DEMOGRAPHICS OF *AECHMOPHORUS* GREBES KILLED IN THREE MORTALITY EVENTS IN CALIFORNIA

DIANA L. HUMPLE[1,2], HANNAHROSE M. NEVINS[3,4], ELIZABETH M. PHILLIPS[3,5], CORINNE GIBBLE[3], LAIRD A. HENKEL[6], KATHERINE BOYLAN[2] & DEREK J. GIRMAN[2]

[1]*PRBO Conservation Science, 3820 Cypress Drive #11, Petaluma, California, 94954, USA (dhumple@prbo.org)*
[2]*Sonoma State University, 1801 E Cotati Ave, Rohnert Park, California, 94928, USA*
[3]*Moss Landing Marine Laboratories, 8272 Moss Landing Road, Moss Landing, California, 95039, USA*
[4]*California Department of Fish and Game Marine Wildlife Veterinary Care and Research Center,*
1451 Shaffer Road, Santa Cruz, California, 95060, USA
[5]*Cooperative Institute for Marine Resources Studies, Oregon State University, 2030 Marine Science Drive, Newport, Oregon, 97365, USA*
[6]*California Department of Fish and Game Office of Spill Prevention and Response, 20 Lower Ragsdale Drive, Suite 100, Monterey, California, 93940, USA*

SUMMARY

HUMPLE, D.L., NEVINS, H.M., PHILLIPS, E.M., GIBBLE, C., HENKEL, L.A., BOYLAN, K. & GIRMAN, D.J. 2011. Demographics of *Aechmophorus* grebes killed in three mortality events in California. *Marine Ornithology* 39: 235–242.

The demographic consequences of oil spills or other mortality events for breeding seabird populations depend on the age and sex composition of the affected population. Differential wintering by sex and age classes occurs in many migratory bird species, and can have important conservation implications. We examined the age and sex ratios of Western Grebes *Aechmophorus occidentalis* and Clark's Grebes *A. clarkii* that died in three mortality events in southern, central and northern California. The most significant of these events for grebes was the Ventura Oiled Bird Incident (VOBI), an oil spill in southern California in 2005 in which approximately 2500 grebes were killed. The other two events examined included a harmful algal bloom in the Monterey Bay of central California in 2007, and the *Kure* oil spill in Humboldt Bay, northern California in 1997. Age and sex of birds was determined by examining gonads and the bursa of Fabricius and assessing chromobox-helicase-DNA-binding sex genes. Bursal involution was evaluated against gonad maturity to determine its value in ageing grebes. While we found bursal involution to be a useful age estimator, some observed patterns warrant further study in grebes. Age and sex ratio patterns varied among the three mortality events and between species, and indicate some degree of differential wintering or local partitioning. For example, Western Grebes killed during VOBI were predominantly adult (79%) with no differences in overall sex ratio; immatures had a male-biased sex ratio. Opportunities to examine demographic patterns in oiled wildlife are frequently overlooked but can be critical to fully understanding and predicting impacts of oil spills, and to directing recovery activities. Because oil spill events tend to be unselective, they also offer a rare opportunity to assess demographic parameters in wintering seabirds.

Key words: Western Grebe, Clark's Grebe, oil spill, Ventura Oiled Bird Incident, differential wintering, harmful algal bloom, bursa of Fabricius, sex ratio, age ratio

INTRODUCTION

Oil spills in coastal California are a conservation concern for many seabird species and are among the top threats to wintering populations of *Aechmophorus* grebes. Western Grebes *A. occidentalis* and Clark's Grebes *A. clarkii* are sister taxa that breed in freshwater lakes throughout western North America and predominantly spend the nonbreeding season in the nearshore marine environment, where they are vulnerable to oiling (Storer & Nuechterlein 1992). In California, Western Grebes are one of the main species impacted by oil spills (California Department of Fish and Game [CDFG] 1969a,b; Straughan 1970, 1971; American Trader Trustee Council 2001; Hampton *et al.* 2003; Carter 2003; CDFG *et al.* 2004; CDFG & US Fish and Wildlife Service [USFWS] 2008; Humple 2009; Oiled Wildlife Care Network [OWCN] / PRBO Conservation Science unpubl. data). Understanding demographic parameters — such as sex and age ratios — of impacted populations is critical to assessing immediate and potential long-term effects of oil

spills (Wiens *et al.* 1984, Heubeck *et al.* 2003) and to determining appropriate restorative actions (Nur & Sydeman 1999).

Differences between the sexes or age classes in wintering distribution or migration has been documented in many migratory bird species (Ketterson & Nolan 1976, Belthoff & Gauthreaux 1991, Olson & Arsenault 2000, Nebel *et al.* 2002, Álvarez & Pajuelo 2004, Humple *et al.* 2007a, Schamber *et al.* 2007). There are several theories explaining differential wintering in migratory birds, including behavioral dominance, resource partitioning, physiological constraints, and the preference of one age class or sex to winter closer to breeding grounds (Myers 1981, Ketterson & Nolan 1983, Fernandez & Lank 2006). This phenomenon can have important implications if mortality events disproportionately affect a certain sex or age class in a given wintering or migratory area (Coulson *et al.* 2001). Potential repercussions for populations affected by oil spills include reduced future productivity at associated breeding areas. For example, if the age ratio in a wintering population is

