GENETIC STRUCTURE AND DEMOGRAPHIC IMPACTS OF OIL SPILLS IN WESTERN AND CLARK'S GREBES

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GENETIC STRUCTURE AND DEMOGRAPHIC IMPACTS OF OIL SPILLS IN WESTERN AND CLARK'S GREBES

Thesis by
Diana L. Humple

EXECUTIVE SUMMARY

The Western Grebe (*Aechmophorus occidentalis*) and its sister species, the Clark's Grebe (*A. clarkii*), are highly vulnerable to oil spills in their nonbreeding nearshore habitat along the Pacific coast. There is a paucity of knowledge on population structure, migratory connectivity, and demographic structure of grebes impacted by oil spills. The conservation applications of these topics and the prevalence of impacted grebes during oil spills in California lent to the pursuit of such knowledge.

The first component of this project was the development of microsatellite markers for use in genetic studies of population structure and migratory connectivity. Following initial development – in collaboration with Genetic Identification Services – and subsequent primer selection and optimization, 11 loci were screened across a panel of 16 grebes of each species. These were also successfully tested on the five other North American grebe species. Loci that were previously developed for Red-necked Grebe were screened in Western and Clark's Grebes. These efforts and results are summarized in Chapter One.

The six most diverse loci were selected and a preliminary assessment of population structure was conducted, assessing across a latitudinal gradient 1) two disparate breeding colonies (Eagle Lake in California and Lake Wabuman in Alberta, Canada) and 2) three disparate wintering regions (coastal Washington, coastal northern California, and coastal southern California). These analyses are summarized in Chapter Two. Differentiation was not detected among these five groups. Differentiation between Western and Clark's Grebes was also assessed, and no significant differentiation was detected. Although this suggests relatively high levels of gene flow as well as some potential hybridization in these nascent species, it also points to the need to include additional and more diverse loci, as even weakly differentiated populations can be differentiated with enough loci.

Chapter Two also summarizes non-genetic approaches to understanding structure and migratory connectivity in grebes. North American band recovery data provided by the Bird Banding Lab was analyzed to provide insights into these patterns. The data indicate that moderate connectivity may occur in Western Grebe, at least for Manitoba breeders. The data also reveal within-winter movements between regions, and examples of lack of site fidelity for both breeding and wintering sites.

In Chapter Three, age and sex ratios were assessed from birds killed in three marine events in coastal California, to test if distributional wintering of age or sex classes occurs. Such information is important for understanding and modeling overall impacts from oil spills, as demographic impacts to breeding populations can be greater if particular age or sex classes are affected disproportionately because of differential wintering distributions.

These events included two oil spills (the 1997 *Kure* oil spill in Humboldt bay, and the 2005 Ventura Oiled Bird Incident), and a 2007 red tide algal bloom in Monterey Bay that resulted in a mass stranding and mortality of seabirds due to loss of waterproofing. Data included those collected during necropsies as well as with molecular sexing techniques. Birds were assigned to age class predominantly based on the presence or absence of a bursa of Fabricius after first attempting to validate this method for grebes; the results suggesting its utility but the need for further study to understand some observed inconsistencies in atrophication patterns. Variable patterns in age and sex ratios were found. For the 2005 Ventura Oiled Bird Incident – a significant event for grebe mortality – Western Grebes showed no bias in overall sex ratio but among immatures were male-biased, and they were adult-biased overall. The latter is of concern since this age class contributes to breeding productivity and typically has high survival. In Monterey Bay, Clark's Grebes were male-biased, while the female-bias in Western Grebes was apparently driven by a skewed sex ratio in immatures. In Humboldt Bay, immature Western Grebes were more common and overall they lacked a differential sex ratio.

In summary, the first species-specific microsatellite markers for Western and Clark's Grebes were developed. These were also successfully tested on other grebe species. Genetic differentiation was not detected among the geographic localities included in the preliminary study of Western Grebes, nor between Western and Clark's Grebes. Future studies would require the inclusion of additional markers. The age and sex ratios from birds killed in the three marine events reveal that differential wintering occurs in grebes, an important consideration when assessing demographic impacts of any spill. Observed within-winter movements and the potential for temporal variation necessitate age and sex structure be assessed following each event, rather than applying the results from these events to future ones. The banding and recovery data indicate an overall plasticity in site fidelity. Combining those results with the lack of genetic differentiation detected, post-spill resources may actually be most appropriately directed toward colonies undergoing other threats, as it may not be possible to identify provenance of individuals killed in winter oil spills; or possibly their provenance may be distributed across many regions. However, the moderate migratory connectivity observed for one breeding site supports further investigation into this topic. Attempts to increase our understanding of population structure and migratory connectivity in grebes may require looking to other technologies. While we may not be able to identify such structure with genetics, other approaches may reveal patterns of structure and connectivity that are important for conservation.

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I shall never forget the picture, as I stood in water more than waist deep, of one of these beautiful 'swan grebes' sailing out from a dense wall of cat-tails, causing scarcely a ripple as it glided along, the body submerged, the long white neck sharply outlined against the green background, the glossy crown, the fiery red eyes, and the javelin-like beak. Who could help admiring such a picture of aquatic grace, such specialized mastery of its native element? ... The western grebe is certainly a water nymph of the first class, built for speed and action, the most highly specialized of all our diving birds.

– Arthur C. Bent, 1919, Western Grebe species account, Life Histories of North American Diving Birds, Dover Publications, Inc., New York.

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CHAPTER 1

CHARACTERIZATION OF MICROSATELLITE LOCI FOR WESTERN

GREBE (*AECHMOPHORUS OCCIDENTALIS*) AND CLARK'S GREBE (*A.*

CLARKII) AND THEIR APPLICATION TO OTHER GREBE SPECIES

ABSTRACT

Eleven tetranucleotide-repeat microsatellite loci were isolated from the Western Grebe

(*Aechmophorus occidentalis*), a migratory waterbird, and screened on the Western Grebe

and its sister species, Clark's Grebe (*A. clarkii*). These were developed to assess

population structure and migratory connectivity in separate studies. All were

polymorphic with two to ten alleles per locus. We also report on the potential utility for

Aechmophorus grebes of three trinucleotide markers developed and published for Red-

necked Grebe (*Podiceps grisegena*; Sachs and Hughes 1999). Additionally, the 11 loci

developed for *Aechmophorus* grebes were tested on the five other North American grebe

species (*Podicipedidae*); initial results suggest these loci may prove useful in future

studies of those species.

INTRODUCTION

The connectivity between wintering and breeding populations of most migratory

birds is poorly understood. Such information is critical for understanding and mitigating

the factors limiting migratory bird populations. Because of the challenges of directly evaluating dispersal, philopatry, and migratory connectivity, researchers have turned to genetics as a tool to increase understanding of this connectivity and of population structure (Webster et al. 2002, Boulet and Norris 2006). Because birds have high levels of vagility, their genetic structure is best examined using multiple molecular markers with high levels of variation. These dispersal capabilities may result in greater gene flow among populations than that found in species with reduced dispersal capabilities, making differentiation difficult to detect with more conserved genetic markers. Microsatellite loci or simple sequence repeats (SSR) are often the most appropriate markers to examine population structure and migratory connectivity in migratory species (Selkoe and Toonen 2006).

STUDY SPECIES AND METHODS

Western Grebes (*Aechmophorus occidentalis*, Lawrence, 1858) and Clark's Grebes (*A. clarkii*, Lawrence, 1858) are closely related sister taxa that, between 1886 and 1985, were considered different phases of the Western Grebe (Storer & Nuechterlein 1992). Both are migratory, breeding in lake systems throughout western North America and wintering predominantly in the near-shore environment along the Pacific Coast. We developed, selected and characterized 11 microsatellite loci for both *Aechmophorus* grebe species to assist future studies of breeding population structure and migratory connectivity, largely unknown in these species. We also screened each of the five additional North American grebe species to test the potential utility of these markers on

other *Podicipedidae* species: one Red-necked Grebe (*Podiceps grisegena*, Boddaert, 1783) from northern coastal California; one Horned Grebe (*P. auritus*, Linnaeus, 1758) and one Eared Grebe (*P. nigricollis*, Brehm, 1831) from central coastal California; one Pied-billed Grebe (*Podilymbus podiceps*, Linnaeus, 1758) from northern coastal California; and a Least Grebe (*Tachybaptus dominicus*, Linnaeus, 1766) from Bolivia. Additionally, we screened *Aechmophorus* grebes with additional loci that Sachs and Hughes (1999) developed for Red-necked Grebe and had tested on other grebe species.

Samples were provided by collaborators or collected ourselves from available carcasses or via catch and release. Muscle, liver, and/or toe samples were taken from carcasses and either frozen or stored temporarily in Queen's lysis buffer (Dawson et al. 1998), and blood was taken from live birds and stored on Whatman FTA® cards. We extracted DNA from muscle and liver with a phenol chloroform iso-amyl alcohol (PCI) extraction process (following Taberlet and Bouvet 1991); from toes using a Qiagen Tissue Extraction Kit; and from blood with Whatman Purification Reagent® and TE (slightly modified from Gutierrez-Corchero et al. 2002).

We provided 150µg of DNA, taken from muscle samples collected in winter from four Western Grebe carcasses in southern California, to Genetic Identification Services (GIS) of Chatsworth, CA. GIS constructed genomic libraries of eight tetranucleotide motifs, out of which two were highly enriched (78% and 60%) and two were moderately enriched (44% and 32%) for microsatellite loci. We examined DNA sequences of 69 candidate loci of the 166 sequences of clones from the libraries provided to us by GIS, and chose 25 based on appropriate motif repeat composition for potential genetic variation and potential for primer design. For these we designed primers with Primer3

(Rozen and Skaletsky 2000) or used those recommended by GIS. We ran annealing temperature and $MgCl_2$ gradients to determine individual optimization of reaction conditions.

Amplification of loci via polymerase chain reaction (PCR) was carried out on an MJ Research PTC-100 or PTC-200 thermocycler. We used one of two PCR types: either one using fluorescent dye-labeled locus-specific forward primers (6FAM, NED, PET and VIC; Applied Biosystems); or one using an M13-tail, with a fluorescent dye in a third non-specific forward 18-bp M13 primer (TGTAAAACGACGGCCAGT, FAM, Applied Biosystems) and a complementary "tail" to the M13 primer at the 5' end of the forward locus-specific primer (Schuelke 2000). The PCR profile for the first approach was 4 min at 94°C; 35 cycles of 40 s at 94°C, 40 s at the specific annealing temperature for the microsatellite (see Table 1), and 30 s at 72°C; and a final 10 min at 72°C. The M13 PCR profile was 5 min at 94°C; 30 cycles of 30 s at 94°C, 40 s at the specific annealing temperature for the microsatellite (see Table 1), and 40 s at 72°C; 10 cycles of 30 s at 94°C, 40 s at 53°C, and 40 s at 72°C; and a final 10 min at 72°C. Each PCR reaction (10μL) contained 5-100ng of extracted DNA template, 2.0-4.0mM $MgCl_2$ (see Table 1), 1X Mg-free buffer (Promega), 0.1 mM of each dNTP, 0.6μM of each primer, 0.25U *Taq* polymerase or *go-Taq* (Promega), and water. The M13 PCR reactions varied from above by containing only 0.15 μM of the locus-specific forward primer and the addition of 0.6μM of the non-specific M13 FAM forward primer.

Amplified regions were initially assessed for polymorphism using 8% polyacrylamide gels, and 13 loci were selected based on polymorphism and successful optimization for screening and analysis on an ABI3100 Genetic Analyzer; two were

subsequently rejected due to monomorphism (1 locus) or irregular allelic patterning (1 locus) and are not included herein.

For each of the 11 selected loci, 16 individuals of each *Aechmophorus* species across a broad geographic range were screened. These were predominantly from wintering areas; for Clark's Grebes, samples were from southern California through the San Francisco Bay area, and for Western Grebes, samples were from southern California through British Columbia. Allele size was visualized using GeneMapper® (Version 3.7).

RESULTS AND CONCLUSIONS

Most loci had relatively low levels of polymorphism, having two to 10 alleles each (Table 1) with an average of 4.5 alleles found per locus. We found one private allele in Western Grebe and five in Clark's Grebe, with most alleles (88% across all loci) occurring in both species. Upon optimization, annealing temperatures for most selected loci were consistent (55°C), but there was variability in optimal $MgCl_2$ concentration (Table 1). Expected heterozygosity values ranged from 0.482-0.857, and observed heterozygosity values from 0.188-0.875.

We found comparable allelic diversity of the loci Sachs and Hughes (1999) developed for Red-necked Grebe to what they reported for fewer individuals. We found 2 alleles in each species (Western Grebe, n=10; Clark's Grebe, n=13) for PgAAT41 versus their findings of 1 and 2 alleles (n=5 for each species and subsequent locus); 1 allele for each species (n=12 and n=7) for PgAAT34 versus their findings of 3 and 2 alleles, respectively; and 1 allele for Clark's Grebe (n=9) for PgAAT8, identical to their results.

Due to our overall poor amplification success and low allelic diversity in these loci, and their subsequent limited utility for a population structure study, optimization and screening was not pursued any further.

Amplification of all the microsatellite loci developed for *Aechmophorus* grebes was successful in at least one other species (and genus) of grebe (Table 2). The PCR conditions used were those optimized for *Aechmophorus* with no attempt to optimize conditions for other species. We did not repeat PCR for individual samples that failed to amplify or produce scoreable product. Thus, loci which produced unscoreable product might, after optimization, be appropriate for those species, and possibly even unsuccessful ones may have utility. We leave such pursuit to future studies.

ACKNOWLEDGEMENTS

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TABLES

Table 1 Tetranucleotide microsatellite loci developed for Western Grebe (*A. occidentalis*) and screened on 16 individuals of each

Aechmophorus species (Western Grebe and Clark's Grebes, *A. clarkii*)[1]. ProbeDB accession numbers 10315672-682

Locus Name	Repeat Motif[2]	Primer sequence (5'-3')	T_a (°C)	$MgCl_2$ conc (mM)	Size range (bp)	A. occidentalis			A. clarkii		
						No. of alleles	H_E	H_O	No. of alleles	H_E	H_O
B8	(CATC)$_3$(CATT)$_1$(CATC)$_3$TAT (CATT)$_2$(CATC)$_9$	AAGGAGGGCATTATGTTATGC GCCAGTAATCACCACAAATTG	55	2	277-289	4	0.520	0.375	3	0.607	0.688
B11	(CATC)$_{11}$	CAGGCAGCAAAAGTTCTTG GGGTCTCTGTGATGTGATGA	55	2.5	147-155	3	0.561	0.438	3	0.619	0.500
B102	(CATC)$_7$CGTC(CATC)$_2$	ACCCATCCATCCATACCTG GCCCAAACCATTCTGTGAT	55	2.5	134-146	4	0.525	0.563	4	0.561	0.375
B112b	(CATC)$_{10}$	CAATCCAAAGGCTGGATCTC TCGAAAACCAAAATGTGCAG	55	3	222-230	3	0.539	0.875	3	0.617	0.750
B113	(CATC)$_{11}$	AAGCACACAATTCAGGAAGTAC GATGCCTCCATTTAGGTAAAAG	55	2	211-231	5	0.754	0.813	6	0.785	0.875
C5	TACA (TA)$_2$ (TACA)$_8$(TA)$_5$	GTTTCCTCCAGCCAGTCAC GTTCCTTCAACAGGCACAAG	55	2.5	249-253	2	0.498	0.188	2	0.482	0.188
E202	(GAAG)$_{14}$N$_{32}$ (AAAG)$_{10}$	TCCAGACCGTTTTGCTTCTT CCTCATGGTTCAATGCCTTT	55	2.5	217-253	8	0.822	0.688	10	0.857	0.625
G118	(TAGA)$_{11}$	TTCTTGTCACATTTCATTGCAC TTCAGGGTTGCTCAGTTGTG	55	4	303-323	4	0.545	0.563	5	0.656	0.750
G206	(TAGA)$_8$AAAGA(TAGA)$_2$	AAGGCAAGCAAACAGAGC ATGGACACAATGCGTCTTAG	55	2	274-290	4	0.662	0.688	5	0.740	0.875
G209	(TAGA)$_9$	TGCAATGCCATTTTCTGTTT ACAGCCCAGAGTTAGGCTCA	55	3	340-348	3[1]	0.607	0.733	3	0.541	0.500
G215	(TAGA)$_2$TAGGTATG(TAGG)$_4$ (TAGA)$_2$TGA(TAGA)$_9$	GACAGGACAACCCTTTTATCC TGGTGGTCTCTGACAGGTC	60	2.5	244-260	5[1]	0.696	0.800	5	0.693	0.750

[1]Included only 15 samples of Western Grebe (could not yield scoreable product for final sample)
[2]N=number of nucleotides in between more discernible repeat regions

Table 2. Success of amplification and allele size for 1 individual of each of the 5 other North American grebe species using tetranucleotide microsatellites developed for Western Grebe. Unsuccessful samples denoted by hyphen (–); those which amplified but produced unscoreable product (with no optimization attempted) noted as such.

Locus Name	Allele Size (bp) and Amplification Success				
	Red-necked Grebe	**Horned Grebe**	**Eared Grebe**	**Pied-billed Grebe**	**Least Grebe**[1]
B8	282, 314	277, 281	281	304, 324	313, 317
B11	128, 156	151, 155	Unscoreable product	160, 164	Unscoreable product
B102	126, 134	138	126, 134	Unscoreable product	Unscoreable product
B112b	222, 230	222, 226	242	238, 242	238, 242
B113	223	215, 227	Unscoreable product	227	231, 235
C5	Unscoreable product	257	Unscoreable product	Unscoreable product	Unscoreable product
E202	217	245	233, 245	237, 241	245
G118	–	311	–	303, 307	Unscoreable product
G206	278, 286	286, 290	Unscoreable product	274, 282	Unscoreable product
G209	–	340, 344	–	–	Unscoreable product
G215	216, 220	248, 252	256	252, 256	310, 366

[1]University of Washington Burke Museum # 71530

CHAPTER 2

PRELIMINARY EVALUATION OF POPULATION STRUCTURE, MIGRATORY CONNECTIVITY, AND MOVEMENT PATTERNS IN WESTERN AND CLARK'S GREBES

ABSTRACT

Little is known about Western Grebe (*Aechmophorus occidentalis*) movement patterns, including breeding philopatry, winter site fidelity, migratory connectivity, and within-season movement. Such patterns are important to understanding grebe biology and determining how populations are limited, but are challenging to explore in this species. We conducted a preliminary study of grebe population structure and movement patterns using microsatellite data and banding recovery data. The six microsatellite loci used did not have the power to detect differentiation among two disparate breeding colonies (Eagle Lake, California; and Lake Wabuman, Alberta) and three wintering areas (coastal Washington, northern coastal California, and southern coastal California). Additionally, no significant differentiation was picked up between the Western Grebe and its closely related sister taxa, the Clark's Grebe (*A. clarkii*). Future studies should include additional and more variable loci. Limited band recovery data suggest that, although migratory connectivity is not strong as birds from a given breeding locale migrating to multiple

wintering regions, moderate levels of connectivity do occur. Birds from Manitoba – the

only breeding population for which there is a sample size of birds banded and

encountered – winter in highest frequency in the coastal and Puget Sound regions of

Washington and British Columbia. These recovery data show Western Grebes to have

plasticity in both their breeding and winter site fidelity, a trait predicted by the variability

in breeding site condition and coastal winter food availability. Additionally, we observed

relatively large-scale movements within winter seasons. These are among the only

insights into Western Grebe movements, outlining the need for larger scale studies of

movement and population structure, especially in light of current conservation issues

faced by this species.

