Pacific Salmon, *Oncorhynchus* spp., and the Definition of "Species" Under the Endangered Species Act

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Introduction

In conjunction with a review of the biological status of Pacific salmon,[1] *Oncorhynchus* spp., initiated in 1978 by the National Marine Fisheries Service (NMFS) and the U.S. Fish and Wildlife Service (FWS), three policy position papers were drafted for the consideration of Columbia River salmon under the Endangered Species Act (ESA or "the Act"). The draft papers were intended to provide guidance at three critical stages in ESA evaluations: Determination of what constitutes a "species" under the Act (and what, therefore, may merit protection), determination of thresholds for listing as threatened or endangered, and

[1] The term "Pacific salmon" has traditionally referred to species of the genus *Oncorhynchus*, five of which (*O. gorbuscha*, *O. keta*, *O. kisutch*, *O. nerka*, and *O. tshawytscha*) occur in North America. The recent decision to move the western trouts from the genus *Salmo* to *Oncorhynchus* calls this usage into question. In this document, "Pacific salmon" is used to include anadromous forms of *O. clarki* and *O. mykiss*, as well as the five above mentioned species.

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ABSTRACT—For purposes of the Endangered Species Act (ESA), a "species" is defined to include "any distinct population segment of any species of vertebrate fish or wildlife which interbreeds when mature." Federal agencies charged with carrying out the provisions of the ESA have struggled for over a decade to develop a consistent approach for interpreting the term "distinct population segment." This paper outlines such an approach and explains in some detail how it can be applied to ESA evaluations of anadromous Pacific salmonids.

The following definition is proposed: A population (or group of populations) will be considered "distinct" (and hence a "species") for purposes of the ESA if it represents an evolutionarily significant unit (ESU) of the biological species. A population must satisfy two criteria to be considered an ESU:

1) It must be substantially reproductively isolated from other conspecific population units, and

2) It must represent an important component in the evolutionary legacy of the species.

Isolation does not have to be absolute, but it must be strong enough to permit evolutionarily important differences to accrue in different population units. The second criterion would be met if the population contributes substantially to the ecological/genetic diversity of the species as a whole.

Insights into the extent of reproductive isolation can be provided by movements of tagged fish, natural recolonization rates observed in other populations, measurements of genetic differences between populations, and evaluations of the efficacy of natural barriers. Each of these methods has its limitations. Identification of physical barriers to genetic exchange can help define the geographic extent of distinct populations, but reliance on physical features alone can be misleading in the absence of supporting biological information. Physical tags provide information about the movements of individual fish but not the genetic consequences of migration. Furthermore, measurements of current straying or recolonization rates provide no direct information about the magnitude or consistency of such rates in the past. In this respect, data from protein electrophoresis or DNA analyses can be very useful because they reflect levels of gene flow that have occurred over evolutionary time scales. The best strategy is to use all available lines of evidence for or against reproductive isolation, recognizing the limitations of each and taking advantage of the often complementary nature of the different types of information.

If available evidence indicates significant reproductive isolation, the next step is to determine whether the population in question is of substantial ecological/genetic importance to the species as a whole. In other words, if the population became extinct, would this event represent a significant loss to the ecological/genetic diversity of the species? In making this determination, the following questions are relevant:

1) Is the population genetically distinct from other conspecific populations?

2) Does the population occupy unusual or distinctive habitat?

3) Does the population show evidence of unusual or distinctive adaptation to its environment?

Several types of information are useful in addressing these questions. Again, the strengths and limitations of each should be kept in mind in making the evaluation. Phenotypic/life-history traits such as size, fecundity, and age and time of spawning may reflect local adaptations of evolutionary importance, but interpretation of these traits is complicated by their sensitivity to environmental conditions. Data from protein electrophoresis or DNA analyses provide valuable insight into the process of genetic differentiation among populations but little direct information regarding the extent of adaptive genetic differences. Habitat differences suggest the possibility for local adaptations but do not prove that such adaptations exist.

The framework suggested here provides a focal point for accomplishing the major goal of the Act—to conserve the genetic diversity of species and the ecosystems they inhabit. At the same time, it allows discretion in the listing of populations by requiring that they represent units of real evolutionary significance to the species. Further, this framework provides a means of addressing several issues of particular concern for Pacific salmon, including anadromous/nonanadromous population segments, differences in run-timing, groups of populations, introduced populations, and the role of hatchery fish.

consideration of the possible role of artificial propagation in recovery plans for listed "species."

