Population structure in the eastern North Pacific gray whale: Implications for management of aboriginal whaling

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ABSTRACT

The eastern North Pacific gray whale (*Eschrichtius robustus*) was removed from the Endangered Species List in 1994, and as a result several aboriginal groups in Washington and British Columbia have proposed to resume whaling. In particular, the Makah are currently in litigation with the National Marine Fisheries Service regarding this issue. Although the majority of whales in this population migrate to summer feeding grounds in the Bering, Chukchi, and Beaufort Seas, a small number of individuals (~200) spend the summers feeding in the waters of Oregon, Washington, and British Columbia. The relationship of these "southern feeding group" whales to the rest of the population is unknown. This information is key to making appropriate management decisions, because these whales would represent the primary target of the aboriginal hunt. We compared mitochondrial sequence data from 53 southern feeding group individuals to sequences from 87 individuals representing the larger population. We found small but significant differences in haplotype frequencies between the two groups (Fst = 0.0189, P = 0.00090; ϕ_{ST} = 0.0169, P = 0.0030), with estimated migration rates << 1%. Moreover, estimates of Θ ($Ne\mu$ for mtDNA data) were significantly different between the two groups (P = 0.0249), indicating that the maternal lineages of the southern feeding group are demographically independent of those from the rest of the population. Combined, these data show that the southern feeding group of gray whales qualifies as a separate management unit (MU), which should be considered when making conservation decisions.

INTRODUCTION

The eastern North Pacific gray whale (*Eschrichtius robustus*) represents one of the few populations that have been removed from the Endangered Species List, with the classification changing from "Endangered" to "Recovered" in 1994. Commercial whaling targeting gray whales in the eastern Pacific began in ~1845 (Henderson 1984), and reduced the population from an estimate of 12,000-15,000 individuals to as low as 1,500-1,900 individuals by 1900 (Henderson 1984; Reilly 1992; Butterworth et al. 2002). International protection began in 1937, when the United States and Norway ended their gray whale hunts, but it was not until 1951 that all modern whaling countries agreed to stop hunting gray whales (Reeves 1984). Systematic surveys from 1967-1998 showed that the population increased at an annual rate of ~ 2.6%, reaching as many as 30,000 individuals (Shelden & Laake 2002; Rugh et al. 2005). Current estimates hover around 20,000 individuals, and there are even some suggestions that the population has reached carrying capacity (Moore et al. 2001; Wade 2002; Rugh et al. 2005).

During the late fall and early winter, whales migrate to the lagoons of Baja California and the Gulf of California, which represent the winter breeding and calving grounds for this population (Swartz 1986; Findley & Vidal 2002; Swartz et al. 2006). During the spring, the majority of whales migrate to their northern feeding grounds in the Bering, Chukchi, and Beaufort Seas (Moore & Ljungblad 1984). However, a small subset of the population (~200 individuals) remains in more southerly feeding grounds along the coasts of Oregon, Washington, and British Columbia (Pike 1962; Hatler & Darling 1974; Darling 1984; Calambokidis et al. 2002; Swartz et al. 2006). These two subsets of the population will be referred to as the northern and southern feeding groups, respectively.

Subdivision with respect to summer feeding ground use is common in baleen whales, and results from maternally-directed site fidelity to different feeding grounds. For example, in humpback whales (*Megaptera novaeangliae*) and North Atlantic right whales (*Eubalaena glacialis*) calves nurse for ~ 11 months (and occasionally longer), and learn migration routes and the location of summer feeding grounds through cultural transmission from their mother (e.g. Baker et al. 1990; Malik et al. 1999). Thus, if there is differential use of feeding grounds by mothers, these preferences will be passed on to their offspring and result in substructuring with respect to summer feeding ground use. Gray whale calves nurse for a much shorter period of time (~ 6 months) (Swartz 1986). Although this is still long enough to learn migratory routes and the location of summer feeding grounds, it is not yet clear whether or not gray whales show this maternally-directed site fidelity. Because of its maternal inheritance, patterns of mitochondrial DNA (mtDNA) diversity should reflect any maternally-based patterns of movement and distribution. Therefore, analysis of mtDNA is ideal for testing hypotheses of maternally-based site fidelity and subsequent population structure in baleen whales.