INTRODUCTION

For most migratory birds, little is known about the links between breeding and

wintering populations (Marra *et al.* 2006) – termed "migratory connectivity" (Webster *et*

al. 2002). This lack of knowledge prevents identification and mitigation of the factors

that limit migratory bird populations. Many seabird speciess – including both nearshore

and pelagic species – are particularly vulnerable to oil spills (Carter 2003). Such spills

typically occur in the winter when migratory species are far from their breeding sites, yet

have consequences for breeding populations or colonies. Knowledge of migratory

connectivity could be applied as a standardized tool in evaluating and monitoring the

impacts of both past and future spills to understand how populations respond to these

spills. The Western Grebe (*Aechmophorus occidentalis*, Lawrence, 1858) is among the

more vulnerable species to oiling along the California coast (Carter 2003, PRBO unpubl. data). The majority of Western Grebes winter in near-shore marine waters and estuaries from British Columbia south to Baja California (Bent 1919, Storer and Nuechterlein 1992), and breed inland at colonies on suitable lakes throughout much of western North America. In addition to oil spills, heavy anthropogenic disturbance at breeding colonies pose significant threats to grebe populations (Storer and Nuechterlein 1992).

An important step in understanding population limitations and influencing management actions is to determine the links between wintering and breeding sites. Little can be done to improve management in coastal wintering areas; consequently, when coastal oil spills occur the resulting settlement money intended to reverse losses is typically applied towards breeding areas. However, until provenance of the affected birds is determined, these resources cannot be applied towards the actual breeding populations impacted. Movement patterns in birds are sometimes studied directly with radio or satellite tracking or with banding and encounter data. However, for some species, directly evaluating movements is difficult or impossible. In those cases, researchers often turn to more indirect approaches to connect migratory populations, such as genetics (Haig *et al.* 1997, Kimura *et al.* 2002, Webster *et al.* 2002, Clegg *et al.* 2003, Hull and Girman 2005, for review see Boulet and Norris 2006). Because satellite and radio tracking are still in an experimental state for Western Grebes due to species-specific tag attachment challenges, we chose to use a genetic approach to answer these questions. We also analyzed available banding and encounter data to determine if any measures of connectivity can be observed.

For this study, microsatellite markers were chosen to address migratory connectivity in Western Grebes. Such markers are likely the most appropriate genetic tool for fine-scaled analyses of population structure and genetic connectivity among breeding and wintering migratory populations (Selkoe and Toonen 2006). Microsatellite regions (also known as tandem repeat non-coding regions or simple sequence repeats) in the nuclear DNA mutate at a much higher rate than more conserved regions due to the lack of selective pressure and the mutation processes themselves. As a result, differentiation between populations can be detected at a higher rate than if examining more conserved regions or loci responsible for phenotypic differences. Consequently, populations with reduced gene flow can often be distinguished from each other with microsatellites. Although mitochondrial DNA mutates quickly, historical hypsithermal and glaciation events that restricted the ranges of many western North American birds are thought to limit its utility here (Hull and Girman 2005).

As a first step we developed the molecular tools to make such analyses possible (see Chapter 1). The second step was to conduct a preliminary study assessing the utility of these markers for population-level assessment. These results were intended to determine genetic structure among a subset of breeding sites and wintering areas. This could in turn inform future larger-scale, range-wide studies of population structure and help assess the ability to meet longer-term goals of genetic assignment of wintering individuals to breeding regions of origin. Analysis was conducted on birds from two disparate breeding colonies and three disparate wintering sites along a latitudinal gradient. It was hypothesized that Western Grebes would reveal genetic structuring between breeding sites of different bioregions and latitudes, indicating reduced gene flow

between disparate regions; and if true, it was further hypothesized that patterns in the latitudinal wintering structure might be detected.

The second focus of this chapter was to compare microsatellite data between the Western Grebe and its sister species, the Clark's Grebes (*A. clarkii*, Lawrence, 1858). The microsatellite data from the preliminary population structure analysis for Western Grebe, and from the 16 Western Grebes screened for marker development (in Chapter 1), were compared to data from the 16 Clark's Grebes screened for marker development to assess interspecies differentiation.

The third focus of this chapter is a review of what is known about Western Grebe movements, and an assessment of their movements and migratory connectivity using banding and encounter data provided by the U.S. Geological Survey's Bird Banding Lab (BBL). The latter is a follow-up to a similar analysis of Western Grebe banding and encounter data through 1989 (Eichhorst 1992). The number of encounters has since doubled, with the vast majority of new encounters from birds injured or oiled along coastal California; the majority of birds banded before were from a breeding study of grebes in Manitoba. It was therefore appropriate to revisit what movement, connectivity, and fidelity insight might be found in the banding and encounter data.

Focal Species and Relevance

Western Grebes are highly vulnerable to oiling on their winter range and are one of the top species impacted in coastal California oil spills (S. Hampton, CDFG-OSPR, *pers. comm.*; PRBO unpublished data). In fact, oil spills are considered one of their

primary population threats (Storer and Nuechterlein 1992). Overall concerns about the

species stem from their vulnerability to anthropogenic events on both wintering and

breeding sites, and from observations of declines throughout parts of their range;

coastally wintering populations of Western Grebes in Washington and British Columbia

are in evident decline (Sauer *et al.* 1996, Badzinski *et al.* 2006), where they are currently

a candidate for listing in Washington (WDFW 2008) and are considered critically

imperiled in British Columbia (BC Ministry of Environment 2000). However, as with

many migratory bird species, little is known about their migratory connectivity (i.e., the

movements and connections of populations between breeding and wintering regions; see

Boulet and Norris 2006). Additionally, Western Grebes have an isolated breeding

distribution, relegated primarily to large lake systems throughout their range, but little is

known about their level of natal or breeding philopatry (Storer and Nuechterlein 1992).

The closely related sister species, the Clark's Grebe *A. clarkii*, was previously

considered a different color phase of the Western Grebe, both collectively called

"Western Grebe" between 1886 and 1985 (Storer and Nuechterlein 1992); prior to 1886

they were described as different species. Although they occasionally hybridize,

observations of strong positive assortative mating in colonies consisting of both species

(Ratti 1979) led to their reconsideration as separate species (Storer and Nuechterlein

1992). Clark's Grebes are sympatric with Western Grebes throughout a slightly restricted

portion of the latter's range. These sister taxa are extremely closely related, and likely

only recently diverged (Eichhorst 1994); some dispute the appropriateness of the species

split. The primary differences between them include some plumage variation (particularly

facial), bill color, their "Advertising Call" (Storer and Nuechterlein 1992; a one- versus

two-note call highly important for courtship and general communication), and minor differences in diving ecology and related morphology (Feerer 1977, Nuechterlein and Buitron 1989, Livezey and Storer 1992). Much of their ecology and especially their vulnerability to anthropogenic and other impacts are shared. While it is therefore appropriate to include Clark's Grebes in some of our discussion, and although data prior to the 1986 split does not differentiate between the two species, the emphasis of this chapter is on Western Grebes. This is due to: 1) the restricted range and reduced numbers of Clark's Grebes, resulting in fewer available data and samples; 2) restrictions on resources available for laboratory and field work.

The high vulnerability of Western and Clark's Grebes to oil spills is due in part to their strong association with the near-shore coastal environments from Baja California north to British Columbia, where most individuals winter (Storer and Nuechterlein 1992) and where spills frequently occur; and is also due to their absolute association with the water, as they never set foot on land in their entire life history unless they are compromised. The Western Grebe is generally among the most common seabird species oiled in California spills, including the 1986 *Apex Houston* spill in central California, the 1990 *American Trader* spill near Huntington Beach, the 1997 *Kure* spill in Humboldt Bay, the 1997 *Torch* spill near Pedernales, the 1999 *Stuyvesant* spill in Humboldt Bay, the release of oil by the sunken vessel *S.S. Jacob Luckenbach* in the Gulf of the Farallones between 1997 and 2003, and the 2007 *Cosco Busan* spill in San Francisco Bay (American Trader Trustee Council 2001, Carter *et al.* 2003, Hampton *et al.* 2003, Ivey 2004, CDFG, CSLC, & USFWS 2004, PRBO/OWCN unpubl. data, and Steve Hampton, pers. comm.). Most dramatically reflecting this vulnerability, during the 2005 Ventura

Oiled Bird Incident – a mystery spill in southern California – Western Grebes comprised approximately 90% of the birds oiled, with over 1200 dead or debilitated birds collected (OWCN/PRBO unpublished data), a number which may be only a fraction of the true mortality. During this event, tissue samples from carcasses were collected and banked to support future research on migratory connectivity.

Little information exists on connectivity between coastal wintering and inland breeding sites in grebes. It is therefore currently unknown which breeding colonies are impacted when oil spills cause mortality in California or other coastal waters. Determining a link between populations affected by winter coastal oil spills and their specific breeding regions will enhance our understanding of the demographic impacts of such oil spills, will allow biologists to monitor appropriate breeding colonies in order to assess how they respond to oil spills, and would direct critical restoration activities towards the actual breeding colonies affected. The Natural Resource Damage Assessment (NRDA) process in California results in funding available to restore wildlife populations impacted by oil spills. However, as restoration activities in the coastal wintering range of this species are limited, the most effective efforts to counter deleterious impacts of spills to grebes focus on breeding sites (Ivey 2004). If it was known that a given winter spill event predominantly affected breeding grebe populations in a specific region, then appropriate efforts could be focused there for future biological monitoring as well as population restoration.

Review of Western Grebe Movements and Population Structure

Although the breeding ecology and courtship behaviors of Western Grebes have been well studied, their movements are very poorly understood (Storer and Nuechterlein 1992). Little has changed on that front since the publication of that species account.

Population Structure and Philopatry

Knowledge of population structure is integral to determining a population's vulnerability, or the potential for immigration and gene flow. In the Western Grebe, it is unknown whether groups of individuals at specific breeding sites form relatively distinct demographic units or whether there is movement of individuals among breeding sites from year to year, which would disrupt the formation of distinct genetic populations. This would be even more disrupted if there was low natal philopatry.

Storer and Nuechterlein (1992) report on the occurrence of natal philopatry (the fidelity of a bird towards its location or region of hatching) in Western Grebes. Natal philopatry was confirmed by observations of some birds with visible nasal tags returning to their natal marshes in subsequent breeding seasons; however, the frequency of philopatry was not presented, and is often challenging to disassociate from mortality.

Few references to breeding philopatry (i.e., individuals returning to the same breeding site in subsequent years) exist for Western Grebes (Eichhorst 1992, Eichhorst 1994). A few records of nonphilopatric individuals have been reported, where birds were encountered at a different breeding site than in a previous season (see Eichhorst 1992 for details). However, such observations and the apparent lack of data on philopatric

individuals in the literature (Eichhorst 1992) does not suggest that philopatry is uncommon; it is instead an artifact of both a paucity of breeding studies with field-observable marked individuals, and of how encounters are reported to the BBL. Eichhorst (1992) summarized data provided by the BBL, which did not include birds encountered within the same 10-minute block by the same research program in which the bird as originally banded, as such encounters are not reported to the BBL.

One previous study examined genetic population structure in the Western Grebe. Eichhorst (1994) used mitochondrial DNA and found homogeneity in haplotypes among broad geographic regions within the species and no geographic structuring. However, such patterns – or lack thereof – are often found for western North American birds when using mitochondrial DNA due to high levels of gene flow during evolutionarily recent geological events (e.g., Pleistocene glaciation or the mid-Holocene hypsithermal period), and for this reason, mitochondrial DNA may not be the most appropriate marker to detect intraspecific population structure (Kimura *et al.* 2002, Clegg *et al.* 2003) in the Western Grebe.

Migratory Connectivity

There are several potential patterns of migratory connectivity in birds: 1) strong connectivity, where all or most the individuals of a breeding population migrate to the same area to winter; 2) moderate connectivity, where a large proportion of the individuals of a breeding population migrate to one wintering area, while others distribute themselves to varying degrees to different wintering areas; and 3) no connectivity, where there is no association between breeding and wintering sites, with individuals from a given breeding

population distributing throughout their winter range (Webster *et al.* 2002, Marra *et al.* 2006, Boulet and Norris 2006). Moderate connectivity is probably most typical (Boulet and Norris 2006).

Due to their affiliation with inland lake systems for breeding and with marine nearshore waters for wintering, Western and Clark's Grebes tend to have more of an east-west migration pattern (or possibly northeast-southwest) than is typical for many migratory Nearctic species, which generally show the more traditional north-south pattern (Gill 1994). In terms of actual migratory routes, Bent (1919) presents undocumented claims that spring migration includes a northerly coastal migration of some grebes before they head inland, and that during fall migration they predominantly either head southwesterly along the Rocky Mountains or south along the Pacific coastal (presumably after a westerly migration directly to the coast). The few published banding encounters provide some insight into fall migration routes (Eichhorst 1992): a Manitoba breeder that was recovered in fall of the same year at Okanagan Lake, British Columbia; another Manitoba breeder recovered in fall of a subsequent year in Montana; and two birds banded in fall in Wyoming and recovered in winter in Nevada.

Previous efforts to understand the migratory connectivity among breeding and wintering populations of Western Grebes are limited to Eichhorst's (1992) study examining band recovery data through 1989, although insight was predominantly limited to birds breeding in Manitoba where most banding occurred. These results are also summarized in Storer and Nuechterlein (1992). Numerous grebes banded in Manitoba were recovered on coastal wintering areas, from southern British Columbia to southern California across essentially the full extent of their wintering range. Eichhorst (1992)

found that most were concentrated in the Vancouver-Seattle area and to a lesser degree in the San Francisco Bay Area, although he pointed out that the absence or low densities in other areas may reflect biases in encounter rates more than wintering probabilities. A single bird banded in winter (San Francisco Bay) was recovered at a breeding site (Saskatchewan). Typically low sample sizes of band recoveries limit the application of banding encounter data towards understand migratory connectivity in most avian species (Marra *et al.* 2006), as does the bias towards geographic regions where banding or encounters occur.

METHODS AND ANALYSIS

Preliminary Assessment of Population Structure

Genetic Sampling

To collect genetic samples, two primary methods were used: 1) collaboration with other researchers or rehabilitators, and 2) capture and release of live birds at breeding colonies. This combination of approaches was designed to provide reasonably large sample sizes for select wintering and breeding localities. Appendix 1 summarizes all samples collected as part of this project, including those not used in the analyses herein, which are banked in Sonoma State University's Core DNA Laboratory.

Through collaboration with various researchers, beached bird surveyors, and rehabilitation centers, samples were acquired from much of the coastal wintering range of the species and from a few breeding sites as well. The winter samples in particular were acquired through collaborative methods because of the vast availability of birds – especially carcasses – to those groups during winter months, and due to the greater logistical challenges and costs associated with capturing birds at sea. Contributors who had access to Western and Clark's Grebe carcasses provided toe lobes, flight feathers, muscle or liver, and those with access to live birds provided blood samples on FTA cards and body feathers. Some samples were obtained by working directly with collaborators (see Chapter 3) to necropsy carcasses from oil spills and other marine events.

Breeding samples were predominantly obtained through catch and release of birds at select inland breeding colonies, due to a paucity of opportunities to get such samples from any collaborators. Samples were collected from two of the largest California colonies, Lake Almanor and Eagle Lake; Upper Klamath Lake, Oregon; Lake Cachuma, Santa Barbara County, California; and Mendota Waterfowl Management Area, California (Appendix 1). The nearly complete breeding failure at Clear Lake (Robison *et al.* 2009; D. Anderson *pers. comm.*) eliminated opportunities for sampling at this important California colony.

Birds were captured on the open water at night using a small motorboat (typically a 14' rigid-hull inflatable Zodiac), a 200,000 candlepower spotlight, and a salmon-landing dip net. Independently swimming but not full-grown juveniles were typically targeted as they were more efficient to capture than adults. Efforts were carefully timed during the breeding season to minimize impact on breeding colonies and individuals.

Blood was collected from the tarsus using a 25g 5/8" hub needle, with 2-3 drops of blood collected per bird and placed on a buffered FTA card. Additionally, several body feathers were plucked and preserved for potential use in future stable isotope analyses and as a backup genetic sample; flight feathers cannot be safely collected from live grebes. Adult birds were given a federal metal band while juveniles still too small for these bands were released unbanded. Birds were released near the site of capture and when possible near their parents. Appropriate U.S., California, and Oregon permits for sampling and importing samples from Canada were first obtained.

Selection of Samples and Populations for Preliminary Population Structure Analysis

Six microsatellite loci were used in our analysis of population structure. Selection was based on the number and variability in alleles found when screening Western Grebes across the panel of 16 individuals (Chapter 1) and the observed patterns of allelic occurrence when this panel was plotted geographically.

Five sampling localities were analyzed to determine whether patterns of latitudinal genetic distribution within breeding and wintering range could be detected (Fig. 1 and App. 2). The breeding localities included two disparate colonies: Eagle Lake, in northeastern California; and Lake Wabuman, Alberta, Canada. These sites are 950 miles apart, are in different physiogeographic regions, and are separated by the Rocky Mountains. The wintering localities also included three disparate areas: coastal Washington state; northern coastal California; and southern coastal California. Eighteen

samples from the breeding colonies and 10 samples for the wintering areas were analyzed.

Additionally, samples from 16 Clark's Grebes from various wintering and breeding sites in California (see App. 2 and Chapter 1) were analyzed to evaluate genetic differentiation between *Aechmophorus* species.

Extraction, PCR, and Genetic Analyzer Methods

Whole genomic DNA was extracted from muscle and liver with a phenol chloroform iso-amyl alcohol (PCI) extraction process (following Taberlet and Bouvet 1991); from toes using a Qiagen Tissue Extraction Kit; from blood with Whatman Purification Reagent® and TE (slightly modified from Gutierrez-Corchero *et al.* 2002); and from flight feathers using a modified Qiagen Extraction procedure (J. Hull, *pers. comm.*), which involved the additional steps of initially placing a scored calamus in 20μL proteinase K for an overnight digestions at 57ºC and adding an additional 20μL for a second overnight digestion, and then following the remaining Qiagen extraction protocols to complete the extraction.