biased towards adult birds, a spill in that region would likely have a greater and more immediate impact on breeding populations and productivity than events that cause mortality in immature birds (Anker-Nilssen *et al.* 1988, Nevins & Carter 2003, Humple *et al.* 2007) because immature birds experience higher natural mortality than adults (Gill 2001) and generally will not contribute as much to population productivity. Furthermore, as many seabirds undergo delayed reproduction, significant mortality of immature birds can result in reduced recruitment (Votier *et al.* 2008) and delayed impacts on breeding populations. Differences in vulnerability between sexes could also affect subsequent productivity (Stenzel *et al.* 2011). In addition, the local partitioning of birds by sex or age class within a wintering region can have similar repercussions when mortality events occur. Despite the potential population-level impacts of oil spills on *Aechmophorus* grebes in California, their wintering age and sex structure has not been previously examined.
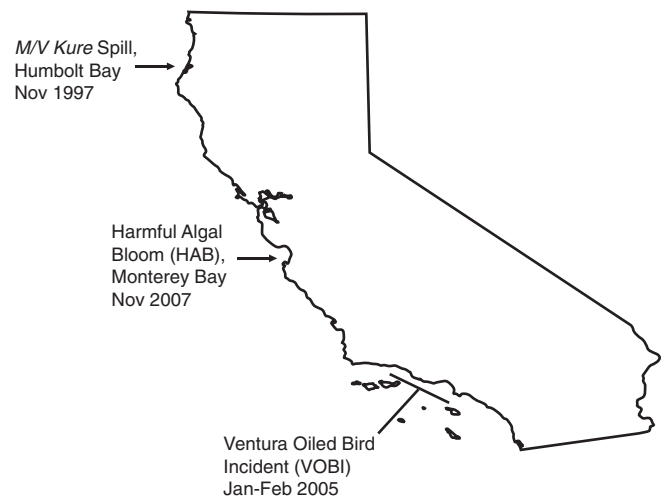
Most studies of differential wintering are limited to species that exhibit distinct sexual dimorphism or age-specific plumages or morphometric patterns that can be easily discerned from visual observation. Oil spills provide two unique opportunities to assess differential wintering and overall age and sex structure of a population. First, as oil spills affect birds using nearshore waters in a relatively unselective way, the patterns of age and sex ratios of birds collected during these events can be considered representative of the local populations (Álvarez & Pajuelo 2004). Second, large sample sizes are usually collected, and age and sex can be determined based on an internal examination in addition to morphometrics or plumage, especially in species for which external characteristics are uninformative. In addition, sex can be determined genetically from tissue samples.

In this study we examined the age and sex structure of Western and Clark's grebes killed in three mortality events (Fig. 1):

1) An oil spill in November 1997 when the fuel tank of the *M/V Kure* was punctured at a loading dock and spilled approximately 17 000 L (4500 gallons) of intermediate fuel oil into Humboldt Bay in northern California. Response personnel collected 960 birds in total, including 33 Western and 2 Clark's grebes, and mortality was estimated to be 75 birds for both grebe species combined (CDFG & USFWS 2008, S. Hampton, pers. comm.), the lowest of the three events examined here.

2) The Ventura Oiled Bird Incident (VOBI) in southern California in January and February 2005, when unusually heavy rains caused a natural oil seep on Santa Paula Creek, Ventura County, California, to spill into marine coastal areas. As a result, 1326 dead or debilitated Western Grebes, 74 Clark's Grebes and 13 unidentified grebes were collected from southern coastal California (Santa Barbara County south to Redondo Beach in Los Angeles County). Western Grebes accounted for 87% of all birds collected during this event. Approximately 1160 *Aechmophorus* grebes were found dead or died in care, and the remaining grebes were rehabilitated and released (OWCN/PRBO, unpubl. data). Total *Aechmophorus* mortality was estimated at approximately 2500 birds (S. Hampton, pers. comm.).

3) An unusual mass seabird stranding and mortality event related to a harmful algal bloom (HAB) in Monterey Bay, central California, in November 2007. Originally identified as a "mystery spill" due to the strange nature of plumage fouling of affected

birds, laboratory results later determined that the novel event was caused by an algal bloom of the dinoflagellate *Akashiwo sanguinea*, which produced a proteinaceous foam that coated the birds' feathers, resulting in loss of waterproofing and hypothermic conditions comparable to petroleum-induced effects (Jessup *et al.* 2009). At least 90 Clark's Grebes and 200 Western Grebes were collected (CDFG unpubl. data); overall mortality estimates have not been produced. The impacts and unselective nature of this event were similar to those of an oil spill and thus provide an opportunity for comparison of demographic patterns among regions and events. Although harmful algal blooms are unusual, there has been at least one other documented mass seabird mortality event caused by the same type of HAB, in Oregon and Washington in 2009 (Phillips *et al.* 2011).

Bursa and gonad maturity are often examined during necropsies to age carcasses that are otherwise difficult to classify (e.g. due to oiling of plumage or lack of other reliable criteria). The bursa of Fabricius (*bursa fabricii*) is an immune organ that typically involutes or atrophies during the first year or two of a bird's life, making it useful for estimating age. It has been found to be a good indicator of age in a broad range of avian species (e.g. Hohman & Cypher 1986, Broughton 1994, Mather & Esler 1999), and the method has been frequently applied (e.g. Anderson *et al.* 1969, McCloskey & Thompson 2000, Nevins & Carter 2003, Phillips *et al.* 2010). The presence of a prominent bursa indicates a young bird, its absence indicates an adult or subadult bird whose bursa has undergone involution, and an intermediate-sized bursa generally indicates an immature (or in some cases subadult) bird manifesting a currently involuting bursa. The bursa can be examined on live birds in some larger species that also have cloacal openings to the bursa (McNeil & Burton 1972), or on carcasses when performing necropsies or preparing skins (Broughton 1994). Western and Clark's grebes are difficult to age using subtle changes in plumage, especially when examining oiled, fouled or scavenged birds. The bursa may therefore be the most consistent method for ageing grebe carcasses (Storer & Nuechterlein 1992). However, there is species-specific variation in bursa involution rate, and associated challenges in classifying some individuals or species based solely on bursa (Fredrickson 1968, McNeil & Burton 1972, Siegel-Causey 1990); therefore, it is important to assess the utility of this ageing technique

M/V Kure Spill, Humbolt Bay Nov 1997

Harmful Algal Bloom (HAB), Monterey Bay Nov 2007

Ventura Oiled Bird Incident (VOBI) Jan-Feb 2005

Fig. 1. Locations of three coastal California mortality events from which age and sex structure of Western and Clark's grebes was assessed.

before applying it to a novel species. Furthermore, it should be used in combination with other ageing criteria when possible. Although the bursa has been used to age *Aechmophorus* grebes (Livezey & Storer 1992), there is a lack of published validation of its species-specific utility. Thus, we sought to compare bursa size to gonad maturity to determine its usefulness in age assessment for these grebe species.

