Aquatic organisms provide insight into environmental problems: From genes to communities

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Aquatic organisms provide insight into environmental problems: 
From genes to communities

by

Michael George Peterson

A dissertation submitted in partial satisfaction
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in

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of the

University of California, Berkeley

Committee in charge:

Professor Vincent H. Resh, Chair
Professor Patrick O’Grady
Professor Stephanie M. Carlson
Professor Mary E. Power

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Aquatic organisms provide insight into environmental problems: From genes to communities

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Abstract

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Professor Vincent H. Resh, Chair

An understanding of environmental responses to current and emerging problems and the capacity to forecast future threats requires appropriate indicators. The temporal scale of impact to animal health and species biodiversity can vary from centuries in the case climate change, to immediate, such as chemical spills. This dissertation uses genetics, physiology, and community ecology to understand current and emerging environmental threats and appropriate biomonitoring responses across a range of temporal scales.

Patterns of genetic diversity across a species range can suggest past glacial refugia and recolonization, dispersal barriers, and evidence of vulnerabilities to future change. However, population genetic structure and historical refugia among co-occurring species is not well characterized for many groups of aquatic organisms. Therefore, I compared phylogeographies of five, large bodied co-occurring aquatic insect species (four stoneflies: Calineuria californica, Hesperoperla pacifica, Pteronarcys californica, and Pteronarcys princeps and one caddisfly: Dicosmoecus gilvipes) across their ranges.

Widespread and persistent environmental contaminants may be monitored in the marine environment using top predators such as marine mammals. I sampled serum, inner blubber, and outer blubber and determined predictive equations between tissues for a suite of persistent organic pollutants in northern elephant seals (Mirounga angustirostris), which indicated strong relationships, particularly between serum and inner blubber POP concentrations.

Although benthic macroinvertebrate communities respond to natural environmental fluctuations they can still serve as indicators of acute anthropogenic stressors. Multiple years of macroinvertebrate sampling (2004, 2010-2013) in an urban creek quantified the community response to an accidental, 3000 L, oil spill using a Before-After Control-Impact approach. Taxa richness and sensitive taxa decreased immediately, but recovered one year later, likely aided by drift from upstream reference sites and winter rains. Additionally, I identified five indicator taxa that will enhance future biomonitoring efforts.

To better understand population dynamics and response to drought in seasonal wetlands, I investigated successional changes in macroinvertebrate occurrence and abundance in northern California. The early-season predator assemblage was dominated by dytiscid beetles and late-season by Lestes damselflies. The phenology of taxa and life history strategies may affect how macroinvertebrate populations respond to increased annual variation in hydroperiod as a result of future climate change.
DEDICATION

This dissertation is dedicated to Kathleen and Jack Peterson
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INTRODUCTION

The use of biological indicators has a long history in conservation biology and environmental monitoring (Bonada et al., 2006; Rigét et al., 2015). However, accurate interpretation of results from monitoring biological indicators requires knowledge of the natural history and the temporal nature of the indicators themselves. Some organisms or assemblages of organisms are appropriate indicators because they are sensitive to either broad or specific types of pollution, while others show broad tolerances to both specific and more general ecological impacts. In many cases, the ideal biological indicator or biomonitoring tool would be an animal that could clearly indicate the presence or magnitude of a specific environmental problem. Because we generally lack such an ideal tool, a range of biological indicators has been used to examine or predict responses to the wide range of anthropogenic disturbances in biological systems (Bonada et al., 2006; Hoffmann & Daborn, 2007; Domisch et al., 2013). Continued expansion of classic ideas of bioindicators, such as indicator species, to include genetics and subsets of communities, along with expanded knowledge of seasonal or life-stage variation, will be valuable when tracking changes and identifying future vulnerability in our environment under current and emerging environmental problems.

Threats to biodiversity, ecosystem resilience, and organism health vary in their pervasiveness, source, and magnitude of consequences. In freshwater and marine habitats environmental problems range from events occurring narrowly in space and time, such as a chemical spill (Lytle & Peckarsky, 2001), to diffuse events, such as global climate change (Barnosky et al., 2012) and bioaccumulation of persistent organic pollutants (Desforges et al., 2016). The responses of organisms to these threats vary according to their individual life history traits, behaviors, and tolerances. Consequently, to address the diversity of environmental problems, ecologists require a diversity of biological monitoring strategies.

Global climate change is a diffuse, long-term, and pervasive threat to biodiversity in freshwater ecosystems, and its gradual nature presents challenges for predicting responses (Moss et al., 2009; Bálint et al., 2011). Prediction of geographic locations where a species may be vulnerable to losses of intraspecific biodiversity requires knowledge of phylogenetic history and current population structuring. Climate change is predicted to increase air temperature, and subsequently water temperatures in rivers (Isaak et al., 2010). For riverine fauna having evolved with physiological mechanisms that favor narrow temperature and consequently oxygen constraints, such as some aquatic insects, increased temperature may result in range contraction and loss of intraspecific biodiversity (Bálint et al., 2011; Pauls et al., 2013). In particular, species with strong population genetic structure, whether the result of poor dispersal ability or historical barriers to migration, may be more susceptible to loss of cryptic evolutionary lineages (Pauls et al., 2013). Moreover, because of dispersal restrictions at high elevations, alpine populations may be particularly vulnerable to these temperature changes (Moritz et al., 2008; La Sorte & Jetz, 2010).

Spatial distribution of genetic diversity can provide insight into population structure and capacity for responses to future climatic conditions. For aquatic insects, genetic markers can reveal the spatial connectivity among stream populations (Hughes et al., 2009). Moreover, the distribution of genetic diversity across a species range holds information regarding how these organisms responded to glacial cycles or current physical barriers (Theissinger et al., 2011; Pessino et al., 2014). Population structuring may exist as a legacy of glacial separation among populations, even in species with continuous present-day distributions. Moreover, co-occuring
species may have similar present-day distribution, but different glacial refugia and recolonization histories (Theissinger et al., 2011). Common river insects, such as stoneflies and caddisflies, are important not only for their own contributions to species diversity, but as important food web links between the primary productivity of algae and higher trophic level vertebrates. Therefore, the degree of aquatic insect vulnerability to climate change is important for the wider river ecosystem.

Among water pollutants, persistent organic pollutants (POPs), such as DDT (dichlorodiphenyltrichloroethane) and PCBs (polychlorinated biphenyls), are widespread contaminants and often monitored because they bioaccumulate in longer-lived marine predators (Weijs et al., 2009). However, temporal variations in animal physiology present challenges for interpretation of POP concentrations. For example, marine mammals often exhibit higher concentrations of POPs in blubber and serum after fasting when compared with pre-fast concentrations and responses of tissues to fasting periods can vary among and within tissues (Debier et al., 2006). Despite bans on DDT and PCBs, as well as regulatory efforts to limit other POPs, these compounds are pervasive in aquatic food webs (Lopez et al., 2012), and biomagnify with trophic level (Borgà et al., 2001; Goerke et al., 2004). For marine mammals, elevated concentrations of POPs can influence the endocrine system (De Swart et al., 1996), neural function, and immune responses (Desforges et al., 2016). Furthermore, bioaccumulation of POPs in marine mammals may have consequences to marine mammal populations, either because of the combined impacts of weakened immune function and infectious disease, or because POPs can transfer from mother to offspring and expose young animals to a suite of contaminants during critical periods of development (Hall et al., 2009; Debier et al., 2012).

The unique life history of the northern elephant seal (Mirounga angustirostris) presents an opportunity to understand internal POP concentrations at extremes of body condition (Le Boeuf et al., 2000). Elephant seals forage on fish and squid far from POP sources in the deep-ocean, yet breed and molt on beaches in California (USA). While elephant seals forage for months at a time, once they arrive on land they fast, losing 25-40% of their mass, as they breed or molt (Costa et al., 1986; Worthy et al., 1992). Their “extremes” in natural body condition may impact temporal fluctuations in POP concentrations some tissues more than others (Louis et al., 2014), and understanding the relationships among internal tissues is critical to monitoring POP concentrations over space and time.

Rivers, streams, and wetlands also suffer from a variety of local disturbances and benthic macroinvertebrates are often important bioindicators (Bonada et al., 2006; Resh, 2008; Milner et al., 2016). However, natural spatially and temporally variability of the benthic community can complicate biomonitoring approaches (Matthews et al., 1991). Macroinvertebrate occurrences and abundances are often seasonally dependent, especially when stream flow (Bèche et al., 2006) or wetland hydroperiod (Sim et al., 2013) is seasonally predictable. The ability to determine and discern anthropogenic disturbance from natural disturbance is a major focus in assessments of stream health (Resh et al., 1988; Barbour et al., 1999), and seasonal variability remains a challenge to the accurate application of biological indicators.

Urbanization is a common factor that can increase threats to riverine fauna (Paul & Meyer, 2001; Cuffney et al., 2010), particularly through habitat alteration and repeated pollutant spills from urbanized areas (Walsh et al., 2005). Oil spills, while often thought to be a problem in the marine environment, remain a consistent threat to stream fauna as well (Rosenberg & Wiens, 1976; Lytle & Peckarsky, 2001). The magnitude of effects on stream biota can be severe, and is influenced by seasonality of macroinvertebrate communities (Guiney et al., 1987; Lytle &
Peckarsky, 2001) and concurrent hydrological conditions (Pontasch & Brusven, 1988). Therefore, understanding stream recovery in relation to seasonal benthic macroinvertebrate assemblages is important to the development of future biomonitoring tools. While the specific type of biological indicator may vary with the timescale of the environmental problem, natural history information is critical to development of successful monitoring approaches and interpretation of responses. The specific life histories of individual organisms can complicate or present opportunities for understanding how organisms respond to perturbations. The biological traits of organisms, such as dispersal ability, may impact their resilience to disturbances over space and time, even in relation to glacial cycles or future global climate change. Seasonality in life history events, such as the timing of reproduction and fasting, may impact vulnerability to high contaminant concentrations (Peterson et al., 2014). Finally, the seasonality in the occurrences and abundances of taxa presents challenges for biomonitoring programs because the timing of stream sampling can impact biological indexes and interpretation (Reece et al., 2001). Future biomonitoring approaches and conservation of biological diversity in relation to global climate change rely on increased knowledge about individual species, life history traits, and the temporal nature of responses to environmental conditions.

Overview of Chapters

The chapters in this dissertation were arranged by levels of biological organization, from genetic to physiological to community response.

In Chapter 1, I examined mitochondrial and nuclear genes of five large bodied, co-occurring aquatic insect species (the caddisfly Dicosmoecus gilvipes, and the stoneflies Calineuria californica, Hesperoperla pacifica, Pteronarcys californica, and Pteronarcys princeps) across their ranges. I compared phylogenies to examine the degree of concordance in population structure, as well as similarities in the locations of glacial refugia and cryptic diversity among species.

In Chapter 2, I measured concentrations of DDTs, PCBs, Chlordanes, PBDEs, and other persistent organic pollutants in the blood and blubber of female and male northern elephant seals (Mirounga angustirostris) before and after a long foraging trip. I used the contaminant concentrations to establish predictive relationships between concentrations of persistent organic pollutants in the blubber and blood serum, and compared the relationships between life history phases.

In Chapter 3, I examined spatial and seasonal variation in the composition of the benthic macroinvertebrate community in an urban, Mediterranean-climate stream, and the responses of benthic macroinvertebrates to an accidental diesel oil spill. By comparing the composition of the benthic community over multiple years and an acute disturbance, I was able to propose indicator taxa with criteria important for future biomonitoring studies.

In Chapter 4, I described the seasonal variation of macroinvertebrate occurrences and abundances in a seasonal wetland in northern California. I determined the early colonizers and dominant predators, and I examined the sequential fluctuations in community composition in relation to water quality factors and the presence of a vertebrate predator.

My dissertation contributes to the utility of current biological monitoring and the development of future biological indicators of global climate change, anthropogenic contaminants, and oil spills, which are current and emerging environmental problems.
LITERATURE CITED


CHAPTER 1

Phylogeographic comparison of five large-bodied aquatic insect species across the western United States
Phylogeographic comparison of five large-bodied aquatic insect species across the western United States

ABSTRACT

Glacial legacy, barriers to migration, and species dispersal abilities are important determinants of intraspecific genetic diversity. Although genetic comparisons can elucidate the distribution of genetic variants among populations, for many groups of organisms the concordance or discordance of population genetic structure and historical refugia among co-occurring species remain unclear. Therefore, I compared phylogeographies of five co-occurring aquatic insect species, including four stoneflies (*Calineuria californica*, *Hesperoperla pacifica*, *Pteronarcys californica*, and *Pteronarcys princeps*) and one caddisfly (*Dicosmoecus gilvipes*), across their species ranges. Study species had large body size and large wing sizes that suggest strong flying ability and enhanced dispersal potential relative to other aquatic insects. However, riverine habitat restrictions and mating behaviors may inhibit dispersal, even among large-bodied species. Mitochondrial (COI and COII) and nuclear (wingless) gene sequences were used to examine population genetic structure relative to potential past and present barriers to dispersal in the western United States. North-south population genetic structure was present for each species but was more pronounced for 2 stoneflies (*Calineuria californica* and *Pteronarcys californica*) and the caddisfly. For these three species, phylogenies indicated concordant clades north and south of San Francisco Bay, which is a large, saltwater estuary in California. In addition, basal phylogenetic nodes and regional centers of haplotype diversity suggest common historical refugia in northern California or southern Oregon, similar to that found in previous studies of salamanders. For 1 stonefly (*Calineuria californica*) and the caddisfly (*Dicosmoecus gilvipes*), there were also found distinct populations in the Sierra Nevada Mountains, suggesting potential barriers to gene flow. Despite being widespread species, the presence of population genetic structure suggests vulnerability in losses of intraspecific diversity under climate change scenarios, particularly for populations at high elevations.

Key words: Plecoptera, Trichoptera, glacial refugia, life-history traits, biodiversity, North America, dispersal, population genetic structure

INTRODUCTION

A suite of physical, genetic, and historical factors governs the geographic distribution of genetic diversity within a species. Geographic barriers, whether glacial or landscape features, may impact dispersal among populations (Avise et al. 1987, Green et al. 1996, Avise 2000), as can the dispersal abilities of a particular species (Hughes 2007, Lehrian et al. 2010). Even when species distributions appear continuous, the genetic legacy of past events can result in cryptic hotspots of intraspecific diversity within the species range (Balint et al. 2011). Whether because of historical barriers or biological behaviors, strong population genetic structure may isolate certain populations, and make species more susceptible to loss of cryptic biodiversity as a result of range contractions associated with global climate change (Pauls et al. 2013).

Physical barriers such as mountain ranges and inhospitable habitat, can restrict dispersal, resulting in populations that are geographically close but still have reduced gene flow (Wake
Glacial oscillations in temperate climates can also act as barriers, reinforcing differences between populations and perhaps even leading to speciation (Brunsfeld et al. 2001). Periods of glacial advancement fragment populations as species retreat to 1 or more isolated refugia, which can increase inter-population diversity through drift, mutation, or natural selection operating on local populations (Hughes et al. 2009, Kuchta et al. 2009). As glaciers retreat and refugial populations become reconnected, genetic diversity may increase relative to pre-glaciation levels (Avise 2000).

Biological behaviors, physiological tolerances, and life-history traits can also either enhance or restrict dispersal potential (Bunn and Hughes 2007). For example, salamanders (Campbell Grant et al. 2010) and the aquatic stages of some insects (Finn et al. 2007) can physiologically tolerate time in the terrestrial environment and are therefore able to enhance dispersal by crawling between small headwater streams. In contrast, behaviors related to mating, such as territoriality, or other energetic tradeoffs, such energy expended for wing-growth versus egg production, can act to restrict gene flow (Zera and Harshman 2001, Hanski et al. 2006). Moreover, some marine invertebrate larvae that are physiologically able to survive longer in the pelagic zone can drift farther and increase gene flow among populations (Doherty et al. 1995, Dawson et al. 2014), while aquatic insects that emerge in autumn or winter can be constrained by cool temperatures during their window of dispersal (Briers et al. 2003, Lehrian et al. 2010). Regardless of cause, increased dispersal ability allows for colonization of new habitats and maintenance of gene flow among populations of a species. In contrast, limitations to dispersal can decrease gene flow among isolated populations and increase genetic differences among populations through genetic drift or natural selection (Slatkin 1985, Bilton et al. 2001).

Gene flow among populations of aquatic insects from different river drainages requires individuals to disperse across terrestrial landscapes and potentially past physical barriers. Thus, the primary opportunity for dispersal of aquatic insects between 2 rivers is during the winged, adult life history stage. However, even with wings to promote dispersal, some insects have common behaviors (e.g., territoriality of dragonflies, Kormondy 1961) or short adult lifespans (e.g., mayflies, Merritt et al. 2008) that may restrict actualized dispersal. Because long-distance dispersal is extremely difficult to study using marked individuals (e.g., McCauley 2010), genetic markers have been widely used to understand evolutionary history and infer species dispersal (Pauls et al. 2006, Hughes et al. 2009).

Genetic markers can be used to identify geographic diversity among populations and infer the history of dispersal for a species (Theissinger et al. 2011, Pessino et al. 2014). I used 5 common aquatic insect species, representing the orders Plecoptera and Trichoptera, which co-occur in the western United States to examine population genetic structure across their species ranges. Phylogenetic and population genetic methods were used to assess the distribution of genetic diversity in association with current geographic barriers, historical glaciation patterns, as well as biological behaviors and traits. Our objectives were to use these species to: 1) assess geographic concordance in population genetic structure among species, 2) examine the degree of cryptic genetic diversity within each species, and 3) infer the phylogeographic history of each species.

**METHODS**

*Study organisms*
The stoneflies *Hesperoperla pacifica* (Perlidae), *Calineuria californica* (Perlidae), *Pteronarcys californica* (Pteronarcydae), and *Pteronarcys princeps* (Pteronarcydae), and the caddisfly *Dicosmoecus gilvipes* (Limnephilidae) are common species occurring west of the Rocky Mountains, USA. These species are large in body size, have similar ranges, and perform a range of ecological functions within the stream ecosystem (Merritt et al. 2008). *Hesperoperla pacifica* and *C. californica* are predatory stoneflies that co-occur in western North America, including as far north as British Columbia (Fig. 1.1). The southern limits of their ranges do vary somewhat, however. Only *C. californica* is found in southern California and *H. pacifica* is more common in Arizona, Rocky Mountain streams, and Montana (Sheldon 1980, Stewart and Stark 2008).

*Pteronarcys californica* and *P. princeps* are shredders that feed on the microbial communities associated with submerged leaf-litter (Merritt et al. 2008). *Pteronarcys californica* is found primarily in large, lower elevations rivers along the Pacific coast, and in the Great Basin, while *P. princeps* is primarily found in small streams of California, particularly at higher elevations (Baumann 1979). In contrast, *D. gilvipes* is a grazer of the periphyton on submerged rocks and is found at both low and high elevations in California, Oregon, and Washington (Resh et al. 2011).

These species all have anatomical traits that would be likely to promote dispersal. Compared to other aquatic insects, they are relatively large bodied (>20 mm) and large winged (20-30 mm), which suggests strong flying ability and consequently high dispersal potential (Stark and Gaufin 1974). However, stoneflies in general are thought to have relatively short lifespans (2-4 weeks) and high incidence of brachyptery (Stewart and Stark 2008), both of which may limit long-distance travel. Brachyptery is relatively rare in caddisflies (Holzenthal et al. 2007) and emergence windows for *D. gilvipes* can be comparatively longer (~8 weeks) than for stoneflies, suggesting greater possibility for dispersal.

Each of these insects, however, has behaviors related to mating systems that may limit their dispersal. For example, many stonefly mating systems, including the species in the current study, rely on auditory communication between male and female individuals (Maketon and Stewart 1984, Sandberg 2011). Adult males will “drum” on logs and other riverside structures, creating cadences of rhythmic sounds, and females receiving this sound can respond with different cadences. This short-distance communication would tend to discourage widespread dispersal. While information on our specific study-organisms is limited, females of some stonefly species are sexually immature for 1-2 weeks of their adult stage, which may allow time for dispersal before mating (Petersen et al. 2006).