Amplification by polymerase chain reaction (PCR) was carried out on an MJ Research PTC-100 or PTC-200 thermocycler. We used one of two PCR types: either one using fluorescent dye-labeled locus-specific forward primers (6FAM, NED, PET and VIC; Applied Biosystems); or one using an M13-tail, with a fluorescent dye in a third non-specific forward 18-bp M13 primer (TGTAAAACGACGGCCAGT, FAM, Applied Biosystems) and a complementary "tail" to the M13 primer at the 5' end of the forward

locus-specific primer (Schuelke 2000). The PCR profile for the first approach was 4 min at 94°C; 35 cycles of 40 s at 94°C, 40 s at the specific annealing temperature for the microsatellite (see Table 1), and 30 s at 72°C; and a final 10 min at 72°C. The M13 PCR profile was 5 min at 94°C; 30 cycles of 30 s at 94°C, 40 s at the specific annealing temperature for the microsatellite (see Table 1), and 40 s at 72°C; 10 cycles of 30 s at 94°C, 40 s at 53°C, and 40 s at 72°C; and a final 10 min at 72°C. The PCR reactions (10μL) contained 5-100ng of extracted DNA template, 2.0-4.0mM $MgCl_2$ (see Table 1), 1X Mg-free buffer (Promega), 0.1 mM of each dNTP, 0.6μM of each primer, 0.25U *Taq* polymerase or *go-Taq* (Promega), and water. The M13 PCR reactions varied from above by containing only 0.15 μM of the locus-specific forward primer and the addition of 0.6μM of the non-specific M13 FAM forward primer.

Electrophoresis was done in an ABI 3100 genetic analyzer, generally using 2μL PCR product, 0.25μL of -500Liz size standard, and 8μL formamide per sample. Allele size was visualized using GeneMapper® (Version 3.7). For some samples, product from two loci with different fluorescently-labeled dye color and/or allele size range were multiplexed using 2μL PCR product from each locus. To reduce error rates, allele scores were all confirmed in a second visual scoring round using GeneMapper and data were proofed.

Population Assessment

Genetic structure among the five Western Grebe sampling localities was assessed using the six most variable loci of the 11 developed (Table 1 and Chapter 1). Analyses of

species differentiation between Western and Clark's Grebes were carried out in two ways: between 16 of each species across the panel (Chapter 1) across all 11 loci (App. 3); and between the 16 Clark's Grebes from the panel and 71 Western Grebes from the panel and the genetic structure study combined, across the six most variable loci.

To assess genetic structure, Weir and Cockerham's (1984) θ estimator of F_{st} was calculated using Arlequin V 3.01 (Excoffier *et al.* 2006) for all pairwise comparisons among sites and estimated overall F_{st} among all sites. F_{st} is the total variance in genotype frequencies among localities (Western Grebe) and between species. Significance of F_{st} was evaluated as described by Excoffier *et al.* (1992) using a non-parametric permutation approach and 1,000 permutations.

Genetic diversity was also evaluated for each locality and species in several ways: 1) as the number of alleles per locus (A); 2) as observed (H_o) and expected heterozygosity (He); and 3) as the number of private alleles per site (P_a), using GenAIEx v. 6.0 (Peakall and Smouse 2006). Population assignment tests were performed using the semi-Bayesian method, which assumes an equal prior probability density of the allelic frequencies for each locus in each population (Rannala & Mountain 1997) using the "leave one out" procedure in, with GenAIEx v. 6.0 (Peakall and Smouse 2006). Additionally Hardy–Weinberg equilibrium and linkage disequilibrium were tested for using Arlequin V 3.01 (Excoffier *et al.* 2006) with the Monte Carlo method (1000 batches and 10,000 iterations per batch).

Assessment of Grebe Movements from Banding Data

The BBL provided comprehensive banding and encounter data for all Western and Clark's Grebes reported from 1934 to 2008. Movements and localities were determined from GPS coordinates provided with Google Earth (Version 4.3.7204.0836 beta). This study primarily focused on assessing new encounters since Eichhorst (1992), which summarized banding and encounter data through 1989; the database contained 120 encounters, including 62 new ones. Because Western and Clark's Grebes were not split until 1985, individuals in the database as "Western Grebe" prior to the split may have included some Clark's Grebes, so were reclassified as "WCGR" (Western/Clark's Grebe).

Encounter data were classified into one of three types: 1) "between-season" recoveries, in which the banding and encounter records for an individual were from different seasons and locations (e.g., breeding vs. wintering, breeding vs. migration, or wintering vs. migration locations) indicating migratory connectivity; 2) "between-year" recoveries, in which banding and encounter records for an individual were from the same season but different year, indicating site fidelity; and 3) "within-season" recoveries, in which banding and encounter records for an individual were for the same season and year and revealed within-season movements. The classification process considered both date and location, as grebes sometimes breed outside of the traditional breeding season, and non- or late-breeders may occur in coastal areas in summer. Recoveries of birds banded following oiling and subsequent rehabilitation were also assessed separately.

We assessed winter distribution from encounters for the only breeding region (Manitoba, Canada) for which there was a sample size of birds banded; these were

predominantly the same recoveries reported in Eichhorst (1992). A χ^2 test was done to determine if the number of grebes recovered in the five wintering regions (coastal Washington/British Columbia, coastal Oregon, northern coastal California, central coastal California, and south coastal California) fit the expected proportion (0.2 for each) across each region. To then determine if the proportion of grebes recovered in the region with the most recoveries differed from expected, a simple proportions test was conducted.

RESULTS

Preliminary Assessment of Population Structure

Equilibrium Tests

A few observations of linkage disequilibrium occurred among populations and loci, but showed little consistency of pattern. Among the five Western Grebe populations (6 loci), there were three populations each with a single pair of loci in linkage disequilibrium ($P < 0.05$; 4% of all locus/population combinations); no given pair of loci was in disequilibrium across more than one population. The other two populations had no linkage disequilibrium. Examining this for all 11 loci for each of the species-specific panels of 16 individuals in each, where each species was treated as a single "population", Western Grebe showed four instances of linkage disequilibrium ($P < 0.05$; 8% of all locus combinations) and Clark's Grebe one instance ($P = 0.04$; 2% of all locus combinations). One consistent pattern of linkage disequilibrium occurred, between the loci B102 and B113; only one of these loci (B113) was included in the population

structure analyses, and therefore assumptions of linkage disequilibrium were not violated in that study.

Tests of Hardy-Weinberg equilibrium (HWE) revealed 1-2 loci/population out of HWE ($P < 0.05$), but the loci were not consistent between different populations. Examining this among the five Western Grebe localities, two had no loci out of HWE, and three populations had one locus each out of HWE (B8 in one, E202 in two). Examining this for all 11 loci for each of the species-specific panels of 16 individuals, where each panel was treated as a single "population", Western Grebe showed two loci that were out of HWE (B112b and C5) and Clark's Grebe showed four loci out of HWE (B102, C5, E202, and G118).

Western Grebe Populations

Table 1 presents the characteristics of the six loci selected for preliminary population structure analysis (B8, B113, E202, G206, G209, and G215), number of samples for each, allelic diversity and allele size, and optimized loci-specific PCR conditions. When compared to the original panel screened of 16 Western Grebes, all previously identified alleles were found within our five populations plus four new alleles (one in B8, one in G206, and two in G209). Four private alleles were found (Table 2), each occurring in only one of the five populations examined and in very low frequency (each a single allele from a single heterozygote individual).

Heterozygosity results for each population are presented in Table 3. When examining overall heterozygosities for all populations and loci combined, expected and observed were nearly identical, with $H_O=0.647$ (SE 0.032) and $H_E=0.670$ (SE 0.023).

Very low pairwise F_{st} values (Table 4) were found among populations, and overall F_{st} was not significantly different from zero ($F_{st} = 0.003$, $P = 0.32$).

Clark's vs. Western Grebes

Observed heterozygosity (H_o), expected heterozygosity H_E), and number of alleles per locus for each species when comparing the panels of 16 to one another can be found in Table 1; and see above (*Western Grebe Population Structure*) for these values in Western Grebe for the 65 birds included in the greater population structure study. One private Western Grebe allele and five private Clark's Grebe alleles were found when examining the panel of 16 individuals of each species (Chapter 1). However, all the Clark's Grebe private alleles for loci later included in the population structure analysis (5/6 private alleles) were found in Western Grebe once the sample size of the latter was increased from 16 to 71 (Table 5); and five new Western Grebe private alleles were found that were not in the panel of 16 Clark's Grebe.

Pairwise F_{st} values between Clark's Grebe and Western Grebe was 0.012 ($P = 0.11$) when comparing the panels of 16 across all 11 loci; and nearly identical at 0.011 and approaching significant ($P = 0.06$) when comparing the full compliment of 71 Western Grebe samples size to 16 Clark's Grebe across six loci. When assessing population assignment of the 16 birds of each species from the panel across all 11 loci, there was a 72% success rate of population assignment back to the correct species (see Figure 3); this included a 75% success rate for Clark's Grebe and a 69% success rate for Western Grebe. This overall rate was nearly identical when comparing the 16 Clark's

Grebe to 71 Western Grebe across just 6 loci (69%); however, the Clark's Grebe success rate decreases to 50% and the Western Grebe success rate increased to 73%.

Assessment of Grebe Movements from Banding Data

A total of 3,052 Western and Clark's Grebes were reported banded from 1934 through 2008. These include 1,394 Western/Clark's Grebes (WCGR; individuals in database as "Western Grebes" during 1934 to 1985, when the two species were lumped as such); 1,410 Western Grebe (1986-2008); and 248 Clark's Grebe (1986-2008). The majority of WCGR are likely Western Grebes as Clark's Grebes are less abundant overall and rare in the Canadian prairie provinces (Storer and Nuechterlein 1992) where many of those bandings occurred. An additional consideration is that the BBL lacks a way to classify intergrades/hybrid grebes, and banders must instead place such individuals into the species category they most resemble.

Of the above total, 1,877 (62%) were banded following rehabilitation and prior to release (BBL bird status code 5 or 7), with 958 of these (31% of total) rehabilitated as a result of oiling (BBL extra info code 40). In fact, all but nine of the Clark's Grebe were banded as a result of rehabilitation, reflecting the trend in recent years and the paucity of modern field studies of this species that includes banding.

During the same time period, a total of 120 grebe encounters were reported to the BBL (3.9% of those banded), comparable to the previously reported encounter rate for a reduced time period and sample size (3.8%; Eichhorst 1992)). Sixty-two encounters were new since Eichhorst (1992) published those results: 11 Clark's Grebes and 51 (post-

species-split) Western Grebes. Fifty-three of the recent encounters were banded by the International Bird Rescue Research Center (IBRRC) in California prior to release after rehabilitation – including from oil spills – and were predominantly coastal non-breeding birds when initially debilitated.

Encounters included 46 between-season recoveries, 16 between-year (same season) recoveries, and 58 within-season (same year) recoveries (see App. 3 Tables 1 and 2). A caveat to these classifications is that it was not always possible to accurately differentiate between breeding and migration (e.g., if birds were banded or encountered as migrants but in their breeding range) or between winter and migration (e.g., if birds occurred in coastal zones), as grebes can breed at nontraditional times of year and be in coastal, nonbreeding sites – but not necessarily migrating – in the middle of summer. Another caveat is that for rehabilitated birds, banding location represents release location, not capture location (Michelle Bellizzi, IBRRC, pers. comm.); some moderate within-season movements or between-year recoveries for rehabilitated birds may be deceiving as birds may be returning to their previous wintering locale if different from their release site.

Between Season Encounters (Migratory Connectivity)

Appendix 4 (Table 1) and Figure 2 summarize between season recoveries of Western Grebes, and Appendix 4 (Table 2) provides details of each record. These include 45 recoveries of which only five are new since Eichhorst (1992) summarized the previously available data. As noted by Eichhorst (1992), the majority of between-season

recoveries came from Manitoba breeding birds. Manitoba, especially Delta Marsh, is among the few places where large numbers of grebes were historically banded (in the1970s). Assessment of these data reveal that Manitoba breeders migrated to wintering sites throughout much of the winter range of the species, although they were not recovered in equal proportions throughout their range (χ^2 = 34, df = 4, P < 0.0001). More of the Manitoba birds (60%) were recovered in the coastal Washington / British Columbia area (χ^2 = 27.56, df=1, P < 0.0001) than the other regions, followed by the San Francisco Bay area (23%).

Other interesting connectivity records include a bird wintering in south coastal California and occurring during breeding season in North Dakota (a new record since Eichhorst 1992), one wintering in central coastal California and occurring during breeding season in Saskatchewan, and one wintering off Baja California and occurring during breeding season in Durango, Mexico. Most new records since Eichhorst (1992) provide more insight into potential migratory routes than connectivity between wintering and breeding sites. These include: two birds banded in winter in southern coastal California, one recovered the subsequent fall in the Mojave Desert bioregion and another recovered the same spring/early summer inland in Riverside Co., California; a bird banded during the breeding season in Minnesota and recovered three years later in spring in Colorado; and a bird banded during the breeding season in Manitoba and recovered fall of the following calendar year in southcentral New Mexico.

Between Year Encounters (Site Fidelity)

The BBL data include 16 records of "between-year" encounters that serve as indices of fidelity to either wintering or breeding sites. Appendix 4 (Table 1) summarizes these results, and Appendix 4 (Table 2) provides details of each record. Providing insight into breeding philopatry, this dataset includes: four Manitoba breeders, two recovered elsewhere in the province (one fairly close to its originally banding site), one recovered in Alberta, and one recovered in Nevada; three birds banded at Lake Osakis, Minnesota in the breeding season, two recovered elsewhere in the state and one at Lake Osakis; one Saskatchewan bird recovered in inland British Columbia; and a bird from Utah recovered the following season at a different Utah lake. It is unknown in some of these recoveries (e.g., the Nevada and British Columbia recoveries) if the bird was still migrating.

Seven records provide insight into winter site fidelity. Of four encounters for birds banded and released in the San Francisco Bay area, one bird banded during the San Francisco Bay oil spill of 1971 was recovered two winters later in coastal Washington, and three were recovered in the San Francisco Bay area (one, three and five winters later). Three birds banded in winter in southern coastal California were recovered in the same area: two the following winter and one bird two winters later.

Within Season Encounters (General Movement Patterns)

All 58 within-season encounters were from the nonbreeding season, and all but two were birds banded following rehabilitation. Appendix 4 (Table 1) summarizes these data, and Appendix 4 (Table 2) provides details on each record. All definitively identified

Clark's Grebe encounters (n=11) fell into this movement category. While no breeding

season within-season recoveries were reported, this is likely because such recoveries are

generally made by the researchers who originally banded the bird (as part of a continuing

project) and are not reported to the BBL.

Nineteen birds were encountered in a different region than where they were

released and 39 were encountered in the same region. Encounters were two days to six

months following release. The more substantial of these movements include: one bird

released in San Francisco Bay and recovered a month later off the north coast (Humboldt

Co.); a bird released in San Francisco Bay area and recovered two months later at Clear

Lake; seven birds released in the San Francisco Bay area and recovered in the Monterey

Bay area two weeks to six months later; one bird released in San Francisco Bay and

recovered off Morro Bay (southcentral coastal California) two weeks later; four birds

released in the San Francisco Bay area and recovered off southern coastal California 1

week to 2 months later; and one bird released off southern California and recovered in the

San Francisco Bay area 4 months later. The majority of these movements were not

consistent with expected temporal migratory patterns that birds would be more likely to

head north in spring, south in fall, and remain relatively local midwinter (see Appendix 4

Table 2).

Recoveries of Rehabilitated Oiled Grebes

Although these are included in some of the results above, and can be found in

Appendix 4 (see footnote in Table 2 which denotes birds banded as a result of oil spill

rehabilitation), it is worth reporting the recoveries of birds banded after rehabilitation

following being oiled. One-third of all recoveries were of grebes banded after receiving

rehabilitation following an oil spill and released coastally. Of these, there were four

between-season recoveries: one released in the San Francisco Bay area was found dead

the following summer in Saskatchewan; and of three released off southern California, one

was found dead the following summer in North Dakota and two were found dead

presumably during spring migration in inland southern California that same year. There

were five between-year encounters: three were birds released in the San Francisco Bay

area, with one recovered dead one winter later in the same area, one recovered alive and

injured five years later in the same area, one recovered dead two winters later off coastal

Washington; and two were birds released in southern coastal California and recovered in

the same area, one the following winter (dead) and one two winters later (injured).

Additionally, 31 oiled and rehabilitated birds were recovered within the same

season. Five were recovered alive but subsequently died or were euthanized; one was

observed alive (with the band read through a scope); one had an unknown fate; and the

rest were found dead. They were recovered 1 day to 6 months from their release date,

some near their release location and others far away (e.g., southern California to San

Francisco Bay).

DISCUSSION AND FUTURE NEEDS

Population Structure

The goal of this preliminary study was to determine if preliminary patterns of differentiation could be detected between the breeding sites as well as among wintering sites. This would suggest population structure and migratory connectivity in the Western Grebe, and that the markers we developed were appropriate for evaluating these at a lrger scale in the future.

No differentiation was detected among the localities examined. These included two breeding colonies and three disparate wintering areas; within each group, the localities were latitudinally disparate from one another. The lack of latitudinal differentiation detected does not discount the potential utility of this method for Western Grebes at a larger scale or with additional markers, so it is premature to conclude that panmixia occurs in the Western Grebe. However, the results confirm that there is not strong genetic differentiation among wintering Western Grebes localities, nor between breeding sites in Alberta and northern California. Even given the low numbers of loci and sample sizes used here, differentiation would still be detected if gene flow was low. Microsatellite studies can even detect differentiation for populations with relatively high levels of gene flow (Selkoe and Toonen 2006) provided enough variable loci are used. Eichhorst (1994) also failed to find differentiation among Western Grebe populations using mitochondrial loci. Differentiation has been shown to typically be lower in birds than other vertebrate taxa due to their increased vagility (Barrowclough 1983, Avise 1994).

A few study design components limit the strength of the conclusion regarding genetic structure in Western Grebes. Resources limited the number of loci, allelic diversity of these loci, and sample size of individuals per population. We included 6 loci in the population structure analyses, but often more than 10 loci are recommended in microsatellite studies of population structure; the preferred number is inversely proportional to the amount of genetic differentiation between populations, or proportional to the amount of gene flow (Selkoe and Toonen 2006). The loci developed for *Aechmophorus* grebes had only low to moderate levels of polymorphism, although the most polymorphic of the 11 loci developed were included. Kalinowsky (2002) found that the use of highly polymorphic loci (e.g., > 8 alleles) provides a better measure of differentiation than using less polymorphic loci, but found that using a small number of highly polymorphic loci was comparable to using a high number of low polymorphic loci. To detect genetic differentiation among populations, the key factor is including enough alleles (whether across few or many loci) in a study. Finally, we included 10-18 samples from each locality; 20-30 are typically recommended (Selkoe and Toonen 2006).