Our objective was to evaluate and compare the sex and age structure of wintering grebes affected in three mortality events in California (Fig. 1). We also sought to evaluate the use of the bursa of Fabricius as an ageing indicator relative to gonad maturity in *Aechmophorus* grebes, using grebes killed in these three events as well as others opportunistically collected in central California.

METHODS

While other oil spills have impacted grebes in California, the mortality events in this study were chosen because of their impact on grebes and because of the availability of carcasses originally stored as evidence. During each of the three mortality events studied, dead and debilitated birds were systematically searched for and collected in coastal and estuarine areas by the Oiled Wildlife Care Network (OWCN), International Bird Rescue, and/or California Department of Fish and Game Office of Spill Prevention and Response (CDFG-OSPR). All birds were given unique log numbers at the time of collection with a prefix indicating whether they arrived at the facility alive or dead (see PRBO & UCD-WHC 2009). Dead birds were frozen soon after collection, or after dying in captivity for those collected alive. Ten percent of carcasses from VOBI, and all carcasses from Monterey Bay HAB and *Kure* events, were retained and available for necropsies. A subset of the available carcasses (4%) was not necropsied because of their poor condition (e.g. due to scavenging or decomposition). Necropsies were conducted in 2008 and 2009, between 1.5 and 11 years after each of the events, at the CDFG-OSPR Marine Wildlife Veterinary Care and Research Center (MWVCRC) in Santa Cruz, CA. We also examined a fourth sample of unoiled grebe carcasses from the Monterey Bay area that were found dead and collected by the Coastal Ocean Mammal and Bird Education and Research Surveys (BeachCOMBERS) Program, Moss Landing Marine Laboratories, or were provided by local rehabilitation centers (Society for Prevention of Cruelty to Animals of Monterey County and International Bird Rescue in Fairfield, CA). These carcasses were necropsied between 2008 and 2010 to determine whether patterns of bursa and gonad maturity in females were comparable to those affected in the three mortality events used in this study.

Necropsies and measurements followed standardized MWVCRC protocols used for all avian species (modified from van Franeker 2004). Following external examination, birds were dissected and their gonads and bursa examined. Length and width of the ovary or left testis and diameter of largest ovarian follicle (DLF) were measured; the development of the oviduct was scored (1–4); the presence (prominent, apparent or membranous) or absence of the bursa was determined; and, when present, bursa length and width were measured. Age was classified after analyses allowed us to develop a set of rules for bursa and gonad maturity (see *Statistical analyses*).

We used genetic techniques to determine the sex of additional VOBI birds whose carcasses were not available for necropsies, using randomly selected muscle samples collected during the spill in 2005 by the Wildlife Processing Unit (managed by PRBO and OWCN).

Sex was determined by assessing two chromobox-helicase-DNA-binding genes (CHD-W and CHD-Z), highly conserved intron loci anchored in two exogenic regions that are effective in sex determination throughout the class Aves (Griffiths *et al.* 1998). Polymerase chain reaction (PCR) with primers for each gene was conducted in the Sonoma State University laboratory. PCR products were run on high-density (8%) acrylamide gels, and the number of bands was assessed; individuals with one band (homozygous) were classified as male and those with two bands (heterozygous) as female. Calibration of these approaches was conducted on five individuals that were also necropsied, and sex classification was identical between the two techniques. To assess sex ratio in VOBI grebes, data from genetically sexed individuals were combined with data from birds sexed by gonad examination.

Statistical analyses

We excluded the single Clark's Grebe from Humboldt Bay and any *Aechmophorus* grebes identified only to genus from statistical analyses. Gonad measurements were converted into the following sexual maturity indices for analysis (from van Franeker 2004): testis index of male gonad maturity = testis length × width; follicle-oviduct index of female gonad maturity = diameter of largest follicle (0.1 mm for those with undeveloped follicles) × oviduct development code; and bursa index = bursa length × width (0 if no visible bursa).

Sex ratios were calculated as the proportion of females to males. A two-sided goodness of fit model was used to determine whether the observed distribution of sex ratio deviated from 1:1. A Pearson χ^2 test was used to determine whether sex ratios differed among the three events for each species. When sample sizes allowed (>10 birds per group), the proportional sex ratios of birds that arrived alive (and subsequently died) and birds that arrived dead were compared with a Pearson χ^2 test to determine whether there was a sex bias in vulnerability to oil.

Logistic regression analysis was used to test whether gonad maturity indices predicted presence or absence of bursa, and linear regression analysis 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 two broad classifications, adult and immature; a third intermediate classification, subadult, was possible to use only in females. Females were classified as adult if they had mature gonads and atrophied bursae; as subadult if they had bursae but one or both gonad components (DLF and oviduct score) were mature; as immature if they had undeveloped gonads, with or without bursae; and as unknown-aged if they had atrophied bursae and conflicting gonad criteria (one mature, one immature). Males were classified as adults if they had atrophied bursae or as immature if they had prominent bursae. Although we expected mature males to have larger testes than young males, we observed no clear cutoff in the testis index, and consequently did not use gonad maturity indices to age males. Thus, male age classifications are based only on bursal examination, and we could not assign any to the subadult classification.

Age ratios were calculated as the proportion of immature to adult birds. Lack of data on productivity and age-specific survival prevented testing for whether age ratios deviated from an expected age structure. A Pearson χ^2 test was used to determine whether age ratios differed among events for each grebe species. To evaluate whether seemingly conflicting patterns in female bursal and gonad

measurements were due to observer error or represented biological reality, we used a Pearson χ^2 test to compare the rate at which female grebes had criteria of bursa presence and mature gonads in the VOBI and Monterey Bay HAB events among observers with little, moderate or extensive necropsy experience.

A Pearson χ^2 test was used to evaluate whether the sex ratio differed within each age class for Western Grebes from VOBI and the Monterey Bay HAB, where sample sizes were greater than 20 birds in each group. All analyses were done using the software program R (Vers. 2.9.1; R Development Core Team 2009).