The relationship between the northern and southern feeding groups is unknown. It is currently assumed that both of these groups use the same breeding ground, and therefore represent the same breeding population (e.g. Swartz et al. 2006). Given the known patterns in other baleen whale species, it seems likely that the northern and southern feeding groups result from maternally-directed site fidelity to different feeding grounds by gray whale mothers. Photo-identification data are consistent with this hypothesis, showing that the majority of whales sighted in the southern feeding areas are re-sighted there in subsequent years, and therefore show the expected site fidelity (Darling 1984; Calambokidis et al. 2002). However, preliminary genetic analyses based on mtDNA were inconclusive (Steeves et al. 2001).

Understanding the relationship between the northern and southern feeding groups is becoming of increasing importance due to the desire of some aboriginal communities in Washington and British Columbia to resume hunting the gray whale. Several aboriginal groups historically hunted gray whales in this area, but voluntarily stopped hunting as whale numbers decreased and/or were required to stop

when the population received international protection (O'Leary 1984; Russell 2004). The one exception was aboriginal whaling in Chukotka, Russia, which was allowed to continue. In 1999 the Makah (in Washington State) resumed whaling, but have since been prevented from doing so by litigation. Specifically, the Makah were given the right to hunt gray whales at traditional sites under the Treaty of Neah Bay in 1855. However, they have been prevented from doing so under the court ruling (in 2004) that in order to continue their hunt they must follow the necessary procedures for obtaining authorization to "take" whales under the Marine Mammal Protection Act (MMPA). The Makah have applied for a waiver from the MMPA regulations, and this request is still in litigation. The outcome of the Makah lawsuit will have large implications for the resumption of whaling by other aboriginal communities in the area as well (Russell 2004).

The majority of the proposed aboriginal whaling will take place in the waters of Washington and British Columbia – the feeding ground of the much smaller southern feeding group. The negative consequences of ignoring potential population structure when making management decisions are well known (e.g. Daugherty et al. 1990; Taylor 1997; Frankham et al. 2002). Therefore, if informed management decisions are to be made regarding resuming this hunt, it is first necessary to understand the relationship of this southern feeding group to the rest of the larger population. Here, we conducted analyses of the mitochondrial DNA of gray whales from the both the southern and northern feeding groups in order to better understand their relationship, and therefore guide management decisions.

MATERIALS AND METHODS

Skin samples were collected from whales representing the southern feeding group in Clayoquot Sound, British Columbia from 1995-2008, using a crossbow and modified bolt (e.g. Lambertsen 1987; Palsbøll et al. 1991) or a pneumatic rifle biopsy system (Barrett-Lennard et al. 1996). Tissue samples were stored in a 20% dimethyl sulfoxide (DMSO) solution (Seutin et al. 1991). Approximately 40 mg from each sample was used for subsequent DNA extraction procedures. The skin was frozen in liquid nitrogen, ground to a fine powder, and transferred to a tube with 500 µl of lysis buffer (4 M urea, 0.2 M NaCl, 0.5% *n*-lauroyl sarcosine, 10 mM 1,2-cyclohexanediaminetetraacetic acid, 100 mM Tris-HCL, pH 8.0). Samples were rotated in the lysis buffer at room temperature for ≥ 5 days, after which time they were subjected to three aliquots of proteinase K, each at a concentration of 0.5 U of proteinase K per milligram of tissue. The addition of proteinase K was as follows: after adding the first aliquot, samples were rotated at room temperature overnight; after adding the second aliquot the samples were placed in a 65°C waterbath for 1 hour, then transferred to a 37°C incubator for 1 hour; after adding the third aliquot, the samples were rotated at room temperature overnight. Approximately 250 µl of the tissue/lysis buffer solution was subsequently extracted using Qiagen DNeasy Tissue Extraction Kits (Qiagen Inc., Mississauga, Ontario, Canada). DNA quantity was estimated using PicoGreen (Singer et al. 1997). Extracted samples included those previously analyzed by Steeves et al. (2001), which were re-extracted and analyzed here along with the newly collected samples.

