms is generally expressed as a bioconcentration factor (BCF) and is defined as the steady-state ratio of the chemical in the organism to that in its environment [19]. Bioconcentration factors are used in environmental studies to estimate the bioaccumulation potential of chemicals in aquatic organisms. Under equilibrium conditions, a chemical's K_{OW} is considered a good predictor of an aquatic BCF [20]. An estimated laboratory-derived bioconcentration factor, reported as the log BCF, for molinate in the crab embryos from the present study was 2.5.

Results from this simple laboratory exposure study indicate that molinate was taken up by the crab embryos at rates similar to other CUPs studied in invertebrates. Bioconcentration factors have been reported for several OCPs in clams, oysters, fish, and other aquatic organisms, but few values have been reported for CUPs. Laboratory-derived BCFs for chlorpyrifos after 7 d of exposure in oysters were 2.3 [6], while BCFs for dichloropropionanilide (DCPA) in clams were 2.1 [2]. Bioconcentration factors of the more water-soluble pesticides are quite a bit lower than the more hydrophobic pesticides, such as dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE), whose BCFs in oysters ranged from 5.0 to 5.5 [2]. The current thinking is that dietary uptake of lipophilic chemicals into aquatic organisms may not be significant for chemicals with log $K_{OW} < 5$ [21]. Therefore, water uptake via gills or across membranes is expected to be the dominant process controlling the uptake of molinate ($\log K_{OW} = 2.9$) and other CUPs by crab embryos. Although molinate and other less hydrophobic CUPs are not expected to biomagnify, the potential still exists for accumulation within aquatic organisms through respiration, diffusion, and possibly food [21].

Pesticide concentrations in crab embryos

Eighteen CUPs and eight OCPs were detected in both species of crab embryos (*H. oregonensis* and *P. crassipes*) collected from the two sites (Table 3). The log K_{OW} values of the detected pesticides ranged from 2.4 to >6 (Table 1). Total percent lipid in the embryos at the different collection sites (Table 2) ranged from 1.4 to 11.7% and depended on the stage of the clutch and the crab species.

The CUPs detected included eight insecticides, seven herbicides, and three degradates (Table 3). Cyfluthrin, a pyrethroid insecticide, was detected at the highest average concentration and was approximately ×50 higher than any other pesticide detected at either site (Table 3). Malathion, an OP insecticide, was the most frequently detected (32%) with the second highest average concentration (Fig. 1 and Table 3). The herbicides (cycloate and DCPA) and the pyrethroid insecticides (bifenthrin, cyfluthrin, and permethrin) were detected in 14 to 21% of the samples (Fig. 1). Fipronil and its three most persistent degradates (fipronil desulfinyl, fipronil sulfide, and fipronil

Table 3. Average (± standard deviation) concentration (μg/g lipid wt) of current-use (CUP) and organochlorine (OCP) pesticides detected in crab embryos (*Hemigrapsus oregonensis* and *Pachygrapsus crassipes*) collected from Bodega Bay and Stege Marsh (CA, USA)[a]

Compound	Type of Use	Bodega Bay	Stege Marsh
CUPs			
Bifenthrin	I	0.124 (0.120)	0.103 (0.097)
Chlorpyrifos	I	ND	0.097 (0.00)
Cyfluthrin	I	106 (170)	ND
Cycloate	H	0.273 (0.102)	0.98 (0.94)
DCPA	H	0.069 (0.00)	0.153 (0.129)
EPTC	H	ND	0.249 (0.132)
Ethalfluralin	H	ND	0.125 (0.00)
Fipronil	I	ND	0.150 (0.114)
Fipronil desulfinyl	D	ND	0.090 (0.049)
Fipronil sulfide	D	ND	0.160 (0.118)
Fipronil sulfone	D	ND	0.036 (0.005)
τ-fluvalinate	I	1.04 (0.929)	ND
Malathion	I	2.21 (1.47)	ND
Molinate	H	ND	0.013 (0.00)
Pendimethalin	H	ND	0.112 (0.050)
Permethrin	I	ND	0.172 (0.215)
Phosmet	I	0.634 (0.00)	ND
Trifluralin	H	ND	0.094 (0.101)
OCPs			
α-Chlordane	Leg	ND	0.011 (0.017)
p,p′ DDD	D	0.073 (0.189)	0.05 (0.074)
p,p′ DDE	Leg	1.97 (3.22)	0.402 (0.203)
p,p′ DDT	D	0.146 (0.417)	0.119 (0.274)
Dieldrin	Leg	0.003 (0.002)	0.078 (0.076)
Heptachlor	Leg	0.004 (0.004)	0.007 (0.007)
trans-Nonachlor	Leg	0.006 (0.006)	0.069 (0.110)
Oxychlordane	Leg	0.006 (0.005)	0.112 (0.078)