Of the above approaches to increasing the capability of microsatellite studies to detect genetic differentiation, Selkoe and Toonen (2006) recommend adding loci as the most appropriate option. We recommend the inclusion of more than six loci for future work to appropriately validate this approach for Western Grebes. Although we have five additional loci that we developed (Chapter 1), their allelic diversity was fairly low (4.2 alleles/loci across 16 individuals) and may not improve our ability to pick up the differentiation signal. We recommend including the most diverse of those and also developing additional, more polymorphic, markers. Although dinucleotide repeat regions

were avoided in this study because of their difficulty in scoring, it may be useful to
develop some for their increased variability. Other recommendations for future research
are increasing sample size (over 40 samples are banked for each of the five regions from
this study), and including an even more geographically distant breeding population (e.g.,
North Dakota or Minnesota). Additionally, inclusion of the Western Grebe subspecies
that is resident to the Mexican Plateau (Storer and Nuechterlein 1992) would be useful as
if differentiation is not detectable between that subspecies and the northerly migratory
subspecies, we are unlikely to detect any differentiation among populations of the
migratory subspecies.

    The tendency towards natal philopatry in many migratory birds (Greenwood and
Harvey 1982), and the relative breeding isolation of Western Grebe colonies relegated to
large and isolated lake systems throughout the West, contributed to our expectation that
differentiation would be found among the latitudinal regions examined. However, the
interannual variation in environmental conditions, and the sensitivity of Western Grebes
towards variable water levels and timing of water level changes at traditional breeding
sites, prevents breeding at certain sites in some years (Ivey 2004, Storer and Nuechterlein
1992). When conditions are not appropriate for breeding, grebes presumably move to
other more appropriate breeding sites, or perhaps forego breeding altogether. This
response suggests grebes are somewhat plastic in their choice of breeding locale. These
same fluctuations in environmental conditions causes their breeding phenology to vary
widely between years at a given site; birds that arrive to conditions not yet appropriate for
breeding may postpone breeding for months until conditions improve (*pers. obs.*). Such
plasticity has been shown for other avian species that experience similarly fluctuating

conditions (Greenwood and Harvey 1982). This suggests that both natal and breeding philopatry cannot be inflexibly high, and that we would therefore not expect to find high genetic differentiation among populations. In an evolutionary sense, there is likely a correlation between such plasticity and weak levels of migratory connectivity (Webster *et al.* 2002).

Genetic Differences between Western and Clark's Grebes

Nonsignificant low levels of differentiation were detected between Western and Clark's Grebes using microsatellite data, and the species performed poorly in "population" assignment tests. While not traditional to conduct population assignment tests for groups (in this case, species) that do not show differentiation, near-significant differences in F_{ST} and the potential utility of such an approach if successful compelled us to do so. Many grebe carcasses are not identifiable to species in studies where this information would inform critical population assessments (e.g., during documentation of oil spill impacts or beached bird surveys, when heads have been removed by scavenging or carcasses are excessively oiled or degraded). Our population assignment results varied from only 50% to 75% correct assignment to species; if we could identify the loci reliable for identification, even some error rate would still provide a useful index of species ratios in such events; but this was not found. Molecular approaches have been used successfully to discriminate between other closely related sister taxa, including among some avian species (Zink and Johnson 1984, Avise 1994).

There have been relatively few genetic studies comparing Western to Clark's Grebes, especially when considering the question regarding origin of speciation for these

very closely related and recently diverged sister taxa (Eichhorst 1994), the unusually sympatric nature of their breeding range, and discussion among some ornithologists as to whether or not Western and Clark's Grebes should be considered separate species (personal obs.). They are included in one ongoing study examining the phylogenetic relationship between all grebes (Van Tuinen and Ogawa, unpublished data). The DNA-DNA hybridization studies conducted (for reviews see Eichhorst 1994 and Storer and Nuechterlein 1992) reached inconsistent conclusions on this topic, although Ahlquist *et al.* (1987) found the genetic distance between Western and Clark's Grebes to be comparable to other very closely related species. Eichhorst (1994) examined divergence between Western and Clark's Grebes and found no detectable mtDNA differences between the species, as well as polyphyletic patterns that likely reflect very recent separation. As Eichhorst (1994) points out, these results do not reject the consideration of these two taxa as separate species, because they only involve mitochondrial DNA, and only 2.1% of the mitochondrial DNA at that. Instead, the lack of observed genetic divergence observed between the species was interpreted as an indication of the high degree of historical connectedness between these two species or their shared ancestor, and of their likely recent divergence (possibly as recent as 6,000 years). It is not uncommon for distances between closely related subspecies to be similar to distances between subpopulations within a species (Avise 1994).

Despite the similarities found for Western and Clark's Grebes, differentiation may still be detectable for populations within species. In fact, species differences are not necessarily always greater than population differences within the species in question (Avise 1994), especially when examining neutral markers. Depending on the speciation

mechanism, two Western Grebe populations could have a longer history of isolation than Clark's Grebes and one of the populations of Western Grebes.

Assessing Movements with Banding and Encounter Data

The banding and encounter data remain the primary insight we have into movements, philopatry, and migratory connectivity in Western and Clark's Grebes. There are caveats to interpreting these data: they are biased towards the limited geographic locales where banding has occurred and towards areas where encounters are probable; many of the records are from the period when Clark's and Western Grebes were not differentiated; and, since rehabilitated birds are not necessarily released at capture locations, some movements may be an artifact of birds attempting to return to their original wintering areas.

As found by Eichhorst (1992), our analysis of the banding and encounter data for Manitoba breeders reveals the highest migratory connectivity with the Washington coast / British Columbia / Puget Sound region. The second most common wintering region for the Manitoba birds, based on numbers of recoveries, is the San Francisco Bay region, with some records revealing movements between Manitoba and the Oregon and northern California coasts as well. Eichhorst (1994) suggested that connectivity may be equally high to other regions as to Washington / Puget Sound and the San Francisco Bay area, but that the probability of encounters are lower in most regions as they are less populated by humans. However, based on the amount of inaccessible shoreline in the Puget Sound region, and on the human-populated nature of some other wintering areas for which little connectivity to Manitoba was observed (e.g., southern coastal California), the recovery

rate for the Washington / Puget Sound region likely reflects higher connectivity to that area. However, with recent and significant declines of wintering grebes off the coasts of Washington and British Columbia (Sauer *et al.* 1996, Badzinski *et al.* 2006, WDFW 2008), and given that most of these Manitoba birds were banded in the 1970s, it is unknown if such high connectivity still occurs there today.

While these data indicate birds from Manitoba spread out over such a large wintering area (a pattern called *synhiemy;* Salomonsen 1955), it is premature to conclude that migratory connectivity is low in grebes. Manitoba does show moderate levels of connectivity to the Washington / Puget Sound region even with the occurrence of synhiemy. These data do allow us to reject strong connectivity (or *allohiemy*; Salomonsen 1955) in the Western Grebe, which stipulates that all individuals from a breeding area migrate to a given wintering region (Webster *et al.* 2002, Marra *et al.* 2006, Boulet and Norris 2006). It is probable that with a large enough sample size and improved study design, connectivity may be found to be moderate for other regions as well, which would have important conservation implications.

Despite the somewhat anecdotal nature of the between-season banding and recovery data, additional insights into connectivity and migratory stopover sites can be found. The Mexico encounter (from 1940) is particularly noteworthy since Western and Clark's Grebes breeding in the Mexican Plateau are considered predominantly resident (Storer and Nuechterlein 1992), although they have been inadequately studied.

Both winter site fidelity (e.g., Kricher and Davis, Jr. 1986, Wilson *et al.* 1991; Sherry and Holmes 1996; Robertson and Cooke 1999) and breeding site fidelity (Greenwood and Harvey 1982) are commonly reported in other migratory birds. The

between-year banding and encounter data for Western Grebes reveal that these birds are somewhat plastic in their site fidelity to both breeding and wintering areas. We have records of birds wintering or breeding at different sites in different years (e.g., an individual banded in the San Francisco Bay area one winter and recovered off coastal Washington two winters later; and an individual banded in Manitoba and recovered in a subsequent breeding season in Alberta). Although these individuals display a lack of site fidelity, such data may bias our understanding as the dataset excludes subsequent encounters of bird in the same area where it was originally banded if the encounter was made by the same researchers. Some observations of breeding site fidelity of grebes in Manitoba have been made (Storer and Nuechterlein 1992, Eichhorst 1994). We cannot determine if the encounter records of non-philopatric birds represent a typical pattern or if they are relatively unusual. Successfully attaching radio or satellite tags to Western Grebes has proven challenging, but once those or similar approaches become viable, that may be yet another appropriate way to assess such movement questions, particularly if genetic approaches prove too challenging. While both approaches are expensive, genetic analysis is often more cost effective at a larger scale than attaching transmitters. If not done at the appropriate scale for a species such as the Western Grebe that lacks strong connectivity, transmitters are likely to also produce fairly anecdotal data on bird movements.

These encounter data reveal that some birds move significant distances within a given winter season, even in short periods of time, such as between the San Francisco Bay area and southern coastal California. The majority of these movements do not fit expected temporal migratory patterns of directional movement north in late winter or

spring and south in fall, and many birds moved long distances midwinter. It is probable

that many of these recoveries indicate true within-season movements. Bent (1919)

suggested they move around in the nearshore environment in response to location of fish

resources, a pattern not found for birds in habitats or regions with consistently abundant

resources (e.g., Warnock and Takekawa 1996, Esler *et al.* 2000). We would therefore

expect some amount of plasticity here. A future endeavor will be to work with

International Bird Rescue Research Center (IBRRC), the organization banding most of

these grebes in winter, to analyze the original capture locations of the released birds for

which we have banding and recovery location data. This will allow us to determine if any

of these observed winter movements were the result of birds attempting to return to their

original wintering site. Although this may explain some of the more modest movements

(e.g., San Francisco to Monterey Bay), it is not likely to explain the larger-scale

movements.

There are inherent biases to using band recoveries to assess movement (or

survival, in the case of oil spills or rehabilitation) in grebes. These recoveries come

almost entirely from carcasses found on beaches, and are more likely to be encountered

in more human-populated regions. Nonetheless, the insights provided are among the only

available and do help to fill in the picture of the mysterious and interesting movements of

grebes.

Conclusions

Our data support a hypothesis of relatively high levels of gene flow among the

populations examined. Further study using a higher number of variable loci might reveal

genetic differentiation not detected herein, albeit possibly more broadly than originally intended. Alternatively, genetic differentiation might occur at a scale other than that assessed here; similar analyses using samples from more disparate geographic locales (e.g., North Dakota in the eastern most portion of their range) might allow the detection of genetic differentiation. The inclusion of additional loci might also allow us to genetically differentiate Western and Clark's Grebes, allowing species assignment for unidentifiable carcasses.

Since even populations exhibiting low levels of gene flow can be difficult to differentiate without enough variable molecular markers, it can be quite challenging to differentiate populations with moderate to high levels of gene flow. This is true even when they do form relatively distinct units with only a few immigrants per generation. Additional confounding factors can decrease our ability to detect differentiation. For instance, if natal site fidelity in grebes were male-biased, as it is in most avian systems (Greenwood and Harvey 1982), the genetic differentiation signal may be muted (Greenwood 1980, Avise 1994). Finally, if breeding site fidelity is high but natal philopatry low, this could result in low genetic differentiation among populations (Avise 1994). It is possible that we may not be capable of determining genetic structure even after increasing our geographic scale or number of loci included, but that other approaches – such as stable isotopes and satellite tagging – may reveal patterns of structure and connectivity that are still important for conservation.

Analysis of banding and encounter data suggest moderate levels of migratory connectivity in Manitoba breeders to the Puget Sound / coastal Washington areas, but with some individuals migrating to wintering areas throughout the entirety of their

wintering range. This has yet to be assessed for other important breeding areas. The between-year and within-season banding and encounter data for Western Grebes reveal that these birds are somewhat plastic in their site fidelity to both breeding and wintering areas. However, the moderate migratory connectivity observed for Manitoba supports further investigation into this topic.

Our results suggest that gene flow may be high enough and migratory connectivity weak or plastic enough that it may be difficult to determine the provenance of grebes killed in winter oil spills. In fact, the provenance of a given wintering population may actually be to multiple breeding regions. We hoped such a study could inform agencies as to which breeding regions they should direct resources toward following a significant oil spill. Despite the lack of genetic differentiation observed in this study, conservation biologists and natural resource managers may still find it appropriate to direct resources towards the breeding populations most likely to have been impacted by a given oil spill as determined by other means. Alternatively, it may be most appropriate to direct resources towards the breeding colonies with the greatest management needs across multiple regions.

ACKNOWLEDGEMENTS

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TABLES AND FIGURES

Figure 1. Selected sites for preliminary analysis of population structure in Western Grebes (blue/straight arrowhead=wintering, purple/curved arrowhead=breeding), overlaid on Breeding Bird Survey map for Western/Clark's Grebes (Sauer *et al.* 2006; the most northerly shaded area are beyond scope of BBS).

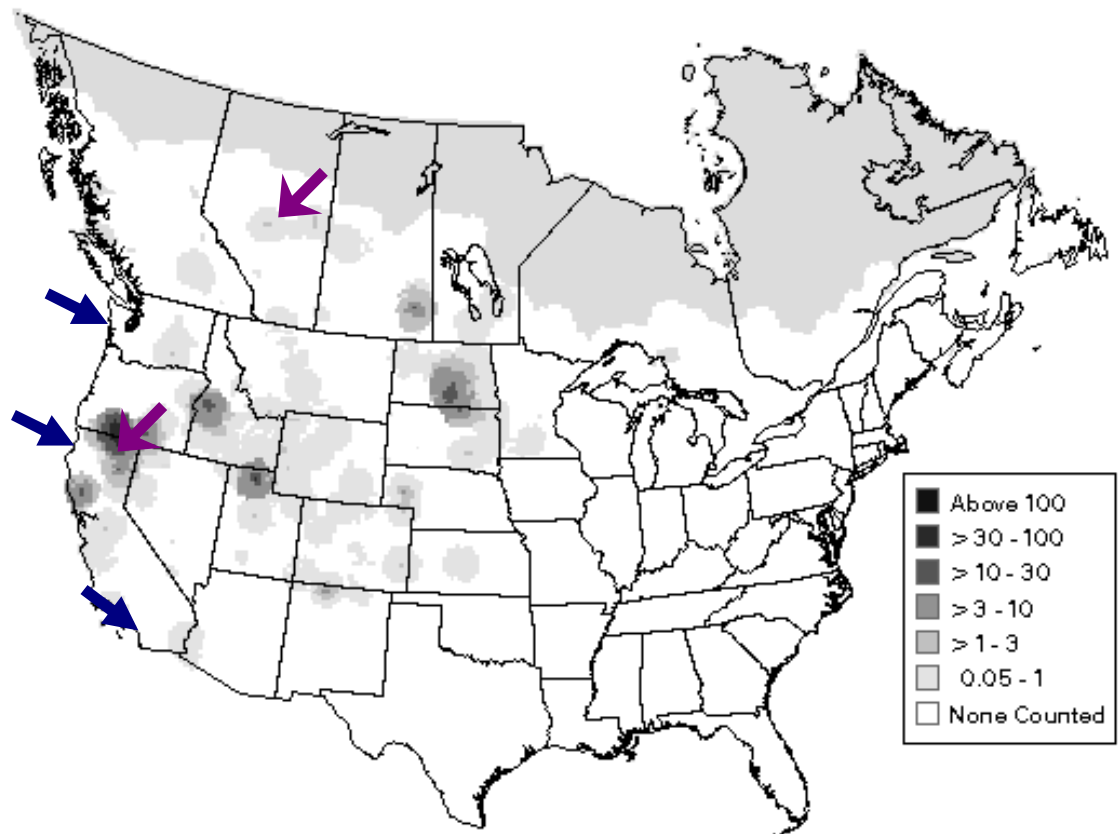**Figure 2.** Between-season recoveries of banded birds from data provided by the Bird

Banding Lab, 1934-2008. Straight lines should not be interpreted as migratory routes.

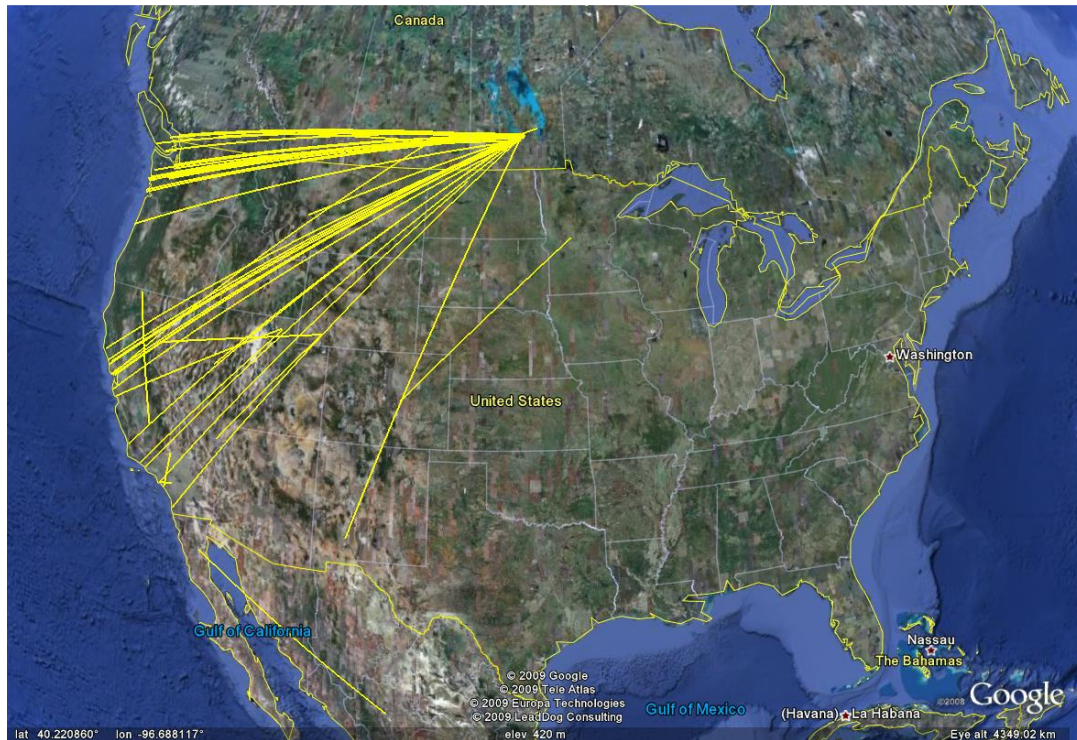Most birds were initially banded in Manitoba; see Appendix 3 for details.