In contrast, *D. gilvipes*, like many limnephilid caddisflies, relies on pheromone communication between females and males (Resh and Wood 1985). From observational studies, caddisflies as a group are thought to be better flyers than stoneflies (Jackson and Resh 1989, Wiggins 2002), and limnephilids have been found to fly farthest relative to other caddisflies from a Swedish stream (Svensson 1974). However like drumming, pheromones are short-distance strategies for finding mates and when combined with short adult lifespans, these factors may cause adults to remain close to the stream where they emerged, and consequently limit their dispersal ability.

Closely-related, co-occurring species were chosen as an outgroups for each study species. The caddisfly *Allocosmoecus partitus* served as outgroup for *D. gilvipes* because these species co-occur in the western United States and are both limnephilids. The stonefly *Doroneuria baumanni* is also in the family Perlidae and commonly occurs with *C. californica* throughout the
study area. The stonefly *P. princeps* is sister-species to and has overlapping geographic
distribution with *P. californica* and was used as an outgroup. Although the stonefly
*Hesperoperla hoguei* is a sister-species of *H. pacifica* it is rare, therefore both *H. hoguei* and *D. baumanni* were used as outgroups for *H. pacifica*.

**Study locations**

Late-instar larvae of each species were collected from streams along the West Coast of
the United States (Fig. 1.1), which included locations representing the majority of the latitudinal
range for each species. Collections for each species included >100 specimens from >12 sites per
species (Table 1.1), with specimens of *P. californica* and *P. princeps* treated as 1 group because
they co-occur with each other and with *D. gilvipes, C. californica,* and *H. pacifica*. This
approach enabled broad-level comparison of genetic diversity across the entire study area. All
specimens were identified to species level prior to DNA extraction using the keys provided for
stoneflies in Stewart and Stark (2008) and for caddisflies in Wiggins (2004). A subset of
*Pteronarcys* specimens were confirmed by Dr. Richard Baumann. All specimens were stored in
95% ethyl alcohol immediately after collection, and voucher specimens of each species used in
this study were kept for reference and are located at University of California, Berkeley Essig
Museum of Entomology.

**Molecular laboratory methods**

Several legs from large specimens or the head from smaller specimens were removed
from the insect body with tweezers and dried. DNA was extracted using the Qiagen DNeasy
Extraction kit (Qiagen, Inc., Alameda, United States). Two mitochondrial genes, Cytochrome c
oxidase subunit I (COI) and subunit II (COII), and 1 nuclear gene, *wingless*, were amplified
using primers described in previous insect phylogenetic studies (Table 1.2). COI and COII were
chosen based on previous studies (e.g., Beckenbach et al. 1993, Simon et al. 1994, Lessios 2008,
Yassin et al. 2010) that suggest their rate of evolution is rapid enough to differentiate between
populations and closely related species. Furthermore, COI is commonly employed in species-
level barcoding for aquatic insects (Sweeney et al. 2011). *Wingless* was chosen as a more
conservative marker of intraspecific diversity (Brower and DeSalle 1998), and used the primers
demonstrated in European limnephilid caddisflies (Pauls et al. 2008) and the European stonefly
*Dinocras cephalotes* (Elbrecht et al. 2014). All specimens were sequenced for COI and COII;
33% of specimens for each species were sequenced for *wingless*, with multiple representatives
from all geographic areas.

PCR for COI was performed using the following protocol: 5 min initial denaturing step at
94°C, followed by 15 cycles of 30 s at 94°C, 30 s at 45°C and 45 s at 72°C, and then 20 cycles of
30 s at 94°C, 30 s at 55°C, and 45 s at 72°C, and a final extension step of 72°C for 5 min. For
each 2 µl of extracted template DNA, the reaction consisted of 17.5 µl nano-pure H₂O, 2.5µl
iTaq (Biorad) buffer, 2.5 µl MgCl₂ (25 µM), 0.5 µl dNTPs (10 µM), 1.5 µl of each primer (10
µM), and 0.15 µl iTaq polymerase. PCR for COII was performed using the following protocol: 5
min initial denaturing step at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 53°C, and 45 s
at 72°C, and a final extension step of 72°C for 5 min. For each 1 µl of extracted template DNA,
the reaction consisted of 17.5 µl nano-pure H₂O, 2.5µl iTaq buffer, 3.0 µl MgCl₂ (25 µM), 0.5 µl
dNTPs (10 µM), 1.5 µl of each primer (10 µM), and 0.15 µl iTaq polymerase. PCR for *wingless*
was performed using the following protocol: 5 min initial denaturing step at 94°C, followed by
30 cycles of 30 s at 94°C, 30 s at 59°C, and 45 s at 72°C, and a final extension step of 72°C for 5
min. For each 2 µl of extracted template DNA, the reaction consisted of 13.5 µl nano-pure H₂O, 2.5 µl iTaq buffer, 3.0 µl MgCl₂ (25 µM), 0.5 µl dNTPs (10 µM), 1.5 µl of each primer (10 µM), and 0.15 µl iTaq polymerase.

PCR amplification was confirmed using agarose gel electrophoresis. Successfully amplified PCR products were cleaned using Exonuclease I - Shrimp Alkaline Phosphatase (ExoSAP) with the following ratio of reagents (Thermo Fisher Scientific, Waltham, Massachusetts): 5 µl PCR product, 0.5 µl Exonuclease I, 0.5 µl Exonuclease I Buffer, 0.5 µl FastAP, and 1.0 µl nano-pure H₂O. ExoSAP conditions were 15 min at 37°C, followed by 15 min at 80°C. Cleaned PCR products were sent to the University of California, Berkeley DNA Sequencing Facility and sequenced in both directions.

**Sequence alignment and phylogenetic analyses**
Sequences were assembled and edited in Geneious Pro, Version 8 (Kearse et al. 2012) and alignments were created with the MAFFT plugin (Katoh and Standley 2013). Sequence lengths were edited and the number of base pairs analyzed was consistently 655 for COI, 680 for COII and 400-410 for wingless. All sequences were submitted to Genbank.

Individual gene sequences and concatenated sequences for each sample were used to create phylogenetic trees based on maximum likelihood and Bayesian inference. The program PhyML (Guindon and Gascuel 2003) plugin for Geneious Pro was used to perform maximum likelihood analyses with the substitution model HKY85, selected using ModelTest (Posada 2008). Support for relationships on each phylogeny was assessed by performing 500 bootstrap replicates (Felsenstein 1985). For Bayesian trees, we used the program Mr. Bayes v 3.2.5 (Ronquist and Huelsenbeck 2003) in Geneious Pro with substitution model HKY85, 1,100,000 MCMC chain length, and 100,000 burn in length. Clades with >70% of bootstraps in maximum likelihood analyses and >0.9 posterior probability for Bayesian analyses were considered to be well supported.

**Population genetic analyses**
Nucleotide diversity and haplotype diversity were calculated to describe intra-population genetic variation in Arlequin v.3.5.1.2 (Excoffier and Lischer 2010). In addition, Arlequin was used to estimate population genetic structure using analysis of molecular variance and global φST (AMOVA, Kimura-2-parameter distance, 10,000 bootstrap replicates). Further, to determine relationships among haplotypes across sampling sites, relationships within each species were reconstructed using a median—joining network in PopArt (Leigh and Bryant 2015). Ancestral populations of species were inferred from the concordance of basal branches of Bayesian and maximum likelihood phylogenetic trees. Historical refugia were inferred by the centers of highest haplotype diversity in median-joining haplotype networks.

**RESULTS**

**Geographic distribution of genetic diversity**
Although the study species are similar in distribution they do show some differences in occurrence across their reported ranges. Of the *Pteronarcys* stoneflies, *P. californica* was only found in lowland streams and *P. princeps* was primarily found in mountain streams of the Sierra Nevada Mountains (Fig. 1.1). *Calineuria californica*, *D. gilvipes*, and *H. pacifica* were collected from both lowland and montane streams. *Calineuria californica* and *H. pacifica* were found in
southern California and Arizona, respectively, which was further south than the other species and occurred in streams that were geographically remote (>500 km) from other collection sites. *Dicosmoecus gilvipes*, *P. californica*, and *H. pacifica* were found in Montana streams, while *C. californica* was only found as far east as eastern Washington (Fig. 1.1).

Population genetic structuring was strong for *D. gilvipes* ($\phi_{ST} = 0.93$, $p < 0.001$, Table 1.3). Both Bayesian and maximum likelihood trees of concatenated genes support a separate population in two coastal streams near Big Sur, which is south of San Francisco and Monterey Bays in California (Fig. 1.2). Within the northern clade, Bayesian trees suggested further north-south distinctions between a Montana/Washington/Oregon clade and a northern California/Oregon clade (Fig. 1.2); maximum likelihood trees do not show this relationship. Additionally, both Bayesian and maximum likelihood trees of concatenated genes also indicated a well-supported population in the Sierra Nevada Mountains. Bayesian and maximum likelihood trees of only *wingless* gene sequences also showed strong support for a Sierra Nevada Mountain population of *D. gilvipes*, but no support for different populations among the remaining streams.

*Calineuria californica* had similar overall population genetic structure to *D. gilvipes* ($\phi_{ST} = 0.93$, $p < 0.001$, Table 1.3), but with additional populations in Washington State and the San Gabriel Mountains of southern California, a site where no other study species were found. Concatenated trees indicate distinct populations along a north-south gradient, including well-supported clades in northern Washington, Oregon/northern California, California central coast, and southern California (Fig. 1.2). Additionally, both approaches indicate a well-supported Sierra Nevada Mountain clade. Similar to *D. gilvipes*, *wingless* gene trees showed strong support for a Sierra Nevada Mountain population of *C. californica*, and no support for different populations among the remaining streams.

*Pteronarcys californica* demonstrated the most pronounced population structure among the study species ($\phi_{ST} = 0.98$, $p < 0.001$, Table 1.3). Broad north-south structure similar to that of *D. gilvipes* and *C. californica* was found *P. californica*, with San Francisco Bay as the break point. Among specimens collected south of San Francisco Bay, concatenated trees indicate 2 populations, 1 at coastal sites near Big Sur, California, and 1 composed of specimens from Coyote Creek, California, which occurs 150 km inland (Fig. 1.1). *Wingless* gene trees indicated northern (Washington and Montana) and southern (California and Oregon) populations of *P. californica*.

Comparatively less population genetic structure was found for *H. pacifica* than for the other species ($\phi_{ST} = 0.68$, $p < 0.001$). Concatenated gene trees showed strong support for a distinct population composed of some specimens from 2 streams in northern Washington, and a distinct population composed of some, but not all, specimens of Scott Creek, California, which occurs south of San Francisco Bay. Trees also supported a clade that included all specimens from Ash Creek, Arizona, which was the most remote site (600 km miles from the nearest site and 3500 km from the farthest), and 2 specimens from the Sierra Nevada Mountains in California. *Wingless* gene trees showed no support for population structure in *H. pacifica*.

In summary, north-south population genetic structure was present in each species, but was more subtle in *H. pacifica*. In addition, concatenated gene trees revealed genetic diversity between Sierra Nevada Mountain and lowland populations for *D. gilvipes*, *C. californica*, and the two species of *Pteronarcys*. *Calineuria californica* and *D. gilvipes* each had distinct populations composed of specimens from Sierra Nevada Mountain streams. For *Pteronarcys*, gene trees indicated strong support for separation between *P. californica* specimens and *P. princeps* of Sierra Nevadan Mountain streams.
**Historical refugia**

Bayesian and maximum likelihood trees demonstrated basal branching of California clades relative to Oregon, Washington, and Montana clades for *C. californica*, *D. gilvipes*, and *P. californica* (Fig. 1.2). Haplotype networks indicate northern California and in some cases southern Oregon streams as the centers of high haplotype diversity (Fig. 1.3, A-C). Additionally, mean haplotype richness was higher in northern California and Oregon sites than Washington or Montana sites for these species. *Hesperoperla pacifica* showed basal branching for some California specimens, but with less resolution. Haplotype networks for *H. pacifica* also showed less clarity of haplotype richness patterns, yet the centers of highest diversity occurred in both northern California and southern Oregon streams (Fig. 1.3, D).

**DISCUSSION**

**Glacial legacy**

Broad concordance in population genetic structure suggests that the co-occurring species *C. californica*, *P. californica*, and *D. gilvipes* likely have had similar historical and current geographic barriers, despite representing 4 families in 2 orders. In particular, genetic structure along the north-south axis of the range of each of the study species suggests the genetic legacy of glacial refugia and population expansion. For example, phylogenetic trees of *D. gilvipes*, *C. californica*, and *P. californica* show north-south population breakpoints and, in the case of *C. californica*, several population breakpoints from southern California to Washington. In addition, haplotype distribution in these 3 species indicates fewer haplotypes in Washington and Montana, as well as in the Sierra Nevada Mountains, suggesting post-glacial expansion into those regions. *Hesperoperla pacifica* had distinct populations in the northern and southern portions of their range, but with only subtle indications of refugia and expansion.

Other taxa (e.g., salamanders) studied in western North America (Steele et al. 2005), as well as aquatic insects from central Europe (Pauls et al. 2006, Engelhardt et al. 2011), indicate similar genetic differences attributable to the isolation of ancestral populations in multiple southern refugia during periods of glacial maxima. During the last glacial maxima in Europe multiple aquatic species, including vertebrates and invertebrates, persisted in 1 or more refugia in southern Europe, and expanded to new habitats as glaciers receded northward. Similar patterns may have resulted from the last period of glaciation (2.5 million to 11,000 years ago) in northwest North America, where glaciers may have isolated populations of many northern species in 1 or more southern refugia (Wake 1997, Steele et al. 2005).

Phylogenies and haplotype networks suggest ancestry in northern California or southern Oregon for *D. gilvipes*, *C. californica*, and *P. californica*. Phylogenetically basal populations for *D. gilvipes*, *C. californica*, and *P. californica* indicate a California ancestral population. Moreover, the comparatively high haplotype richness found in northern California and southern Oregon relative to Washington and Montana in *D. gilvipes*, *C. californica*, *P. californica*, and to a lesser degree *H. pacifica*, also suggest ancestral populations persisted in this geographic region. Regions of highest haplotype diversity are often regarded as past refugia and represent the full array of genetic diversity and source of expansionary populations. In contrast, populations that were founded more recently often have signatures of founder effects or bottlenecks (Theissinger et al. 2011, Pessino et al. 2014). Previous studies suggest the Klamath-Siskiyou mountains in
northern California is a potential Pleistocene glacial refugia for aquatic-associated rough-skinned newts, Pacific giant salamanders, and black salamanders (Kutcha and Tan 2005, Steele and Storfer 2006, Reilly et al. 2013). Some studies of salamanders have suggested multiple refugia, including the Columbia River in northern Oregon/Washington (Steele and Storfer 2006) or the Sacramento River in California (Reilly et al. 2013). While our sampling sites did not allow for fine resolution of refugia, all 3 species likely used refugia in the northern California region, which suggests the potential for concordant refugial geography with co-occurring amphibians.

**Geographic barriers**

Population structure of *D. gilvipes*, *C. californica*, and *P. californica* align with several geographic barriers that may restrict insect dispersal on the Pacific Coast of the United States. The arid environment of the California Central Valley provides a likely barrier between coastal and inland populations. The California Central Valley was historically a freshwater lake, until 600,000 years ago (Sarna-Wojcicki et al. 1985, Dupre 1990), which would have acted as a barrier to east-west migration between mountain streams and coastal streams in California (Kuchta et al. 2009). Previous studies of *Ensatina* salamanders, whose inferred biogeography as a “ring species” demonstrates the concept of dispersal around a central barrier, show evidence of the potential of the California Central Valley to impact the distribution of genetic diversity (Wake 1997, Kuchta et al. 2009). For the aquatic species in the present study, the distinct Sierra Nevada Mountain clades versus lowland clades for *C. calineuria* and *D. gilvipes* may represent restricted dispersal across or around the California Central Valley (Fig.1.1), yet the location of populations in this study lacks the resolution to investigate whether a ring pattern is present.

Because the historical freshwater lake of the California Central Valley originally fed a large river with an outlet at Monterey Bay, the Monterey region is a historical north-south break point for many coastal taxa, including reptiles (Feldman and Spicer 2006) and amphibians (Kuchta and Tan 2006, Rissler et al. 2006). However, *C. californica*, *P. californica*, and *D. gilvipes* differ from the results of the amphibian studies because they did not demonstrate a break point at Monterey Bay, but rather at San Francisco Bay, a much younger geographic feature (Sarna-Wojcicki et al. 1985, Dupre 1990). San Francisco Bay, a saline estuary that is inhospitable to many taxa including riverine insects, presents a potential barrier to current dispersal north-south along the west coast of California. For example, *H. pacifica* had a distinct population in Scott Creek, California, which lies north of Monterey Bay and south of San Francisco Bay, but the lack of a coastal location south of Monterey Bay where this species was found limits interpretation of a broader break point.

Multiple well-supported Sierra Nevada Mountain clades for *C. californica* and *P. princeps*, as well as a single well-supported clade for *D. gilvipes* in the Sierra Nevada Mountains align with results from European studies that indicate montane habitats are important for intraspecific biodiversity (Pauls et al. 2006, Engelhardt et al. 2011, Taubmann et al. 2011). Other montane locations also harbored distinct populations for *C. californica* and *H. pacifica*. *Calineuria californica* had a distinct population in the San Gabriel Mountains, and *H. pacifica* had a distinct population composed of individuals from montane sites in Arizona and the Sierra Nevada Mountains.

**Dispersal traits and behaviors**

Despite being co-distributed, differences in population genetic structure exist among study species, suggesting differences in biological traits or dispersal potential. For these species,
winged flight, large body size, and large range size suggest strong dispersal potential; however, behaviors may constrain realized dispersal. Previous observational studies of stonefly adult behavior after emergence suggest that adults remain near their emergence location (Briers et al. 2002). Moreover, isotopic tracer experiments in other stonefly species indicate that most adults remain within 200 meters of their emergence stream (Macneale et al. 2005). The stonefly species in this study have narrow time windows for mating (~2 - 4 weeks), and their acoustic mating systems keeps females near the stream environment, which provides strong tradeoffs with dispersal. Little is known about the adult lifespan of individual stonefly species; however, the stoneflies in this study are estimated to live 2 weeks as adults (Stewart and Stark 1995). Previous genetic studies in caddisflies indicate some macropterous species may not be successful dispersers (Myers et al. 2001). Even so, many limnephilid caddisflies, such as D. gilvipes, have longer emergence windows and estimated longer lifespans (Wiggins 2004), which may provide increased time for dispersal and gene flow among populations to occur. However, as a poikilotherm, the autumn emergence of D. gilvipes, particularly in high-elevation habitats (Erman 1989) may inhibit dispersal potential relative to the summer emergence of C. californica, P. californica, and H. pacifica.

Where dispersal-related traits and behaviors appear consistent between species, other factors may explain differences in population genetic structure. For example, H. pacifica and C. californica are both in the family Perlidae, and have nearly identical body size, wing size, as well as emergence timing and emergence duration at several locations (Peckarsky 1979, Sheldon 1980, 1999). However, these species showed strikingly different population genetic structure and association of distinct populations with current geographic barriers. While this may be the result of other, unrecognized differences in dispersal potential, an alternative hypothesis may be that H. pacifica has dispersed at a slower rate since the last glacial maxima than C. californica because of different habitat constraints or different corridors of migration. Faster colonization or tolerance of wider habitat conditions during postglacial recolonization may have allowed C. californica populations to disperse and differentiate to a greater degree than H. pacifica.

Cryptic biodiversity

Sierra Nevada and northwest Washington specimens of C. californica may be representatives of a cryptic species. In some cases, genetic markers can identify cryptic diversity among certain geographic regions that may not be reflected in morphologically based taxonomic keys (Pfrender et al. 2010, Zhou et al. 2010). In our study, while concatenated gene trees and individual mitochondrial gene trees support 5 distinct C. californica populations (3-10% divergence from each other), the more conservative nuclear gene trees only corroborated strong support for a distinct Sierra Nevada/northwest Washington clade within C. californica (0.5-2% divergence). The genus Calineuria is monotypic, whereas other stonefly genera in this study comprise 2 sister species each (H. pacifica and hoguei; P. californica and princeps), suggesting that there may be undescribed diversity in the Calineuria genus. In Pteronarcys, P. princeps occurs in the Sierra Nevada Mountains, while P. californica occurs at lower elevations. Therefore, I hypothesize that C. californica may be a species-complex with similar distinctions.