RESULTS

Western Grebes outnumbered Clark's Grebes in all three events, accounting for approximately 95% of all *Aechmophorus* grebes collected during VOBI, 69% of those collected in the Monterey Bay HAB and 94% of those collected in Humboldt Bay. Necropsies were conducted on 151 grebe carcasses from VOBI, 78 grebes from Monterey Bay HAB and 27 grebes from *Kure*. An additional 53 grebes from VOBI were sexed genetically. Necropsies were also conducted on 62 unoiled grebes, unrelated to these three events, obtained from BeachCOMBERS and rehabilitation centers in central California.

We found no biased sex ratio in Western Grebes collected in VOBI or Humboldt Bay, while there was a female bias in the Monterey Bay HAB ($G = 4.48$, $P = 0.034$; Table 1). The sex ratios in Western Grebes differed among the three events (Pearson's $\chi^2 = 50.3$, $P < 0.0001$). We found no biased sex ratio in Clark's Grebes from VOBI but a male bias in those from the Monterey Bay HAB ($G = 6.06$, $P = 0.014$), although there were no significant differences between events (Pearson's $\chi^2 = 0.008$, $P = 0.9$). No differences in sex ratio were found between Western Grebes that were brought in alive to the rehabilitation center and subsequently died (live on arrival, "LOA") and those brought in dead ("DOA"; predominantly collected dead, otherwise died en route) for any event (VOBI: Pearson's $\chi^2 = 0.03$, $P = 0.86$, 15 DOA, 171 LOA; Monterey Bay HAB: Pearson's $\chi^2 = 2.20$, $P = 0.14$, 27 LOA, 24 DOA; and *Kure*: Pearson's $\chi^2 = 0.05$, $P = 0.83$, 12 DOA, 11 LOA).

In females, bursa presence was predicted by gonad maturity (follicle-oviduct index; $Z = -3.71$, $P = 0.0002$), and bursa index decreased significantly with gonad maturity (adjusted $R^2 = 0.1066$, $F = 13.76$, $P = 0.0003$). Seventy-two percent of females were consistently aged when using solely bursa or gonads. In males, bursa presence was weakly predicted by gonad maturity (testis index; $Z = -1.76$, $P = 0.08$); however, bursa index did not change significantly with testis index (adjusted $R^2 = 0$, $F = 0.1$, $P = 0.75$). Testis index overlapped in range almost completely between males with a prominent bursa (index = 18–77) and males with no bursa (index = 16–90).

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. Females aged as immature based on undeveloped gonads but that had no visible bursae (already involuted) accounted for 21% of the females assigned to the immature age class. Although immature males could also have early-atrophied bursae, we could not assess whether involution timing relative to gonad maturation is similar between sexes. Fifteen percent of females (VOBI, n = 12; Monterey Bay HAB, n = 5) had visible bursae and mature gonads (DLF and/or oviduct score) and were classified as subadult. This proportion was comparable to that of unoiled females from central California from opportunistic sampling unrelated to these three events (17%, n = 24). The rate at which females were classified as subadult based on these criteria did not vary significantly with observer experience (Pearson's $\chi^2 = 0.58$, $P = 0.75$); it was 17% for observers with considerable experience (n = 18), 10% for observers with moderate experience (n = 20) and 17% for observers with minimal prior experience (n = 65).

Age ratios varied among events and between species (Table 2). Western Grebes were adult-biased in VOBI, showed no age bias in the Monterey Bay HAB and were immature-biased in Humboldt Bay. However, the age ratios of Western Grebes were not significantly different among events (Pearson's $\chi^2 = 4.56$, $P = 0.1$). The sex ratio of VOBI Western Grebes was significantly different between immature and adult age classes (Pearson's $\chi^2 = 8.78$, $P = 0.003$); there was no sex bias in adult birds (n = 100, $G = 0.36$, $P = 0.55$) but a male bias in immature birds (82% male, n = 22,

TABLE 1
Proportional sex ratios (females:males) of Western and Clark's grebes killed during three mortality events in California

Species, spill/event	Sex ratio (F:M)	n	G^a	P
Western Grebe				
Southern California (VOBI)	0.48:0.52	186	6.06	0.77
Central California (Monterey Bay HAB)	0.65:0.35	51	4.48	0.034[b]
Northern California (*Kure*)	0.52:0.48	23	0.04	0.83
Clark's Grebe				
Southern California (VOBI)	0.50:0.50	14	0	1
Central California (Monterey Bay HAB)	0.24:0.76	21	6.06	0.014[b]

[a] *G*-statistic from two-sided goodness of fit test.
[b] Significant at $P < 0.05$.

TABLE 2
Proportional age ratios (immature:adult) of Western and Clark's grebes killed during three mortality events in California[a]

Species, spill/event	Age ratio (immature:adult)	n
Western Grebe		
Southern California (VOBI)	0.21:0.79	135
Central California (Monterey Bay HAB)	0.53:0.47	51
Northern California (*Kure*)	0.91:0.09	23
Clark's Grebe		
Southern California (VOBI)	0.30:0.70	10
Central California (Monterey Bay HAB)	0.32:0.68	19

[a] Female grebes tentatively classified as subadult (VOBI, Western n = 12, Clark's n = 1; Monterey Bay HAB, Western n = 5, Clark's n = 1) were excluded from this table as subadults could not be classified in males.

$G = 9.64$, $P = 0.002$; Figure 2). The sex ratio of Western Grebes from the Monterey Bay HAB also differed significantly between immature and adult age classes (Pearson's $\chi^2 = 9.94$, $P = 0.002$), where adult birds again had no significant sex bias (n = 20, $G = 1.83$, $P = 0.18$), but immature birds were 75% female (n = 28, $G = 7.33$, $P = 0.007$; Figure 2). In Clark's Grebes, slightly more adult birds were killed in both VOBI and the Monterey Bay HAB (Table 2), and the age ratios were not significantly different between events (Pearson's $\chi^2 = 2.56$, $P = 0.1$).