A 345 bp portion of the mitochondrial DNA control region was amplified using the primers t-PRO and Primer-2 from Yoshida et al. (2001). PCR cycling conditions consisted of: (*i*) an initial denaturation step of 5 minutes at 94°C; (*ii*) 30 cycles of 94°C for 30 seconds, 57°C for 1 minute, and 72°C for 1 minute; and (*iii*) a final extension step of 60°C for 45 minutes. Reactions were carried out in 20 µl volumes containing 1X PCR Buffer (20 mM Tris-HCl pH 8.0, 50 mM KCl), 0.05 U μl^{-1} *Taq* polymerase (Invitrogen), 1.5 mM $MgCl_2$, 0.2 mM each dNTP (Invitrogen), and 10 ng of DNA. After amplification, primers and unincorporated dNTPs were degraded using EXOSAP-IT (Dugan et al. 2002), and products were sequenced using the DYEnamic dye terminator kit (GE Healthcare,

Piscataway, NJ, USA). Products were size-separated and visualized on a MegaBACE 1000 (GE Healthcare). Sequences were edited using MEGA 4 (Kumar et al. 2008).

To compare the data from southern feeding group whales to those of the northern feeding group, we compared our mitochondrial sequence data to those reported in Goerlitz et al. (2003). These samples were collected from 83 individuals in the winter calving and breeding lagoons around Baja California. The rationale is that although whales with different summer feeding distributions may congregate on the same area in the winter, the probability of one of these samples representing a southern feeding group individual is low; given that the total population size estimate is ~ 20,000 individuals (Swartz et al. 2006), and the estimate for the southern feeding group is ~ 200 (Calambokidis et al. 2002). This approach would also make our results conservative – where true differentiation will likely be greater than that observed due to this potential for some southern feeding group whales to be represented in the winter sample set.

Sequences were aligned with CLUSTALX (Thompson et al. 1994). Alignments were conducted under a range of gap opening and extension penalties and compared by eye to establish the optimal alignment. The sequences were very similar, and all alignments were the same under the tested conditions. Haplotype and nucleotide diversity (π) (Nei 1987) were estimated using Arlequin ver. 3.1 (Excoffier et al. 2005). Variations between mtDNA sequences were recorded and identical sequences were grouped into haplotypes. Final haplotype assignments were confirmed with FaBox ver. 1.35 (Villesen 2007). Population differentiation of the mtDNA sequences between the southern feeding group and the winter samples was estimated using the analysis of molecular variance approach described in Excoffier et al. (1992) as implemented in the program Arlequin. The significance of the resulting estimates of F_{ST} and ϕ_{ST} was tested using 1000 permutations. Relationships between haplotypes were visualized via a median-joining network using the program Network 4.5.1.6 (Fluxus Technology Ltd.).

To gain insight into the nature of the observed population structure, we estimated effective population sizes, migration rates, time since divergence, and growth rates for the two feeding groups using the Isolation with Migration program (IM, Nielsen & Wakely 2001; Hey & Nielsen 2004; Hey et al. 2004). However, repeated trials with various parameter options suggested that there was not enough information in our data set to obtain accurate estimates for all of these values (data not shown). Instead, we focused on estimating just the effective population sizes and migration rates using the program MIGRATE (Beerli & Felsenstein 2001; Beerli 2006). The Bayesian inference approach was implemented, using a transition/transversion ratio of 11.22 and an α estimate of 0.09 for the gamma distribution of mutation rate heterogeneity among sites (both estimated using TREE-PUZZLE, Schmidt et al. 2002). We used the Metropolis method of generating posterior distributions. The program was run with uniform prior distributions and one long chain. To ensure consistency between runs, MIGRATE was run four times with a burn-in of 100,000 steps, and a run length of 10,000,000 steps with data recorded every 500 steps. The likelihood ratio test option of MIGRATE was also used to test the hypothesis that the northern and southern feeding groups have different effective population sizes. Specifically, the hypothesis tested was $\Theta_{southern} = \Theta_{northern}$, where $\Theta = N_e\mu$ for mitochondrial data, where μ is the mutation rate per site per generation.

RESULTS

DNA was extracted and mtDNA control regions sequenced from 57 summer resident gray whales. The sequencing protocol resulted in 336 bp of comparable sequence between individuals. Twenty-seven polymorphic sites were identified, which resulted in 18 haplotypes in the summer

resident whales (Tables 1 and 2). None of the variable sites identified in the southern feeding group were new - all were also represented by the sequences described by Goerlitz et al. (2003) (Table 2).