Target genes and distribution of streams

Assessments of population genetic structure rely on the target genes and distribution of study sites to represent the actual connectivity of populations within the species. For example, previous studies have demonstrated diversity among riverine insect populations using
mitochondrial DNA alone (Schultheis et al. 2012, Previšić et al. 2014). However, other studies indicate that the inclusion of both mitochondrial and nuclear genes, because they differ in mutation rates, can provide a more comprehensive analysis of population connectivity (Elbrecht et al. 2014). In the present study, the use of both mitochondrial and nuclear genes enabled a comparison of gene trees for each gene to corroborate conclusions drawn from concatenated gene trees. With regard to sampling locations, the rivers and streams sampled in the present study encompassed the majority of the north-south range of these species, a span of nearly 2000 km, and for some species as far east as the Rocky Mountains of Montana, a span of 1200 km. Additional genetic structure could exist for H. pacifica, P. californica, and D. gilvipes in the eastern portion of their range, which includes streams of the Rocky Mountains and Great Basin. In addition, H. pacifica may also have additional genetic structure in Arizona, where only 1 stream was sampled for this study, and in the Sierra Nevada Mountains, where only 8 specimens from 2 streams were collected.

Implications for conservation of biodiversity with climate change

Despite being widespread aquatic species with large ranges, each of the study species has genetic structure that suggests vulnerability to losses of intraspecific genetic diversity under climate change scenarios. Calineuria californica, D. gilvipes, and P. californica had strong genetic structure, which may increase risk to future losses of intraspecific biodiversity (Bálint et al. 2011). In addition, the distribution of genetic diversity within species suggest similar refugial and expansion patterns in response to previous climate shifts among at least 3 of the study species, which suggest these species may respond similarly to future climate changes. Because these organisms are widespread and numerically important to river ecosystems along the Pacific Coast of the United States, the resilience of these species is likely to be important for stream food webs. For example, D. gilvipes in an Oregon stream accounted for 55-96% of the macroinvertebrate biomass (Tait et al. 1994), and their abundance can have strong impacts on stream communities (Power et al. 2008).

Our study identified geographically and genetically isolated populations of H. pacifica and C. californica at high elevations (>1,800 m) in the southern latitudes of their species range, which are vulnerable to range contraction and population extirpation. For example, predicted increases in air and consequently water temperatures may alter high-elevation streams and their biodiversity more than in lowland streams (Null et al. 2013, Elsen and Tingley 2015). In addition, previous studies of small, montane mammals demonstrated upward elevational range-shifts that correspond with increased minimum temperatures over the past century (Moritz et al. 2008). Populations near mountaintops are of particular concern because dispersal to higher elevations to remain within physiological requirements is constrained (Grayson 2005, La Sorte and Jetz 2010). Therefore, vulnerability may be highest where we find isolated, high elevation, and genetically distinct populations. Further, while Sierra Nevada Mountain populations are not as geographically isolated, they also represent distinct populations from lowland streams, and are likely at risk to similar thermal or hydrologic changes.

In conclusion, our results identify intraspecific genetic diversity in populations of large-bodied, co-occurring riverine insect species. Genetic indications of concordance in population structure and common glacial refugia for some study species, but not all, suggest the importance of Pleistocene glaciation, even for widespread species. Finally, results of this study identify cryptic diversity within the range of each species, particularly in mountain streams, which may be the intraspecific biodiversity most vulnerable to global climate change.
ACKNOWLEDGEMENTS

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LITERATURE CITED


Table 1.1. Total specimens collected and number of sampling locations for the stoneflies *C. californica, P. californica, P. princeps*, and *H. pacifica*, and the caddisfly *D. gilvipes*, as well as outgroups *A. partitus, D. baumanni, and H. hoguei*.

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Table 1.2. Primers and references for mitochondrial and nuclear genes sequenced in this study.

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<td>5'- ACY TCR CAR CAC CAR TGR AA -3'</td>
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Table 1.3. Haplotype richness and Phi-ST for conCaliforniatenated sequences (COI, COII, and wingless) for *Pteronarcy californica*, *Pteronarcy princeps*, *Dicosmoecus gilvipes*, *Hesperoperla pacifica*, and *Calineuria californica* within river systems in the western United States. Sites are organized by region.

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Table 3. *Continued.* Haplotype richness and Phi-ST for conCaliforniatenated sequences (COI, COII, and wingless) for *Pteronarcyx californica*, *Pteronarcyx princeps*, *Diclosmocaecus gilvipes*, *Hesperoperla pacifica*, and *Calineuria californica* within river systems in the western United States. Sites are organized by region.

<table>
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Table 1.3. Continued. Haplotype richness and Phi-ST for concatenated sequences (COI, COII, and wingless) for *Pteronarcys californica, Pteronarcys princeps, Dicosmoecus gilvipes, Hesperoperla pacifica,* and *Calineuria californica* within river systems in the western United States. Sites are organized by region.

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Figure 1.1. Map of stream sampling locations in western North America. Individual species (*Dicosmoecus gilvipes*, *Calineuria californica*, *Hesperoperla pacifica*, *Pteronarcys californica*, *Pteronarcys princeps*) collected at each site are coded by color.
Figure 1.2. Comparison of concatenated (COI, COII, wingless) maximum likelihood phylogenetic trees for 5 study taxa and outgroups. *Pteronarcys californica* and *P. princeps* were combined for phylogenetic analysis. Color codes are consistent by region and used for populations where bootstrap percentages were ≥ 70% and Bayesian posterior probability values were ≥ 0.9.
Figure 1.3A. Median-joining haplotype networks for *Pteronarcyis californica* and *Pteronarcyis princeps*. Circles represent individual haplotypes, the size of the circle represents the number of individuals sharing that haplotype, and stream locations are coded by color. Short hash-marks perpendicular to haplotype branches indicated number of base pair differences between haplotypes.
Figure 1.3B. Median-joining haplotype networks for *Dicosmoecus gilvipes* and outgroups. Circles represent individual haplotypes, the size of the circle represents the number of individuals sharing that haplotype, and stream locations are coded by color. Short hash-marks perpendicular to haplotype branches indicated number of base pair differences between haplotypes.
Figure 1. 3C. Median-joining haplotype networks for *Calineuria californica* and outgroups. Circles represent individual haplotypes, the size of the circle represents the number of individuals sharing that haplotype, and stream locations are coded by color. Short hash-marks perpendicular to haplotype branches indicated number of base pair differences between haplotypes.
Figure 1.3D. Median-joining haplotype networks for *Hesperoperla pacifica* and outgroups. Circles represent individual haplotypes, the size of the circle represents the number of individuals sharing that haplotype, and stream locations are coded by color. Short hash-marks perpendicular to haplotype branches indicated number of base pair differences between haplotypes.
CHAPTER 2

Northern elephant seal serum and blubber as integrators of contaminants in the deep ocean
Northern elephant seal serum and blubber as integrators of contaminants in the deep ocean

ABSTRACT

Long-lived, upper trophic level marine mammals are vulnerable to bioaccumulation of persistent organic pollutants (POPs). Internal tissues may accumulate and mobilize POP compounds at varying rates, related to the body condition of the animal and characteristics of the compound. Northern elephant seals (Mirounga angustirostris) forage in the northeastern Pacific Ocean on fish and squid in the mesopelagic zone. Elephant seals return to land twice a year to breed and molt, and fast during both periods. Paired blubber and blood samples were collected from adult female and male northern elephant seals in 2012 and 2013 at the Año Nuevo State Reserve (California, USA). For adult females (N = 24), the same seals were sampled before (end of molting fast) and after (start of the breeding fast) their approximately seven month long foraging trip. For males, different seals were sampled before (N = 14) and after (N = 15) the same foraging trip as females. Commonly studied tissues (serum, inner blubber, outer blubber) were examined to describe relationships and determine predictive equations among tissues for a suite of POP compounds, including, ΣDDTs, ΣPCBs, ΣCHLs, and ΣPBDEs. Strong relationships were found among tissues for many, but not all compounds. Additionally, the strongest relationships for ΣDDTs, ΣPCBs, ΣCHLs, and ΣPBDEs were found between serum and inner blubber, which are the tissues with higher metabolic activity. Overall, male and female elephant seals showed striking similarity in tissue relationship for many POPs, despite differences in depuration opportunities, such as gestation and lactation. The ability to estimate POP blubber concentrations from a serum sample, or vice versa, has the potential to enhance toxicological assessment because access to blood or blubber samples varies among studies of marine mammals.

BACKGROUND

Earlier research provided important scaffolding for this study (Peterson et al. 2015). Northern elephant seals (Mirounga angustirostris) are vulnerable to bioaccumulation of persistent organic pollutants (POPs) because they are top predators in the North Pacific Ocean. My collaborative research investigated a suite of POPs in blubber (inner and outer) and blood (serum) of free-ranging northern elephant seals in relation to physiology and ecology. For adult females (N = 24), the same seals were satellite tracked and sampled before and after their approximately seven month long foraging trip. For males, different adults and subadults were sampled before (N = 14) and after (N = 15) the same foraging trip. For females, we calculated blubber burdens for all compounds. For both males and females the highest POP concentrations were found for ΣDDTs and ΣPCBs. In blubber and serum, males had significantly greater concentrations than females for almost all compounds. Moreover, for both males and females, ΣDDT and ΣPBDEs were highly correlated in blubber and serum. While ΣPCBs were highly correlated with ΣDDTs and ΣPBDEs in blubber and serum for males, ΣPCBs showed weaker correlations with ΣDDTs and ΣPBDEs in females. As females gained mass while foraging, concentrations of almost all POPs in inner and outer blubber significantly decreased; however, the absolute burden in blubber significantly increased, indicating ingestion of contaminants while foraging. In addition, three clusters of seal foraging behavior were identified, with emphasis on
geography, diving behavior, and stable carbon and nitrogen isotopes. Behavioral clusters corresponded with differences in $\sum$DDTs, $\sum$PBDEs, MeO-BDE 47, as well as the ratio of $\sum$DDTs to $\sum$PCBs, indicating the potential for foraging behavior to exacerbate or reduce contaminant exposure. The greatest concentrations of $\sum$DDTs and $\sum$PBDEs were observed in the cluster that foraged closer to the coast and had blood samples more enriched in $^{13}$C. Bioaccumulation of POPs by elephant seals supports deep-ocean food webs as a sink for POPs and underscores elephant seals as a potential sentinel for biomonitoring of contamination in deep-ocean food webs.

**INTRODUCTION**

Persistent organic pollutants (POPs) are lipophilic environmental contaminants that are pervasive in marine food webs and bioaccumulate in organisms, which presents particular concern for long-lived, upper trophic level marine mammals. Although close proximity to POP sources can result in higher POP concentrations (Frouin et al. 2011), even foraging strategies that place animals far from contaminant sources do not insulate them from POP exposure (Peterson et al. 2015). Elevated concentrations of POPs are associated with endocrine, immune, and reproductive effects in marine and terrestrial wildlife (Tanabe 2002, Debier et al. 2005, Desforges et al. 2016). Among marine mammals, pinnipeds and odontocetes are vulnerable to biomagnification of POPs as a result of their high trophic position, and therefore are often the target species for POP biomonitoring efforts (Weijs et al. 2010, Yordy et al. 2010b, Lopez et al. 2012).

The internal tissues of marine mammals accumulate and mobilize POPs at varying rates, which present challenges for interpretation. Blood and blubber are commonly studied tissue compartments because they can be sampled non-lethally and are relatively accessible to researchers. Blood is in direct contact with internal tissues of toxicological concern (e.g., liver) and it is responsive to recent foraging (De Swart et al. 1996) and fasting (Louis et al. 2014). Therefore, blood can serve as a relevant indicator of recent contaminant exposure or a reflection of changes in physiological state that may liberate contaminants from storage tissues into circulation. In contrast, blubber is a lipid-rich tissue used for energy storage in both pinnipeds and odontocetes (Koopman et al. 1996, Strandberg et al. 2008). Within the vertical profile of the blubber layer, metabolic activity and fatty-acid mobilization vary, with inner blubber more metabolically active than outer (Strandberg et al. 2008, Fowler et al. 2014). For studies that aim to link contaminant concentrations to recent behavior, such as foraging or fasting, blood and inner blubber will respond more quickly than outer blubber (Louis et al. 2014). For studies that compare groups of animals or POP concentrations over time, outer blubber or full thickness blubber cores may provide a more relevant indicator of longer-term bioaccumulation (Randhawa et al. 2015). Although blubber is usually collected in pinnipeds and cetaceans, blood collection from live animals is less invasive, and many studies may store blood samples long term that can be utilized to track contaminants over time.

Northern elephant seals (*Mirounga angustirostris*) are upper trophic level predators that bioaccumulate POPs as they forage in the northeastern Pacific Ocean. The life history of northern elephant seals includes two foraging migrations per year interspersed with fasting periods for breeding and molting on land, which make them relatively accessible for study among marine mammals (Robinson et al. 2012). Additionally, foraging and fasting life-history
phases create dramatically different body conditions. For example, during the molting fast, which occurs pre-foraging, females lose approximately 25% of their mass (Worthy et al. 1992). While foraging, female elephant seals are at sea for 4 to 7 months and gain an average of 264 kg mass (Robinson et al. 2012). After arriving on land, females will then undergo a breeding fast, which will result in losing approximately 40% of their body mass (Costa et al. 1986). Body condition is an important determinant of POP concentrations in blood and blubber (Peterson et al. 2014), and effective toxicological risk assessment relies upon understanding POP concentrations at extremes in body condition.

Commonly studied tissues with higher (serum, inner blubber) and lower (outer blubber) metabolic activity were examined to describe relationships and determine predictive equations among tissues for POPs in northern elephant seals. The primary focus was to investigate relationships for polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT) and its metabolites, chlordanes (CHLs), and polybrominated diphenyl ethers (PBDEs), but hexachlorobenzene (HCB), hexachlorocyclohexane (α-HCH and β-HCH), and the naturally produced 6-MeO-BDE 47 were also included. Specifically, my objectives were to 1) assess serum POP concentrations as a predictor of inner and outer blubber POP concentrations, and inner blubber POP concentrations as a predictor of outer blubber POP concentrations, 2) compare serum-blubber relationships between males and females, and 3) compare serum-blubber relationships between pre-foraging (late in the molting fast) and post-foraging (early in the breeding fast) seals with markedly different body condition.

METHODS

Animal Sampling

Paired blubber and blood samples were collected from adult female and male northern elephant seals in 2012 and 2013 at the Año Nuevo State Reserve (California, USA, 37.11° N, 122.33° W). The same known-age females (N = 24), ranging in age from four to twelve years, were sampled before (late in the molting fast) and after (early in the breeding fast) the approximately seven month foraging trip (Fig. 2.1). Because repeatedly sampling males is presents significant challenges, blubber cores and blood samples were collected from 29 unique male northern elephant seals at two points in their life history: 14 seals were sampled at the end of the molting fast and 15 seals were sampled at the start of the early breeding fast (Fig. 2.1). Standard procedures for chemical immobilization and collection of blubber and blood from northern elephant seals were used (Le Boeuf et al. 2000, Robinson et al. 2012). A full-thickness blubber core was collected from the lateral pelvic area of each seal using a sterile 6 mm biopsy punch (Miltex, Inc., York, Pennsylvania, USA) and stored in aluminum foil. Blood samples were collected from the extradural vein and stored on ice in the field. Upon return to the lab within several hours, samples were centrifuged and serum aliquots were transferred to glass vials. Blubber cores and serum samples were stored at -20°C until analysis.

Laboratory analysis

Chemical determinations targeted 29 PCB congeners (IUPAC numbers: CB 28, 49, 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 146, 149, 153, 156, 170, 171, 174, 177, 180, 183, 187, 194, 196/203, 199, 206, and 209), seven PBDE congeners (IUPAC numbers: BDE 28, 47, 99, 100, 153, 154, and 183), three DDTs (p,p'-DDD, p,p'-DDE, p,p'-DDT), five chlordanes (CHLs: OxC
(oxychlordane), CC (cis-chlordane), TC (trans-chlordane), TN (trans-nonachlor), CN (cis-nonachlor), hexachlorobenzene (HCB), hexachlorocyclohexane (α-, β-, and γ-HCH), and the naturally-produced methoxylated PBDE, 6-MeO-BDE 47 in all samples.

Extraction, clean-up, and concentration measurement methods for blubber and serum followed protocols described in Vanden Berghe et al. (2012) and Peterson et al. (2015). In brief, for blubber analyses, the skin layer and hair (1.3 cm deep on average, Schwarz et al. 2015), was removed from the outer portion of the biopsy core, and the remaining blubber was cut into inner and outer segments of approximately equal mass. Blubber segments were analyzed separately because previous research determined differences in metabolic activity and stratification in fatty acid profiles among layers (Strandberg et al. 2008, Fowler et al. 2014). Serum samples were split for separate determination of target contaminants and lipids. Four lipid classes in serum (total cholesterol, phospholipids, triacylglycerides, and non-esterified fatty acids) were determined with enzyme kits from Diasys Diagnostic Systems (Holzheim, Germany) and Wako Chemicals (Neuss, Germany), with the concentrations of each lipid class calculated on the basis of standard equivalents. Total lipid concentrations were calculated as the sum of the four lipid classes (Debier et al. 2006, Vanden Berghe et al. 2012). All POP concentrations in serum were lipid-normalized before statistical analyses.

PBDEs, MeO-PBDEs, CHLs, HCB, and HCHs were measured by gas chromatography-electron capture negative ion/mass spectrometry (GC-ECNI/MS) on a 30 m × 0.25 mm × 0.25 µm DB-5 column (J&W Scientific, Folsom, CA, USA) by monitoring two ions m/z = 79 and 81 (for PBDEs and MeO-PBDEs) and two specific ions for each pesticide. DDTs and PCBs were measured by gas chromatography-electron ionization/mass spectrometry (GC-EI/MS) on a 25 m × 0.22 mm × 0.25 µm HT-8 column (SGE, Zulte, Belgium) by monitoring 2 ions for each homologue group.

**Quality control**

Quality control included randomized analysis of procedural blanks, solvent blanks, and standards throughout the extraction process. Recoveries for individual PCB and PBDE congeners ranged between 75 and 104% (RSD < 12%). For each analyte, the mean procedural blank value was used for subtraction to determine final analyte concentrations. After blank subtraction, the limit of quantification (LOQ) was set at 3 x SD of the procedural blank. For analytes that were not detected in procedural blanks, LOQs were calculated for a ratio S/N (signal to noise) equal to 10. A standard reference material SRM 1945 (PCBs, OCPs, and PBDEs in whale blubber) was used to test the accuracy of the method. Measured values did not deviate more than 15% from the certified values.

**Statistical analysis**

The relationship between each pair of tissues (inner blubber:serum, outer blubber:serum, outer blubber:inner blubber) was examined individually for each class of contaminants (ΣDDTs, ΣPCBs, ΣCHLs, and ΣPBDEs), for individual contaminants (HCB, α-HCH, β-HCH), and the naturally-produced 6-MeO-BDE 47, before elephant seals left for a foraging trip (late in the molting fast), and upon return from foraging (early in the breeding fast). In order to quantify the relationships between concentrations of POPs in paired tissues, I used mixed effects models in the statistical program R, version 3.0.2 (R Development Core Team 2012) and used F-tests (R package afex) to determine significance. I first ran a global model for each pair of tissue POP concentrations with sex as a factor and an interaction between the predictor tissue POP
concentration × sex. If the interaction was significant, I conducted subsequent analyses for each sex separately. All POP concentrations were natural-log transformed prior to analysis to meet the assumptions of general linear models. When sex was not a significant predictor, I removed the term for the predictive equation.

In addition, I calculated partition coefficients for outer blubber/serum and inner blubber/serum for each contaminant in male and female elephant seals. Partitioning coefficients were calculated for each individual as the ratio between concentrations in the two tissues. For females, I used paired t-tests to compare late molting and early breeding contaminant partitioning coefficients for ΣDDTs, ΣPCBs, ΣCHLs, and ΣPBDEs.