[a] ND = not detected; D = degradate; H = herbicide; I = insecticide; Leg = organochlorine insecticide.
See Table 1 for definitions of acronyms.

sulfone) were detected in 7 to 11 % (Fig. 1) of the samples, but at relatively low concentrations (Table 3). No published study to date has measured CUPs in crab embryos, and only a limited number of studies have analyzed crab tissue [3,7,21,22]. Dugan et al. [7] detected diazinon, an OP used extensively in agricultural areas in central California, at relatively high concentrations in sand crabs. Similarly, another OP, chlorpyrifos, was detected in burrowing crabs collected from an urban estuary near Brisbane, Australia [3]. Although many of the OPs and other CUPS have a log K_{OW} <5, they are still taken up by crabs exposed to these compounds.

Eight legacy OCPs were detected in crab embryos collected from both sites (Table 3). Several OCPs, including dieldrin, oxychlordane, and trans-nonachlor, were detected in greater than 50% of the samples (Fig. 1), however, DDT and its degradates (dichlorodiphenyldichloroethane [DDD] and DDE) were detected at the highest average concentrations compared to all other OCPs (Table 3). Although banned in the United States for 30 years, DDT and its degradates are persistent in the environment and are biologically available for uptake by aquatic organisms. The highly persistent and bioaccumulative metabolite of DDT, *p,p′*DDE, was the most frequently detected and contributed to 89% of the OCP burden in the crab embryos (Fig. 1). This was similar to pesticide profiles observed in embryos from burrowing mud crabs, *Chasmagnathus granulata* [12], and in the tissues of gravid and non-gravid sand crabs [7]. Total DDT concentrations measured in crab embryos from the present study were an order of magnitude higher than crab embryos collected in Brazil from *C. granulata* [12], as well as in whole body *C. granulata* samples from Argentina [23]. However, the range of total DDT concentrations (43 to 13,000 ng/g lipid weight) from this study was similar to a series of studies on sand crabs along the California coast, where lipid normalized concentrations ranged from 180 to 6,500 ng/g [7,24].

Site differences

Due to the very different habitat preferences (*H. oregonensis* prefer rocky intertidal areas, whereas *P. crassipes* prefer vegetated marsh areas in the mid- to upper-intertidal), a comparison between species may reflect environmental differences in the pesticides as well as differences in accumulation, thus not allowing for a true species-specific comparison. Therefore, all pesticide comparisons will be made on a site basis, and concentration data will be combined for both crab species (Table 3).

Crab embryos collected from the two sites exhibited very different pesticide profiles. Embryos from Stege Marsh, which is adjacent to a highly industrialized area, were dominated by OCPs (Fig. 1). In contrast, Bodega Bay crabs, which were exposed to runoff from a suburban golf course community, had higher concentrations of CUPs compared to OCPs (Table 2) and were dominated by pyrethroids and OP insecticides (cyfluthrin and malathion) (Fig. 2 and Table 3). Total OCPs (ΣOCP) in crabs collected from Stege Marsh were greater than total CUPs (ΣCUP) and contributed 74% on average to the total mass of pesticide measured. However, total CUPs in crabs from Bodega Bay were greater than total OCPs (Table 2) and contributed 70% on average to the total pesticide concentrations in the embryos. Although a few similar CUPs were detected at each site, the pesticide profile in embryos varied spatially (Fig. 2), which can be attributed to differences in land-uses between sites. This pattern is more evident when comparing the site-specific relative abundances of CUPs at each site compared to the OCPs.