Table 1. Characteristics of microsatellite loci selected for preliminary Western Grebe population structure assessment across five

localities and for Western/Clark's Grebe genetic differentiation study.

Locus Name	Repeat Motif	Primer sequence (5'-3')	# grebes screened	T_a (°C)	MgCl$_2$ conc. (mM)	Size range (bp)	A. occidentalis (n=16)			A. clarkii (n=16)		
							No. of alleles	H_E	H_O	No. of alleles	H_E	H_O
Loci selected for population structure and species differentiation analysis:												
B8	CATC	Fwd: AAGGAGGGCATTATGTTATGC Rev: GCCAGTAATCACCACAAATTG	64	55	2	277-289	4	0.520	0.375	3	0.607	0.688
B113	CATC	Fwd: AAGCACACAATTCAGGAAGTAC Rev: GATGCCTCCATTTAGGTAAAAG	65	55	2	211-231	5	0.754	0.813	6	0.785	0.875
E202	GAAG	Fwd: TCCAGACCGTTTTGCTTCTT Rev: CCTCATGGTTCAATGCCTTT	62	55	2.5	217-253	8	0.822	0.688	10	0.857	0.625
G206	TAGA	Fwd: AAGGCAAGCAAACAGAGC Rev: ATGGACACAATGCGTCTTAG	61	55	2	274-290	4	0.662	0.688	5	0.740	0.875
G209	TAGA	Fwd: TGCAATGCCATTTTCTGTTT Rev: ACAGCCCAGAGTTAGGCTCA	58	55	3	340-348	3[1]	0.607	0.733	3	0.541	0.500
G215	TAGA	Fwd: GACAGGACAACCCTTTTATCC Rev: TGGTGGTCTCTGACAGGTC	61	60	2.5	244-260	5[1]	0.696	0.800	5	0.693	0.750
Additional loci used in species differentiation analysis:												
B11	CATC	Fwd: CAGGCAGCAAAAGTTCTTG Rev: GGGTCTCTGTGATGTGATGA	16	55	2.5	147-155	3	0.561	0.438	3	0.619	0.500
B102	CATC	Fwd: ACCCATCCATCCATACCTG Rev: GCCCAAACCATTCTGTGAT	16	55	2.5	134-146	4	0.525	0.563	4	0.561	0.375
B112B	CATC	Fwd: CAATCCAAAGGCTGGATCTC Rev: TCGAAAACCAAAATGTGCAG	16	55	3	222-230	3	0.539	0.875	3	0.617	0.750
C5	TACA	Fwd: GTTTCCTCCAGCCAGTCAC Rev: GTTCCTTCAACAGGCACAAG	16	55	2.5	249-253	2	0.498	0.188	2	0.482	0.188
G118	TAGA	Fwd: TTCTTGTCACATTTCATTGCAC Rev: TTCAGGGTTGCTCAGTTGTG	16	55	4	303-323	4	0.545	0.563	5	0.656	0.750

Table 2. Private alleles by Western Grebe locality.

Locality	Locus	Allele	Freq
Wintering, southern coastal CA	G215	268	0.050
Breeding, Eagle Lake, CA	G215	244	0.031
Breeding, Lake Wabuman, AB	G206	294	0.031
Breeding, Lake Wabuman, AB	G209	352	0.029

Table 3. Mean sample size, number of alleles/locus, and observed (Ho) and expected (He) heterozygosity for each locality across all 6 loci. N=mean number of samples/locus, Na=mean number of alleles/locus.

Locality		N	Na	Ho	He
Wintering, coastal WA	Mean	10.000	4.333	0.550	0.631
	SE	0.000	0.615	0.067	0.059
Wintering, northern coastal CA	Mean	9.333	4.667	0.721	0.686
	SE	0.333	0.760	0.073	0.062
Wintering, southern coastal CA	Mean	9.833	4.667	0.594	0.643
	SE	0.167	0.494	0.088	0.058
Breeding, Eagle Lake, CA	Mean	15.667	5.667	0.659	0.693
	SE	0.955	0.955	0.074	0.047
Breeding, Lake Wabuman, AB	Mean	16.667	5.833	0.709	0.698
	SE	0.494	0.872	0.056	0.041

Table 4. Pairwise Fst values for 5 Western Grebe localities using 6 loci.

winWA=wintering coastal Washington; winNOCA=wintering northern coastal

California; winSOCA=wintering southern coastal California; brEALA=breeding Eagle

Lake, California; brLAWA=breeding Lake Wabuman, Alberta.

	winWA	winNOCA	winSOCA	brEALA	brLAWA
winWA	0.00000				
winNOCA	-0.00027	0.00000			
winSOCA	-0.01287	0.00753	0.00000		
brEALA	-0.01247	-0.01114	-0.00316	0.00000	
brLAWA	0.00479	-0.00579	-0.00478	-0.01395	0.00000

Table 5. Private alleles by species. For loci used in population structure analysis, n = 71

for WEGR; and for loci only used in initial screening (Ch. 1), n = 16 for WEGR.

Species	Locus	N	N in comparison spp population	Allele	Freq
WEGR	B8	71	16	273	0.014
WEGR	B8	71	16	289	0.021
WEGR	G206	71	16	294	0.007
WEGR	G209	71	16	336	0.016
WEGR	G209	71	16	352	0.008
WEGR	G215	71	16	268	0.008
CLGR	G118	16	16	323	0.031

APPENDICES

Appendix 1. Western and Clark's Grebe samples acquired. Numbers shown are for quantities of Western Grebe / Clark's Grebe / Unidentified Western or Clark's Grebe.

Region	Provider or Collaborator (Source)	# Samples	Sample Type
Breeding Sampling			
Lake Wabuman, Alberta, Canada	Bev Gringas, Environment Canada (oil spill)	42/0/0	Flight feathers
Eastern Washington	Biodiversity Research Institute (mercury sampling)	1/0/0	Blood
Eagle Lake, Northeastern CA	D. Humple / PRBO (research)	50/0/1	Blood, body feathers
Lake Almanor, Northeastern CA	D. Humple / PRBO (research)	11/2/0	Blood, body feathers
Tule Lake, Northeastern CA	John Hodge, USFWS (net entanglement)	5/0/0	Body feathers
Central Valley, CA (Mendota Waterfowl Management Area)	D. Humple /PRBO; IBRRC (rehab)	0/14/1	Blood, body feathers
Clear Lake, CA	Dan Anderson, UC Davis (research)	6/2/0	Tissue
Lake Cachuma, Santa Barbara, CA	D. Humple / PRBO (research)	3/1/0	Blood, body feathers
Winter /Migration Sampling			
Southern coastal California	OWCN/PRBO, CDFG / Moss Landing Marine Lab / D. Humple (oil spill, rehab)	380/39/8	Tissue, flight feathers
Southeastern California – Mojave	Ernie Acosta, CDFG / D. Humple (salt pond strandings)	17/2/3	Toe, flight feathers
Central coastal California	Hannah Nevins / BeachCOMBERS; Michelle Bellizzi, International Bird Rescue Research Center; Melanie Piazza, San Rafael WildCare, PRBO/OWCN (red tide event; rehab; beached bird censuses; oil spill)	131/44/130	Tissue, toe, flight feathers, blood
Northern coastal California	Jane Dolliver, COASST; CDFG/OSPR; Moss Landing Marine Lab; D. Humple (oil spills, beached bird censuses)	30/1/8	Tissue, toe
Coastal Oregon	Jane Dolliver, COASST (beached bird censuses)	63/2/5	Toe
Coastal Washington	UC Davis Wildlife Health Center; Dave Nysewander, Washington Department of Fish and Wildlife; Jane Dolliver, COASST (beached bird censuses, research)	49/0/9	Toe, tissue
Coastal British Columbia	Sean Boyd, CWS/Environment Canada (fishing line entanglement)	2	Flight feathers, toe
Total Number of Samples		**849/115/149**	**1113**

Appendix 2. Samples used in population structure analysis and original screening of markers (Chapter 1), including source location, season, sample type and provider. Feathers are flight feathers (secondaries).

DNA ID	Location	Season	Sample Type	Provider / Source
Population Structure Analysis (WEGR)				
40[1]	Coastal WA	Winter	Muscle	OWCN
41[1]	Coastal WA	Winter	Muscle	OWCN
42	Coastal WA	Winter	Muscle	OWCN
43	Coastal WA	Winter	Muscle	OWCN
44	Coastal WA	Winter	Muscle	OWCN
45	Coastal WA	Winter	Muscle	OWCN
46	Coastal WA	Winter	Muscle	OWCN
47	Coastal WA	Winter	Muscle	OWCN
48	Coastal WA	Winter	Muscle	OWCN
49	Coastal WA	Winter	Muscle	OWCN
1[1]	N. coastal CA	Winter	Liver	*Stuyvesant* Spill, CDFG/MLML/D. Humple
2[1]	N. coastal CA	Winter	Liver	*Stuyvesant* Spill, CDFG/MLML/D. Humple
3	N. coastal CA	Winter	Liver	*Stuyvesant* Spill, CDFG/MLML/D. Humple
4[1]	N. coastal CA	Winter	Liver	*Stuyvesant* Spill, CDFG/MLML/D. Humple
5	N. coastal CA	Winter	Muscle	*Stuyvesant* Spill, CDFG/MLML/D. Humple
221	N. coastal CA	Winter	Liver	*Kure* Spill, CDFG / MLML / D. Humple
222	N. coastal CA	Winter	Liver	*Kure* Spill, CDFG / MLML / D. Humple
223	N. coastal CA	Winter	Liver	*Kure* Spill, CDFG / MLML / D. Humple
224	N. coastal CA	Winter	Liver	*Kure* Spill, CDFG / MLML / D. Humple
225	N. coastal CA	Winter	Liver	*Kure* Spill, CDFG / MLML / D. Humple
6[1]	S. coastal CA	Winter	Muscle	Ventura Oil Spill Incident, PRBO / OWCN
7[1]	S. coastal CA	Winter	Muscle	Ventura Oil Spill Incident, PRBO / OWCN
8[1]	S. coastal CA	Winter	Muscle	Ventura Oil Spill Incident, PRBO / OWCN
13	S. coastal CA	Winter	Muscle	Ventura Oil Spill Incident, PRBO / OWCN
14	S. coastal CA	Winter	Muscle	Ventura Oil Spill Incident, PRBO / OWCN
15	S. coastal CA	Winter	Muscle	Ventura Oil Spill Incident, PRBO / OWCN
16	S. coastal CA	Winter	Muscle	Ventura Oil Spill Incident, PRBO / OWCN
17	S. coastal CA	Winter	Muscle	Ventura Oil Spill Incident, PRBO / OWCN
18	S. coastal CA	Winter	Muscle	Ventura Oil Spill Incident, PRBO / OWCN
19	S. coastal CA	Winter	Muscle	Ventura Oil Spill Incident, PRBO / OWCN
79[1]	Eagle Lake, CA	Breeding	Blood	D. Humple
80[1]	Eagle Lake, CA	Breeding	Blood	D. Humple
204	Eagle Lake, CA	Breeding	Blood	D. Humple
205	Eagle Lake, CA	Breeding	Blood	D. Humple
206	Eagle Lake, CA	Breeding	Blood	D. Humple
207	Eagle Lake, CA	Breeding	Blood	D. Humple
208	Eagle Lake, CA	Breeding	Blood	D. Humple
209	Eagle Lake, CA	Breeding	Blood	D. Humple
210	Eagle Lake, CA	Breeding	Blood	D. Humple
211	Eagle Lake, CA	Breeding	Blood	D. Humple

DNA ID	Location	Season	Sample Type	Provider / Source
212	Eagle Lake, CA	Breeding	Blood	D. Humple
213	Eagle Lake, CA	Breeding	Blood	D. Humple
214	Eagle Lake, CA	Breeding	Blood	D. Humple
215	Eagle Lake, CA	Breeding	Blood	D. Humple
216	Eagle Lake, CA	Breeding	Blood	D. Humple
217	Eagle Lake, CA	Breeding	Blood	D. Humple
218	Eagle Lake, CA	Breeding	Blood	D. Humple
219	Eagle Lake, CA	Breeding	Blood	D. Humple
231	Lake Wabuman, AB	Breeding	Feathers	Lake Wabuman spill, Environment Canada
232	Lake Wabuman, AB	Breeding	Feathers	Lake Wabuman spill, Environment Canada
233	Lake Wabuman, AB	Breeding	Feathers	Lake Wabuman spill, Environment Canada
234	Lake Wabuman, AB	Breeding	Feathers	Lake Wabuman spill, Environment Canada
235	Lake Wabuman, AB	Breeding	Feathers	Lake Wabuman spill, Environment Canada
236	Lake Wabuman, AB	Breeding	Feathers	Lake Wabuman spill, Environment Canada
237	Lake Wabuman, AB	Breeding	Feathers	Lake Wabuman spill, Environment Canada
238	Lake Wabuman, AB	Breeding	Feathers	Lake Wabuman spill, Environment Canada
239	Lake Wabuman, AB	Breeding	Feathers	Lake Wabuman spill, Environment Canada
240	Lake Wabuman, AB	Breeding	Feathers	Lake Wabuman spill, Environment Canada
241	Lake Wabuman, AB	Breeding	Feathers	Lake Wabuman spill, Environment Canada
242	Lake Wabuman, AB	Breeding	Feathers	Lake Wabuman spill, Environment Canada
243	Lake Wabuman, AB	Breeding	Feathers	Lake Wabuman spill, Environment Canada
244	Lake Wabuman, AB	Breeding	Feathers	Lake Wabuman spill, Environment Canada
245	Lake Wabuman, AB	Breeding	Feathers	Lake Wabuman spill, Environment Canada
246	Lake Wabuman, AB	Breeding	Feathers	Lake Wabuman spill, Environment Canada
247	Lake Wabuman, AB	Breeding	Feathers	Lake Wabuman spill, Environment Canada
248	Lake Wabuman, AB	Breeding	Feathers	Lake Wabuman spill, Environment Canada
Initial Marker Screening (WEGR) [1]				
71	coastal OR	Winter	Toe	COASST (Beached Bird Survey)
72	coastal OR	Winter	Toe	COASST (Beached Bird Survey)
75	coastal BC	Winter	Toe	Environment Canada
76	coastal BC	Winter	Toe	Environment Canada
77	central coastal CA	Winter	Toe	MLML/BeachCOMBers (beached bird survey)
78	central coastal CA	Winter	Toe	MLML/BeachCOMBers (beached bird survey)
Initial Marker Screening (CLGR) [1]				
55	S. coastal CA	Winter	Muscle	Ventura Oil Spill Incident, PRBO / OWCN
60	S. coastal CA	Winter	Muscle	Ventura Oil Spill Incident, PRBO / OWCN
62	S. coastal CA	Winter	Muscle	Ventura Oil Spill Incident, PRBO / OWCN
63	S. coastal CA	Winter	Muscle	Ventura Oil Spill Incident, PRBO / OWCN
64	S. coastal CA	Winter	Muscle	Ventura Oil Spill Incident, PRBO / OWCN
65	S. coastal CA	Winter	Muscle	Ventura Oil Spill Incident, PRBO / OWCN
66	S. coastal CA	Winter	Muscle	Ventura Oil Spill Incident, PRBO / OWCN
68	S. coastal CA	Winter	Muscle	Ventura Oil Spill Incident, PRBO / OWCN
95	central coastal CA	Winter	Blood	IBRRC / Rehabilitation
96	S. central coastal CA	Winter	Blood	IBRRC / Rehabilitation
97	Central Valley CA	Breeding	Blood	IBRRC / Rehabilitation
98	central coastal CA	Winter	Blood	IBRRC / Rehabilitation
100	S. central coastal CA	Winter	Blood	IBRRC / Rehabilitation

DNA ID	Location	Season	Sample Type	Provider / Source
101	central coastal CA	Winter	Blood	IBRRC / Rehabilitation
104	central coastal CA	Winter	Blood	IBRRC / Rehabilitation

[1]Footnoted samples listed under WEGR Population Structure were also used in WEGR panel for initial screening

Appendix 3. Tetranucleotide microsatellite loci developed for Western Grebe (*A. occidentalis*) and screened on 16 individuals of each

Aechmophorus species (Western Grebe and Clark's Grebes, *A. clarkii*)[1].

Locus Name	Repeat Motif[2]	Primer sequence (5'-3')	T_a (°C)	$MgCl_2$ conc. (mM)	Size range (bp)	*A. occidentalis*			*A. clarkii*		
						No. of alleles	H_E	H_O	No. of alleles	H_E	H_O
B8	(CATC)₃(CATT)₁(CATC)₃TAT (CATT)₂(CATC)₉	AAGGAGGGCATTATGTTATGC GCCAGTAATCACCACAAATTG	55	2	277-289	4	0.520	0.375	3	0.607	0.688
B11	(CATC)₁₁	CAGGCAGCAAAAGTTCTTG GGGTCTCTGTGATGTGATGA	55	2.5	147-155	3	0.561	0.438	3	0.619	0.500
B102	(CATC)₇CGTC(CATC)₂	ACCCATCCATCCATACCTG GCCCAAACCATTCTGTGAT	55	2.5	134-146	4	0.525	0.563	4	0.561	0.375
B112B	(CATC)₁₀	CAATCCAAAGGCTGGATCTC TCGAAAACCAAAATGTGCAG	55	3	222-230	3	0.539	0.875	3	0.617	0.750
B113	(CATC)₁₁	AAGCACACAATTCAGGAAGTAC GATGCCTCCATTTAGGTAAAAG	55	2	211-231	5	0.754	0.813	6	0.785	0.875
C5	TACA (TA)₂ (TACA)₈(TA)₅	GTTTCCTCCAGCCAGTCAC GTTCCTTCAACAGGCACAAG	55	2.5	249-253	2	0.498	0.188	2	0.482	0.188
E202	(GAAG)₁₄N₃₂ (AAAG)₁₀	TCCAGACCGTTTTGCTTCTT CCTCATGGTTCAATGCCTTT	55	2.5	217-253	8	0.822	0.688	10	0.857	0.625
G118	(TAGA)₁₁	TTCTTGTCACATTTCATTGCAC TTCAGGGTTGCTCAGTTGTG	55	4	303-323	4	0.545	0.563	5	0.656	0.750
G206	(TAGA)₈AAAGA(TAGA)₂	AAGGCAAGCAAACAGAGC ATGGACACAATGCGTCTTAG	55	2	274-290	4	0.662	0.688	5	0.740	0.875
G209	(TAGA)₉	TGCAATGCCATTTTCTGTTT ACAGCCCAGAGTTAGGCTCA	55	3	340-348	3[1]	0.607	0.733	3	0.541	0.500
G215	(TAGA)₂TAGGTATG(TAGG)₄ (TAGA)₂TGA(TAGA)₉	GACAGGACAACCCTTTTATCC TGGTGGTCTCTGACAGGTC	60	2.5	244-260	5[1]	0.696	0.800	5	0.693	0.750

[1]Included only 15 samples of Western Grebe (could not yield scoreable product for final sample)
[2]N=number of nucleotides in between more discernible repeat regions

Appendix 4. Western and Clark's Grebe banding and encounter data (from dataset provided by the Bird Banding Lab), 1934-2008.