RESULTS

Serum and blubber samples from all elephant seals had detectable concentrations of ΣPCBs, ΣDDTs, ΣCHLs, and ΣPBDEs. The concentrations of POPs used in this study for northern elephant seal serum, inner blubber, and outer blubber have been reported previously (Peterson et al. 2015). Late in the molting fast, serum concentrations (ng g⁻¹ lipid) for males ranged from 823-3778 (ΣDDTs), 437-1679 (ΣPCBs), 187-468 (ΣCHLs), and 11-68 (ΣPBDEs); inner blubber concentrations (ng g⁻¹ lipid) ranged from 1054-7107 (ΣDDTs), 584-4396 (ΣPCBs), 247-1061 (ΣCHLs), and 13-112 (ΣPBDEs); outer blubber concentrations (ng g⁻¹ lipid) ranged from 1355-8730 (ΣDDTs), 733-4610 (ΣPCBs), 320-1262 (ΣCHLs), and 18-112 (ΣPBDEs). Upon return from foraging, early in the breeding fast, serum concentrations (ng g⁻¹ lipid) for males ranged from 390-7532 (ΣDDTs), 236-2317 (ΣPCBs), 111-350 (ΣCHLs), and 5-135 (ΣPBDEs); inner blubber concentrations ranged from 705-12689 (ΣDDTs), 448-3942 (ΣPCBs), 237-684 (ΣCHLs), and 13-264 (ΣPBDEs); outer blubber concentrations (ng g⁻¹ lipid) ranged from 770-15061 (ΣDDTs), 517-3975 (ΣPCBs), 233-729 (ΣCHLs), and 14-284 (ΣPBDEs).

For females late in the molting fast, serum concentrations (ng g⁻¹ lipid) ranged from 623-2360 (ΣDDTs), 445-997 (ΣPCBs), 120-271 (ΣCHLs), and 7-43 (ΣPBDEs); inner blubber (ng g⁻¹ lipid) ranged from 902-3354 (ΣDDTs), 580-1806 (ΣPCBs), 242-532 (ΣCHLs), and 14-76 (ΣPBDEs); outer blubber concentrations (ng g⁻¹ lipid) ranged from 865-2677 (ΣDDTs), 501-1053 (ΣPCBs), 211-352 (ΣCHLs), and 12-52 (ΣPBDEs). For females early in the breeding fast, serum concentrations (ng g⁻¹ lipid) ranged from 365-1912 (ΣDDTs), 194-470 (ΣPCBs), 74-175 (ΣCHLs), and 4-27 (ΣPBDEs); inner blubber concentrations (ng g⁻¹ lipid) ranged from 598-3086 (ΣDDTs), 422-1029 (ΣPCBs), 158-373 (ΣCHLs), and 10-61 (ΣPBDEs); outer blubber concentrations (ng g⁻¹ lipid) ranged from 875-2803 (ΣDDTs), 539-1046 (ΣPCBs), 207-327 (ΣCHLs), and 13-55 (ΣPBDEs).

Serum and inner blubber

Late molting seals did not have a significant interaction between sex × serum concentrations of ΣDDTs, ΣPCBs, ΣCHLs, and ΣPBDEs on the concentrations of these POP classes in inner blubber \((p≥0.18)\). Similarly, early breeding seals did not have a significant interaction between serum POP concentrations × sex on the POP concentrations in inner blubber, except for PBDEs \((F_{1,35}=6.45, p=0.015)\). Therefore, I removed the interaction from all models except for early breeding PBDEs, where I analyzed males and females separately.

Serum POP concentrations were significantly and positively related to inner blubber POP concentrations for all POP classes, both late in the molting fast (ΣDDTs: \(F_{1,35}=156.92, p<0.001\); ΣPCBs: \(F_{1,35}=63.83, p<0.001\); ΣCHLs: \(F_{1,35}=76.84, p<0.001\); ΣPBDEs: \(F_{1,35}=116.72, p<0.001\)
and early in the breeding fast (ΣDDTs: \( F_{1,36}=227.55, p<0.001; \) ΣPCBs: \( F_{1,36}=133.32, p<0.001; \) ΣCHLs: \( F_{1,36}=49.38, p<0.001 \)), while accounting for any potential effect of sex (Fig. 2.2). There was not a significant sex effect for any of the models where males and females were analyzed together (Late molting ΣDDTs: \( F_{1,35}=0.01, p=0.95; \) ΣPCBs: \( F_{1,35}<0.01, p=0.96; \) ΣCHLs: \( F_{1,35}=1.27, p=0.27; \) ΣPBDEs: \( F_{1,35}=0.01, p=0.91 \)). Early breeding: ΣDDTs \( F_{1,36}=0.75, p=0.39; \) ΣPCBs: \( F_{1,36}=3.35, p=0.08; \) ΣCHLs: \( F_{1,36}=2.13, p=0.15 \)). Early in the breeding fast, concentrations of ΣPBDEs in serum of females (\( F_{1,22}=24.55, p<0.001 \)) and males (\( F_{1,13}=422.54, p<0.001 \)) were significantly and positively related to concentrations of ΣPBDEs in inner blubber. For ΣDDTs, ΣPCBs, and ΣCHLs at both sampling periods, and ΣPBDEs at late molting, all relationships had \( R^2 \) values >0.7, and relationships for all POP classes were stronger at early breeding than at late molting (Table 2.1). In addition, the highest \( R^2 \) values at each life history phase were found for ΣDDTs. The specific equations to predict POP concentrations in inner blubber from POPs concentrations in serum for ΣDDTs, ΣPCBs, ΣCHLs, and ΣPBDEs are found in Table 2.1.

While ΣDDTs, ΣPCBs, ΣCHLs, and ΣPBDEs concentrations in serum were significantly related to those in inner blubber, I did not find a significant relationship for 6-MeO-BDE 47 late in the molting fast, or HCB and α-HCH at early in breeding (Supplementary materials, S2). In addition, serum:inner blubber relationships were strong for β-HCH at both time periods (\( R^2 \geq 0.86 \)). Test statistics and further explanation of results for these compounds can be found in supplemental materials. The specific equations to predict POP concentrations in outer blubber from POPs concentrations in serum for ΣDDTs, ΣPCBs, ΣCHLs, and ΣPBDEs are found in Appendix 2.1.

**Serum and outer Blubber**

A significant interaction between POP concentrations in serum \( \times \) sex on POP concentrations in outer blubber was detected for ΣDDTs, ΣPCBs, and ΣCHLs during both late molting (ΣDDTs: \( F_{1,34}=5.27, p=0.028; \) ΣPCBs: \( F_{1,34}=24.8, p<0.001; \) ΣCHLs: \( F_{1,34}=21.89, p<0.001 \)) and early breeding (ΣDDTs: \( F_{1,35}=7.48, p=0.010; \) ΣPCBs: \( F_{1,35}=7.68, p=0.009; \) ΣCHLs: \( F_{1,35}=10.38, p=0.003 \)). In addition, I detected the interaction in early breeding ΣPBDEs (\( F_{1,35}=12.58, p<0.001 \)), but not late molting ΣPBDEs (\( F_{1,34}=3.57, p=0.067 \)). Therefore, I removed the interaction from the model for late molting ΣPBDEs, but for all other POP classes we conducted separate statistical analyses on males and females.

Serum POP concentrations were significantly and positively related to outer blubber POP concentrations for all POP classes, during both sampling periods, for females (late molting: ΣDDTs: \( F_{1,22}=33.95, p<0.001; \) ΣPCBs: \( F_{1,22}=6.77, p=0.016; \) ΣCHLs: \( F_{1,22}=11.71, p=0.002 \); early breeding: ΣDDTs: \( F_{1,22}=66.43, p<0.001; \) ΣPCBs: \( F_{1,22}=7.44, p=0.012; \) ΣCHLs: \( F_{1,22}=7.22, p=0.013; \) ΣPBDEs: \( F_{1,22}=24.80, p<0.001 \)) and males (late molting: ΣDDTs: \( F_{1,12}=83.77, p<0.001; \) ΣPCBs: \( F_{1,12}=97.86, p<0.001; \) ΣCHLs: \( F_{1,12}=62.53, p<0.001 \); early breeding: ΣDDTs: \( F_{1,13}=188.90, p<0.001; \) ΣPCBs: \( F_{1,13}=97.18, p<0.001; \) ΣCHLs: \( F_{1,13}=50.00, p<0.001; \) ΣPBDEs: \( F_{1,13}=368.10, p<0.001 \); Fig. 2.2). For late molting seals, concentrations of ΣPBDEs in serum were significantly related to concentrations of ΣPBDEs in outer blubber (\( F_{1,35}=45.13, p<0.001 \)), when accounting for sex (\( F_{1,35}=2.37, p=0.13 \)). There was not a significant sex effect for ΣPBDEs, indicating that similar ΣPBDE concentrations in serum corresponded to similar ΣPBDE concentrations in outer blubber for males and females (Fig. 2.2). Serum-blubber relationships were stronger for males (\( 0.94 \geq R^2 \geq 0.79 \)) than females (\( 0.75 \geq R^2 \geq 0.23 \)) for ΣDDTs, ΣPCBs, ΣCHLs, and ΣPBDEs at both sampling periods, but the differences between the sexes
were more pronounced for ΣPCBs and ΣCHLs at both life history stages than for DDTs and PBDEs (Fig. 2.2). For each contaminant class, the slope of the relationship for males was steeper than females, indicating that at higher serum concentrations individual males had proportionately higher outer blubber contaminant concentrations than females in comparison with seals having lower serum concentrations. The specific equations to predict POP concentrations in outer blubber from POPs concentrations in inner blubber for ΣDDTs, ΣPCBs, ΣCHLs, and ΣPBDEs are found in Table 2.1.

In contrast with ΣDDTs, ΣPCBs, ΣCHLs, and ΣPBDEs, serum concentrations of the compounds 6-MeO-BDE 47, HCB, and α-HCH were not always significantly related to inner blubber concentrations (Fig. 2.3). Late in the molting fast, 6-MeO-BDE 47, HCB, and α-HCH concentrations in female serum samples were not significantly related to concentrations in outer blubber samples. In addition, HCB was not significantly related at the early breeding fast. However, β-HCH did show a strong relationship between serum and outer blubber concentrations at both time periods ($R^2 \geq 0.78$). Test statistics and further explanation of results for these compounds can be found in supplemental materials. The specific equations to predict POP concentrations in outer blubber from POPs concentrations in inner blubber for 6-MeO-BDE 47, HCB, α-HCH, β-HCH and are found in Appendix 2.1.

**Inner blubber and outer blubber**

A significant interaction was observed between POP concentrations in inner blubber × sex for ΣDDTs, ΣPCBs, ΣCHLs, and ΣPBDEs in outer blubber during both late molting (ΣPCBs: $F_{1,34}=18.37$, $p<0.001$; ΣCHLs: $F_{1,34}=31.60$, $p<0.001$) and early breeding (ΣDDTs: $F_{1,35}=9.47$, $p=0.004$; ΣPCBs: $F_{1,35}=13.37$, $p<0.001$; ΣCHLs: $F_{1,35}=26.23$, $p<0.001$; ΣPBDEs: $F_{1,35}=7.80$, $p=0.008$), with the exception of ΣDDTs and ΣPBDEs during late molting (ΣDDTs: $F_{1,34}=2.32$, $p=0.14$; ΣPBDEs: $F_{1,34}=2.11$, $p=0.16$). Therefore, in the case of late molting, I removed the interaction from the models for ΣDDTs and ΣPBDEs, and for ΣPCBs and ΣCHLs I analyzed males and females separately.

Inner blubber POP concentrations were significantly and positively related to outer blubber POP concentrations for ΣDDTs, ΣPCBs, ΣCHLs, and ΣPBDEs during both sampling periods, for both females (late molting: ΣPCBs: $F_{1,22}=17.40$, $p<0.001$; ΣCHLs: $F_{1,22}=42.37$, $p<0.001$; early breeding: ΣDDTs: $F_{1,22}=26.36$, $p<0.001$; ΣPCBs: $F_{1,22}=15.15$, $p<0.001$; ΣCHLs: $F_{1,22}=5.02$, $p=0.036$; ΣPBDEs: $F_{1,22}=24.05$, $p<0.001$) and males (late molting: ΣPCBs: $F_{1,12}=181.40$, $p<0.001$; ΣCHLs: $F_{1,12}=290.40$, $p<0.001$; early breeding: ΣDDTs: $F_{1,13}=791.90$, $p<0.001$; ΣPCBs: $F_{1,13}=552.00$, $p<0.001$; ΣCHLs: $F_{1,13}=124.8$, $p<0.001$; ΣPBDEs: $F_{1,13}=489.40$, $p<0.001$; Fig. 2.2). For late molting seals, concentrations of ΣDDTs and ΣPBDEs in serum were significantly related to concentrations of ΣDDTs and ΣPBDEs in outer blubber ($F_{1,35}=409.70$, $p<0.001$ and $F_{1,35}=143.80$, $p<0.001$ respectively), when accounting for sex ($F_{1,35}=0.64$, $p=0.43$ and $F_{1,35}=4.26$, $p<0.047$, respectively; Fig. 2.2). The significant sex effect for ΣPBDEs in outer blubber showed the male and females had the same slope, but males had higher concentrations of ΣPBDEs in outer blubber than females for similar concentrations in inner blubber.

Additionally, the relationships between inner and outer blubber were stronger for males than females for ΣDDTs, ΣPCBs, ΣCHLs, and ΣPBDEs at both sampling periods (Fig. 2.2). For female concentrations of ΣCHLs in inner blubber and outer blubber, the relationship was stronger in late molting ($R^2=0.66$) relative to early breeding ($R^2=0.19$), whereas the relationships between concentrations of ΣPCBs and ΣPBDEs in inner blubber and outer blubber were similar between the two life history stages (Fig. 2.2). For ΣDDTs, ΣPCBs, and ΣCHLs from both time
periods, and early breeding ΣPBDEs, the slope of the relationship for males was steeper than females, indicating that males with higher inner blubber concentrations had proportionately higher concentrations than females in outer blubber when compared with males and females with lower inner blubber concentrations. The specific equations to predict POP concentrations in outer blubber from POPs concentrations in inner blubber for ΣDDTs, ΣPCBs, ΣCHLs, and ΣPBDEs are found in Table 2.1.

For 6-MeO-BDE 47, α-HCH, β-HCH, and HCB, inner blubber POP concentrations were significantly and positively related to outer blubber POP concentrations during both sampling periods. Late in the molting fast, 6-MeO-BDE 47 showed strong inner:outer blubber relationships ($R^2 \geq 0.84$), as did females and males for β-HCH in early breeding ($R^2 \geq 0.96$). Early in the breeding fast, there was no interaction between inner blubber POP concentrations × sex for these four compounds. Test statistics and further explanation of results for these compounds can be found in supplemental materials. The specific equations to predict POP concentrations in outer blubber from POPs concentrations in inner blubber for 6-MeO-BDE 47, α-HCH, β-HCH, and HCB are found in Appendix 2.1.

**Partitioning coefficients**

For females, partitioning coefficients for outer blubber/serum and inner blubber/serum for ΣDDTs, ΣPCBs, ΣCHLs, and ΣPBDEs were lower during the late molting fast than the early breeding fast ($t \geq 2.43$, $df = 23$, $p \leq 0.02$). At the late molting fast, the median (min-max) outer blubber/serum partitioning coefficients for females and males, respectively, were $1.23$ (0.86-1.72) and $1.26$ (0.96-2.19) for ΣDDTs, $1.17$ (0.83-1.28) and $1.42$ (1.12-2.61) for ΣPCBs, $1.41$ (1.05-1.98) and $1.39$ (1.13-2.63) for ΣCHLs, and $1.43$ (0.55-3.89) and $1.63$ (1.12-3.06) for ΣPBDEs. Furthermore, during the early breeding fast, the median (min-max) partitioning coefficients for females and males, respectively, were $2.24$ (1.47-3.24) and $1.74$ (1.30-2.45) for ΣDDTs, $2.49$ (1.56-3.24) and $1.71$ (1.22-2.18) for ΣPCBs, $2.25$ (1.60-3.04) and $1.95$ (1.59-2.51) for ΣCHLs, and $2.97$ (1.06-5.49) and $2.10$ (1.83-3.09) for ΣPBDEs.

For inner blubber/serum partitioning coefficients during the late molting fast, the median (min-max) partitioning coefficients for females and males, respectively, were $1.36$ (1.02-1.89) and $1.33$ (1.10-2.69) for ΣDDTs, $1.69$ (1.12-2.37) and $1.65$ (1.12-2.74) for ΣPCBs, $1.91$ (1.44-2.20) and $1.64$ (1.23-3.12) for ΣCHLs, and $1.78$ (0.84-2.36) and $1.71$ (1.27-3.34) for ΣPBDEs. During the early breeding fast, inner blubber/serum partitioning coefficients were $1.74$ (1.29-2.70) and $1.49$ (1.18-2.24) for ΣDDTs, $2.31$ (1.51-2.94) and $1.70$ (1.34-2.28) for ΣPCBs, $208$ (1.48-2.94) and $1.94$ (1.54-2.45) for ΣCHLs, and $2.33$ (1.01-6.01) and $2.11$ (1.73-292) for ΣPBDEs.

**DISCUSSION**

Free-ranging elephant seals demonstrated strong, predictive relationships among serum, inner blubber, and outer blubber POP concentrations even after the influence of recent foraging at sea or weeks of fasting on land. Previous research has shown PCB concentrations to fluctuate asynchronously among serum, inner blubber, and outer blubber in response to recent foraging or fasting activities (Debier et al. 2012, Peterson et al. 2014, Louis et al. 2016a). Late in the molting fast, elephant seals had been fasting for several weeks and their more metabolically active tissues, serum and inner blubber, likely reflected recent mobilization of POPs from the inner blubber layer to serum (Louis et al. 2014) and redistribution of POPs among internal organs.
Mobilization of fatty acids from blubber is complex and based on the lipophilicity of fatty acids (Hall et al. 2008, Louis et al. 2016b) and the vertical composition of the blubber itself (Koopman et al. 1996). Early in the breeding fast, elephant seals had recently returned from an extensive (4-7 month) foraging trip; therefore, serum and inner blubber likely represented POPs ingested with food, as well as ongoing redistribution of POPs from other internal tissues. Outer blubber, on the other hand, is less metabolically active than inner blubber (Strandberg et al. 2008, Debier et al. 2012), and may reflect a longer-term signal of POP bioaccumulation.

Serum and inner blubber are the two most metabolically active tissues in this study, and for nearly all contaminant pairings, males and females could be included in the same predictive equation, highlighting commonality in physiological mechanisms related to these two tissues. The lack of detectable interactions between serum POP concentrations × sex, or an effect of sex, except for ΣPBDEs during early breeding, indicates that these tissues relate similarly in males and females despite concurrent gestation of a pup at late molting, and concurrent lactation at early breeding, by females. Serum also performed well as a predictor of inner blubber POP concentrations for male and female elephant seals. The relatively strong predictive relationships found in this study between serum and inner blubber for ΣDDTs, ΣPCBs, ΣCHLs, and ΣPBDEs at both sampling periods. However, lower female partitioning coefficients during the late molting fast compared with the early breeding fast for inner blubber/serum show tissue concentrations in serum and inner blubber are changing relative to each other during the foraging trip. This indicates that accurate prediction between serum and inner blubber may require different equations for different time periods.

Unlike serum:inner blubber relationships, females and males had significantly different relationships for serum:outer blubber and inner blubber:outer blubber, which may be attributed to differences in elimination capacities (Debier et al. 2003), the impact of lactation on specific POP compounds (Debier et al. 2012, Vanden Berghe et al. 2012), or different foraging locations. Predictive equations for males performed well among all tissues and at both sampling periods. In contrast, the strength of female tissue relationships was inconsistent across POP classes. When there was a significant difference in the slope of the relationships, male elephant seals were always characterized by steeper slope compared with females, resulting in greater differences between male and female outer blubber concentrations at higher concentrations of serum or inner blubber than at lower concentrations. Steeper slopes in male tissue relationships could reflect the contamination associated with their prey type or foraging locations, which are more related to coastal areas than for many females (Le Boeuf et al. 2000).

Similar to inner blubber/serum, lower partitioning coefficients for outer blubber/serum at late in the molting fast, prior to the foraging trip, compared with early in the breeding fast, after the foraging trip, show that tissue concentrations in serum and outer blubber are changing relative to each other over the foraging trip. Previous research with elephant seals shows decreases in POP concentrations over a foraging trip for serum, inner blubber, and outer blubber (Peterson et al. 2015). Increased partitioning coefficients for outer blubber/serum suggests that while each tissue decreases in POP concentrations during foraging (Peterson et al. 2015), the decrease in serum is of greater magnitude than inner or outer blubber.