Seven herbicides were detected in crab embryos from Stege Marsh at concentrations ranging from less than 0.01 to 1 μg/g lipid weight (Table 3). A few of the herbicides, including cycloate, EPTC, and molinate, were detected frequently in sediment collected from Stege Marsh in 2005 (data not shown). Known herbicide contamination was a direct result from a former agrochemical production plant. Past contamination of the marsh explains the herbicide profile and the high relative abundance of herbicides, such as cycloate and EPTC, compared to the other CUPs detected in embryos from Stege Marsh (Fig. 2). In contrast, embryos from Bodega Bay had a much simpler herbicide profile, compared to Stege Marsh, in that cycloate and DCPA were the only herbicides detected (Fig. 2). Dichloropropionanilide occurs frequently throughout California and tends to be relatively persistent in both water and sediment [25]. Another study conducted in the agriculturally dominated Central Valley of California detected DCPA in clam tissue at 0.150 μg/g [2], similar to concentrations measured at sites in the present study (Table 3).

Seven insecticides were detected in embryos from Stege Marsh, while six were detected at Bodega Bay (Fig. 1). Three OP insecticides (chlorpyrifos, malathion, and phosmet), four pyrethroids (bifenthrin, cyfluthrin, τ-fluvalinate, and permethrin), and fipronil and its three major degradates were detected in crab embryos from the two sites. Embryos from Stege Marsh had a higher number of insecticides detected, but concentrations were much lower compared to embryos collected from Bodega Bay (Table 3 and Fig. 2). Bodega Bay embryo CUP profiles were dominated by two major insecticides: the OP malathion and the pyrethroid cyfluthrin (Fig. 2). In a previous study during

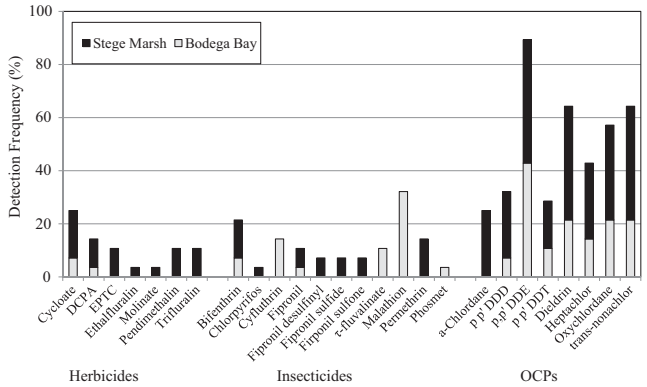

Fig. 1. Total percent frequency of detection (%) for pesticides measured in composite crab embryos from both species (*Hemigrapsus oregonensis* and *Pachygrapsus crassipes*) from Bodega Bay and Stege Marsh (CA, USA) ($n = 28$). The pesticides are ordered by type; herbicides first followed by current-use insecticides and finally legacy organochlorine insecticides. See Table 1 for definitions of acronyms.

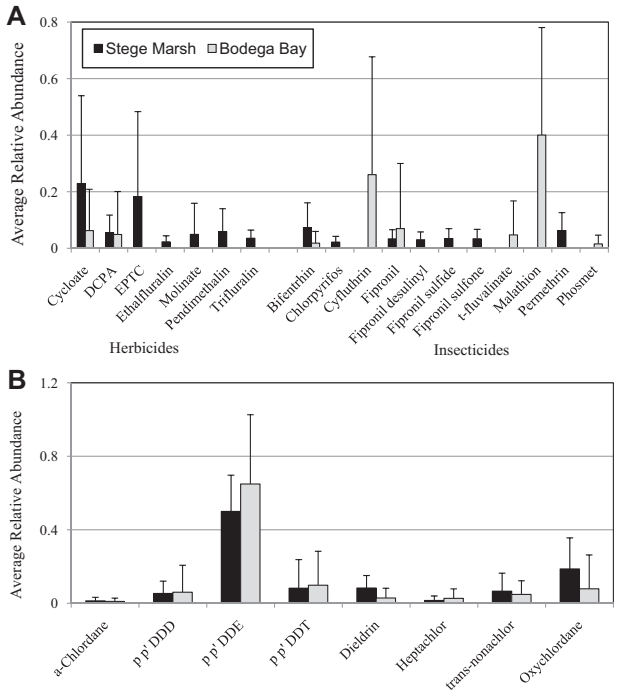

Fig. 2. Pesticide profiles depicted as an average relative abundance (calculated as individual pesticide concentration divided by the sum of all pesticides) for (**A**) current-use pesticides (CUPs) and (**B**) organochlorine pesticides (OCPs) detected in crab embryos from both species (*Hemigrapsus oregonensis* and *Pachygrapsus crassipes*) collected from Stege Marsh ($n = 13$) and Bodega Bay (CA, USA) ($n = 15$). See Table 1 for definitions of acronyms.