Table 1. Summarized accounts of banding and encounter data by region and type of recovery.

Species[1]	Location of Banding	Banding Season	Location of Encounter	# recoveries	Season/Timing of Encounter
Between-Season Recoveries					
WCGR	Lake Manitoba, MB	Breeding	Straits of British Columbia	4	Wintering / migration (September through May)
WCGR	Lake Manitoba, MB	Breeding	Inland British Columbia	1	Migration (October)
WCGR	Lake Manitoba, MB	Breeding	Coastal Washington / Puget Sound	13	Wintering / migration (October through April)
WCGR	Lake Manitoba, MB	Breeding	Coastal Oregon	1	Wintering / migration (October)
WCGR	Lake Manitoba, MB	Breeding	Northern coastal CA	1	Wintering (January)
WCGR	Lake Manitoba, MB	Breeding	Central coastal / Bay Area CA	7	Wintering / migration / nonbreeding (throughout year)
WCGR	Lake Manitoba, MB	Breeding	South coastal CA	3	Wintering / migration (February / April)
WCGR	Lake Manitoba, MB	Breeding	Southwestern MT	1	Migration? Encountered Sept 26
WEGR	Lake Manitoba, MB	Breeding	Southcentral NM	1	Migration? Encountered September 1st
WCGR	Lake Manitoba, MB	Breeding	Nevada	1	Unknown
WCGR	Lake Manitoba, MB	Breeding	Lake Winnipeg	1	Migration / breeding (18 Sept; 2 yrs 3 mths > banding)
WCGR	Southeastern SK	Breeding	Southwestern BC	1	Migration / breeding (July of following year)
WCGR	Central coastal / Bay Area CA	Wintering	Southeastern Saskatchewan	1	Breeding
WEGR	Lake Osakis, MN	Breeding	Central CO	1	Breeding / Migration (April)
WCGR	Seedskadee NWR, WY	Migration?	Nevada	2	Wintering (Dec & Feb)
WCGR	Willard Bay, UT	Breeding	Southcentral/southern coastal CA	2	Wintering (Dec & Mar)
WEGR	South coastal CA	Wintering	Northwestern North Dakota	1	Breeding / migration (September)
WCGR	Tule Lake, CA	Migr? (Oct)	South San Joaquin Valley, CA	1	Migration/Wintering
WEGR	South coastal CA	Wintering	Inland southern CA (Riverside Co.)	1	Migration / breeding (June)
WEGR	South coastal CA	Wintering	Inland southern CA (Mojave Desert)	1	Migration (October)
WCGR	Baja California, MX	Nonbreeding	Durango, MX	1	Breeding/Migration (September)
				Subtotal=46	

Species[1]	Location of Banding	Banding Season	Location of Encounter	# recoveries	Season/Timing of Encounter
Between-Year Recoveries					
WCGR	Lake Manitoba, MB, Canada	Breeding	Elsewhere in Manitoba	2	1 nearby (6/6); 1 on Lake Winnipeg (6/20)
WCGR	Lake Manitoba, MB, Canada	Breeding	Eastcentral AB, Canada	1	Encountered May 23rd (breeding?)
WCGR	Lake Manitoba, MB, Canada	Breeding	Southern (Clark Co.) NV	1	Banded 6/20/78; encountered 5/31/80
WEGR	Lake Osakis, MN	Breeding	Elsewhere in Minnesota	2	1 nearby (6/01; >10 yrs) 1 in SE MN (4/22/96, >5 yrs)
WCGR	Southeastern SK, Canada	Breeding	Southern inland BC	1	breeding? Encountered July 1965, 1 yr later
WEGR	Lake Osakis, MN	Breeding	Lake Osakis, MN	1	following breeding season
WCGR	Willard Bay, UT	Breeding (?)	Utah Lake, UT	1	Banded 8/30/73 (breeding/migr); encountered 5/24/74
WCGR	San Francisco Bay, CA	Wintering	Southcentral coastal WA	1	Banded 4/71, encountered 1/73
WEGR/WCGR	San Francisco Bay, CA	Wintering	San Francisco Bay Area, CA	3	encountered 1, 3, and 5 winters later
WEGR	South coastal (L.A. Co.), CA	Wintering	South coastal (L.A. Co.), CA	3	2 birds encountered next winter; 1 bird 2 winters later
				Subtotal=16	
Within-Season Recoveries					
WCGR	Puget Sound region, WA	Winter	Puget Sound region, WA	1	Same season (2 months later)
WEGR	North coast (Humboldt Co.) CA	Winter	North coast (Humboldt Co.)	1	Same season (1 week later)
WEGR	North coast (Humboldt Co.) CA	Winter	N. central coast (Mendocino Co.)	1	Same season (2 months later)
WEGR	San Francisco Bay, CA	Winter	North coast (Humboldt Co.)	1	Same season (1 month later)
WCGR	Clear Lake CA	Fall/Winter	Clear Lake CA	1	Same season (1 week later)
WEGR	San Francisco Bay Area, CA	Winter	Clear Lake CA	1	Same season (2 months later)
WEGR/WCGR	San Francisco Bay Area, CA	Winter	San Francisco Bay Area, CA	11	Same season (2 days to 6 months later)
WEGR/WCGR	San Francisco Bay Area, CA	Winter	Monterey Bay Area, CA	5	Same season (2 weeks to 6 months later)
WEGR	San Francisco Bay Area, CA	Winter	South coastal CA	3	Same season (1-2 weeks later)
WEGR/WCGR	Central coastal CA	Winter	Central coastal CA	5	Same season (2 days to 6 months later)
WEGR	South coast (Orange Co.), CA	Winter	San Francisco Bay Area, CA	1	Same season or migration (4 months later)
WEGR	South coast (L.A. Co.), CA	Winter	South coast (L.A. Co.), CA	11	Same season (3 days to 5 months later)
WEGR	South coast (L.A. Co.), CA	Winter	South coast (San Diego Co.), CA	2	Same season (10 days to 2 months later)
WEGR	South coast (Ventura Co.), CA	Winter	South coast (Ventura Co.), CA	3	Same season (1 day to 1 month later)
CLGR	San Francisco Bay Area, CA	Winter	San Francisco Bay Area	5	Same season (5 days to 2 months later)

Species[1]	Location of Banding	Banding Season	Location of Encounter	# recoveries	Season/Timing of Encounter
CLGR	San Francisco Bay Area, CA	Winter	San Mateo coast, CA	1	Same season (1 week later)
CLGR	San Francisco Bay Area, CA	Winter	Monterey Bay Area, CA	2	Same season (2-3 months later)
CLGR	San Francisco Bay Area, CA	Winter	S. central coast (Morro Bay), CA	1	Same season (2 weeks later)
CLGR	San Francisco Bay Area, CA	Winter	South coast (L.A. Co.), CA	1	Same season (2 months later)
CLGR	South coast (L.A. Co.), CA	Winter	South coast (L.A. Co.), CA	1	Same season (1 week later)
				Subtotal=58	

[1]Until 1985 both WEGR and CLGR were considered two phases of the same species and called WEGR in banding data; those data are recorded here as WCGR (Western/Clark's Grebe).

Appendix 4 (continued)

Table 2. All records of encounter data for Western and Clark's Grebes, organized by type of recovery and banding date. Codes described in footnotes.

Spec	Bandnumb	Permit	Stat	Banding Location	b_lat	b_lon	bprec	Band Date	Encounter Location	Enc Date	e_lat	e_lon	eprec
Between-Season Recovery													
WCGR	4621041	4243	3	Willard Bay, UT	41.42	-112.08	10	6/14/1934	Monterey Bay Area, CA	Dec 1936	36.92	-122.08	10
WCGR	4621267	4243	3	Willard Bay, UT	41.42	-112.08	10	6/27/1934	South coast (Santa Barbara Co.), CA	March 1935	34.42	-119.58	10
WCGR	40683569	5095	3	Baja California (inland), MX	31.28	-115.30	12	7/5/1940	Durango, MX	9/10/1940	25.01	-104.99	12
WCGR	37714377	4837	7	Tule Lake, CA	41.92	-121.42	10	10/15/1941	San Joaquin Valley, CA	Nov 1941	36.25	-119.42	10
WCGR	59708307	855	3	Southeastern SK, Canada	50.42	-104.58	10	5/9/1964	Southwestern (inland) BC, Canada	July 1965	49.75	-120.25	10
WCGR	100723975	3622	3	Lake Manitoba, MB, Canada	50.25	-98.25	10	6/15/1974	Southcentral (inland), BC, Canada	10/20/1974	49.58	-119.75	10
WCGR	100723993	3622	3	Lake Manitoba, MB, Canada	50.25	-98.25	10	7/15/1974	Central coast (San Mateo Co.), CA	1/1/1977	37.25	-122.42	10
WCGR	100708601	3622	3	Lake Manitoba, MB, Canada	50.25	-98.25	10	9/7/1974	Puget Sound area, WA	11/1/1976	47.58	-122.58	10
WCGR	100708619	3622	3	Lake Manitoba, MB, Canada	50.25	-98.25	10	9/18/1974	Northcentral coast (Lincoln Co.), OR	10/14/1974	44.75	-124.08	10
WCGR	102740781	3622	3	Lake Manitoba, MB, Canada	50.25	-98.25	10	7/23/1976	San Francisco Bay Area, CA	1/23/1977	38.25	-123.08	10
WCGR	108730924	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/15/1977	Puget Sound area, WA	3/24/1978	47.42	-122.42	10
WCGR	108730929	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/15/1977	San Francisco Bay Area, CA	4/4/1980	37.92	-122.75	10
WCGR	108728118	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/20/1977	Puget Sound area, WA	4/15/1979	47.75	-122.58	10
WCGR	108728133	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/20/1977	North Bay Area (Sonoma Co.), CA	11/15/1977	38.42	-123.08	10
WCGR	108728142	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/20/1977	Puget Sound area, WA	3/6/1979	47.08	-124.08	10
WCGR	108728148	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/20/1977	South coast (Grays Harbor Co.), WA	2/6/1979	46.42	-124.08	10
WCGR	108728164	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/20/1977	South coast (Grays Harbor Co.), WA	10/16/1977	46.92	-123.75	10
WCGR	108728172	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/20/1977	Central coast (San Mateo Co.), WA	3/10/1979	46.92	-124.25	10
WCGR	108728200	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/20/1977	North Bay Area (Sonoma Co.), CA	7/5/1979	38.42	-122.92	10
WCGR	108728158	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/20/1977	Strait of Georgia area, BC, Canada	May 1982	49.25	-123.08	10
WCGR	108728208	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/25/1977	San Francisco Bay, CA	2/6/1979	37.92	-122.42	10
WCGR	108728253	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/25/1977	Lake Winnipeg, MB, Canada	9/18/1979	50.92	-96.92	10
WCGR	108728296	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/25/1977	NV	Jan 1979	0.00	0.00	10
WCGR	108728304	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/25/1977	Strait of Juan de Fuca, BC, Canada	5/21/1979	48.42	-123.75	10
WCGR	108728309	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/25/1977	Puget Sound area, WA	2/12/1978	47.25	-122.75	10
WCGR	108728343	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/25/1977	South coast (San Diego Co.), CA	4/9/1978	32.75	-117.25	10
WCGR	59706403	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/28/1977	Puget Sound area, WA	2/19/1978	47.42	-122.42	10
WCGR	59706424	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/28/1977	Puget Sound area, WA	1/2/1980	47.08	-124.08	10
WCGR	108728389	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	7/9/1977	San Francisco Bay, CA	3/17/1983	37.92	-122.42	10
WCGR	108730766	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	5/25/1978	Southcentral coast (SLO Co.), CA	1983	35.08	-120.58	10
WCGR	108730770	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	5/25/1978	Puget Sound area, WA	4/17/1979	47.25	-122.58	10

Spec	Bandnumb	Permit	Stat	Banding Location	b_lat	b_lon	bprec	Band Date	Encounter Location	Enc Date	e_lat	e_lon	eprec
WCGR	108730788	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	5/25/1978	Canyon Ferry Lake area, SW MT,	9/26/1982	46.58	-111.75	10
WCGR	110734132	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/21/1978	South coast (Grays Harbor Co.), WA	2/6/1980	46.92	-124.08	10
WCGR	110734153	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/21/1978	South coast (Ventura Co.), CA	12/4/1982	34.25	-119.25	10
WCGR	59706474	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/26/1978	South coast (Grays Harbor Co.), WA	4/2/1984	46.42	-124.08	10
WCGR	110734159	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/26/1978	Strait of Juan de Fuca, BC, Canada	March 1983	48.58	-124.75	10
WCGR	110734233	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	7/11/1978	Strait of Georgia, BC, Canada	9/15/1983	49.08	-123.75	10
WCGR	110734256	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	7/11/1978	North coast (Mendocino Co.), CA	1/9/1984	38.92	-123.58	10
WCGR	112700805	9545	7	Seedskadee NWR, WY,	41.42	-109.58	10	10/31/1979	Southern (Clark Co.), NV	12/5/1979	36.25	-115.08	10
WCGR	112700806	9545	7	Seedskadee NWR, WY	41.42	-109.58	10	10/31/1979	Pyramid Lake area, NW NV	Feb 1981	39.92	-119.58	10
WCGR	111732318[1]	21214	7	San Francisco Bay Area, CA	37.92	-122.58	10	1/18/1980	Southeastern SK, Canada	6/23/1980	50.25	-104.08	10
WEGR	76780606	22053	3	Lake Manitoba, MB, Canada	50.25	-98.25	10	6/26/1989	Southcentral (Sierra Co.), NM	9/1/1990	32.92	-107.25	10
WEGR	83781688	20989	3	Lake Osakis, MN,	45.92	-95.08	10	5/20/1991	Central (El Paso Co.), CO	4/11/1995	38.92	-104.75	10
WEGR	158752741[1]	21214	7	South coast (L.A. Co.), CA	33.75	-118.25	10	5/17/2006	Northwestern (Renville Co.), ND	8/7/2006	48.58	-101.58	10
WEGR	171791261[1]	21214	7	South coast (Orange Co.), CA	33.58	-117.92	10	3/28/2007	Mojave Desert, CA	10/3/2007	35.25	-117.75	10
WEGR	158752949[1]	21214	7	South coast (L.A. Co.), CA	33.75	-118.25	10	3/18/2008	Inland Riverside Co., southern, CA	6/28/2008	33.92	-117.42	10
Between-Year Recovery													
WCGR	59708307	855	3	Southeastern SK, Canada	50.42	-104.58	10	5/9/1964	Southern (inland) BC, Canada	July 1965	49.75	-120.25	10
WCGR	62794529[1]	9316	7	San Francisco Bay, CA	37.92	-122.42	10	4/9/1971	Southcentral coastal WA	1/26/1973	46.92	-124.08	10
WCGR	62794536[1]	9316	7	San Francisco Bay, CA	37.92	-122.42	10	4/18/1971	San Francisco Bay area, CA	3/12/1972	37.75	-122.42	10
WCGR	62744112	6267	3	Willard Bay, UT	41.42	-112.25	10	8/30/1973	Utah Lake, UT,	5/24/1974	40.25	-111.75	10
WCGR	100723985	3622	3	Lake Manitoba, MB, Canada	50.25	-98.25	10	6/15/1974	Near Lake Manitoba, MB, Canada	6/6/1982	49.92	-98.25	10
WCGR	108728170	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/20/1977	Eastcentral AB, Canada	5/23/1979	54.75	-111.75	10
WCGR	108728107	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/20/1977	Southern (Clark Co.) NV	5/31/1980	36.08	-114.92	10
WCGR	110734135	3622	3	Lake Manitoba, MB, Canada	50.58	-98.08	10	6/21/1978	Lake Winnipeg, MB, Canada	6/20/1983	50.92	-96.92	10
WEGR	83781650	20989	3	Lake Osakis, MN	45.92	-95.08	10	5/2/1991	Southeastern (Wright Co.) MN	4/22/1996	45.25	-93.75	10
WEGR	83781686	20989	3	Lake Osakis, MN	45.92	-95.08	10	5/20/1991	Douglas Co., MN	June 2001	45.92	-95.25	10
WEGR	83781691	20989	3	Lake Osakis, MN	45.92	-95.08	10	5/20/1991	Lake Osakis, MN,	7/10/1992	45.92	-95.08	10
WEGR	80782293	21214	7	San Francisco Bay, CA	37.75	-122.42	10	5/31/2001	San Francisco Bay Area, CA	June 2004	37.75	-122.42	10
WEGR	136796144[1]	21214	7	San Francisco Bay Area, CA	37.75	-122.42	10	3/30/2002	San Francisco Bay Area, CA	4/9/2007	37.75	-122.42	10
WEGR	164786701[1]	21214	7	South coastal (L.A. Co.), CA	33.75	-118.25	10	1/2/2003	South coastal (L.A. Co.), CA	1/18/2005	33.92	-118.42	10
WEGR	158799841	21214	7	South coastal (L.A. Co.), CA	33.75	-118.25	10	10/21/2004	South coastal (L.A. Co.), CA	May 2006	33.42	-117.58	10
WEGR	158752715[1]	21214	7	South coastal (L.A. Co.), CA	33.75	-118.25	10	10/13/2005	South coastal (L.A. Co.), CA	2/12/2007	33.70	-118.05	0
Within-Season Recovery													
WCGR	70731272	8566	2	Clear Lake, CA	39.1	-122.9	10	11/22/1968	Clear Lake, CA	11/29/1968	38.9	-122.58	10
WCGR	62794526[1]	9316	7	San Francisco Bay , CA	37.9	-122.4	10	4/9/1971	San Francisco Bay , CA	4/10/1971	37.8	-122.42	10
WCGR	58708735[1]	9316	7	San Francisco Bay , CA	37.9	-122.4	10	5/2/1971	San Francisco Bay , CA	7/13/1971	37.8	-122.25	10
WCGR	80752496	9739	7	Puget Sound region, WA	47.3	-122.4	10	1/10/1976	Puget Sound region, WA	4/1/1976	47.3	-122.42	10
WCGR	102713434	8045	3	San Francisco Bay Area, CA	37.8	-122.3	10	1/28/1978	Monterey Bay Area, CA	2/16/1978	37.8	-122.42	10