The sequenced region from the summer resident whales was slightly different than those in Goerlitz et al. (2003), and therefore to align all sequences for analyses 15 bp were excluded from one end of the sequences from the Goerlitz et al. (2003) sequences, and 45 bp were excluded from the opposite end of the southern feeding group sequences. This resulted in a total of 291 bp that could be compared between the two sample sets. Trimming the sequences in this manner did not remove any variable sites within the southern feeding group samples, but did remove the variation differentiating sequences 1, 2 and 28 from Goerlitz et al. (2003), which were subsequently collapsed into one haplotype for these analyses. For the purposes of this study, these 'collapsed' sequences are referred to as haplotype 1.

Fifteen of the 29 haplotypes (52%) were shared between both groups, 11 (38%) were only found in the northern feeding group, and three (10%) were found only in the southern feeding group (Table 2). Estimates of differentiation for haplotype frequencies between groups were small but significant, with values of 0.01890 for F_{ST} ($P = 0.00090$) and 0.01688 for ϕ_{ST} ($P = 0.0030$). The median-joining network shows that although there is some evolutionary differentiation between the haplotypes from the two feeding groups, for the most part the haplotypes from each are scattered throughout the network (Fig. 1). Haplotype and nucleotide diversity (π) were estimated at 0.9279 and 0.019910, respectively for the southern feeding group. These values are very similar to estimates obtained based on samples from the winter breeding/calving ground, which were 0.95 and 0.02, respectively (Goerlitz et al. 2003).

The results from the MIGRATE analyses are shown in Table 3. Estimates for each value are very similar across iterations, suggesting that the program was run long enough to reach convergence on the estimates. The estimates of Θ for the northern and southern feeding groups are clearly different. This observation was confirmed by the likelihood ratio test, which rejected the hypothesis of $\Theta_{southern} = \Theta_{northern}$ ($P = 0.024878$). The 95% confidence intervals for the migration rate estimates are extremely large, making them uninformative. This result is not surprising, however, because the approach implemented by MIGRATE is known to recover precise and accurate estimates of Θ even in situations where there is not enough information in the data to recover meaningful migration rate estimates (Beerli 2006).

DISCUSSION

The conservation and/or management of wildlife populations requires knowledge of how individuals are subdivided into separate entities that have relatively independent demographic processes, which are often referred to as "management units". Such information is required to identify how each unit, and the population as a whole, will respond to exploitation and/or unintentional impacts. Moritz (1994) was the first to provide a working definition of a management unit (MU) in a population genetics context, and defined them as "…populations with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles." While this definition has been widely applied in population genetics studies, it has recently been argued that management units should be defined based on criteria demonstrating demographic isolation rather than simply rejecting the hypothesis of panmixia (Palsbøll et al. 2007). This idea makes intuitive sense, because the true question for management is whether or not the units will respond differently to the pressures of concern (e.g. exploitation and/or unintentional mortality).

The data presented here show that the southern feeding group of gray whales represents a distinct management unit under both of these criteria. The analysis showing statistically significant differentiation of mitochondrial haplotypes demonstrates qualification as an MU under the criteria of Mortiz (1994). Moreover, the analysis showing that the effective sizes of both groups are different ($\Theta_{southern} \neq \Theta_{northern}$) shows that the maternal lineages of the southern feeding group are demographically independent of those of the northern feeding group. Indeed, if they were not an independent unit, then estimates of Θ from the two data sets should converge on the same value. Thus, the southern feeding group qualifies as a separate management unit under the criterion of Palsbøll et al. (2007). Combined, these data show that the southern feeding group requires separate management consideration with regards to resuming aboriginal (or any) whaling.