the late 1990s, pyrethroids and OPs (specifically malathion) were detected consistently in waters adjacent to golf courses, as well as in samples collected from golf course greens [26]. Of the pyrethroids detected in embryos from Bodega Bay, cyfluthrin had the second highest overall relative abundance compared to all other compounds (Fig. 2), with concentrations from composite embryo samples ranging from 2 to 400 μg/g lipid weight. Bifenthrin and τ-fluvalinate were also detected in embryos from Bodega Bay, but at much lower concentrations compared to cyfluthrin (Fig. 2 and Table 3). In embryos from Stege Marsh, bifenthrin and permethrin were the only two pyrethroids detected, and permethrin was detected more frequently compared to bifenthrin (Fig. 1).

Although OPs are being replaced by the pyrethroids for home-use, such as termiticides and for landscape application, they are still actively used on golf courses; and as the use of pyrethroids continues to increase, especially in urban environments, their frequency of detection is increasing, particularly in sediment samples collected from urban creeks throughout California [27,28]. More importantly, bifenthrin and cyfluthrin are the two pyrethroids that are most commonly detected in urban creeks [29]. Pyrethroids are also considered to be acutely toxic to benthic invertebrates at very low concentrations [28], and sublethal effects on crab embryo development at sub ppb levels has been measured [10]. As pyrethroid use continues to increase, especially in suburban areas, it is conceivable that these compounds will be found more often and at increasing concentrations in crab embryos. Currently, little information is available on the effects of pyrethroids on crab embryo development, however, this study highlights the need for future studies that attempt to answer this question.

Crab embryos collected from the two sites exhibited similar OCP profiles but very different CUP profiles. The similarities in the OCP profiles between the two sites indicated past contamination and no new inputs, whereas the differences in CUP profiles were directly related to differences in land-use practices. Specifically, Stege Marsh embryos were dominated by herbicides, particularly cycloate and EPTC, from previous contamination by a former agrochemical plant. In contrast, CUP profiles in embryos from Bodega Bay were dominated by malathion and cyfluthrin, indicative of inputs from suburban and golf course applications. The apparent differences in CUP profiles between the two sites further supports the idea that crab embryos are effective indicators of pesticide contamination, especially in areas with different land-use practices.

Crab embryos as bioindicators of ecosystem health

A wide variety of pesticides ranging in concentration from 0.1 to 400 μg/g lipid weight were detected in the crab embryos collected from two very different sites in California. Current-use and legacy pesticides, including several lipophilic herbicides, OPs, pyrethroids, DDT and its degradates, as well as several other ubiquitous OCPs, were detected in composite embryo samples from both sites. This is one of the first studies to analyze crab embryos for a large suite of both current-use and legacy pesticides. No matter the mechanisms, crab embryos effectively accumulate a wide range of pesticides, increasing their effectiveness as an environmental monitoring tool. More importantly, future studies should be geared toward using these organisms as simple bioindicators of ecosystem health.

Marine invertebrates have frequently been used for the assessment of marine pollution [11,30,31], because they are abundant in many near-shore environments and are relatively immobile compared to other organisms, such as fish [15]. Typically, invertebrate embryos or larvae are several orders of magnitude more sensitive to toxicants than adults and have been used in many toxicity tests [9,29,32,33]. In the present study, shore crab embryos were chosen as an appropriate test subject because of their limited home ranges and their ability to adapt to contaminated sites.

Many studies have shown the reproductive effects of DDE, DDT, and other OCPs, as well as chlorpyrifos and several pyrethroids on embryos of crabs and sea urchins [8,10,34]. Because this is the first study, to our knowledge, that reported a wide variety of pesticides in crab embryos, continued work

must be done to determine biological effects. Future work will involve analyses of pesticides in sediments and crab embryos concurrent with measurements of reproductive abnormalities. Preliminary results indicate that embryos do accumulate organic contaminants in the environment and have the potential to be good indicators of ecosystem health, especially when contaminant body burden analyses are paired with reproductive impairment assays.

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