Female serum and inner blubber were strong predictors of outer blubber for ΣDDTs and ΣPBDEs, but weaker predictors for ΣPCBs and ΣCHLs. Female lactation physiology results in disproportionate mobilization and transfer to milk of some POP compounds over others (Debier et al. 2012, Vanden Berghe et al. 2013), which may explain the weaker performance of predictive equations for ΣPCBs and ΣCHLs. ΣPCBs represents the sum of a suite of congeners,
each with compound-specific log $K_{ow}$ (octanol-water partitioning coefficient; Hawker and Connell 1988). When females fast, lower lipophilic congeners are more readily mobilized from inner blubber to serum (Debier et al. 2012) and from serum to milk during lactation (Debier et al. 2003, 2012, Vanden Berghe et al. 2012). As a result, there can be fasting effects on individual congeners, which may make serum ΣPCB concentrations an unreliable predictor of inner or outer blubber ΣPCB concentrations in certain scenarios. In the current study, differences in lipophilicity of specific congeners may distort the ability to predict outer blubber concentrations from serum or inner blubber for ΣPCBs in both seasons ($R^2 \leq 0.44$) and ΣCHLs in early breeding ($R^2 \leq 0.25$).

Significant positive relationships between blood and fat compartments have been observed and quantified for POPs in the tissues of a range of animals, including polar bears (Bernhoft et al. 1997), turtles (Keller et al. 2004), dolphins (Yordy et al. 2010c), and Hawaiian monk seals (Lopez et al. 2012). Hawaiian monk seals and northern elephant seals are both pinnipeds, the taxonomic group that includes seals, sea lions, and walrus. Hawaiian monk seals of various ages and both sexes had strong relationships between blubber and serum for ΣDDTs, ΣPCBs, and ΣCHLs (Lopez et al. 2012). Strong correlations among vertebrates that encompass a wide range of dietary patterns and life history strategies suggest that POP concentrations in blood may be used to predict POP concentrations in blubber for many animals.

Our study of a pinniped species is similar in approach to a study on odontocetes (taxonomic group that includes toothed cetaceans) that determined predictive equations for ΣDDTs, ΣPCBs, ΣCHLs, and ΣPBDEs in the bottlenose dolphin (Tursiops truncatus) at Sarasota Bay, Florida, USA (Yordy et al. 2010c). Bottlenose dolphin predictive equations for plasma and full-thickness blubber were established for males and juveniles, and partitioning coefficients (blubber:plasma) were calculated for males, juveniles, and females (Yordy et al. 2010c). Northern elephant seals differ from bottlenose dolphins in several crucial aspects, namely that they lack a lipid-rich internal melon tissue that can accumulate POPs (Yordy et al. 2010a), they undergo extreme fasting periods that mobilize POPs from blubber to serum (Debier et al. 2006), and the inter-offspring interval is higher for dolphins relative to elephant seals, which provides a different frequency of opportunity for contaminant elimination by female elephant seals. However, both northern elephant seals and bottlenose dolphins are relatively long-lived, top marine predators with relatively large fat compartments (blubber) that can bioaccumulate POPs.

Both studies found significant, positive relationships among POP concentrations in male blood and blubber. Predictive equations for bottlenose dolphins showed strong relationships for all POP classes ($R^2 \geq 0.91$), but did not include females. Whereas bottlenose dolphin predictive equations for males and juveniles had slopes from 0.91-1.08, male elephant seal equations showed a greater range in slope, ranging from 0.78-1.42. Additionally, blubber:plasma partitioning coefficients reported for bottlenose dolphin males were higher for ΣDDTs and ΣCHLs, but lower for ΣPCBs and ΣPBDEs, than either inner blubber:serum or outer blubber:serum partitioning coefficients for elephant seals males. Elephant seal females had higher partitioning coefficients during the early breeding fast than female bottlenose dolphins, but lower partitioning coefficients for ΣDDTs, ΣPCBs, and ΣCHLs. Early in breeding, elephant seal females have just returned from a long foraging trip, and have very recently given birth to a pup and begun lactation.

Higher partitioning coefficients early in the breeding fast may represent the response of serum to recent foraging and greater dilution of POPs in serum compared with blubber. The differences in partitioning coefficients between the two species may also be attributed to
different compound-specific exposures related to foraging locations and prey. Elephant seal females at both time periods had higher partitioning coefficients for ΣPBDEs than bottlenose dolphins, which may reflect lower exposure, and consequently lower serum ΣPBDEs concentrations, during foraging than Sarasota Bay bottlenose dolphins, which have higher concentrations for all contaminants than northern elephant seals (Yordy et al. 2010c).

**Implications for biomonitoring**

The ability to estimate POP blubber concentrations from serum, or vice versa, has the potential to enhance toxicological assessment in marine mammals. In cases where blubber samples are more attainable than blood (Elfes et al. 2010), the ability to calculate blood concentrations increases the capacity for toxicological risk assessment because blood interacts with vital organs (e.g., liver). In some cases, blood samples are more attainable or are banked from past collections and can be leveraged for new studies. Because blubber is the largest reservoir for POPs, the ability to link blood to the blubber reserve of POPs may be important for assessing life-time risk or estimating peak serum concentrations.

Despite different factors, such as lactation, that could affect relationships among tissues, male and female elephant seals showed striking similarity in tissue relationship for many POPs. Given the timing of lactation, we would expect female and male northern elephant seals to have more differences in physiology during the breeding fast, yet even in that time period, many relationships between serum and inner blubber can be computed for males and females together. Males do not have the opportunity to reduce POPs or change POP proportions in this way. Furthermore, the detection of positive relationships in two marine mammals, in the current study and in Yordy et al. (2010c), with clear behavior and life history differences raises the question: are predictive equations species-specific or can we determine generalities among species? If future research that involves direct handling of animals can produce additional blood-blubber relationships, then it may be possible to construct more generalized equations for groups of similar marine mammals, such as for pinnipeds. Generalized relationships, even for a few POPs, would enhance monitoring of contaminants in wildlife, particularly in situations where free-ranging animals are difficult to sample.

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LITERATURE CITED


Figure 2.1. The foraging and fasting time periods in one year of a female (left) and male (right) northern elephant seal. Individual females are on land for approximately four-five weeks during the breeding fast and five-six weeks during the molting fast. Males and females are on land for a similar lengths of time during the molting fast, but males arrive on land earlier and stay longer than females during the breeding fast. Note that individual seals are on land for less time than the full periods shown above because seals do not all arrive to the colony at the same time.
A. Late molting fast

Figure 2.2. Relationships between ΣDDTs, ΣPCBs, ΣCHLs, and ΣPBDEs concentrations in serum and inner blubber (upper panels), serum and outer blubber (middle panels), and inner blubber and outer blubber (lower panels) of adult female and male northern elephant seals at two time periods, late in the molting fast (A, top) and early in the breeding fast (B, bottom). Regression lines indicate significant relationships (p < 0.05) between two tissues during each sampling period; if there was a significant difference in the slope or y-intercept between females and males, the relationship and R² value for both sexes are shown. Males are shown by solid circles and black lines, whereas females are open symbols and solid gray lines. Dashed lines represent the relationship for both males and females when they were not significantly different.
B. Early breeding fast

Figure 2.2. Continued. Relationships between ΣDDTs, ΣPCBs, ΣCHLs, and ΣPBDEs concentrations in serum and inner blubber (upper panels), serum and outer blubber (middle panels), and inner blubber and outer blubber (lower panels) of adult female and male northern elephant seals at two time periods, late in the molting fast (A, top) and early in the breeding fast (B, bottom). Regression lines indicate significant relationships (p < 0.05) between two tissues during each sampling period; if there was a significant difference in the slope or y-intercept between females and males, the relationship and R² value for both sexes are shown. Males are shown by solid circles and black lines, whereas females are open symbols and solid gray lines. Dashed lines represent the relationship for both males and females when they were not significantly different.
A. Late molting fast

Figure 2.3. Relationships between 6-MeO-BDE 47, α-HCH, β-HCH, and HCB concentrations in serum and inner blubber (upper panels), serum and outer blubber (middle panels), and inner blubber and outer blubber (lower panels) of adult female and male northern elephant seals at two time periods, late in the molting fast (A, top) and early in the breeding fast (B, bottom). Regression lines indicate significant relationships (p < 0.05) between two tissues during each sampling period; if there was a significant difference in the slope or y-intercept between females and males, the relationship and R^2 value for both sexes are shown. Males are shown by solid circles and black lines, whereas females are open symbols and solid gray lines. Dashed lines represent the relationship for both males and females when they were not significantly different.
Figure 2.3. Continued. Relationships between 6-MeO-BDE 47, α-HCH, β-HCH, and HCB concentrations in serum and inner blubber (upper panels), serum and outer blubber (middle panels), and inner blubber and outer blubber (lower panels) of adult female and male northern elephant seals at two time periods, late in the molting fast (A, top) and early in the breeding fast (B, bottom). Regression lines indicate significant relationships (p < 0.05) between two tissues during each sampling period; if there was a significant difference in the slope or y-intercept between females and males, the relationship and $R^2$ value for both sexes are shown. Males are shown by solid circles and black lines, whereas females are open symbols and solid gray lines. Dashed lines represent the relationship for both males and females when they were not significantly different.
Table 2.1. Predictive equations, including only significant terms, for concentrations of DDTs, PCBs, CHLs and PBDEs between serum and inner blubber, serum and outer blubber, and inner and outer blubber. The $R^2$ refers to the goodness of fit of the data on the line produced by the predictive equation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Late Molting (N=14 males, 24 females)</th>
<th>$R^2$</th>
<th>Early Breeding (N=15 males, 24 Females)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum-Inner Blubber</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Sigma$DDTs</td>
<td>$F+M \ln[\Sigma$DDT$<em>{Inner}] = 1.107 \times \ln[\Sigma$DDT$</em>{Serum}] - 0.467$</td>
<td>0.86</td>
<td>$F+M \ln[\Sigma$DDT$<em>{Inner}] = 0.911 \times \ln[\Sigma$DDT$</em>{Serum}] + 1.101$</td>
<td>0.91</td>
</tr>
<tr>
<td>$\Sigma$PCBs</td>
<td>$F+M \ln[\Sigma$PCB$<em>{Inner}] = 0.921 \times \ln[\Sigma$PCB$</em>{Serum}] + 1.010$</td>
<td>0.71</td>
<td>$F+M \ln[\Sigma$PCB$<em>{Inner}] = 0.774 \times \ln[\Sigma$PCB$</em>{Serum}] + 2.080$</td>
<td>0.87</td>
</tr>
<tr>
<td>$\Sigma$CHLs</td>
<td>$F+M \ln[\Sigma$CHL$<em>{Inner}] = 0.971 \times \ln[\Sigma$CHL$</em>{Serum}] + 0.739$</td>
<td>0.78</td>
<td>$F+M \ln[\Sigma$CHL$<em>{Inner}] = 0.851 \times \ln[\Sigma$CHL$</em>{Serum}] + 1.450$</td>
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</tr>
<tr>
<td>$\Sigma$PBDEs</td>
<td>$F+M \ln[\Sigma$PBDE$<em>{Inner}] = 0.888 \times \ln[\Sigma$PBDE$</em>{Serum}] + 0.883$</td>
<td>0.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Serum-Outer Blubber</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>$\Sigma$DDTs</td>
<td>$F \ln[\Sigma$DDT$<em>{Outer}] = 0.782 \times \ln[\Sigma$DDT$</em>{Serum}] + 1.761$</td>
<td>0.61</td>
<td>$F \ln[\Sigma$DDT$<em>{Outer}] = 0.683 \times \ln[\Sigma$DDT$</em>{Serum}] + 2.850$</td>
<td>0.75</td>
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<td></td>
<td>$M \ln[\Sigma$DDT$<em>{Outer}] = 1.212 \times \ln[\Sigma$DDT$</em>{Serum}] - 1.350$</td>
<td>0.87</td>
<td>$M \ln[\Sigma$DDT$<em>{Outer}] = 0.986 \times \ln[\Sigma$DDT$</em>{Serum}] + 0.677$</td>
<td>0.94</td>
</tr>
<tr>
<td>$\Sigma$PCBs</td>
<td>$F \ln[\Sigma$PCB$<em>{Outer}] = 0.370 \times \ln[\Sigma$PCB$</em>{Serum}] + 4.291$</td>
<td>0.23</td>
<td>$F \ln[\Sigma$PCB$<em>{Outer}] = 0.389 \times \ln[\Sigma$PCB$</em>{Serum}] + 4.410$</td>
<td>0.25</td>
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<td></td>
<td>$M \ln[\Sigma$PCB$<em>{Outer}] = 1.34 \times \ln[\Sigma$PCB$</em>{Serum}] - 1.973$</td>
<td>0.89</td>
<td>$M \ln[\Sigma$PCB$<em>{Outer}] = 0.857 \times \ln[\Sigma$PCB$</em>{Serum}] + 1.471$</td>
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<td>$\Sigma$CHLs</td>
<td>$F \ln[\Sigma$CHL$<em>{Outer}] = 0.436 \times \ln[\Sigma$CHL$</em>{Serum}] + 3.321$</td>
<td>0.35</td>
<td>$F \ln[\Sigma$CHL$<em>{Outer}] = 0.287 \times \ln[\Sigma$CHL$</em>{Serum}] + 4.190$</td>
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<td></td>
<td>$M \ln[\Sigma$CHL$<em>{Outer}] = 1.42 \times \ln[\Sigma$CHL$</em>{Serum}] - 2.055$</td>
<td>0.84</td>
<td>$M \ln[\Sigma$CHL$<em>{Outer}] = 0.777 \times \ln[\Sigma$CHL$</em>{Serum}] + 1.904$</td>
<td>0.79</td>
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<td>$\Sigma$PBDEs</td>
<td>$F+M \ln[\Sigma$PBDE$<em>{Outer}] = 0.814 \times \ln[\Sigma$PBDE$</em>{Serum}] + 0.980$</td>
<td>0.60</td>
<td>$F \ln[\Sigma$PBDE$<em>{Outer}] = 0.532 \times \ln[\Sigma$PBDE$</em>{Serum}] + 2.04$</td>
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<tr>
<td></td>
<td>$M \ln[\Sigma$PBDE$<em>{Outer}] = 0.946 \times \ln[\Sigma$PBDE$</em>{Serum}] + 0.941$</td>
<td>0.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Inner Blubber-Outer Blubber</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Sigma$DDTs</td>
<td>$F+M \ln[\Sigma$DDT$<em>{Outer}] = 0.932 \times \ln[\Sigma$DDT$</em>{Inner}] + 0.420$</td>
<td>0.94</td>
<td>$F \ln[\Sigma$DDT$<em>{Outer}] = 0.603 \times \ln[\Sigma$DDT$</em>{Inner}] + 3.027$</td>
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<td></td>
<td>$M \ln[\Sigma$DDT$<em>{Outer}] = 0.967 \times \ln[\Sigma$DDT$</em>{Inner}] + 0.393$</td>
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<tr>
<td>$\Sigma$PCBs</td>
<td>$F \ln[\Sigma$PCB$<em>{Outer}] = 0.500 \times \ln[\Sigma$PCB$</em>{Inner}] + 3.21$</td>
<td>0.44</td>
<td>$F \ln[\Sigma$PCB$<em>{Outer}] = 0.475 \times \ln[\Sigma$PCB$</em>{Inner}] + 3.531$</td>
<td>0.41</td>
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<td></td>
<td>$M \ln[\Sigma$PCB$<em>{Outer}] = 1.120 \times \ln[\Sigma$PCB$</em>{Inner}] - 1.021$</td>
<td>0.94</td>
<td>$M \ln[\Sigma$PCB$<em>{Outer}] = 0.920 \times \ln[\Sigma$PCB$</em>{Inner}] + 0.561$</td>
<td>0.98</td>
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<tr>
<td>$\Sigma$CHLs</td>
<td>$F \ln[\Sigma$CHL$<em>{Outer}] = 0.521 \times \ln[\Sigma$CHL$</em>{Inner}] + 2.560$</td>
<td>0.66</td>
<td>$F \ln[\Sigma$CHL$<em>{Outer}] = 0.235 \times \ln[\Sigma$CHL$</em>{Inner}] + 4.271$</td>
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<td>$M \ln[\Sigma$CHL$<em>{Outer}] = 1.098 \times \ln[\Sigma$CHL$</em>{Inner}] - 0.782$</td>
<td>0.96</td>
<td>$M \ln[\Sigma$CHL$<em>{Outer}] = 0.951 \times \ln[\Sigma$CHL$</em>{Inner}] + 0.317$</td>
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<tr>
<td>$\Sigma$PBDEs</td>
<td>$F \ln[\Sigma$PBDE$<em>{Outer}] = 0.906 \times \ln[\Sigma$PBDE$</em>{Inner}] + 0.150$</td>
<td>0.65</td>
<td>$F \ln[\Sigma$PBDE$<em>{Outer}] = 0.624 \times \ln[\Sigma$PBDE$</em>{Inner}] + 1.301$</td>
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<tr>
<td></td>
<td>$M \ln[\Sigma$PBDE$<em>{Outer}] = 0.906 \times \ln[\Sigma$PBDE$</em>{Inner}] + 0.303$</td>
<td>0.93</td>
<td>$M \ln[\Sigma$PBDE$<em>{Outer}] = 0.988 \times \ln[\Sigma$PBDE$</em>{Inner}] + 0.070$</td>
<td>0.97</td>
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</table>
Table 2.2. Predictive equations, including only significant terms, for concentrations of 6-MeO-BDE 47 (MeOBDE), α-HCH, β-HCH, and HCB between serum and inner blubber, serum and outer blubber, and inner and outer blubber. The $R^2$ refers to the goodness of fit of the data on the line produced by the predictive equation.

<table>
<thead>
<tr>
<th></th>
<th>Late Molting (N=14 males, 24 females)</th>
<th>Early Breeding (N=15 males, 24 Females)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum-Inner Blubber</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6MeOBDE47</td>
<td>F Not significant</td>
<td>F $\ln[\text{MeOBDE}<em>{\text{Inner}}] = 0.440 \times \ln[\text{MeOBDE}</em>{\text{Serum}}] + 1.18$</td>
</tr>
<tr>
<td></td>
<td>M Low detectability</td>
<td>M $\ln[\text{MeOBDE}<em>{\text{Inner}}] = 0.440 \times \ln[\text{MeOBDE}</em>{\text{Serum}}] + 0.871$</td>
</tr>
<tr>
<td>α-HCH</td>
<td>F+M $\ln[\alpha-HCH_{\text{Inner}}] = 0.340 \times \ln[\alpha-HCH_{\text{Serum}}] + 1.539$</td>
<td>0.19</td>
</tr>
<tr>
<td>β-HCH</td>
<td>M $\ln[\beta-HCH_{\text{Inner}}] = 0.820 \times \ln[\beta-HCH_{\text{Serum}}] + 1.20$</td>
<td>0.86</td>
</tr>
<tr>
<td>HCB</td>
<td>F $\ln[\Sigma HCB_{\text{Inner}}] = 0.282 \times \ln[\Sigma HCB_{\text{Serum}}] + 2.071$</td>
<td>0.16</td>
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<tr>
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<td>M $\ln[\Sigma HCB_{\text{Inner}}] = 0.282 \times \ln[\Sigma HCB_{\text{Serum}}] + 2.284$</td>
<td>0.58</td>
</tr>
<tr>
<td><strong>Serum-Outer Blubber</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6MeOBDE47</td>
<td>F Not significant</td>
<td>F $\ln[\text{MeOBDE}<em>{\text{Outer}}] = 0.350 \times \ln[\text{MeOBDE}</em>{\text{Serum}}] + 0.891$</td>
</tr>
<tr>
<td></td>
<td>M Low detectability</td>
<td>M $\ln[\text{MeOBDE}<em>{\text{Outer}}] = 0.350 \times \ln[\text{MeOBDE}</em>{\text{Serum}}] + 0.518$</td>
</tr>
<tr>
<td>α-HCH</td>
<td>M $\ln[\alpha-HCH_{\text{Outer}}] = 0.732 \times \ln[\alpha-HCH_{\text{Serum}}] + 0.897$</td>
<td>0.53</td>
</tr>
<tr>
<td>β-HCH</td>
<td>F $\ln[\beta-HCH_{\text{Outer}}] = 0.639 \times \ln[\beta-HCH_{\text{Serum}}] + 1.682$</td>
<td>0.78</td>
</tr>
<tr>
<td>HCB</td>
<td>M $\ln[\beta-HCH_{\text{Outer}}] = 0.938 \times \ln[\beta-HCH_{\text{Serum}}] + 0.810$</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>M $\ln[\beta-HCH_{\text{Outer}}] = 0.430 \times \ln[\beta-HCH_{\text{Serum}}] + 1.62$</td>
<td>0.53</td>
</tr>
<tr>
<td><strong>Inner Blubber-Outer Blubber</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6MeOBDE47</td>
<td>F+M 0.083</td>
<td>F+M $\ln[\Sigma MeOBDE_{47\text{Outer}}] = 0.889 \times \ln[\Sigma MeOBDE_{47\text{Inner}}] - 0.183$</td>
</tr>
<tr>
<td>α-HCH</td>
<td>F $\ln[\alpha-HCH_{\text{Outer}}] = 0.362 \times \ln[\alpha-HCH_{\text{Serum}}] + 1.00$</td>
<td>0.23</td>
</tr>
<tr>
<td>β-HCH</td>
<td>M $\ln[\beta-HCH_{\text{Outer}}] = 1.144 \times \ln[\beta-HCH_{\text{Serum}}] + 0.340$</td>
<td>0.91</td>
</tr>
<tr>
<td>HCB</td>
<td>F Not significant</td>
<td>F+M $\ln[\Sigma HCB_{\text{Outer}}] = 0.746 \times \ln[\Sigma HCB_{\text{Inner}}] + 0.916$</td>
</tr>
</tbody>
</table>
Appendix 2.1. Tissue relationships for lower concentration contaminants.