DISCUSSION

Age and sex structure

Our examination of the age and sex structure of wintering populations of Western and Clark's grebes killed in multiple mortality events indicate that these grebes likely have some degree of differential wintering in California. We found that Western Grebes killed in VOBI in southern California were predominantly adult (79%), while those from the Monterey Bay HAB showed no bias towards either age class (53% adult), and those from the *Kure* spill in Humboldt Bay were mostly immature birds (91%). This pattern suggests that adult Western Grebes may winter further south than immature birds in California coastal waters. We found evidence that differential wintering of the sexes in Western Grebes may be limited to the immature age class: 75% of immature birds in the Monterey Bay HAB and 18% of immature birds in VOBI were female. The adult age classes from both events showed no sex bias, and the marginally significant overall female-biased sex ratio in the Monterey Bay HAB was driven by this female bias in immatures. In Humboldt Bay, Western Grebes showed no differential sex ratio. In comparison, Phillips *et al.* (2011) found no sex or age bias in Western Grebes killed by a HAB event in Oregon in October 2009. Although sample sizes of Clark's Grebes were small, we did observe a male bias (76%) in the Monterey Bay HAB that was not observed in VOBI, and an adult bias (68% and 70%, respectively) in both events.
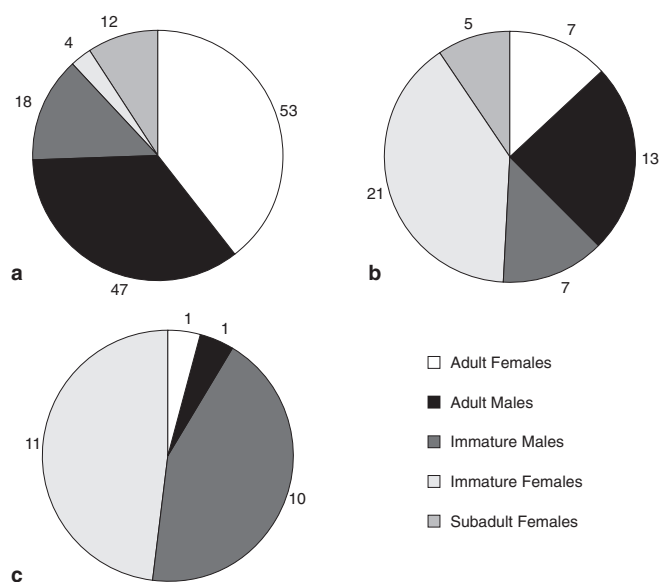
Interpretation of sex and age structure in wintering grebes is complicated by a number of factors. Grebes migrate in a predominantly east-west pattern from inland breeding areas to coastal wintering grounds, in contrast to the north-south distributions observed in many studies that form the basis of differential wintering theories (Belthoff & Gauthreaux 1991). As a consequence, birds wintering in geographically disparate coastal regions may actually be equidistant from the same breeding colonies, confounding the theories that differential wintering involves certain sex or age classes preferring to be closer to breeding sites. Also, wintering populations may represent a combination of multiple breeding populations because migratory connectivity may not be strong in these species (Eichhorst 1992, Humple 2009). However, migratory patterns, migratory connectivity and distributional mechanisms are poorly understood in grebes (Storer & Nuechterlein 1992, Eichhorst 1992, Humple 2009), and increased knowledge of migratory connectivity patterns is needed (Carter 2003).

Another mechanism that may influence wintering patterns of adults is the potential initiation of courtship in Western and Clark's grebes during the nonbreeding season. Courtship behaviors are observed on the coast (Storer & Nuechterlein 1992, D. Humple pers. obs.), but it remains unknown whether successful pair formation begins at this stage, whether it is prevalent, or whether pair bonding is maintained in the nonbreeding season as in some waterfowl (Savard 1985, Smith *et al.* 2000). If nonbreeding courtship is an important component of pair formation, or nonbreeding pair bond maintenance occurs, an equal distribution of adult males and females in their wintering areas should occur, as we observed. In addition, natal breeding dispersal distances differ between the sexes in some avian species, and females often disperse further than males (Greenwood 1980). If there is a relationship between breeding and wintering locales, this dispersal pattern may influence sex-specific wintering distributions in immature birds; however, this cannot be evaluated from the current state of knowledge (but see Eichhorst 1992, Humple 2009).

Observed patterns in age ratios could reflect differential overwintering survival. In VOBI, which occurred 2 to 3 months later in the season than the other events, Western Grebes included 79% adults, a higher proportion than in the other two events. Hence, the adult bias in VOBI could be related to increased winter mortality of immature birds (Gill 2001) rather than differential wintering. Other potential biases include differential survival of age or sex classes during rehabilitation, and biases in age- or sex-related fitness between birds that wash up dead and those captured live. We found no difference in sex ratios between grebes brought in alive versus dead, suggesting a lack of fitness-related sex-bias in stranding. There are also differences in habitat among the three events (predominantly estuarine for Humboldt Bay versus open water for the other two events) that could influence local partitioning patterns such that classes may distribute themselves or flock unequally within a region (Iverson *et al.* 2004). This may also be true for species ratios: Clark's Grebes are less common than Western Grebes throughout their range (Storer & Nuechterlein 1992) and were found in relatively low proportions in VOBI (5%) and Humboldt Bay (4%). In Monterey Bay, Clark's Grebes were in higher relative abundance in the HAB event (31%), but accounted for only an estimated 5–10% in prior surveys (Henkel 2004); differences may be due to inter- or intra-annual variation in species patterns or localized impacts of the event. Biased age ratio patterns could also reflect annual productivity differences among regions

Fig. 2. Proportion of sex and age classes for Western Grebes killed in: (a) the 2005 Ventura Oiled Bird Incident (n = 122), (b) the 2007 Monterey Bay harmful algal bloom (n = 48), and (c) the 1997 *Kure* spill in Humboldt Bay (n = 23).

Legend:
- □ Adult Females
- ■ Adult Males
- ▨ Immature Males
- ▢ Immature Females
- ▨ Subadult Females

in the preceding summers. We need to know more about dispersal and winter survival in grebes, indicating the need for banding and tracking studies.