On receipt of petitions (April-June 1990) to list several populations of Pacific salmon as threatened or endangered "species" under the Act, NMFS sought public comments on draft Policy Position Paper #1, "Definition of Species" (a summary of a longer document by Utter (1981)). Based on that paper and public comments on it, discussions of the issue by the ESA Technical Committee, and ideas discussed at a Vertebrate Population Workshop convened in June 1990 by FWS and NMFS, Waples (1991a) prepared a NOAA Technical Memorandum that formed the basis for NMFS' "Interim Policy on Applying the Definition of Species Under the Endangered Species Act to Pacific Salmon" (56 FR 10542; March 13, 1991). After considering public comments on the Interim Policy and the supporting NOAA Technical Memorandum, NMFS published a Final Policy on this issue (56FR 58612; Nov. 20, 1991). Those comments were also considered in preparation of this paper.

Background of the Endangered Species Act

The stated purposes of the Endangered Species Act of 1973, as amended (16 U.S.C. 1531 et seq) are to "provide a means whereby the ecosystems upon which endangered species and threatened species depend may be conserved, [and] to provide a program for the conservation of such endangered species and threatened species." "Species" is used in a more expansive way in the ESA than biologists or taxonomists generally use the term. In the original (1973) version of the Act, a "species" was defined to include "any subspecies of fish or wildlife or plants and any other group of fish or wildlife of the same species or smaller taxa in common spatial arrangement that interbreed when mature." Use of this language established that the scope of the Act extends beyond the traditional biological definition of species to include smaller biological units. Amendments in 1978 (Public Law 95-632 (1978), 92 Stat. 3751) provided the current language in the Act: A "species" is defined to include "any subspecies of fish or wildlife or plants, and any distinct population segment of any species of vertebrate fish or wildlife which interbreeds when mature." Unfortunately, although the Act thus allows listing of populations that are "distinct," it does not explain how population distinctness shall be evaluated or measured.

Nevertheless, there is some guidance relevant to this issue. A review of legislative history indicates that an important motivating factor behind the Act was the desire to preserve genetic variability, both within and between species. For example, the House of Representatives described the rationale for House Resolution 37, a forerunner to the Act, in the following terms (H.R. Rep. 412, 93d Cong., 1st Sess., 1973):

"From the most narrow possible point of view, it is in the best interests of mankind to minimize the losses of genetic variations. The reason is simple: They are potential resources. They are keys to puzzles which we cannot yet solve, and may provide answers to questions which we have not yet learned to ask."

On the other hand, in 1979 the General Accounting Office (GAO)[2] recommended that the authority to list vertebrate populations under the ESA be removed. Although this recommendation was not adopted, the Senate Report to the 1979 amendments stated that "the committee is aware of the great potential for abuse of this authority and expects the FWS to use the ability to list populations sparingly and only when biological evidence indicates that such action is warranted" (Sen. Rep. 151, 96th Cong., 1st Sess., 1979). Finally, the ESA (Sec. 4(b)(1)(A)) specifies that listing decisions should be based "solely on the basis of the best scientific and commercial data available."

Although not entirely self-contradictory, the charge to conserve irreplaceable genetic resources but to do so sparingly (and scientifically) clearly establishes a certain tension in the process of "species" determination for vertebrate populations under the ESA. The approach adopted here is an attempt to balance these themes in a framework consistent with both the letter and intent of the ESA.

Definition: A vertebrate population will be considered distinct (and hence a "species") for purposes of conservation under the Act if the population represents an evolutionarily significant unit[3] (ESU) of the biological species. An ESU is a population (or group of populations) that:

1) Is substantially reproductively isolated from other conspecific population units, and

2) Represents an important component in the evolutionary legacy of the species.

Note that the dual criteria reflect two common meanings of "distinct": The criterion of reproductive isolation emphasizes the concept of "separate" or "apart from," whereas the criterion of evolutionary importance focuses on characteristics that are "different" or "unique."

Isolation does not have to be absolute, but it must be strong enough to allow evolutionarily important differences to accrue in different population units. Population characteristics that are important in an evolutionary sense must have a genetic basis; therefore, the second criterion would be satisfied if the population in question contributes substantially to the overall genetic diversity of the species. Because ecological diversity may foster local adaptations (and hence genetic diversity), a population that occupies unusual or distinctive habitat or in other ways represents an important ecological adaptation for the species may also be an ESU.