Serum and inner blubber

During the late molt, we did not detect a significant interaction between POP concentrations in serum \(\times\) sex on POP concentrations in inner blubber for HCB, \(\alpha\)-HCH, or \(\beta\)-HCH (HCB: \(F_{1,34}=0.91, p=0.348\); \(\alpha\)-HCH: \(F_{1,34}=0.64, p=0.430\); \(\beta\)-HCH: \(F_{1,34}=0.77, p=0.39\)). Serum concentrations were significant and positively related to inner blubber concentrations, accounting for the effect of sex (HCB: \(F_{1,35}=9.76, p=0.003\); \(\alpha\)-HCH: \(F_{1,35}=2.09, p=0.16\); \(\beta\)-HCH: \(F_{1,35}=2.81, p=0.102\)). Males were excluded from tests for 6-MeO-BDE 47 due to low detectability of this compound in samples; no significant relationship was found for 6-MeO-BDE 47 in females.

In early breeding, we did not detect a significant interaction for 6-MeO-BDE 47 or \(\beta\)-HCH (6-MeO-BDE 47: \(F_{1,34}=2.20, p=0.15\); \(\beta\)-HCH: \(F_{1,34}=0.11, p=0.75\)). During the early breeding fast, serum concentrations of 6-MeO-BDE 47 and \(\beta\)-HCH were significant and positively related to inner blubber concentrations (Appendix 1), while accounting for sex (6-MeO-BDE 47: \(F_{1,36}=6.54, p=0.015\); \(\beta\)-HCH: \(F_{1,35}=1.95, p=0.17\)). No significant relationship was found for HCB in early breeding. While the relationships between serum concentrations and inner blubber concentrations of \(\beta\)-HCH at early breeding and late molting were strong \((R^2 \geq 0.58)\), weaker relationships were found for \(\alpha\)-HCH \((R^2 \leq 0.38)\).

Serum and outer blubber

During the late molt, we detected a significant interaction between POP concentrations in inner blubber \(\times\) sex on POP concentrations in outer blubber for HCB, \(\alpha\)-HCH, \(\beta\)-HCH (HCB: \(F_{1,34}=12.37, p=0.001\); \(\alpha\)-HCH: \(F_{1,34}=11.29, p=0.002\); \(\beta\)-HCH: \(F_{1,34}=5.51, p=0.025\)). In contrast, early breeding seals did not have a significant interaction between serum POP concentrations \(\times\) sex on outer blubber POP concentrations. During the early breeding fast, serum concentrations of HCB, \(\alpha\)-HCH, and \(\beta\)-HCH were significant and positively related to outer blubber concentrations (Appendix 1), while accounting for sex (HCB: \(F_{1,34}=7.57, p=0.009\); \(\alpha\)-HCH: \(F_{1,34}=5.34, p=0.03\); \(\beta\)-HCH: \(F_{1,34}=4.59, p=0.04\)). Similar to serum-inner blubber, the relationship between serum concentrations and outer blubber concentrations of \(\beta\)-HCH was strong \((R^2 \geq 0.90)\).

Inner and outer blubber

During the late molt, we detected a significant interaction between POP concentrations in inner blubber \(\times\) sex on POP concentrations in outer blubber for HCB, \(\alpha\)-HCH, \(\beta\)-HCH (HCB: \(F_{1,34}=12.37, p=0.001\); \(\alpha\)-HCH: \(F_{1,34}=11.29, p=0.002\); \(\beta\)-HCH: \(F_{1,34}=5.51, p=0.025\)), but not for 6-MeO-BDE 47 (\(F_{1,34}=0.029, p=0.865\)). For 6-MeO-BDE 47, inner blubber POP concentrations were significantly and positively related to outer blubber concentrations (Appendix 2) when accounting for sex (\(F_{1,34}=0.083, p=0.776\)). At the early breeding fast, we did not detect a significant interaction for 6-MeO-BDE 47, HCB, \(\alpha\)-HCH, \(\beta\)-HCH (6-MeO-BDE 47: \(F_{1,35}=0.64, p=0.430\); HCB: \(F_{1,35}=1.28, p=0.266\); \(\alpha\)-HCH: \(F_{1,35}=1.43, p=0.240\); \(\beta\)-HCH: \(F_{1,35}=0.713, p=0.404\)). For seals at the early breeding fast, inner blubber concentrations for all four compounds were significantly related to outer blubber concentrations (Appendix 2), when accounting for sex (6-MeO-BDE 47: \(F_{1,36}=3.30, p=0.08\); HCB: \(F_{1,36}=0.52, p=0.473\); \(\alpha\)-HCH: \(F_{1,36}=0.60, p=0.445\); \(\beta\)-HCH: \(F_{1,36}=2.10, p=0.156\)).
CHAPTER 3

Benthic macroinvertebrate response to seasonal and annual variability, and response to an accidental oil spill, in an urban Mediterranean-climate stream
Benthic macroinvertebrate response to seasonal and annual variability, and response to an accidental oil spill, in an urban Mediterranean-climate stream

ABSTRACT

Benthic macroinvertebrates show strong seasonal fluctuations in both occurrence and abundances in relation to variability in hydrologic conditions. Coastal northern California is located in a Mediterranean-climate area, and is characterized by predictable wet and dry seasons for precipitation, and consequently stream flows. In urbanized streams of this climate region, stream flow characteristics may magnify or dampen seasonal fluctuations and increase risks of anthropogenic disturbances such as acute oil spills. The timing of spills may have seasonally relevant consequences corresponding to the seasonal fluctuations in fauna. Monthly sampling over multiple years (2004, 2010-2013) was used to examine seasonal variability and responses in the taxonomic composition of benthic macroinvertebrates at three sites in Strawberry Creek, an urban stream on the University of California, Berkeley (USA) campus, widely used for teaching and research purposes. Although taxa occurrences were similar between wet and dry seasons, taxa abundances varied at the two sites having higher taxonomic richness, whereas the site with decreased richness lacked these fluctuations. The effects of an accidental, acute oil spill occurring during the wet season 2011 were examined using a Before-After Control-Impact approach. Three days after the oil spill, macroinvertebrate richness and abundance decreased dramatically compared with pre-spill levels. Abundances and richness increased progressively over the subsequent two months, and the taxonomic composition of the benthic macroinvertebrate community recovered to pre-spill family richness one year post-spill. Resilience of the benthic macroinvertebrate community was likely aided by drift from an upstream reference site with relatively high taxa richness and the flushing effects of rains and increased flows. Seasonal comparisons and the response of taxa abundances to chemical disturbance enabled identification of five complementary indicator taxa that are common, abundant, and easily identified, which will enhance future biomonitoring efforts and student involvement in research projects in this stream.

Key words: seasonal variation, benthic macroinvertebrates, Mediterranean-climate streams, oil spill, urban creek

INTRODUCTION

The degree of seasonality in benthic macroinvertebrate communities is often driven by seasonal fluctuations in hydrology (Burgherr et al., 2002; Bogan & Lytle, 2007). Mediterranean-climate areas of the world are characterized by long dry summers and cool wet winters, which produce streams characterized by periods of low flows during the dry season, followed by elevated flows during and after the wet season (Gasith & Resh, 1999; Bonada & Resh, 2013). Consequently, these streams show strong seasonality in organism abundance and taxonomic composition (Bêche et al., 2009; Bonada & Resh, 2013; Resh et al., 2013). However, although the timing of seasonal precipitation and floods is predictable, the magnitude of precipitation and consequently its annual variability is high, which may result in high year-to-year variation in abundance and composition (McElravy et al., 1989; Resh et al., 2013).
Urbanization around streams often decreases stream biodiversity (Hachmoller et al., 1991; Paul & Meyer, 2001), but effects of urbanization on the seasonality of benthic macroinvertebrate abundances remain unclear. For example, increased urbanization generally increases impervious surfaces within watersheds, which results in increased flashiness of stream flows in response to storms (Walsh et al., 2005). In contrast, urban environments can also increase stream permanence as a result of increased input from human use, such as sprinklers or treated wastewater, during dry summer months (Luthy et al., 2015). Beyond chronic threats from urban environments, urban streams also face acute threats from spills of oil, surfactants, or other chemicals. For stream organisms in Mediterranean-climate regions, urban threats are particularly common because there are dense human populations. The synchrony of chemical spills with seasonal biotic assemblages may have different consequences related to seasonality of stream organisms (Crunkilton & Duchrow, 1990; Lytle & Peckarsky, 2001). Moreover, the timing of events in terms of seasonality of flow may ameliorate or exacerbate the effects of such disturbances.

Seasonal variability presents challenges for bioassessment programs because the timing of stream sampling can impact biological indexes and interpretation (Reece et al., 2001; Šporka et al., 2006). Benthic macroinvertebrate assemblages vary based on life histories of the individual taxa and disturbance in the environment. Therefore, biological assessments may yield different abundance, richness metrics, and sensitive taxa estimates depending on the time of year and must separate the effects of anthropogenic disturbances from those of natural variability in taxa assemblages (Reynoldson et al., 2001).

This study examined seasonal and annual variability in benthic macroinvertebrates, and response to an acute chemical disturbance over multiple years. The objectives were to: 1) describe the faunal changes in terms of seasonal and annual variability in an urban Mediterranean stream, 2) provide a set of criteria for identifying a subset of stream taxa that are common, abundant, and respond to disturbance, and therefore may be useful to citizen-scientist and educational groups as bioindicators of future disturbance, and 3) used a Before-After Control-Impact approach to assess the response of macroinvertebrates to a diesel oil spill over both the short- (<3 months) and long-term (one and two years later).

METHODS

Study Site

Strawberry Creek (37.87° N, 122.27° W) is a Mediterranean-climate stream located within the California floristic province, an area widely considered a biodiversity hotspot (Ball et al., 2013). The stream is located on the University of California, Berkeley, USA campus and has an important educational component, with several thousand undergraduate students having laboratories or field exercises based on information gathered from the creek. The stream has two forks that form a confluence on the campus of University of California, Berkeley, after which the stream continues through multiple culverts and an urban park for 5 km before entering San Francisco Bay. Average annual precipitation in a typical water year for this region is 61.7 cm, but >90% of precipitation occurs from October-April (Fig. 3.1). Wet seasons are characterized by brief peaks in stream gauge height in response to precipitation, while dry seasons are characterized by consistent low flows (Fig. 3.1). Past studies of Strawberry Creek have estimated the lag time between peak precipitation and peak flows to be approximately 20 min (Charbonneau & Resh, 1992).
Sampling

We sampled benthic macroinvertebrates at three riffle sites in Strawberry Creek, with one site on the south fork, one on the north fork, and one site below the confluence (Fig. 3.2). Sampling occurred semi-monthly from March 2004-July 2005, and monthly from October 2010 – September 2013. Sampling protocols were similar to the United States Environmental Protection Agency rapid bioassessment protocol (Barbour et al., 1999), with some modifications given the seasonal low flow and urban conditions of Strawberry creek, such as narrow stream width and channelized portions of stream. Three 1-m cross-sections were sampled at each riffle site using a 500μm D-frame net. At each transect, approximately 0.5 m$^2$ of the river substrate upstream of the net was dislodged by kicking. Benthic samples were preserved in the field with 95% ethanol. Specimens were sorted from organic matter with a magnifying glass and lamp, and all specimens were identified to family, with some common taxa identified to species (Merritt et al., 2008).

Size-spectra analysis

A subset of samples from each site were used to compare size-spectra among sites, between wet and dry seasons, and both before and after the accidental oil spill. The body length of each organism was measured and used to estimate ash-free dry mass (Benke et al., 1999; Méthot et al., 2012). Biomass estimates were then used to calculate the normalized biomass of each size class using the following process (Chang et al., 2013). The normalized biomass was calculated as the sum of biomass from all individuals belonging to a size class divided by the width of the size class, and then transformed using a natural log. The normalized biomasses were then regressed on the size classes to estimate the size spectra. For statistical analyses, we compared the slopes of the lines for wet and dry seasons using a liner model testing for differences in slope ($R$, Version 3.2.1). Size classes that contained no organisms at a specific site and season were excluded from the regression analyses.

Seasonality analysis

Samples refer to benthic macroinvertebrates collected at a particular site on each unique date. Taxa abundances were used to calculate relative abundances, percent detection for each taxa at each site and season, and community metrics. Four samples with low abundances (<20 individuals) were removed from the dataset, prior to multivariate analyses.

We used ordination and clustering techniques to assess seasonal and site differences of natural-log transformed macroinvertebrate abundances (counts) and relative abundances (proportions) in the statistical program R, Version 3.2.1 (vegan package). Hierarchical clustering was used to examine differences by site and by season based on relative abundances and percent detection of each taxa. Non-metric multi-dimensional scaling (NMDS, Kruskal 1964) with Sorensen (Bray-Curtis) distance was used to examine seasonal differences at each site based on relative abundances. Hierarchical and non-metric clustering (Riffle, Matthews et al., 1991) were used in R to examine the similarity between wet and dry season samples within each site. In addition, the PRE scores generated by non-metric clustering program Riffle, which represent the relative ability to predict a variable based on the cluster membership, were used in selecting potential indicator taxa.

Oil spill analysis
On December 10, 2011 approximately 3000 L of diesel oil from an accidental spill entered the north fork of Strawberry Creek via a stormwater culvert. Many academic buildings at the University of California, Berkeley are associated with research projects necessitating backup power generation and fuel in case of short-term power outages. This spill resulted from a failure of a diesel fuel storage tank in an academic building, which then entered a culvert system that outlet into Strawberry Creek. Although neither the north fork site, nor the south fork site were not exposed to this spill (Fig. 3.2), the below confluence site was downstream of the spill and showed immediate visual and olfactory evidence of substantial oil exposure. Hydrocarbons with signatures of diesel fuel were found in downstream sediment and soil when finally measured 12 days post-spill (California Department of Fish and Game, Lab Report #L-706-11) and visible oil sheens were observed when dislodging stream cobbles even 6 weeks post-spill.

A Before-After Control-Impact design was used to analyze the impact of the oil spill. Sites upstream of the spill, located in the north and south forks, were used as control sites and one site downstream of the spill, the below confluence site, was used as the impact site (Fig 3.2). All sites had been sampled monthly for 15 consecutive months prior to the spill and for 21 consecutive months after the spill, including at 3 and 14 days immediately post-spill. Macroinvertebrate abundance, family richness, Simpson’s diversity index, size spectra, and functional feeding group diversity were compared before and after the spill at each site. Before-after comparisons were conducted at 2 weeks, 1-month, 2-month, 3-month, 6-month, and 1 year intervals. In addition, the seasonally relevant time period (December/January) was compared across all sampling years (2004, 2010, 2011, 2012, 2013, 2014).

RESULTS

Seasonal variation in precipitation

The timing of wet and dry seasons varied annually during this study. Although >90% of annual rainfall occurs between September and April, the frequency of precipitation in October, November, and December months varied during the four years of this study period. November rainfall in 2004, 2010, and 2011 ranged from 4.8-7.5 cm, while 2012 had only 1.9 cm. In 2004 and 2010, December precipitation was 21.0 cm and 19.0 cm, respectively, whereas for 2011, 2012, and 2013, December precipitation was <1.52 cm of rain each year. Therefore, the “wet season” in this study was defined as November-April for 2004 and 2010, and January-April for 2011, 2012, and 2013, to reflect the breaks in the precipitation data. In addition, the total precipitation also varied by year (Fig. 3.1). The years 2004-2005 and 2010-2011 were wetter than an average years, while precipitation was less than average in 2011-2012 and dramatically lower in 2012-2013 (Fig. 3.1).

The Fauna and dynamics

Twenty-eight aquatic insect families and 3 non-insect macroinvertebrate groups (Oligochaeta, Amphipoda, and Collembola) were found in Strawberry Creek, yet only a subset of these taxa were found at all sites (Table 3.1). Of the families present, three taxa were identified to genus: Simulium (Diptera: Simulidae), Baetis (Ephemeroptera: Baetidae), and Rhyacophila (Trichoptera: Rhyacophilidae). Three common taxa were identified to species: Malenka californica (Plecoptera: Nemouridae), Parthina vierra (Trichoptera: Odontoceridae), and Argia vivida (Odonata: Coenagrionidae).
Hierarchical clustering of both percent detection and relative abundances clearly separated samples by site. Additionally, differences in family richness and percentages of common taxa were observed at north fork compared to the south fork and below confluence sites (Table 3.1). The north fork sites were characterized by increased proportions of *A. vivida* and Chironomidae relative to that at other sites, whereas the south fork and below confluence sites consistently had higher proportions of all Ephemeroptera-Plecoptera-Trichoptera (EPT) taxa. Eliminating sample results obtained immediately after the oil spill, family richness (±range) was 13.0 (7-18) families in the south fork, 7.2 (4-12) families below the confluence, and 7.0 (3-9) families at the two north fork sites, indicating gradients among sites. *Simulium*, Chironomidae, *Baetis*, and *M. californica* were commonly found at all sites. While *Gammarus* amphipods were commonly found at each site, amphipod abundance in the south fork was as high as >10x that of other sites.

Less common EPT taxa were only present in south fork and below confluence sites. For example, leptophlebiid mayflies were present in 5.6% of below-confluence samples and 11.2% of south fork samples; heptageniid mayflies were present in 2.7% of samples from both south-fork and below-confluence sites; chloroperlid stoneflies were present in 22% of south-fork samples and not found at the below-confluence site. Caddisflies were extremely rare in the north fork, never representing >0.8% of total specimens collected. For example, the only caddisfly taxa found in the north fork were *Rhyacophila* and Hydropsychidae, which were rare (Table 3.1). In contrast, caddisflies were low in abundance but consistently detected at south fork and below-confluence sites (Table 3.1). For example, *Rhyacophila* and Hydropsychidae were present in >33% of samples from the south-fork and below-confluence locations. Additionally, Odontoceridae was present in south-fork and below-confluence samples, but Lepidostomatidae was only present in the south-fork (Table 3.1). Caddisflies, including *Rhyacophila*, Hydropsychidae, Lepidostomatidae, and Odontoceridae, only occurred at sites that also had *M. californica*. In contrast, for samples at all sites, there were times when *M. californica* was present and no caddisflies were present.

**Seasonal differences**

High frequency sampling demonstrated different taxonomic composition among sampling sites (Table 3.1), therefore we analyzed each site separately for seasonal differences. Seasonal differences in total abundance of individuals were evident at each site (Table 3.2). Dry season months had higher abundance of individuals (Table 3.1), in some cases 10x higher, compared with wet season samples. For size spectra calculated from a subset of samples collected within 2011-2012, no significant difference was found in the slope of size spectra between wet and dry seasons ($F_{1,16}=0.021, p=0.89$).