Spec	Bandnumb	Permit	Stat	Banding Location	b_lat	b_lon	bprec	Band Date	Encounter Location	Enc Date	e_lat	e_lon	eprec
WCGR	111723208	20774	5	central coastal WA	47.1	-124.3	10	3/6/1979	central coastal WA	4/5/1979	46.9	-124.25	10
WCGR	111732347	21214	7	San Francisco Bay Area, CA	37.9	-122.6	10	10/21/1981	San Francisco Bay Area, CA	4/11/1982	37.9	-122.25	10
WCGR	136734814[1]	21793	7	San Francisco Bay Area, CA	37.6	-122.4	10	2/6/1986	San Francisco Bay Area, CA	2/10/1986	37.6	-122.25	10
WCGR	97700390[1]	21510	7	Monterey Bay Area, CA	36.8	-121.8	10	6/3/1986	Monterey Bay Area, CA	6/5/1986	36.8	-121.75	10
WEGR	112701434[1]	21226	7	south coastal (Ventura Co.), CA	34.1	-119.1	10	2/23/1990	south coastal (Ventura Co.), CA	March 1990	34.4	-119.75	10
WEGR	112701429[1]	21226	7	south coastal (Ventura Co.), CA	34.1	-119.1	10	2/23/1990	south coastal (Ventura Co.), CA	2/24/1990	34.4	-119.75	10
WEGR	112701428[1]	21226	7	south coastal (Ventura Co.), CA	34.1	-119.1	10	2/23/1990	south coastal (Ventura Co.), CA	3/3/1990	34.4	-119.75	10
WEGR	150797909[1]	21214	7	San Francisco Bay , CA	37.9	-122.3	10	1/26/1991	South coast (L.A. Co.), CA	2/9/1991	33.8	-118.25	10
WEGR	243777324[1]	21214	7	North coast (Humboldt Co.), CA	41.1	-124.1	10	11/21/1997	N. central coast (Mendocino Co.), CA	11/28/1997	38.9	-123.75	10
WEGR	243777340[1]	21214	7	North coast (Humboldt Co.), CA	41.1	-124.1	10	11/22/1997	North coast (Humboldt Co.), CA	1/11/1998	40.9	-124.25	10
WEGR	153725459[1]	21214	7	central coastal CA	37.1	-122.3	10	11/24/1997	central coastal CA	May 1998	36.8	-121.75	10
WEGR	400758445[1]	21214	7	central coastal CA	37.1	-122.3	10	11/24/1997	central coastal CA	Jan 1998	37.4	-122.42	10
WEGR	153725458[1]	21214	7	central coastal CA	37.1	-122.3	10	11/24/1997	central coastal CA	12/13/1997	37.1	-122.25	10
WEGR	400758471[1]	21214	7	San Francisco Bay , CA	37.9	-122.3	10	3/9/1998	San Francisco Bay , CA	3/11/1998	37.9	-122.25	10
CLGR	80782228	21214	7	San Francisco Bay , CA	37.9	-122.3	10	2/16/2000	San Francisco Bay , CA	3/9/2000	37.9	-122.42	10
WEGR	80782226	21214	7	San Francisco Bay , CA	37.9	-122.3	10	2/16/2000	San Francisco Bay , CA	2/29/2000	37.9	-122.42	10
WEGR	80782275[1]	21214	7	South coastal (L.A. Co.), CA	33.8	-118.3	10	4/11/2001	South coastal (L.A. Co.), CA	6/7/2001	34.1	-119.25	10
CLGR	136796125	21214	7	San Francisco Bay Area, CA	38.1	-122.3	10	12/21/2001	South coast (L.A. Co.), CA	2/14/2002	33.9	-118.42	10
WEGR	165798850	21214	7	San Francisco Bay , CA	37.9	-122.3	10	11/18/2002	north coast (Humboldt Co.), CA	12/31/2002	41.1	-124.08	10
WEGR	400779824[1]	21214	7	San Francisco Bay Area, CA	37.8	-122.4	10	1/2/2003	San Francisco Bay Area, CA	1/19/2003	36.9	-121.92	10
WEGR	158799810[1]	21214	7	South coastal (L.A. Co.), CA	33.8	-118.3	10	5/4/2003	South coastal (L.A. Co.), CA	5/14/2003	33.6	-117.75	10
WEGR	158799858[1]	21214	7	South coastal (L.A. Co.), CA	33.8	-118.3	10	1/15/2005	South coastal (L.A. Co.), CA	1/19/2005	33.6	-118.08	10
WEGR	158799938[1]	21214	7	South coastal (L.A. Co.), CA	33.8	-118.3	10	1/19/2005	South coastal (L.A. Co.), CA	1/31/2005	33.8	-118.08	10
WEGR	158799910[1]	21214	7	South coastal (L.A. Co.), CA	33.8	-118.3	10	1/19/2005	South coastal (L.A. Co.), CA	1/21/2005	33.6	-118.08	10
WEGR	158799967[1]	21214	7	South coastal (L.A. Co.), CA	33.8	-118.3	10	1/21/2005	South coastal (San Diego Co.)	1/31/2005	32.8	-117.08	10
WEGR	158799996[1]	21214	7	South coastal (L.A. Co.), CA	33.8	-118.3	10	1/22/2005	South coastal (L.A. Co.)	2/5/2005	33.8	-118.25	10
WEGR	158799981[1]	21214	7	South coastal (L.A. Co.), CA	33.8	-118.3	10	1/22/2005	South coastal (L.A. Co.)	Feb 2005	33.4	-117.75	10
WEGR	158799985[1]	21214	7	South coastal (L.A. Co.), CA	33.8	-118.3	10	1/22/2005	South coastal (L.A. Co.), CA	6/11/2005	33.6	-118.08	10
WEGR	158799880[1]	21214	7	South coastal (L.A. Co.), CA	33.8	-118.3	10	1/23/2005	South coastal (L.A. Co.), CA	1/29/2005	33.8	-118.08	10
CLGR	158752818	21214	7	San Francisco Bay Area, CA	38.1	-122.4	10	7/2/2006	San Francisco Bay Area, CA	7/12/2006	37.9	-122.58	10
WEGR	158752746	21214	7	South coastal (L.A. Co.), CA	33.8	-118.3	10	7/6/2006	South coastal (San Diego Co.), CA	8/26/2006	32.8	-117.27	0
WEGR	158752752	21214	7	South coastal (L.A. Co.), CA	33.8	-118.3	10	11/9/2006	South coastal (L.A. Co.), CA	11/12/2006	34.1	-118.25	10
CLGR	158752829	21214	7	San Francisco Bay Area, CA	38.1	-122.4	10	4/3/2007	Napa Co., CA	5/16/2007	38.3	-122.42	10
WEGR	171791265[1]	21214	7	South coastal (Orange Co.), CA	33.6	-117.9	10	4/4/2007	San Francisco Bay, CA	8/4/2007	37.9	-122.42	10
WEGR	158752790	21214	7	South coastal (L.A. Co.), CA	33.8	-118.3	10	6/5/2007	South coastal (L.A. Co.), CA	6/15/2007	33.6	-117.92	10
WEGR	169745993	21214	7	San Francisco Bay Area, CA	37.8	-122.4	10	10/20/2007	Clear Lake, CA	12/10/2007	39.1	-122.58	10
WEGR	158752882[1]	21214	7	San Francisco Bay Area, CA	38.1	-122.9	10	11/20/2007	Monterey Bay Area, CA	5/3/2008	36.9	-121.75	10
WEGR	158752891[1]	21214	7	San Francisco Bay Area, CA	38.1	-122.9	10	11/27/2007	San Francisco Bay , CA	2/21/2008	37.9	-122.46	0

Spec	Bandnumb	Permit	Stat	Banding Location	b_lat	b_lon	bprec	Band Date	Encounter Location	Enc Date	e_lat	e_lon	eprec
CLGR	158753054	21214	7	San Francisco Bay Area, CA	38.1	-122.9	10	12/2/2007	Monterey Bay Area, CA	4/6/2008	36.6	-122.25	10
CLGR	158753021	21214	7	San Francisco Bay Area, CA	38.1	-122.9	10	12/2/2007	San Francisco Bay Area, CA	12/7/2007	37.9	-122.58	10
WEGR	158753030	21214	7	San Francisco Bay Area, CA	38.1	-122.9	10	12/2/2007	Monterey Bay Area, CA	12/13/2007	36.9	-121.75	10
WEGR	158753046	21214	7	San Francisco Bay Area, CA	38.1	-122.9	10	12/2/2007	San Francisco Bay Area, CA	12/27/2007	38.1	-122.88	0
WEGR	169763210[1]	21214	7	San Francisco Bay Area, CA	38.1	-122.9	10	12/4/2007	Monterey Bay Area, CA	5/3/2008	36.9	-122.08	10
CLGR	158753132	21214	7	San Francisco Bay Area, CA	38.1	-122.9	10	12/5/2007	Monterey Bay Area, CA	3/6/2008	36.6	-121.92	10
CLGR	158753145	21214	7	San Francisco Bay Area, CA	38.1	-122.9	10	12/6/2007	North Bay, CA	2/5/2008	38.3	-122.92	10
WEGR	158753151	21214	7	San Francisco Bay Area, CA	37.8	-122.4	10	12/10/2007	South coast (Ventura Co.), CA	12/25/2007	34.3	-119.25	10
WEGR	158753157	21214	7	San Francisco Bay Area, CA	37.8	-122.4	10	12/12/2007	Monterey Bay Area, CA	12/30/2007	36.6	-121.92	10
CLGR	158753191	21214	7	San Francisco Bay Area, CA	38.1	-122.4	10	2/25/2008	San Mateo coastline, CA	3/5/2008	37.3	-122.42	10
CLGR	158753190[1]	21214	7	San Francisco Bay Area, CA	38.1	-122.4	10	2/25/2008	Southcentral coast (Morro Bay), CA	3/10/2008	35.4	-120.85	0
WEGR	169763284[1]	21214	7	San Francisco Bay , CA	37.9	-122.3	10	3/9/2008	South coast (Ventura Co.), CA	3/16/2008	34.3	-119.25	10
WEGR	169763302	21214	7	San Francisco Bay Area, CA	38.1	-122.4	10	4/6/2008	San Francisco Bay Area, CA	4/24/2008	37.9	-122.58	10
WEGR	172734552	21214	7	San Francisco Bay , CA	37.9	-122.3	10	5/21/2008	San Francisco Bay , CA	8/13/2008	37.9	-122.25	10
CLGR	172734612	21214	7	South coastal (L.A. Co.), CA	33.8	-118.3	10	5/24/2008	South coastal (L.A. Co.), CA	5/31/2008	33.9	-118.42	10

[1]Birds rehabilitated as a result of oiling.

spec=species; until 1985 both WEGR and CLGR were considered two phases of the same species and called WEGR in banding data; those data are recorded here as WCGR (Western/Clark's Grebe).
permit = BBL permittee under which original banding occurred; **stat** = BBL code "birdstat"; 2=Transported to a different 10-minute block, but otherwise normal wild bird; 3=Normal, wild bird, released in same 10-minute block as captured, held 24 hours or less;   5= Sick, Exhausted, Injured (old or new injury), Crippled, or with a Physical Deformity, held 24 hours or less; 7=Rehabilitated and held longer than 24 hours; **b_lat, b_lon, e_lat, e_lon** = Latitude and longitude (decimal degrees) of banding/encounter. For those given in 10' block, coordinates represent SE corner of the 10' block;
bprec, eprec=BBL code "b_coord_prec" and "e_coord_prec"; precision of latitude and longitude provided; 10=10' block, 1=1' block, 0=exact

CHAPTER 3

AGE AND SEX RATIOS OF WESTERN AND CLARK'S GREBES KILLED IN TWO OIL SPILLS AND AN ALGAL BLOOM IN COASTAL CALIFORNIA

ABSTRACT

Differential wintering of age and sex classes in migratory birds has been found in many species, and can have important conservation implications. If population-limiting events such as oil spills predominantly impact one age or sex class over another due to their greater prevalence in a wintering region, demographic ramifications to breeding populations are possible. We examined age and sex ratios of wintering Western Grebes *Aechmophorus occidentalis* and Clark's Grebes *A. clarkii* killed in two oil spills, the 1997 *Kure* spill in Humboldt Bay and the 2005 Ventura Oiled Bird Incident (VOBI) in southern California; and in a third marine event, the 2007 stranding and mortality from a red tide in Monterey Bay. Mortality of Western Grebes was greatest from VOBI. Birds were sexed and aged by examining gonads and bursa of Fabricius during necropsies and with chromobox-helicase-DNA-binding sex genes. Bursa involution was evaluated against another age proxy, gonad maturity. Bursa was found to be useful for ageing grebes, but some inconsistencies require greater study of its uses and patterns in grebes. Age and sex patterns varied, and indicate some degree of differential wintering or local partitioning. Western Grebes were comprised of 91% immatures in *Kure* and 21% in

VOBI. In the Monterey Bay event, females comprised 65% of Western Grebes (driven by a skewed sex ratio in immatures) and 24% of Clark's Grebes. Although VOBI Western Grebes lacked an overall skewed sex ratio, immatures were male-biased (78%). Opportunities to examine demographic patterns in oiled wildlife are often overlooked but can be critical to fully understanding and predicting impacts of oil spills, and provide one of the few opportunities to assess such demographic parameters in wintering seabirds.

INTRODUCTION

Oil spills are of conservation concern for many seabird species and populations, and are among the top threats to Western Grebes (*Aechmophorus occidentalis*, Lawrence, 1858) and Clark's Grebes (*A. clarkii*, Lawrence, 1858) (Storer and Nuechterlein 1992), sister taxa that breed in freshwater lakes and spend nonbreeding seasons in the near-shore marine environment where they are vulnerable to oiling. In California, Western Grebes are typically one of the main species impacted during oil spills (CDFG 1969a,b; Straughan 1970, 1971; American Trader Trustee Council 2001; Hampton *et al.* 2003; Carter 2003, CDFG, CSLC, & USFWS 2004; CDFG & USFWS 2008; PRBO/OWCN unpubl. data). Understanding demographic parameters of impacted populations, including sex and age ratios, is crucial to assessing overall and potential long-term impacts of oil spills (Heubeck *et al.* 2003).

Differential wintering (or "differential migration"), in which sex or age classes do not migrate in equal distribution to the same wintering areas, has been documented in

many migratory bird species and across taxa (Ketterson and Nolan 1976, Belthoff and

Gauthreaux 1991, Olson & Arsenault 2000, Nebel *et al.* 2002, Alvarez and Pajuelo 2004,

Humple *et al.* 2007a, Schamber *et al.* 2007). Multiple theories exist to explain differential

wintering in migratory birds, including the preference for one age or sex class to be closer

to breeding grounds, behavioral dominance, resource partitioning, and physiological

constraints (Myers *et al.* 1981, Ketterson and Nolan 1983, Fernandez and Lank 2006).

This phenomenon can have important implications if population-limiting events

disproportionately affect any sex or age class (Coulson *et al.* 2001). There are obvious

potential repercussions for populations affected by oil spills, including the potential for a

spill to affect future productivity at breeding areas. For example, if the age ratio in a

region is biased towards adult birds, a spill in that region would likely have a greater and

more immediate impact to breeding populations than events that cause mortality in

immature birds. This is because immatures already experience high mortality and

generally do not contribute to population productivity, whereas adults typically have high

survival and are already contributing to the productivity of a population. Alternatively,

significant mortality in immatures may result in delayed impacts to breeding populations.

Studies of differential wintering are often limited to species that are encountered

by researchers in large enough sample sizes, show dimorphism, and have externally

determinable age characteristics. Oil spills provide unique opportunities to study this

phenomenon, because of the large numbers of certain species found, and opportunity to

determine age and sex in the hand or with necropsies. Western and Clark's Grebes killed

in three marine events in coastal California (Fig. 1) were studied to determine age and sex

structure. The events were chosen because of impacts to grebes and access to samples;

carcasses from other oil spills during the same time period that also had substantial impact to grebes (e.g., the 2007 *Cosco Busan* oil spill in San Francisco Bay; PRBO/OWCN unpublished data) are not yet available for necropsies as they are evidence in potential legal proceedings.

The first event included was the *M/V Kure* oil spill, in which approximately 4500 gallons of oil spilled into Humboldt Bay in northern coastal California in November 1997; 33 Western and 2 Clark's Grebes were collected and mortality was estimated at approximately 75 birds for both species combined (CDFG & USFWS 2008, S. Hampton, pers. comm.). The second and most significant of the events was the January-February 2005 Ventura Oiled Bird Incident (VOBI), in which a mystery source in southern coastal California resulted in the collection of over 1325 Western Grebes (87% of all birds) and 74 Clark's Grebes (5%). Approximately 1090 of these Western Grebes and 68 Clark's Grebes were found dead or died in care, with *Aechmophorus* mortality estimated at 2500 birds (S. Hampton, pers. comm.). The third event included in this study was not a petroleum spill, but an unusual mass seabird stranding and mortality event caused by a red tide algal bloom in Monterey Bay, central coastal California in November 2007. A proteinaceous foam produced by the red tide coated birds' feathers, resulting in loss of waterproofing and hypothermic conditions in birds (Jessup *et al.* 2009) much like petroleum-induced effects. It impacted at least 90 Clark's Grebes and 200 Western Grebes (CDFG unpubl. data). Although not an oil spill, the event impacted the population in a similar fashion, and the age and gender data for birds killed in this event should not be biased.

In order to test whether age and sex ratios were evenly distributed at these wintering sites, we determined sex and age classes of a subset of birds killed in these three marine events. Birds were sexed either genetically or by assessing gonads during necropsies, and were aged by a combination of gonad maturity and the bursa of Fabricius (also called the *bursa fabricii*), an immunosuppressive organ in birds that is helpful for ageing birds as it involutes or atrophies during the first year or so of life (Davis 1947, Glick 1983). The presence of a prominent bursa indicates a young bird, its absence indicates an adult bird or a subadult bird whose bursa has undergone involution, and intermediate-sized bursas generally indicate immature or subadult birds manifesting currently regressing bursas. The bursa can be examined on live birds in some larger species that also have cloacal openings to the bursa (McNeil and Burton 1972), and is frequently examined when performing necropsies or preparing skins. Because of apparent species-specific variation in bursa involution rate and associated challenges in classifying some individuals or species with bursa measurements alone (Fredrickson 1968, McNeil and Burton 1972, Siegel-Causey 1990), it is important to validate the use of this ageing technique before its use in a novel species and to combine it with other ageing criteria when possible. It has been found to be a good indicator of age in a number of species (e.g., Hohman and Cypher 1986, Broughton 1994, Mather and Esler 1999) and the approach has been applied to many others (e.g., Anderson *et al.* 1969, McCloskey and Thompson 2000, Nevins and Carter 2003). Storer and Nuechterlein (1992) consider the bursa the most appropriate method for ageing Western and Clark's Grebes outside the nonbreeding season, as these species are difficult to age by plumage. Bursa has been used to age grebes (Livezey and Storer 1992), but because there are no published accounts of it

having been tested in grebes, we first compared bursa size to gonad maturity. This allowed us to assess age in species for which that is otherwise difficult. This study is among the few to examine age and sex structure of wintering Western and Clark's Grebes.