Bursa of Fabricius

Our assessment of bursa data reveals its applicability in ageing Western and Clark's grebes; however, some patterns we observed—along with our inability to compare our results with known-aged banded birds—suggest its application warrants further validation. Age estimates based on bursa or gonads alone matched in 72% of females. The bursa typically atrophies within 1–2 years of life for many species (Wing 1956) and up to 4 years in longer-lived albatross (Broughton 1994), and is generally assumed to atrophy before reproductive age is reached (Glick 1983, Møller and Erritzøe 2001). Twenty-one percent of the females we classified as immature lacked bursa but had not yet developed mature gonads, supporting the idea that bursal involution occurs before gonad development in grebes. Including bursa as an ageing component also enabled us to estimate the age of an additional 17% of females that would have otherwise been unknown-aged if assessment were based on gonads alone (one characteristic mature, one immature). Although the bursa index itself decreased significantly with gonad maturity in female grebes, it had relatively low explanatory value.

We classified as subadult a subset (15%) of females that had mature gonads and visible bursae. This observation was not related to experience level of the observer (and therefore not likely due to errors in necropsy classification). We found the rate at which females demonstrated this pattern to be consistent between birds killed in these events and the sample of unoiled grebes opportunistically collected, suggesting these observations are not based on bursa regeneration following petroleum exposure, a process that has not been documented but has been speculated (Leighton 1984, Lowenstein & Fry 1985, Nevins & Carter 2003). All birds examined in this study, however, were of compromised health of some sort — whether due to oiling, plumage-fouling, starvation, injury, or disease; not enough is known about the potential regeneration of this immune organ during times of stress to be confident that bursae did not regenerate in these individuals, especially in the absence of other ageing criteria (e.g. plumage). Further study is required.

Because males were aged based on bursa alone, some subadult males were likely misclassified, although we cannot assume that bursa involution rates are similar between the sexes (Møller and Erritzøe 2001). Testis size during winter in *Aechmophorus* grebes may not accurately reflect age or sexual maturity, as it does with some other species (van Franeker 2004), and could not be used here.

Considering these age classification challenges, we recognize that our reported age ratios may be slightly biased, particularly in males, for which we lacked secondary ageing criteria. Although grebes may breed in their second calendar year, age at first breeding is poorly known (Storer & Nuechterlein 1992), and some subadult females in this sample may have been classified as immature (e.g. birds with immature gonads and no bursa). Other oil spill studies have used bursa for ageing, often in combination with other criteria (e.g. Nevins and Carter 2003, Cadiou *et al*. 2004). Because not enough is known in most species—including grebes—about the exact timing of bursa involution, we recommend additional ageing criteria be included in future studies whenever possible and that

opportunities to carefully study bursa in combination with plumage and molt patterns (*sensu* Pyle 2008) be pursued.

CONCLUSIONS

This study is a starting point to understanding age and sex distribution patterns in wintering Western and Clark's grebes. Such information can improve modeling of demographic impacts of oil spills, including the consequences that biased age- or sex-related mortality may have on future productivity. This information also has conservation value beyond the immediate oil spill events by improving our understanding of demographic patterns for the species. The large proportion of adult birds killed in VOBI is of concern, and the large sample size from that event provides compelling support for the patterns we observed. With so little known about grebe demographics, we cannot predict whether subsequent recruitment of younger birds to breeding colonies will compensate for this adult mortality (e.g. Votier *et al*. 2008), but knowledge of demographic processes will assist managers in evaluating oil spill impacts and determining the most effective population restoration programs (Wiens *et al*. 1984, Nur & Sydeman 1999). While the source populations represented in these mortality events remain unknown, band recovery patterns suggest the source of breeding birds in any wintering region is likely a combination of several regions (Eichhorst 1992, Humple 2009). Bursa involution and gonad development patterns, while useful, need further study and refinement for grebes. The high vulnerability to oiling for coastally wintering Western and Clark's grebes, and the unfortunate prevalence of such events, will provide further opportunity for such demographic studies.

REFERENCES

ÁLVAREZ, D. & PAJUELO, M.A.F. 2004. Differential migration in a wintering population of Common Guillemots *Uria aalge* affected by the *Prestige* oil spill. *Ardeola* 51: 451-454.

AMERICAN TRADER TRUSTEE COUNCIL. 2001. Final restoration plan and environmental assessment for seabirds injured by the American Trader oil spill. Report of the American Trader Natural Resource Trustee Council, US Fish and Wildlife Service, California Department of Fish and Game, and National Oceanic and Atmospheric Administration [Available online at: http://www.darrp.noaa.gov/southwest/amtrader/pdf/amtradefinal_restoreplanon.pdf; accessed 2 August 2011]

ANDERSON, B.W., KETOLA, T.E., & WARNER, D.W. 1969. Spring sex and age ratios of Lesser Scaup and Ring-Necked Ducks in Minnesota. *Journal of Wildlife Management* 33: 209-212.

ANKER-NILSSEN, T., JONES, P.H. & RØSTAD, O.W. 1988. Age, sex, and origins of auks (Alcidae) killed in the Skagerrak oiling incident of January 1981. *Seabird* 11: 28-46.

BELTHOFF, J.R. & GAUTHREAUX JR, S.A. 1991. Partial migration and differential winter distribution of House Finches in the eastern United States. *Condor* 93: 374-382.

BROUGHTON, J.M. 1994. Size of the bursa of Fabricius in relation to gonad size and age in Laysan and Black-footed albatrosses. *Condor* 96: 203-207.

CADIOU, B., RIFFAUT, L., MCCOY, K.D., CABELGUEN, J., FORTIN, M., GELINAUD, G., LE ROCH, A., TIRARD, C. & BOULINIER, T. 2004. Ecological impact of the "Erika" oil spill: determination of the geographic origin of the affected Common Guillemots. *Aquatic Living Resources* 17: 369-377.