Hastings (1993) showed that populations behave in a demographically independent manner when migration rates are less than ~ 10%. We have intentionally not converted Θ estimates to N_e estimates (where $\Theta = N_e\mu$ for mtDNA data) because this requires knowledge of the substitution rate (μ). Estimates of μ for the control region of baleen whale mtDNA vary by over an order of magnitude (e.g. Rooney et al. 2001). Moreover, μ, whatever its true value is, is undoubtedly the same for the northern and southern feeding groups, and therefore comparing estimates of Θ is an appropriate and less controversial method for comparing N_e. Regardless, if we apply the μ estimate of 1.8×10^{-8} from Rooney et al. (2001) to our data, the resulting estimates of migration rates are << 1%. Again, this result shows that the southern feeding group is demographically independent.

Previous studies have suggested that the haplotype diversity in the southern feeding group is too high to have resulted from strict maternally-directed site fidelity beginning with a few founders after the cessation of commercial whaling within the past century (Ramakrishnan et al. 2001). Our results are consistent with this interpretation. Under that hypothesis only a few closely related haplotypes should be represented within the southern feeding group, as opposed to the pattern seen in Figure 1. However, the hypothesis of a founding event within the past century is not consistent with the known sighting information. Indeed, gray whales have been seen in the southern feeding grounds throughout their history, including in times of lowest abundance (Swartz et al. 2006, and references therein). Moreover, if a few individuals recently founded the southern feeding group then the estimate of $\Theta_{southern}$ should be substantially smaller, as effective population size estimates are heavily influenced by bottlenecks.

Instead, what the sighting and genetic data suggest is that the southern feeding group of gray whales pre-dates whaling. Under this hypothesis, the haplotype diversity is expected to be high, because those lineages that survived whaling would be a random sample from a much larger population. Substantial gaps would also be expected between existing haplotypes resulting from the removal of haplotypes by whaling. This pattern is exactly what is seen in Figure 1. The similarity of haplotypes, and the degree of haplotype sharing between the northern and southern feeding groups, suggest that there is some degree of migration between the two. However, although reliable estimates of migration rates could not be obtained here, the data clearly show that the rate of migration is low enough that the two groups represent independent demographic entities. The southern feeding group therefore qualifies as a separate management unit, and requires separate management consideration.

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Table 1. Characteristics of the data for the northern and southern feeding groups, and for the combined data set.

Group	Individuals	Sequence Length (bp)	Polymorphic Sites	Haplotypes
Northern	83	306	30	28
Southern	57	336	27	18
Combined	140	291	27	29

Table 2. Variable sites characterizing haplotypes from both sample sets of gray whales. Variable site positions are numbered to correspond with those in Goerlitz et al. (2003). The columns labeled NFG and SFG indicate the number of individuals from the northern feeding group and the southern feeding group, respectively.