Seasonal differences in relative abundances, but not occurrences, of taxa were observed for the south fork and below confluence site. Sampling locations in the south-fork and below-confluence were the only sites that could be separated into dry and wet seasons using NMDS ordination and relative taxa abundances (Fig. 3.4). NMDS separated samples collected in the south-fork and below-confluence into wet and dry seasons, with some overlap (Fig. 3.3), but did not clearly separate north fork samples. Additionally, we did not find differences in taxa occurrences (Table 3.1). In particular, five most common taxa were found in >60% of samples in both seasons (Table 3.1).

The most apparent taxa-level seasonal differences were elevated absolute and relative abundance of *Simulium* in the dry season and *M. californica* in the wet season (Table 3.1). *Simulium* peaked in abundance in May, June, or July of each year, and at peak abundance
Simulium represented >50% of the benthic community at each site. In contrast, during each wet season, Simulium represented <15% of individuals collected at each site. High abundances of Baetis occurred at different times of year at different sites. For example, dramatic increases in Baetis abundance occurred in May or June at the below-confluence sampling location each year, but in January or February on the north fork, and in both wet and dry seasons for the south fork.

**Interannual differences**

Interannual variability in abundance was apparent for some taxa. Winter increases in the caddisfly *P. vierra* were evident in January 2004 and January 2013, but undetected in the winter months of 2010, 2011 and 2012. Peak abundance of individuals for *M. californica* occurred in the dry season each year, but varied in abundance 2-7x among years: June 2004 (n=108 individuals), October 2010 (n=1064), June 2011 (n=288 individuals), July 2012 (n=643), and June 2013 (n=295). Rare taxa, such as Chloroperlidae, Leptophlebiidae, and Lepidostomatidae, were rare in all years.

While NMDS did not demonstrate separation by year, analysis of separate wet and dry seasons showed narrow trends. NMDS of wet season months showed a narrow separation between the wet seasons of 2004-2005 and 2010-2013. NMS of dry season samples show a cluster of samples from 2004, 2012, and 2013 with slight separation from dry season samples from 2010 and 2011.

**Response to accidental oil spill**

Macroinvertebrate abundance and Simpson’s diversity index demonstrated short-term decreases in response to the December 2011 diesel oil spill. For example, immediately following the oil spill, only Chironomidae (n=29), Gammarus (n=16), and *Baetis* (n=1) were found at the impact site, and all macroinvertebrate metrics decreased dramatically relative the previous several months (Fig. 3.3). However, reference sites in both stream forks showed similar macroinvertebrate metrics to those of preceding months (Figure 3.3). Richness, Simpson’s diversity index, and % EPT (with and without Baetidae) remained low two weeks after the oil spill. Six weeks after the spill all metrics increased, and at 10 weeks these metrics had rebounded to pre-spill levels, and the first appearance of the plecopteran (*M. californica*) occurred since prior to the spill (Fig. 3.3). Size spectra could not be compared between pre-spill and post-spill communities because too few size classes were found post-spill. No size classes larger than 3.5 (4-8mm) were present at the impact site following the oil spill.

The benthic macroinvertebrate community showed recovery in all metrics in subsequent wet and dry seasons. Samples taken during the post-spill dry season, in May and June 2012, included all families that were present in the pre-spill years of 2004 and 2010. In addition, ordination of pre-spill and post-spill June macroinvertebrate communities showed no separation of samples. Because the oil-spill occurred in December 2011, the winter months are most seasonally relevant for comparison. Samples taken from December 2012 (one year post-spill) and 2013 were similar to pre-spill winter samples from December 2004 and 2010 for all community metrics (Table 3.2).

**Assessment of indicator taxa**

Rare taxa, such as Chloroperlidae and Lepidostomatidae, were exclusive to the south fork site. However, their infrequent presence in samples complicated interpretation of seasonal trends, and reduced their utility as indicator taxa.
The influences of taxa on ordination, as evidenced by the NMS magnitudes, suggest that the relative abundances of *M. californica*, *Rhyacophila*, *Simulium*, *Hydropsychidae*, *Baetis*, and Chironomidae had the strongest influences on the ordination results. In addition, ordination of samples on just *Rhyacophila*, *M. californica*, *Simulium*, and *Hydropsychidae*, and *Baetis* separated samples into seasons with similar results as using all Strawberry Creek taxa. Moreover, while non-metric clustering did not clearly identify wet and dry seasons based on relative abundances, *M. californica*, *Baetis*, and *Simulium* as 3 of 4 highest PRE scores, and *Rhyacophila* had the highest PRE score among caddisflies.

DISCUSSION

In this study we demonstrated that relative abundances of macroinvertebrate taxa varied among seasons in an urban, Mediterranean-climate stream. The fauna of Strawberry Creek demonstrated seasonal variation at individual sites in total abundance and relative abundance of certain taxa. Previous studies in Mediterranean-climate streams in northern California have found fluctuations in the seasonal variation in taxa occurrences of benthic macroinvertebrate communities, where certain taxa are exclusive to dry seasons and others exclusive to wet seasons (McElravy et al., 1989; Bêche et al., 2006). Even though these studies were within the same region as Strawberry Creek, their benthic macroinvertebrate assemblages include greater taxa richness and exclusive seasonal taxa (e.g. *Gumaga* caddisflies) that are not found in Strawberry Creek. Lower taxa richness in Strawberry Creek relative to other northern California streams may reduce seasonal signals in taxonomic composition. Indeed, the six most common taxa were present in the majority of samples from both seasons. While the seasonal trends in the current study were not as dramatic in determining distinct wet and dry season taxa, relative taxa abundances did fluctuate seasonally for several taxa.

While size-spectra has shown promise as a metric of bioassessment in marine environments (Warwick, 1984; Strayer, 1986) and some lentic systems (Benke et al., 1999; Solimini et al., 2005), size-spectra did not vary among seasons in this study. We believe that this is because common and abundant taxa in Strawberry Creek are relatively small (e.g., chironomids and baetids). Therefore, a limited range of body sizes at each of the study sites likely inhibits size spectra as a useful bioassessment metric here and in other systems with reduced size ranges. However, in systems having a range of faunal sizes, such as crayfish or other crustaceans (Poff et al., 1993), this approach may be demonstrated to be more useful.

*Resiliency to accidental oil spill*

Although oil spills tend to be associated with marine environments in public perception, in many years more oil is spilled in stream and river habitats, and with greater frequency (Etkin, 2001). Oil storage and transport occurs along river corridors, and accidental spills impact stream biota through the direct toxicity of hydrocarbons and alteration of habitat, such as preventing surface breathing or covering rock surfaces (Rosenberg et al., 1986; Crunkilton & Duchrow, 1990). Effects of spills have been shown to linger, and produce benthic communities dominated by one taxa group (Lytle & Peckarsky, 2001). Urbanized watersheds include numerous sources for potential oil spills, therefore, the risk of spills is quite common in urban environments. In addition, the acute threat of spills to aquatic life may be magnified by other chronic water pollution issues in urban streams (Paul & Meyer, 2001).
The ability to compare post-spill biological condition to pre-spill conditions is rare, because spills happen haphazardly and without warning (Lytle & Peckarsky, 2001). In this case, frequent year-round sampling provides this study with a unique comparative perspective. In Strawberry Creek, the response of the benthic community after the accidental oil spill was a drastic decrease in richness and abundance followed by a progression of increased taxa richness and abundance over time. Chironomidae were present at the impact site 3 days after the spill, which aligns with previous studies that suggested tolerance to crude oil in some species of Chironomidae (Rosenberg & Wiens, 1976). *Baetis* mayflies recovered more quickly after the accidental oil spill than other EPT taxa, however *Baetis* were slow to recover in a previous freshwater oil spill study (Lytle & Peckarsky, 2001), suggesting sensitivity of some *Baetis* species to oil perturbation. *Baetis* are generally more tolerant of high turbidity than other mayflies, stoneflies, or caddisflies (Chang et al., 2014), and their population recovered more quickly than the stoneflies and caddisflies at the impact site, indicating higher tolerance to the remnants or oil, higher drift rates, or timely seasonal population increases. *Baetis* also has a multivoltine population in Strawberry Creek, as has been found in other locations (Robinson et al., 1992) and can contribute to quicker recoveries after disturbance. In contrast, the initial absence and slower recolonization of *M. californica* suggests greater sensitivity to oil. In contrast with *Baetis*, *M. californica* is likely univoltine in Strawberry Creek, which is similar to previous research in a temperate stream (Richardson, 2001). Prior to the presence of *M. californica* after the spill, the assemblage of benthic macroinvertebrates at the impact site did not include any shredders. Therefore, the recovery of *M. californica* provides the recovery of an important stream function.

The progression of taxa recovery after the accidental oil spill generally aligns with relative taxon-specific tolerance values reported from California (Ode, 2003) for these taxa. The taxon-specific values rank Baetidae and Hydropsychidae moderately sensitive and Nemouridae, Lepidostomatidae and Rhyacophilidae as more sensitive (Ode, 2003). At 3 days and 14 days after the spill, impact-site samples contained only 1 *Baetis* and no *M. californica*. Four *Baetis* and 1 *M. californica* individual were found at 5 weeks, 16 *Baetis* and 8 *M. californica* at 9 weeks, and 60 *Baetis* and 38 *M. californica* at 12 weeks. While the presence of caddisflies in Strawberry Creek is generally infrequent, only 1 individuals of the caddisfly *P. vierra* was found at the impact site at 12 weeks and only 2 individuals of *P. vierra* at 16 weeks. In the summer months, a single *Rhyacophila* was found in June and August samples. Additional caddisfly taxa were found in later months. Therefore, our observations of recovery after an oil spill may represent general responses of stream biota to anthropogenic disturbances in this stream.

Resilience of the benthic macroinvertebrate community to the accidental oil spill is likely attributable to drift from an upstream site where richness and diversity were maintained. For example, *M. californica* stoneflies are common and abundant in the south fork, but rare and sometimes absent from the north fork. In the weeks after the accidental oil spill, north fork samples contained 2-10% *M. californica*, whereas the south fork samples were characterized by 25-44%. In addition, caddisflies are almost non-existent in the north fork of Strawberry Creek, therefore, the recolonization of caddisflies in the impact area six months after the spill almost certainly occurred via drift from the south fork or flight, rather than downstream drift from the north fork. Thus, the richness and diversity of macroinvertebrates in the south fork was likely important to the degree of resilience in the benthic community below the confluence, highlighting the benefits of previous restoration efforts in the south fork (Charbonneau & Resh, 1992) and the maintenance of habitat in both forks.
Because different life cycle stages occur in different seasons, there could be a lag time between an oil spill disturbance in the wet season and effects in the dry season. For example, other disturbances, such as the magnitude of winter stream flows, are important determinants of macroinvertebrate community composition in the summer (Power et al., 2008). However, community metrics and taxonomic composition from the summer after the oil spill were not different from other summer samples during our study, which provides further evidence of successful recolonization.

Seasonality of a disturbance may impact recovery time of the benthic community. While multiple factors affect the impact of oil spills (e.g., stream flow), the recovery of benthic macroinvertebrate taxa richness has been shown to take longer in other cases (Lytle & Peckarsky, 2001), which may relate to seasonality. In the case of Strawberry Creek, an oil spill in the dry season may have produced effects with longer timescales. Dry season taxa include higher abundances and higher family richness, therefore direct toxicity would have impacted more taxa and more organisms. In addition, low summer flows would provide a weaker mechanism for flushing the stream of lingering fractions of oil. Furthermore, the dry season is likely a critical reproductive time for many of the Strawberry Creek taxa that have summer emergence, which could cause population consequences for the following year.

Assessment of indicator taxa

Criteria are critical for development of complementary indicator taxa. While a wide range of criteria for indicator taxa have been suggested (Rosenberg & Wiens, 1976; Noss, 1990; Hilty & Merenlender, 2000), the appropriate group of indicator taxa for Strawberry Creek would collectively indicate acute disturbances, general ecological condition, and seasonal fluctuations. In order to increase the practicality of biomonitoring, especially because there are common biomonitoring efforts by undergraduate students and volunteers, the taxa selected should be common, abundant, and easily identified. Moreover, the clearest indicator taxa would have responses that were relatively faithful, meaning always present, to a particular season or habitat condition, but also relatively exclusive, meaning that they would be absent when conditions change (Bogan & Lytle, 2007). Although Strawberry Creek does not have taxa that are solely found in the dry or the wet season, the abundances of the proposed indicator taxa do follow seasonal patterns. Finally, indicator taxa should include taxa that range in sensitivity to disturbance, and correlate to changes in the wider benthic macroinvertebrate community.

Our study has identified complementary indicator taxa that fit these criteria. The black fly Simulium, the mayfly Baetis, the caddisflies Rhyacophila and Hydropsychidae, and the stonefly M. californica are found at all four sites investigated in the current study and are cosmopolitan in the region (Ball et al., 2013; Resh et al., 2013). Simulium, Baetis, and M. californica are common and abundant at all sampling locations. In addition, Simulium and M. californica were found to be important seasonal drivers of the benthic community, and M. californica is sensitive to water pollution (Ode, 2003). Baetis demonstrates inconsistent seasonal abundance, but is common at each site and moderately-sensitive to water pollution. Rhyacophila and Hydropsychidae represent two groups of caddisflies that are sensitive and moderately sensitive to pollution (Ode 2003), respectively, and are found consistently at low abundance at south-fork and below-confluence sites, but inconsistently at north fork sites. Further, caddisflies only occurred at sites that also had M. californica, but M. californica was commonly present with no caddisflies, suggesting increased tolerance of M. californica. Therefore, Rhyacophila and Hydropsychidae may be more sensitive to anthropogenic disturbances and be important future indicators habitat improvements.
This approach for identifying subsets of indicator taxa can be used to enhance benthic macroinvertebrate biomonitoring of Mediterranean-climate streams in other locations. Frequent initial collections were critical to describe seasonal fluctuations, as well as to find rare taxa. Additionally, opportunistic comparisons among sites can determine the richness and abundance of the local taxa, and identify candidate taxa that are common, abundant, and range in pollution sensitivity. Volunteers and community groups are common contributors to stream restoration and in some cases, monitoring efforts, and their efforts may be enhanced by a more narrow focus on targeted groups of taxa. Therefore, identification of subsets of taxa that can be useful for assessing the ecological condition of a stream and practical for a wide-range of taxonomic training will broaden the potential for future biological monitoring of future habitat restoration or degradation.

ACKNOWLEDGEMENTS

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LITERATURE CITED


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Table 3.1. Percent occurrence of each taxa in samples collected from the north fork, south fork, and below confluence sites of Strawberry Creek by season (Dry or Wet) from 2004, and 2010-2013. Samples from the first three months after the oil spill were not included.

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<th>Below Confluence</th>
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<th>South Fork</th>
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<tr>
<td>Elmidae</td>
<td>0.11</td>
<td>0.17</td>
<td>0.00</td>
</tr>
<tr>
<td>Dytiscidae</td>
<td>0.06</td>
<td>0.13</td>
<td>0.00</td>
</tr>
<tr>
<td>Pspheneidae</td>
<td>0.00</td>
<td>0.09</td>
<td>0.00</td>
</tr>
<tr>
<td>Chloroperlidae</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Table 3.2. Seasonal metrics, including taxa richness, mean count (mean number of individual specimens collected in each sample), percent Ephemeroptera-Plecoptera-Trichoptera with (%EPT) and without *Baetis* (%EPT-B) included, and percentages (%) of total individuals for common taxa in Strawberry Creek by sampling locations.

<table>
<thead>
<tr>
<th>Location</th>
<th>Season</th>
<th>Taxa Richness</th>
<th>Mean Count</th>
<th>Shannon Diversity</th>
<th>% EPT</th>
<th>% EPT-B</th>
<th>% Trichoptera</th>
<th>% Simulium</th>
<th>% Argia</th>
<th>% M. californica</th>
</tr>
</thead>
<tbody>
<tr>
<td>North Fork</td>
<td>Dry</td>
<td>7.6</td>
<td>487.4</td>
<td>0.7</td>
<td>4.5</td>
<td>1.2</td>
<td>0.1</td>
<td>19.9</td>
<td>12.6</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>Wet</td>
<td>6.2</td>
<td>136.2</td>
<td>0.5</td>
<td>5.2</td>
<td>2.9</td>
<td>&lt;0.1</td>
<td>13.9</td>
<td>20.3</td>
<td>2.8</td>
</tr>
<tr>
<td>South Fork</td>
<td>Dry</td>
<td>12.9</td>
<td>530.0</td>
<td>1.7</td>
<td>39.0</td>
<td>34.0</td>
<td>2.0</td>
<td>13.9</td>
<td>0.8</td>
<td>31.7</td>
</tr>
<tr>
<td></td>
<td>Wet</td>
<td>11.4</td>
<td>229.2</td>
<td>1.3</td>
<td>68.8</td>
<td>65.3</td>
<td>3.9</td>
<td>7.0</td>
<td>1.3</td>
<td>60.0</td>
</tr>
<tr>
<td>Below</td>
<td>Dry</td>
<td>8.9</td>
<td>347.0</td>
<td>1.6</td>
<td>39.5</td>
<td>19.9</td>
<td>0.5</td>
<td>21.3</td>
<td>1.8</td>
<td>15.3</td>
</tr>
<tr>
<td>Confluence</td>
<td>Wet</td>
<td>7.2</td>
<td>109.0</td>
<td>1.2</td>
<td>47.8</td>
<td>19.2</td>
<td>1.3</td>
<td>5.3</td>
<td>2.4</td>
<td>17.8</td>
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</tbody>
</table>

Table 3.3. Percent Ephemeroptera-Plecoptera-Trichoptera (%EPT), %EPT without *Baetis* (%EPT-B), Shannon Diversity Index, and Taxa Richness for December collections in years before (2004, 2010), during (2011), and after (2012, 2013) the accidental oil spill.

<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>%EPT</td>
<td>36.4</td>
<td>81.5</td>
<td>2.4</td>
<td>69.5</td>
<td>42.1</td>
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<tr>
<td>%EPT-B</td>
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<td>33.1</td>
<td>0</td>
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<tr>
<td>Simpson Diversity</td>
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<td>1.12</td>
<td>0.74</td>
<td>1.11</td>
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</tr>
<tr>
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<td>5</td>
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<td>3</td>
<td>9</td>
<td>10</td>
</tr>
</tbody>
</table>
Figure 3.1. Upper panel displays seasonal variation in gauge height over time at the below confluence site in Strawberry Creek during the wet season 2010-2011. The lower panel displays total precipitation near Strawberry Creek for each water year during this study. The dashed line denotes average precipitation (1970-2010, NOAA Archives).
Figure 3.2. Schematic of Strawberry Creek and sampling sites. The entry point of the accidental oil spill is denoted by at star. The path of the oil runs downstream from the entry point through the remainder of Strawberry Creek.
Figure 3.3. Percent Ephemeroptera-Plecoptera-Trichoptera (EPT) of individual macroinvertebrates collected at Below the Confluence (middle, red), at the South Fork reference (upper, black), and north fork reference (lower, gray) immediately following the diesel oil spill. The dashed line represents the time of the accidental oil spill.
Figure 3.4. Ordination by non-metric multidimensional scaling for the A) north fork, B) south fork, and C) below confluence. All points from a particular season (Wet or Dry) are connected to the mean location for that season. Wet season samples (blue) and Dry season samples (right) are indicated with some overlap for the south fork and below confluence sites.
Figure 3.5. Regression of size spectra for the below confluence site by season. The slopes of wet (winter and spring) and dry (summer and fall) seasons were not significantly different.
CHAPTER 4
Seasonal variation of macroinvertebrates in a temporary wetland of northern California
ABSTRACT

Seasonal wetlands are important habitats for biodiversity of both invertebrate and vertebrate fauna. Many aquatic species have life history traits for colonization and development in such temporary aquatic habitat, and these traits influence the annual succession of the macroinvertebrate community. The chronology of taxa appearance and changes in relative abundances during the hydroperiod of seasonal wetlands are important for understanding population dynamics and responses to drought. This study investigated the successional changes in macroinvertebrate abundances in a seasonal wetland in northern California (USA). Water quality parameters were measured regularly, including dissolved oxygen, temperature, pH, surface area, and specific conductance during the wet season (January-July) in 2007-2009. Additionally, macroinvertebrates were collected with net sweeps and the presence and absence of specific life stages of amphibians were visually observed from March-June each year. As the hydroperiod progressed, wetland surface area decreased, while water temperature and specific conductance increased. Moreover, sequential changes in dominant taxa and relative abundances occurred within the macroinvertebrate community, particularly among several macroinvertebrate predators. Among the macroinvertebrate predators collected, the early-season community was dominated by dytiscid beetles, while later-season communities were dominated by *Lestes* damselflies. The phenology of individual aquatic taxa and specific life history strategies may impact the sensitivity of macroinvertebrate populations to increased annual variation in hydroperiod that may result from climate changes in this region.