CALIFORNIA DEPARTMENT OF FISH AND GAME. 1969a. Progress report on wildlife affected by the Santa Barbara Channel oil spill, January 28–March 31, 1969 [Unpublished report]. Sacramento, CA: California Department of Fish and Game.

CALIFORNIA DEPARTMENT OF FISH AND GAME. 1969b. Second progress report on wildlife affected by the Santa Barbara oil spill, April 1–May 31, 1969 [Unpublished report]. Sacramento, CA: California Department of Fish and Game.

CALIFORNIA DEPARTMENT OF FISH AND GAME, CALIFORNIA STATE LANDS COMMISSION & UNITED STATES FISH AND WILDLIFE SERVICE. 2004. Stuyvesant/Humboldt coast oil spill draft damage assessment and restoration plan.

CALIFORNIA DEPARTMENT OF FISH AND GAME & UNITED STATES FISH AND WILDLIFE SERVICE. 2008. *Kure*/Humboldt Bay oil spill: Final damage assessment and restoration plan / environmental assessment. 85 pp.

CARTER, H.C. 2003. Oil and California's seabirds: an overview. *Marine Ornithology* 31: 1-7.

COULSON, T., CATCHPOLE, E.A., ALBON, S.D., MORGAN, B.J.T., PEMBERTON, J.M., CLUTTON-BROCK, T.H., CRAWLEY, M.J. & GRENFELL, B.T. 2001. Age, sex, density, winter weather, and population crashes in Soay Sheep. *Science* 292: 1528-1531.

EICHHORST, B.A. 1992. An analysis of Western Grebe banding and recovery data. *North American Bird Bander* 17: 108-115.

FERNANDEZ, G. & LANK, D.B. 2006. Sex, age, and body size distributions of Western Sandpipers during the nonbreeding season with respect to local habitat. *Condor* 108: 547-557.

FREDRICKSON, L.H. 1968. Measurements of coots related to age and sex. *Journal of Wildlife Management* 32: 409-411.

GILL, F.B. 2001. Ornithology, second edition. New York: W.H. Freeman and Company. pp. 486-487.

GLICK, B. 1983. Bursa of Fabricius. In: Farner, D.S., King, J.R. & Parkes, K.C. (Eds.) Avian biology, vol. 7. New York: Academic Press. pp. 443-500.

GREENWOOD, P.J. 1980. Mating systems, philopatry, and dispersal in birds and mammals. *Animal Behavior* 28: 1140-1162.

GRIFFITHS, R., DOUBLE, M.C., ORR, K. & DAWSON, R.J.G. 1998. A DNA test to sex most birds. *Molecular Ecology* 7: 1071-1075.

HAMPTON, S., FORD, R.G., CARTER, H.R., ABRAHAM, C. & HUMPLE, D. 2003. Chronic oiling and seabird mortality from the sunken vessel *S.S. Luckenbach* in central California. *Marine Ornithology* 31: 35-41.

HENKEL, L.A. 2004. Season abundance of marine birds in nearshore waters of Monterey Bay, California. *Western Birds* 35: 126-146.

HEUBECK, M., CAMPHUYSEN, K., BAO, R., HUMPLE, D., SANDOVAL REY, A., CADIOU, B., BRAGER, S. & THOMAS, T. 2003. Assessing the impact of major oil spills on seabird populations. *Marine Pollution Bulletin* 46: 900-902.

HOHMAN, W.L. & CYPHER, B.L. 1986. Age-class determination of Ring-necked Ducks. *Journal of Wildlife Management* 50: 442-445.

HUMPLE, D.L. 2009. Genetic structure and demographic impacts of oil spills in Western and Clark's grebes [MS thesis]. Rohnert Park, CA: Sonoma State University.

HUMPLE, D., ABRAHAM, C., RAMOS, A., DOPICO, M., FERNÁNDEZ-BOÁN, M., GARCÍA, L., BAO, R., HEUBECK, M. & CAMPHUYSEN, C. J. 2007a. Age and sex structure of auk (*Alcidae)* mortality during the *Prestige* oil spill in Galicia, NW Spain, November 2002–April 2003 [Extended abstract]. In: Proceedings of the 2007 Effects of Oil on Wildlife Conference. Monterey, CA: Oiled Wildlife Care Network. pp. 63-67.

IVERSON, S.A., SMITH, B.D. & COOKE, F. 2004. Age and sex distributions of wintering Surf Scoters: implications for the use of age ratios as an index of recruitment. *Condor* 106: 252-262.

JESSUP, D.A., MILLER, M.A., RYAN, J.P., NEVINS, H.M., KERKERING, H.A., MEKEBRI, A., CRANE, D.B., JOHNSON, T.A. & KUDELA, R.M. 2009. Mass stranding of marine birds caused by a surfactant-producing red tide. *PLoS ONE* 4: e4550.

KETTERSON, E.D. & NOLAN, V. 1983. The evolution of differential bird migration. *Current Ornithology* 1: 357-402.

KETTERSON, E.D. & NOLAN JR, V. 1976. Geographic variation and its climatic correlates in the sex-ratio of eastern wintering Dark-eyed Juncos (*Junco hyemalis hyemalis*). *Ecology* 57: 679-693.

LEIGHTON, F.A. 1984. Adenovirus-like agent in the bursa of Fabricius of Herring Gulls (*Larus argentatus* Pontoppidan) from Newfoundland, Canada. *Journal of Wildlife Diseases* 20: 226-230.

LIVEZEY, B.C. & STORER, R.W. 1992. Morphometric comparison of skeletons of the Western Grebe complex *Aechmophorus* of the United States and Canada. *Condor* 94: 668-679.

LOWENSTEIN, L.J. & FRY, D.M. 1985. Adenovirus-like particles associated with intranuclear inclusion bodies in the kidney of a Common Murre (*Uria aalge*). *Avian Diseases* 29: 208-213.

MATHER, D.D. & ESLER, D. 1999. Evaluation of bursal depth as an indicator of age class in Harlequin Ducks. *Journal of Field Ornithology* 70: 200-205.

MCCLOSKEY, J.T. & THOMPSON, J.E. 2000. Aging and sexing Common Snipe using discriminant analysis. *Journal of Wildlife Management* 64: 960-969.