METHODS

Thorough search and collection for dead and debilitated birds in coastal and estuarine areas was conducted by the Oiled Wildlife Care Network (OWCN), International Bird Rescue Research Center, or California Department of Fish and Game during each of the events. Carcasses were frozen soon after collection, or after birds died or were euthanized in captivity. Necropsies were conducted of 27 grebes that died in the *Kure* spill, 78 grebes that died in the red tide event, and 151 grebes that died in VOBI. All carcasses from *Kure* and the red tide event, and 10% of carcasses from VOBI, were retained following those events and available for necropsies. Necropsies were conducted 1.5-11 years following the events, in 2008-2009, at the California Department of Fish and Game's Marine Wildlife Veterinary Care and Research Center (MWVCRC) in Santa Cruz and in collaboration with Moss Landing Marine Laboratories. These examinations were conducted after California Department of Fish and Game had relinquished the carcasses from evidentiary retention. Carcass condition permitted us to perform necropsies on 96% of carcasses. Clark's Grebes from the *Kure* spill (n=1) and necropsied birds identified only as *Aechmophorus* spp. (n=2) were excluded from this study. All

birds were given unique log numbers at the time of the spill or red tide event with a prefix that indicated if it arrived at the facility alive or dead (PRBO and OWCN 2005).

Necropsies and measurements followed standardized protocols used at MWVCRC for all avian species (modified from van Franeker 2004). Following external examinations, birds were dissected and gonads and bursa of Fabricius examined. Length and width of the ovary or left testis and diameter of largest follicle (DLF) of the ovary were measured; the development of the oviduct (1-4) in females was scored; the presence (prominent, apparent, or membranous) or absence of the bursa was determined; and when present, bursa length and width were measured.

Additionally, 58 birds killed in VOBI were genetically sexed using muscle samples collected from carcasses during the spill response in 2005 by the Wildlife Processing Unit (managed by PRBO Conservation Science and OWCN). These included 53 samples from carcasses not retained following the event and therefore not necropsied. Gender was determined by assessing two chromobox-helicase-DNA-binding genes (CHD-W and CHD-Z), which are highly conserved intron loci anchored in two exogenic regions that have been shown to be effective in sex determination in birds throughout the Class *Aves*, in which females are heterozygous (ZW) and males are homozygous (ZZ) for sex chromosomes (Griffiths *et al.* 1998). Polymerase chain reaction (PCR) was conducted with primers for each gene, a process that has been used effectively for avian sex-determination in the Sonoma State University lab and others. PCR products were run on high-density (8%) acrilimide gels and the number of bands assessed, with those with one band classified as male and those with two as female.

Statistical Analyses

Necropsy measurements were later converted into the indices for analysis (van Franeker 2004): testis index of male maturity = testis length x width; follicle-oviduct index of female maturity = diameter of largest follicle (0.1mm for those with undeveloped follicles) x oviduct development code; and bursa index = bursa length x width (0 for birds with no visible bursa).

Sex ratios were presented as 1) the proportion of females to males, and 2) the number of females per male. A two-sided goodness of fit model was used to see if the observed distribution of sex ratio fit the predicted distribution under the null hypothesis (0.5 to 0.5) if there were no biased sex ratio. When sample sizes allowed, proportional sex ratios were compared with a Chi-square test to determine if they were different for birds that arrived alive (and subsequently died) than birds that arrived dead. Logistic regression was used to test if gonad maturity indices predicted presence or absence of bursa, and linear regression to test the relationship between gonad maturity indices and bursa index. Based on these results, a model for assigning age classifications to birds was developed using only two broad classifications: adult and immature. Birds were classified as adults if they had mature gonads and atrophied bursas (females) or simply atrophied bursas (males), and as immatures if they had prominent bursas (both sexes) or no bursas but undeveloped gonads (females).

Age ratios were calculated as 1) the proportion of immatures to adults, and 2) the number of immatures per adult. Lack of available data on productivity and age-specific survival in these species prevented the development of an age structure model (Nevins and Carter 2003) to determine what the predicted age ratio would be if there were no

differential wintering by age class. A two-sided goodness of fit model was used to see if the observed distribution of sex ratio was significantly biased towards either age class. When allowed by sample size (Western Grebes killed in VOBI and the red tide event), a Chi-square test was used to evaluate if the sex ratio within each age class differed. All analyses were done in R (Vers. 2.9.1; R Development Core Team 2009).

RESULTS

Western Grebes outnumbered Clark's Grebes in each of these events, making up 94-95% of all *Aechmophorus* grebes in VOBI and *Kure* and 69% of those collected in the Monterey red tide event.

In females, bursa presence was predicted by gonad maturity (follicle-oviduct index; $Z = -3.71$, $P = 0.0002$) and DLF ($Z = -4.35$, $P < 0.0001$), and bursa index increased significantly with gonad maturity (Adjusted $R^2 = 0.1066$, $F = 13.76$, $P = 0.0003$) and DLF (Adjusted $R^2 = 0.16$, $F = 23.36$, $P < 0.0001$). In males, bursa presence was weakly predicted by gonad maturity (testis index; $Z = -1.76$, $P = 0.08$). However, testis width did not significantly predict bursa presence ($Z = -1.57$, $P = 0.12$), and bursa index did not change significantly with testis index (Adjusted $R^2 = 0$, $F = 0.1$, $P = 0.75$) nor testis width (Adjusted $R^2 = 0$, $F = 0.03$, $P = 0.859$). Based on these results, age classifications were assigned using a set of rules that combined gonad maturity and bursa in females and relied solely on bursa in males.

Western Grebes from the Monterey Bay red tide event were female-biased (G^2 = 4.48, df = 1, P = 0.034) with 1.86 females per male, and Clark's Grebes from the same event were male-biased (G^2 = 6.06, df = 1, P = 0.014) at 0.32 females per male. Western Grebes from the *Kure* and VOBI, and Clark's Grebes from VOBI, showed no biased sex ratio (Table 1). No difference in sex ratio was found between Western Grebes that were brought in alive to the rehabilitation center and subsequently died (live on arrival, "LOA") and those that arrived dead ("DOA"; note some may have been collected alive) for any event (VOBI: χ^2 = 0.03, df = 1, p = 0.86, 15 DOA, 171 LOA; SCMS: χ^2 = 2.20, df = 1, P = 0.14, 27 LOA, 24 DOA; and *Kure*: χ^2 =0.05, df = 1, P = 0.83, 12 DOA, 11 LOA).

Age ratio varied among events and species (Table 2). There was no significant bias towards either age class for Clark's Grebes of either event, nor for Western Grebes from the red tide event. Western Grebes were significantly biased towards immatures at the *Kure* spill (G^2 = 18.29, df = 1, $P < 0.0001$) and towards adult from VOBI (G^2 = 46.68, df = 1, $P < 0.0001$). Sex ratio of VOBI Western Grebes was significantly different between age classes (χ^2 = 8.78, df = 1, P = 0.003), with adults showing no sex bias (1.13 females per male, n=100, G^2 = 0.36, df = 1, P = 0.55) but immatures including significantly more males than females (0.22 females per male, n=22, G^2 = 9.64, df = 1, P = 0.002; Figure 2). The sex ratio of Western Grebes killed in Monterey Bay also significantly differed between age classes (χ^2 = 9.94, df = 1, P = 0.002), with adults again showing no significant sex bias (0.54 females per male, n = 20, G^2 = 1.83, df = 1, P = 0.18), and immatures including significantly more females than males (3 females per male, n = 28, G^2 = 7.33, df = 1, P = 0.007; Figure 2).

DISCUSSION

Age and Sex Structure

The biased age and sex ratios from these events indicate that Western and Clark's Grebes do undergo some degree of differential wintering. Western Grebes from southern California were adult-biased, showed no bias in sex ratio overall or among adults, and were male-biased within immatures. In Monterey Bay, Clark's Grebes were male-biased, while the female-bias in Western Grebes was apparently driven by the skewed sex ratio in immatures as adults showed no sex bias. In Humboldt Bay, immature Western Grebes were more common than adults, and overall they showed no differential sex ratio.

Interpreting the lack of an observed differential sex ratio in adults is complicated by a number of factors. One factor is the predominantly east-west migration of these inland-breeding and coastal-wintering birds compared to the north-south distributions in many such studies on which the theories of differential wintering are based (Belthoff and Gauthreaux 1991). As a consequence, birds wintering in very disparate regions may be equidistant from the same breeding colonies, which confounds the theories of differential wintering that involve certain classes vying to be closer to breeding sites. A second factor is that some if not all wintering populations in a region are made up of mixed breeding populations (see Chapter 2 and Eichhorst 1992); however, migratory patterns, migratory connectivity and distributional mechanisms are poorly understood in Western and Clark's Grebes (Storer and Nuechterlein 1992). This is poorly understood in most avian species,

including those with similar life history patterns (loons, scoters, other grebes). This niche

of inland-breeders and coastal-winterers may follow different patterns then most other

migratory species. Knowledge of migratory connectivity patterns is needed (Carter 2003).

Another mechanism potential reducing differential wintering of the sexes in adults may

be the initiation of courtship in Western and Clark's Grebes during the nonbreeding

season (pers. obs., Storer and Nuechterlein 1992). Although such courtship behaviors are

observed on the coast, it is undocumented if actual pair formation begins at this stage. If

this was an important component of pair formation, though, one would predict an equal

distribution of adult males and females in their wintering areas.

Bursa of Fabricius

Our assessment of bursa data reveals its utility in Western and Clark's Grebes, but

its applications were not perfect and need some additional validation. Comparing the

bursa data with female gonad maturity, not all birds could be classified as immatures if

they had bursas or as adults if they lacked bursas. Because the bursa atrophies within 1-2

years of life for most species (King 1956), some immatures or subadult birds are

expected to lack bursas but still have immature gonads. Some inconsistencies between

bursa and gonad maturity were observed that could not be explained, and these birds

(15% of females) we classified as unknown age. The relationship between testes and

bursa indices in males was less clear, in part because the continuous instead of

categorical measurements of male gonads prevented easy placement of birds into age

classes the way combined bursa, DLF and oviduct scores allowed in females. Therefore,

males were aged simply by bursa, recognizing that there was likely an error rate to these

classifications. Although grebes may breed in their second calendar year, age at first breeding is not well-documented (Storer and Nuechterlein 1992) and consequently subadult females in this sample were likely classified as immature (birds with immature gonads and no bursa) or were excluded from analyses due to conflicting characteristics; and subadult males with already atrophied bursas were likely misclassified as adults. Although bursa may atrophy by the spring of the second year (McCloskey and Thompson 2000), it is more biologically appropriate to lump potential subadults in with true immatures than to potentially lump immature birds with atrophied bursas with adults. Despite the potential misclassification of some subadult males as adults, the results of this study (that the only grouping with a significant adult-bias was the VOBI Western Grebes with so extremely adult-biased that this is not likely the consequence of including some subadults whose bursas had already undergone atrophication; and that no adult grouping was significantly male-biased) suggest the limited impacts of such misclassifications on overall patterns. Nonetheless, our intention is to more carefully study bursa in the near future in an attempt to increase our understanding of its atrophication in grebes and its application to ageing; further necropsies will be performed on numerous carcasses that have been collected by rehabilitation centers with special attention to inconsistencies.

Conclusions

Assessing birds collected in events such as those discussed here provides unique opportunities to improve both our understanding of the impacts of the event as well as the demographics of local species or populations. Proper documentation of the wildlife that

are impacted by such events, including the carcasses found on beaches (Heubeck *et al.* 2003, PRBO and OWCN 2005, Humple *et al.* 2007b), is critical to such endeavors. Although studies using beached bird censuses and rehabilitation data can also provide important demographic information, birds killed or debilitated by oil spills likely provide a more accurate picture of the demographics of the birds in the region because fitness plays a lesser role in vulnerability to oiling. Unlike starvation and disease, oil spills is unlikely to have a biased impact toward any age class or sex (Nevins and Carter 2003), and we assume that birds killed in these events were representative of the populations using these nearshore waters. However, age or sex ratios from oiled birds may not reflect the actual population if the spill is highly localized within a broader area and if the classes distribute themselves or flock unequally (Iverson *et al.* 2004). This may even be true for species ratios; Clark's Grebes, which are less common than Western Grebes overall (Storer and Nuechterlein 1992) and in each of these events, reached their highest abundance relative to Western Grebes in the Monterey Bay red tide event at 31% but were an estimated 5-10% of birds surveyed in Monterey Bay in 1999-2000 (Henkel 2004). To reduce bias, all available carcasses of appropriate quality were examined and samples were randomly chosen for genetic sexing; additionally, systematic searches of beaches were conducted during all three events. Other potential biases of perhaps greater concern include biases in age or sex classes in survival during rehabilitation; and, especially in species such as grebes that are hesitant to come on land, biases between birds that wash up dead versus beach themselves alive. Although sample sizes were small in some instances, we found no difference in sex ratio between grebes brought in alive versus dead. These topics need further study in grebes and other species in order to

improve our understanding of the biology of the species, of rehabilitation science, and rehabilitation success.

This study is only a stepping-stone to understanding age and sex distributions in wintering Western and Clark's Grebes and to providing data that may improve modeling of demographic impacts of oil spills. However, the large sample sizes of birds from the Ventura Oiled Bird Incident provides compelling support for the patterns observed in southern California, and the large number of adults killed in that event is of concern. These overall patterns suggest that oil spills likely have differential demographic effects in wintering grebes, depending upon which wintering area is affected. It remains unknown which breeding populations are represented by these oiling events. Further studies will help determine if these observations represent local patterns in both time and space, or larger patterns for these two species that are highly vulnerable to oiling.

ACKNOWLEDGEMENTS

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TABLES AND FIGURES

Figure 1. Locations of three coastal events from which age and sex ratios of Western and Clark's Grebes were assessed.

Figure 2. Combined age and sex ratios for Western Grebes killed in the Ventura Oiled Bird Incident (VOBI) and the Monterey Bay red tide plumage-fouling event.
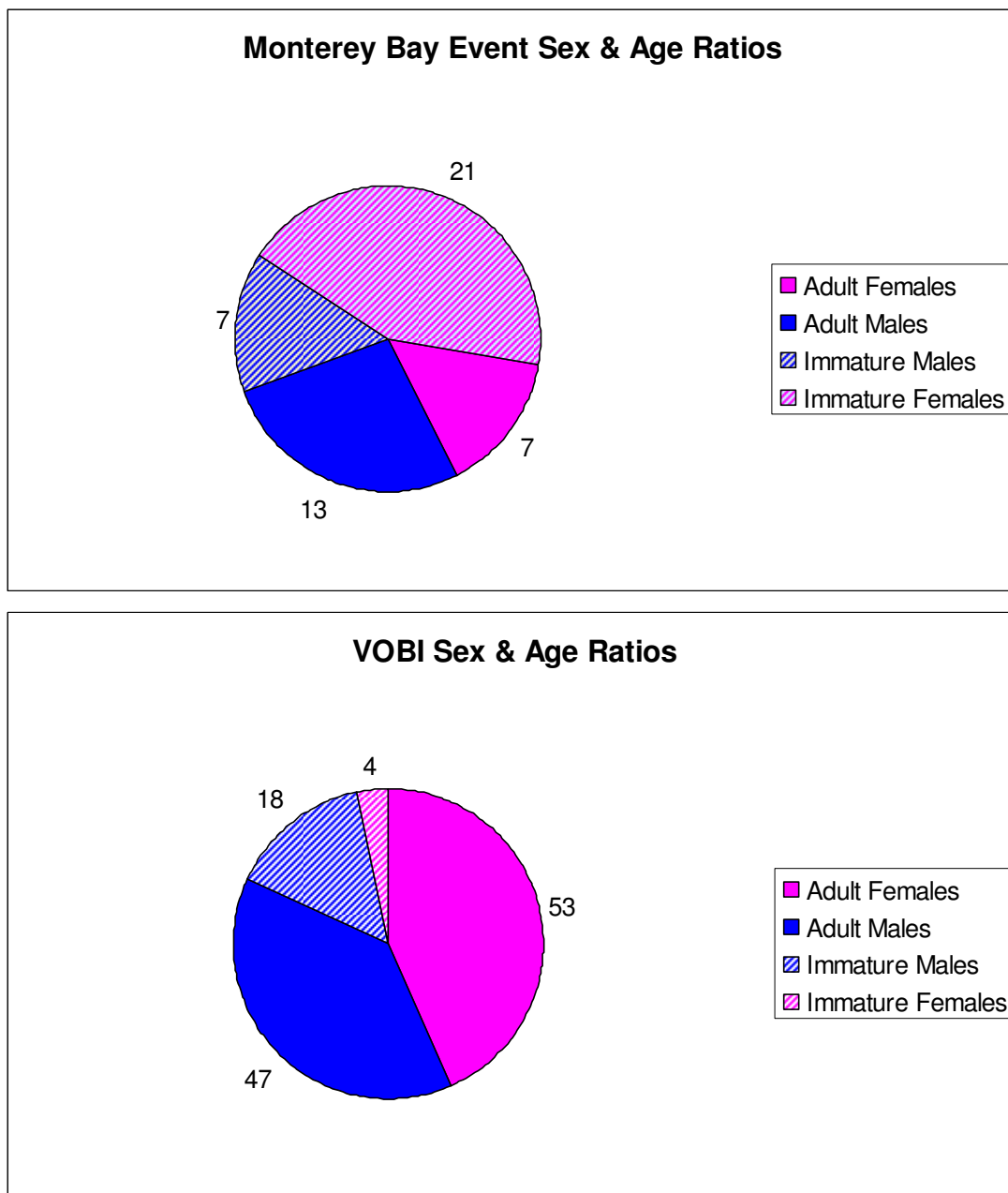
Table 1. Sex ratios of Western and Clark's Grebes killed in three California events.

Spill/Event	Prop. Sex Ratio (F:M)	Sex Ratio (F per M)	N	G^2	P
Western Grebe					
Kure	0.52:0.48	1.08:1	23	0.04	0.83
Monterey red tide	0.65:0.35	1.86:1	51	4.48	0.034*
VOBI	0.48:0.52	0.95:1	186	6.06	0.77
Clark's Grebe					
Monterey red tide	0.24:0.76	0.32:1	21	6.06	0.014*
VOBI	0.50:0.50	1:1	14	0	1

Table 2. Age ratios of Western and Clark's Grebes killed in three California events.

Spill/Event	Prop. Age Ratio (Imm : Adult)	Age Ratio (Imm per Adult)	N	G^2	P
Western Grebe					
Kure	0.91 : 0.09	10.5 : 1	23	18.29	<0.0001
Monterey red tide	0.53 : 0.47	1.13 : 1	51	0.78	0.38
VOBI	0.21 : 0.79	0.27 : 1	135	53.99	<0.0001
Clark's Grebe					
Monterey red tide	0.33 : 0.67	0.58 : 1	19	1.33	0.25
VOBI	0.30 : 0.70	0.43 : 1	10	1.65	0.20