Key words: seasonal wetland, dytiscids, *Lestes, Notonecta*, Northern California,

INTRODUCTION

Natural history studies contribute to our basic understanding of ecology by presenting patterns in nature and by identifying biological and physical factors that may constrain or enhance species abundances (Rosenberg & Resh, 1993). Studies that document the temporal co-occurrence of species in the same habitat (e.g., Poff & Matthews, 1985; Berté & Pritchard, 1986) can provide the precursors for hypotheses that address biotic interactions among species. Moreover, syntheses of natural history information can inform ecological theory (McCreadie & Bedwell, 2013). Consequently, discovery of the degree to which collective groups of species, such as guilds, have similar tolerances, traits, or habitat requirements allows for development of biological indices and other conceptual frameworks related to biological assessment (Karr, 1991; Solimini et al., 2008; Lunde & Resh, 2012a).

Wetlands in central and northern California (USA) are important habitat for invertebrates, such as crustaceans (King et al., 1996), and vertebrates, such as migrating birds (Gilmer et al., 1982; Isola et al., 2000). However, wetland habitat, along with rivers and lakes, have been degraded or destroyed in many places. In California, nearly 90% of historic wetlands have been drained and filled (Dahl, 1990). Moreover, among freshwater ecosystems, less is known about the aquatic biology of many seasonal wetlands, such as ponds, when compared with streams and lakes (Boix et al., 2012; Pérez-Bilbao et al., 2015).
Seasonal wetlands provide aquatic habitat during certain seasons, but are generally dry at other parts of the year. When dry, they do not support a diverse aquatic fauna (Palik et al., 2001; Lunde & Resh, 2012a). When water returns, macroinvertebrates access temporary wetlands via flight from nearby perennial habitat or by having desiccation-resistant life history stages that are able to survive in the sediment or nearby plants during the dry season (Batzer & Wissinger, 1996). Even so, physical and chemical factors in temporary aquatic environments, such as temperature and water chemistry, can constrain the occurrence and abundance of macroinvertebrate taxa (Williams, 1996; Zamora-Muñoz & Svensson, 1996). While abiotic conditions may progress in a seasonally predictable direction, high interannual variability in precipitation (e.g., drought) can lengthen or reduce the hydroperiod (Williams, 1996; Boix et al., 2004; Biggs et al., 2005).

We investigated seasonal variation in the abiotic conditions and phenology of the macroinvertebrate community in a seasonal wetland in northern California over multiple years. The objectives of this study were to investigate 1) examine the seasonal progression of abiotic conditions in a seasonal wetland over multiple years, 2) describe seasonal and spatial variability of abundances and taxonomic composition in the macroinvertebrate community, and 3) to assess the succession of the common macroinvertebrate predators.

METHODS

Sampling location
The focus of this study is a seasonal wetland known as Hog Lake (39.0316 °N, 123.0789 °W), located at the Hopland Research and Extension Center in Mendocino county, in northern California (USA). This seasonal wetland is located in a Mediterranean-climate area that is characterized by hot, dry summers and mild, rainy winters. Approximately 75% of rainfall occurs between November and February, and these storms provide the water to fill Hog Lake each winter. The aquatic habitat generally remains until late June or July each summer. Mean precipitation is 6.8 cm for July-October (1952-2011, Hopland Research and Extension Center). Mean precipitation for November and December (1952-2011) is 11.8 cm and 18.0 cm, respectively. For the specific years of the study, monthly rainfall is shown in Figure 4.1. In addition, the lake was divided into two halves using a rubber barrier from December 2007-August 2008.

Abiotic measurements
Abiotic measurements were taken every 2-4 weeks during January through June in 2007, 2008, and 2009. The physical size of Hog Lake was estimated in two weeks. Physical measurements of the length, width, and circumference of the wetland were measured on a subset of sampling days with a field tape measure and were used to calculate surface area. When possible, the most current images available were used in Google Earth Professional to determine the size of the wetland. Dissolved oxygen (mg/L), specific conductance (μS/cm), temperature (°C), pH, and turbidity (NTU) were taken at two locations (East and west ends of the wetland), in the seasonal wetland. To measure dissolved oxygen, specific conductance, and temperature we used a Yellow Springs Instrument MP 556 (YSI). Turbidity was measured using a HACH 2100P turbidity meter, and pH was measured with an Oakton pHTstr 3 meter. For overall descriptions of the
wetland, abiotic characteristics at the east and west sites were averaged to obtain a single value for each variable prior to analysis.

**Biological sampling**

Samples were collected every 2-4 weeks during the months of March, April, May, and June 2007-2009. Wetland invertebrates were collected with a 500 μm D-frame dipnet from the same two sites (East and west) that abiotic measurements were taken. Five net sweeps at each site were collected in a way that included various depths and microhabitats in the limnetic zone, and therefore captured invertebrates in the benthos and the water column.

All macroinvertebrates collected were stored in 70% ethyl alcohol in the field. In the laboratory, macroinvertebrates were sorted with a 25X magnifying glass and identified to family. Some taxa were identified to genus, including *Limnephilus* (Trichoptera: Limnephilidae) caddisflies, *Lestes* (Odonata:Lestidae) damselflies, and *Notonecta* (Hemiptera:Notonectidae) backswimmers. All aquatic insects collected were identified, as well as the individuals of California fairy shrimp, *Linderiella occidentalis*. Copepods were excluded because of their small sizes (<500 μm D-frame). For some samples, macroinvertebrates were subsampled and identified to family, and in some cases genus, by EcoAnalysts, Inc. For subsamples, total abundance estimates were calculated from the percent subsampled.

Hog Lake provides habitat for frogs (Lunde & Resh, 2012b), newts, and migrating birds. Visual observations of all vertebrates present in the wetland and surrounding vegetation, including amphibians and birds, were recorded for each sampling date. Observations of the California newt, *Taricha torosa*, at the time of sampling were noted, including the presence or absence of life history stages, including egg, larvae, and adult.

**Statistical Analyses**

We used non-metric multi-dimensional scaling (NMS, Kruskal, 1964) and clustering techniques to assess seasonal and site differences of macroinvertebrate abundances (counts) in the statistical program R, Version 3.2.1 (*vegan* package). Our non-metric multi-dimensional scaling analysis (NMS, Kruskal, 1964) used Sorensen (Bray-Curtis) distance to assess seasonal differences in both the abiotic factors and aquatic invertebrate community in Hog Lake. Stress values of <0.2 were considered significant. We used Principle Components Analysis (PCA) to identify the abiotic factors that represented the majority of the seasonal variance and compared tested linear models with those factors. Finally, we used Akaike Information Criterion (AIC) scores to determine the best model fit for abiotic factors with NMS axis 1.

**RESULTS**

**Abiotic factors**

The timing and magnitude of the winter precipitation that filled Hog Lake each year, along with the total precipitation at Hog Lake varied each year (Fig. 4.1). In November and December 2006, Hog Lake received 8.8 cm and 15.1 cm, respectively. In contrast, in 2007, Hog Lake received only 1.5 cm in November, but 15.5 cm in December; in 2008, Hog Lake received 9.9 cm and 8.2 cm. In each case, precipitation filled the wetland to capacity by mid-January (2007 and 2008) or early February (2009) sampling dates. Total precipitation was 58.9 cm for October 2006-
Physical measurements in 2007, 2008, and 2009 all demonstrate that Hog Lake began holding water some time between November and January and was dry by mid-July. The limited precipitation that occurs in this area from July through the end of October (Fig. 4.1) indicates that the seasonal wetland was not filled until increased precipitation began in late November or December. The largest surface area occurred each year in January or February, and steadily decreased until drying out in late June or early July (Fig. 4.2). Full capacity of the wetland corresponded to a surface area of 3,200 m², and mean surface area for March and June were 2,802 m² and 917.5 m², respectively.

Surface area was inversely related to water temperature each year (Fig. 4.2). Temperature ranged from 9.4 °C to 28 °C, and median temperature was nearly 2X higher in May and June compared with March and April. Turbidity varied widely throughout the study, ranging from 1.0-12.8 NTU. Specific conductance and water calcium concentrations also increased throughout the hydroperiod and were correlated ($R>0.8$) (Table 4.1).

Dissolved oxygen ranged widely from 6.1-13 mg/L and showed less directionality than temperature and specific conductance (Table 4.1). Dissolved oxygen in Hog Lake was relatively high (>10 mg/L) on many sampling dates. The pH values were alkaline, ranging from 8-10.

Fauna and dynamics
A total of 16 families and 10,028 individuals were collected over the 12 sampling events of this study. Total abundance and family richness of organisms increased from March to late June each year (Table 4.1). Certain taxa, such as Limnephilus caddisflies, were only present in one month (March 2008). No taxa were present in every sample, however Lestes damselflies occurred in all samples except the earliest March sample in 2008 (Table 4.2).

The dominant predator taxa varied during the wet season. In March, dytiscid beetles were the dominant predator in Hog Lake, representing >90% of the macroinvertebrate predators present. Although dytiscids were dominant in early April samples, representing >80% of the macroinvertebrate predators, Lestes was also observed but in lower numbers. By late April, the relative abundance of Lestes increased relative to that of earlier samples and it was the dominant macroinvertebrate predator in the wetland, followed in relative abundance by dytiscids, Libellulid dragonflies, corixid water boatman, and Notonecta backswimmers, respectively.

Lestes continued to be the most abundant predator through the remainder of the hydroperiod. In May, Notonecta and libellulids were less abundant than Lestes, but were more abundant than all other invertebrate predators. Relatively few dytiscids were found in late May. In June, Lestes continued to be most abundant, followed by Notonecta, libellulids, and dytiscids (Fig. 4.6). Baetid mayflies were rare in March and April, but had high abundances in June (>3000) and made up the majority of the macroinvertebrate abundance.

Eggs from the California newt, Taricha torosa, were present from January through early April, but not at later sampling dates. No larvae were present until mid-April in each year, at which time larvae were present, indicating emergence was occurring. Newt larvae were then present at each sampling date until the end of the season. Adult California newts were also observed, but inconsistently, among sampling trips.

Community analysis
Non-metric multidimensional scaling of macroinvertebrate abundances indicated separation of samples by month in ordination space (Stress< 0.2, Figure 3). Moreover, monthly samples were arranged in order from March to June. When East and West sites were ordinated separately, the NMS plots produced clear separation between March and April samples, yet more overlap between May and June samples occurred (Stress<0.2, Fig. 4.5).

The separation of March samples can be strongly attributed to the presence of *Limnephilus* caddisflies and members of the fly family Dixidae. May and June samples were closer in space in some years than others, and separation between samples was associated with the abundances of many taxa, including the predators *Lestes, Notonecta*, libellulids, and coenagrionid dragonflies, as well as baetid collectors. Although abiotic factors varied by season (Table 4.1), the best regression model between abiotic factors and NMDS axis 1 included only temperature.

**DISCUSSION**

Temporal variation in abiotic conditions and biotic assemblages has been found in lentic habitats, such as perennial wetlands (Suren & Lambert, 2010) and lakes (Reid et al., 1995). However, in temporary environments, such as seasonal wetlands, common challenges for macroinvertebrates, such as the ability to withstand desiccation, become more pronounced. Aquatic insects have many life history strategies that allow them to use temporary habitats, such as rapid larval growth and ovarian diapause (Williams, 1996; Bohonak & Jenkins, 2003). Moreover, oviposition of desiccation tolerant eggs in the terrestrial environment (Jannot et al., 2008) and desiccation-tolerant early-larval stages are life history characteristics also common seasonal wetland fauna (Batzер & Wissinger, 1996).

The presence of relatively high dissolved oxygen concentrations and high water pH are generally indicative high primary productivity (Wilkinson et al., 2015). High rates of photosynthesis can deplete carbon dioxide and consequently, carbonic acid in lentic environments, thereby resulting elevated pH values (Wilkinson et al., 2015). High primary productivity and dissolved oxygen concentrations indicate availability of algae for primary consumers and increased oxygen for metabolism. This is particularly important late in the hydroperiod of a seasonal wetland such as Hog Lake, because for ectothermic fauna, such as macroinvertebrates and newts, increased water temperatures increase rates of metabolism.

Species of caddisflies often vary along gradients of permanence in aquatic habitat (Wissinger et al., 2003; Wiggins, 2004) and often have specific traits that reflect those habitats (Zamora-Muñoz & Svensson, 1996; Wissinger et al., 1999). The presence of *Limnephilus* caddisflies early (March of 2008) in the successional state of Hog Lake, when few other taxa were abundant, suggests that their larval growth occurs in the early months of the hydroperiod and that this taxa may be adapted to an early emergence. In montane wetlands, *Limnephilus* caddisflies pupate in sediment (Wissinger et al., 2003), which could explain why we found relatively large-sized larvae in March, but no specimens in later months. In addition, records from the Essig Museum of Entomology indicate that *Limnephilus nogus* adults were caught in emergence traps at Hog Lake in early May, which suggests an emergence time my earlier than the usual drying of the wetland.

Early season peaks in abundance for dytiscids in March and April suggest faster development or earlier seasonal dispersal to Hog Lake. Dytiscids are generally considered predators, and can use seasonal wetlands with their drought- and cold-resistant eggs (Nilsson &
Söderström, 1988). Dytiscids are early colonizers of seasonal wetlands, and often do so by migrating from perennial habitats (Nilsson & Svensson, 1995; Schäfer et al., 2006). They may also be early colonists at Hog Lake, and their high abundances early in the hydroperiod, but not later, may suggest predation by the other invertebrate (e.g., Lestes) or vertebrate predators (e.g., newts), or by later migration to other habitats.

Increased abundances in libellulids, Notonecta and Lestes as the hydroperiod progressed may suggest different abilities to colonize and become established at Hog Lake, or different adaptations for using temporary habitats. Libellulids and Notonecta were common but not the most abundant invertebrates in May and June. Previous studies with Lestes and Notonecta show strong dispersal abilities and colonization of both permanent and seasonal wetlands (McCauley, 2006, 2008; Baines et al., 2015). Therefore, these taxa could be colonizing from further-distant sources than species that are weaker fliers.

Notonecta and Lestes may co-occur late in the hydroperiod because they have different foraging behaviors. As predators, species within Notonecta and Lestes have been shown to prey on many species of mosquitoes (Blaustein et al., 1995) and microcrustacea (Havel et al., 1993), respectively. Furthermore, Notonecta have been observed to be more successful feeding in open water habitat (Blaustein et al., 1995), while Lestes was more successful in littoral habitat (Havel et al., 1993). While we did not observe emergence events, increasing abundances of Lestes and Notonecta in May and June suggest later emergence relative to that of Limnephilus caddisflies and dytiscid beetles.

Increases in late-season predator abundance could be supported by increased prey density. For example, the surface area and estimated volume of Hog Lake decreased consistently from March to June. As habitat area decreases, organisms are concentrated, which decrease the distances between predators and prey (Boix et al., 2004, 2012). With multiple predators present, larger macroinvertebrate predators likely eat each other as well. In addition, increased late-season water temperature may increase abundances in the limnetic zone, which was where we sampled, through rapid growth rates of earlier instars. High abundance of late-season baetids likely provides an important, high-density food source for the increasing abundances of predators. In addition, predator taxa may be prey for other predator taxa, particularly with decreased habitat size.

Late-season habitats present tradeoffs for aquatic predators. For example, smaller wetland size increases prey density, which should benefit predators (Batzer & Wissinger, 1996). However, as wetlands approach dryness, the risk of not completing life history stages increases. As habitat decreased, temperature also increased, which causes increased metabolism in ectotherms, including the organisms examined in this study. Temperature is an important for seasonal regulation of some aquatic insect species, including odonates (Corbet, 1980). Previous studies indicate the Lestes, in particular, may be adapted for the late stages of the hydroperiod. For example, a species of Lestes in North Carolina had progressively higher coefficients for growth rates in each successive instar (Lutz, 1968; Corbet, 2003), suggesting that as temperatures warm, Lestes may be able to increase growth rate and therefore, develop more quickly when temporary habitats become small and warm.

The presence of amphibians as top predators in seasonal wetlands can influence the composition of the macroinvertebrate community (Wissinger et al., 1999). In Hog Lake, California newts are considered top predators in this study system. The presence of eggs before mid-April, but not after, combined with the presence of larvae in mid-April, but not before, indicate that emergence of newts occurred in April in Hog Lake. This is advantageous for
feeding on macroinvertebrates because their total abundances had increased from March to April. Newt larvae have been found to consume macroinvertebrates and that of their chemical cues affect macroinvertebrate behavior (Bucciarelli & Kats, 2015). Our samples did not indicate a decrease in the total abundance of macroinvertebrates throughout May and June, indicating that predation by newts did not outpace invertebrate production.

However, individual taxa may have been impact by the emergence of this predator. For example, dytiscid abundance in Hog Lake decreased concurrently with the emergence of newt larvae. Other amphibians, notably tiger salamanders in Arizona (USA) and Colorado (USA) prey on limnephilids and dytiscids as a minor proportion of their diet (Holomuzki, 1987). Moreover, in montane wetlands, tiger salamanders preferentially prey on the most mobile or conspicuous caddisfly (Wissinger et al., 2003) and, therefore, the more mobile predators may be at heightened risks of predation. Finally, predators cues can promote aquatic insects, such as Notonecta backswimmers, to disperse at higher rates (McCauley & Rowe, 2010), which could indirectly cause changes in the composition of macroinvertebrates in Hog Lake.

Increased occurrence of drought as a result of climate change may impact populations of aquatic insects in seasonal wetlands. While many macroinvertebrates have been shown to develop more quickly under warmer temperatures, extremes in annual variability of hydroperiod, such as during droughts, may shorten hydroperiods to the exclusion of some taxa (Sim et al., 2013). Moreover, variation in hydroperiod of seasonal wetlands influences the macroinvertebrate community structure by influencing a range of life history traits, including growth, development, and reproduction of individual taxa (Jannot et al., 2008; Céréghino et al., 2012; Pérez-Bilbao et al., 2015). However, permanent wetlands can serve as source populations for seasonal wetlands and even small aquatic habitats can act as refuges for some aquatic insect taxa (Strachan et al., 2014). Consequently, threats to biodiversity may be specific to not just species but also to certain life history strategies of organisms inhabiting seasonal wetlands.

ACKNOWLEDGEMENTS

I thank K. Lunde and M. Chiu for field and analytical assistance throughout this project. I am grateful to J. Ball and many volunteers for assistance with field collections. I thank the Hopland Research and Extension Center for logistical help and long-term weather information. Additionally, I thank the Margaret C. Walker Fund for graduate research in Systematic Entomology and the Edward A. Colman Fellowship in Watershed Management from the Department of Environmental Science, Policy, and Management at the University of California, Berkeley, for support.

LITERATURE CITED


Figure 4.1. Monthly precipitation (cm) at Hog Lake, Mendocino County, California (USA) during 2007, 2008, and 2009.
Figure 4.2. Wetland surface area (left) and water temperature (right) at Hog Lake, by month, from March to June in 2007, 2008, and 2009.
Figure 4.3. Non-metric multi-dimensional scaling ordination of the macroinvertebrate communities by season and the directional influence of each individual taxa (red color).
Figure 4.4. Regression of water temperature and MDS1 ($R^2 = 0.55$, $p = 0.006$).
Figure 4.5. Non-metric multi-dimensional scaling ordination of communities by season and site, and the directional influence of each individual taxa (red color).
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<th>Calcium</th>
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Table 4.2. Mean Abundance of each taxon in samples collected by month from Hog Lake in Mendocino County, California (USA) from 2007-2009.

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