MCNEIL, R. & BURTON, J. 1972. Cranial pneumatization patterns and bursa of Fabricius in North American shorebirds. *Wilson Bulletin* 84: 329-339.

MØLLER, A.P. & ERRITZØE, J. 2001. Dispersal, vaccination and regression of immune defence organs. *Ecology Letters* 4: 484-490.

MYERS, J.P. 1981. A test of three hypotheses for latitudinal segregation of the sexes in wintering birds. *Canadian Journal of Zoology* 59: 1527-1534.

NEBEL, S., LANK, D.B., O'HARA, P.D., FERNANDEZ, G., HAASE, B., DELGADO, F., ESTELA, F.A., EVANS OGDEN, L.J., HARRINGTON, B., KUS, B.E., LYONS, J.E., MERCIER, F., ORTEGO, B., TAKEKAWA, J.Y., WARNOCK, N. & WARNOCK, S.E. 2002. Western Sandpipers (*Calidris mauri*) during the nonbreeding season: spatial segregation on a hemispheric scale. *Auk* 119: 922-928.

NEVINS, H.M., & CARTER, H.R. 2003. Age and sex of Common Murres *Uria aalge* recovered during the 1997-98 Point Reyes Tarball Incidents in central California. *Marine Ornithology* 31: 51-58.

NUR, N. & SYDEMAN, W.J. 1999. Demographics processes and population dynamic models of seabirds: implications for conservation and restoration. *Current Ornithology* 15: 149-188.

OLSON, C.V. & *ARSENAULT*, D.P. 2000. Differential winter distribution of Rough-legged Hawks (*Buteo lagopus*) by sex in western North America. *Journal of Raptor Research* 34: 157.

PHILLIPS, E.M., NEVINS, H.M., HATCH, S.A., RAMEY, A.M., MILLER, M.A. & HARVEY, J.T. 2010. Seabird bycatch in Alaska demersal longline fishery trials: a demographic summary. *Marine Ornithology* 38: 111-117.

PHILLIPS, E.M., ZAMON, J.E., NEVINS, H.M., GIBBLE, C.M., DUERR, R.S. & KERR, L.H. 2011. Summary of birds killed by a harmful algal bloom along south Washington and north Oregon coasts during October 2009. *Northwestern Naturalist* 92: 120-126.

PRBO Conservation Science & University of California Davis Wildlife Health Center). 2009. Protocols for the processing of oiled wildlife in the state of California. Petaluma & Davis, CA: Point Reyes Bird Observatory & University of California Davis [Available online at: http://data.prbo.org/cadc2/uploads/Articles/OilSpill/WPUprotocols_nov09_COMPLETE_with_appendices.pdf; accessed 2 August 2011]

PYLE, P. 2008. Identification guide to North American birds, Part II: *Anatidae* to *Alcidae*. Point Reyes Station, CA: Slate Creek Press. 835pp.

R DEVELOPMENT CORE TEAM. 2009. The R project for statistical computing. R Vienna: Foundation for Statistical Computing. ISBN 3-900051-07-0 [Available online at: http://www.R-project.org; accessed 2 August 2011]

SAVARD, J.L. 1985. Evidence of long-term pair bonds in Barrow's Goldeneye *Bucephala islandica. Auk* 102: 389-391.

SCHAMBER, J.L., SEDINGER, J.S., WARD, D.H. & HAGMEIER, K.R. 2007. Latitudinal variation in population structure of wintering Pacific Black Brant. *Journal of Field Ornithology* 78: 74-82.

SIEGEL-CAUSEY, D. 1990. On use of size of the bursa of Fabricius as an index of age and development. *Journal of Field Ornithology* 61: 441-444.

SMITH, C.M., COOKE, F, ROBERTSON, G.J., GOODIE, R.I. & BOYD, W.S. 2000. Long-term pair bonds in Harlequin Ducks. *Condor* 102: 201-205.

STENZEL, L.E., PAGE, G.W., WARRINER, J.C., WARRINER, J.S., NEUMAN, K.K., GEORGE, D.E., EYSTER, C.R. & BIDSTRUP, F.C. 2011. Male-skewed adult sex ratio, survival, mating opportunity and annual productivity in the Snowy Plover *Charadrius alexandrinus. Ibis* 153: 312-322.

STORER, R.W. & NUECHTERLEIN, G.L. 1992. Western Grebe (*Aechmophorus occidentalis*) and Clark's Grebe (*A. clarkia*). In: Poole, A. & Gill, F. (Eds.) The birds of North America, no. 26. Philadelphia & Washington, DC: Academy of Natural Sciences & American Ornithologists' Union.

STRAUGHAN, D. 1970. Ecological effects of the Santa Barbara oil spill. In: Santa Barbara oil symposium, 16–18 December 1970. Santa Barbara, CA: University of California, Marine Science Institute. pp. 173-182.

STRAUGHAN, D. 1971. Oil pollution and seabirds. In: Straughan, D. (Ed.) Biological and oceanographical survey of the Santa Barbara Oil Spill 1969–1970. Volume 1: biology and bacteriology. Los Angeles: Allan Hancock Foundation & University of Southern California. pp. 307-312.

VAN FRANEKER, J.A. 2004. Save the North Sea Fulmar-Litter-EcoQO Manual, Part 1: Collection and dissection procedures. Alterra-rapport 672. Wageningen: Alterra.

VOTIER, S.C., BIRKHEAD, T.R., ORO, D., TRINDER, M., GRANTHAM, M.J., CLARK, J.A., MCCLEERY, R.H. & HATCHWELL, B.J. 2008. Recruitment and survival of immature seabirds in relation to oil spills and climate variability. *Journal of Animal Ecology* 77: 974-983.

WIENS, J.A., FORD, R.G. & HEINEMANN, D. 1984. Information needs and priorities for assessing sensitivity of marine birds to oil spills. *Bird Conservation* 28: 21-49.

WING, L.W. 1956. Natural history of birds: a guide to ornithology. New York: The Ronald Press Co. 539 pp.