Hap	26	67	68	80	88	89	90	91	93	98	102	104	105	106	124	129	171	220	248	264	265	266	271	284	288	289	290	NFG	SFG	Total
1	A	T	C	T	T	T	C	G	G	T	T	T	T	T	A	A	T	A	C	G	C	G	T	C	G	T	C	6	6	12
3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	2	0	2
4	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	0	2
5	.	C	T	.	C	.	T	.	.	.	.	C	C	.	G	G	C	.	T	.	T	.	C	.	.	.	T	1	0	1
6	.	.	T	.	C	C	T	.	.	.	.	C	.	.	G	.	.	.	T	.	T	A	.	T	.	.	.	2	6	8
7	.	.	T	.	C	C	T	.	.	.	C	C	.	.	G	.	.	.	T	A	T	A	.	T	.	C	.	3	4	7
8	.	.	T	.	C	.	.	A	.	.	.	C	.	.	G	.	C	.	T	.	T	.	C	.	.	.	T	1	0	1
9	.	.	T	.	C	.	T	.	A	.	.	C	.	.	G	.	.	.	T	.	.	.	.	.	.	.	T	1	0	1
10	.	.	T	.	C	.	T	.	.	C	.	C	.	.	G	.	.	G	T	.	T	.	C	.	.	.	T	1	0	1
11	.	.	T	.	C	.	T	.	.	C	.	C	.	.	G	.	.	.	T	.	T	.	C	.	.	.	T	1	0	1
12	.	.	T	.	C	.	T	.	.	C	.	C	.	C	G	.	.	.	T	.	T	.	C	.	.	.	.	4	1	5
13	.	.	T	.	C	.	T	.	.	.	C	C	.	C	G	.	.	.	T	A	T	A	.	T	.	.	.	3	6	9
14	.	.	T	.	C	.	T	.	.	.	C	C	.	C	G	.	.	.	T	A	T	A	C	T	.	.	.	2	0	2
15	.	.	T	.	C	.	T	.	.	.	C	C	.	C	G	.	.	.	T	.	T	A	C	T	A	.	.	1	8	9
16	.	.	T	.	C	.	T	.	.	.	.	C	C	C	G	.	.	.	T	.	T	A	C	T	.	.	.	2	1	3
17	.	.	T	.	C	.	T	.	.	.	.	C	C	.	G	.	C	.	T	.	T	.	C	.	.	.	T	1	1	2
18	.	.	T	.	C	.	T	.	.	.	.	C	.	C	G	.	.	.	T	.	T	A	.	T	.	.	.	7	3	10
19	.	.	T	.	C	.	T	.	.	.	.	C	.	C	G	.	.	.	T	.	T	A	C	T	.	.	.	6	0	6
20	.	.	T	.	C	.	T	.	.	.	.	C	.	C	G	.	.	.	T	.	T	A	C	T	A	.	.	10	2	12
21	.	.	T	.	C	.	T	.	.	.	.	C	.	.	G	.	C	.	T	.	T	.	C	.	.	.	T	4	2	6
22	.	.	T	.	C	.	T	.	.	.	.	C	.	.	G	.	C	.	T	.	T	.	.	.	.	.	T	2	2	4
23	.	.	T	.	C	.	T	.	.	.	.	C	.	.	G	.	.	.	T	.	.	.	.	.	.	.	T	10	2	12
24	.	.	T	.	C	.	T	.	.	.	.	C	.	.	G	.	.	.	T	.	T	.	C	.	.	.	T	3	1	4
25	.	.	T	.	C	.	T	.	.	.	.	C	.	.	G	.	.	.	T	.	T	A	C	T	A	.	.	5	8	13
26	.	.	T	.	C	.	T	.	.	.	.	C	.	.	G	.	.	.	T	.	T	A	C	T	.	C	.	2	0	2
27	G	.	T	.	C	.	T	.	.	.	.	C	.	.	G	.	.	.	T	.	.	.	.	.	.	.	T	1	0	1
29	.	.	T	.	C	.	T	.	.	.	.	C	.	.	G	.	.	.	T	.	.	.	.	.	.	.	.	0	1	1
30	.	.	T	.	C	.	T	.	.	C	.	C	.	C	G	.	.	.	T	.	T	.	C	.	.	.	T	0	2	2
31	.	.	T	.	C	.	T	.	.	.	C	C	.	C	G	.	.	.	T	.	T	A	C	T	.	.	.	0	1	1

Table 3. Results from the MIGRATE analysis. Included is the estimated mode for each parameter, as well as the 95% confidence intervals in parentheses. *M* is the immigration rate *m* divided by the mutation rate μ. For mitochondrial DNA data, the number of immigrants per generation can be calculated by multiplying *M* by Θ. Included are the estimates for each of the four runs, as well as the average across all four runs.

Iteration	$\Theta_{northern}$	$\Theta_{southern}$	$M_{southern\text{-}northern}$	$M_{northern\text{-}southern}$
1	0.0388 (0.0200-0.0800)	0.0158 (0.00650-0.0365)	393 (130-740)	433 (170-860)
2	0.0388 (0.0205-0.0790)	0.0163 (0.00700-0.0365)	373 (130-705)	448 (170-865)
3	0.0403 (0.0180-0.0800)	0.0173 (0.00700-0.0390)	348 (125-700)	428 (155-820)
4	0.0358 (0.0195-0.0840)	0.0168 (0.00700-0.0360)	408 (175-765)	463 (165-900)
Avg	**0.0384 (0.0195-0.0808)**	**0.0166 (0.00688-0.0370)**	**381 (140-728)**	**443 (165-861)**

474 **Figure 1.** Median-joining network for the gray whale sequences. Transitional mutations are indicated
475 with a line, and transversions are indicated with a box. Sizes of the circles are proportional to the
476 haplotype frequencies in the entire data set. Pie charts indicate the proportion of that haplotype found
477 in the northern (black) and southern (gray) feeding groups.
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