The term "evolutionary legacy" is used in the sense of "inheritance," i.e., something received from the past and carried forward into the future. This reflects

[2] GAO. 1979. Endangered species—a controversial issue needing resolution. Rep. to Congress, Gov. Account. Off., Wash., D.C.

[3] The term "evolutionarily significant unit" can be traced to Ryder (1986), who reported that the term was used at a 1985 meeting of zoo biologists and systematists in Philadelphia. The usefulness of this concept in the context of ESA evaluations was stressed at the Vertebrate Population Biology Workshop by A. Dizon (NMFS, Southwest Fisheries Science Center, La Jolla, Calif.). See Dizon et al. (In press) for additional discussion of this and related ideas with an orientation toward marine mammals.

the concern expressed in the Act (Sec. 2(a)(5)) for "better safeguarding, for the benefit of all citizens, the nation's heritage in fish, wildlife, and plants." Specifically, the evolutionary legacy of a species is the genetic variability that is a product of past evolutionary events and which represents the reservoir upon which future evolutionary potential depends. Conservation of these genetic resources helps to ensure that the dynamic process of evolution will not be unduly constrained in the future. Although the Act (Sec. 2(a)(3)) also notes that species "are of esthetic, ecological, recreational, and scientific value to the nation and its people," focussing on these attributes without regard to the underlying genetic basis for diversity is not a sound strategy for long-term species survival. Furthermore, societal values change rapidly on an evolutionary time scale, and "species" with no apparent significance today may be found to be "valuable" at some point in the future. It is better, then, to focus on conserving important genetic resources; if this is accomplished, then the other benefits of biodiversity (including various societal interests) follow naturally.

The framework adopted here provides a focal point for accomplishing the major goal of the Act—to conserve the genetic diversity of species and the ecosystems they inhabit. At the same time, it allows discretion in the listing of populations by requiring that they represent units of evolutionary significance to the biological species. In this framework, reproductive isolation is a necessary but not a sufficient condition for a population to be considered "distinct." Given enough time, an isolate may evolve into an ESU, but isolation by itself does not confer distinctness.

Application to Pacific Salmon

Application of the ESU concept to Pacific salmon requires consideration of reproductive isolation and ecological/genetic diversity. For convenience these concepts are considered separately here, but it is recognized that they are inherently related.

Reproductive Isolation

With Pacific salmon, reproductive isolation[4] is seldom a black-and-white situation; rather, it is a question of degree (e.g., Ricker, 1972). Although the homing instinct is well documented in these species, natural straying does occur (Quinn, 1984), and anadromous[5] spawning populations that are completely isolated from other conspecific populations are probably rare. A relevant question thus becomes: How much exchange with other populations can a salmon population experience and still be considered an ESU? Similarly, it is important to consider whether isolation is a recent phenomenon or whether it represents a long-standing condition.

One approach to this question emphasizes the effects of migration in inhibiting the process of genetic differentiation. The term "gene flow" is commonly used to describe the movement of genes from one population to another; that is, gene flow represents genetically effective migration. An oft-cited maxim based on the work of Sewall Wright (1978) is that gene flow between populations at the rate of one individual per generation is sufficient to prevent the tendency for different alleles to be fixed by chance (genetic drift) in different populations. Migration (the physical movement of individuals) may occur at a higher rate than gene flow if some migrating individuals have reduced reproductive success or fail to reproduce entirely.

Although gene flow at the level of one individual per generation may prevent extreme genetic divergence, it is not sufficient to equalize allele frequencies across populations, and Wright (1978) also pointed out that genetic differentiation is by no means negligible even if gene flow occurs at several times this rate. Furthermore, the above comments apply to a balance between migration and genetic drift of neutral alleles. Selection for locally adapted alleles can offset the homogenizing effects of gene flow. Thus, substantial differences among populations can be maintained at strongly selected loci, while frequencies at neutral loci remain relatively uniform (Slatkin, 1987).

Another way to consider the migration problem is to view it as a question of replacement. In this context, the relevant question is this: If all individuals in the population in question were permanently removed, would the area naturally be repopulated by individuals of the same biological species, and if so, within what time frame? Presumably, an area that would be naturally repopulated at or near the previous abundance level in a short time would be unlikely to harbor an ESU. This will be a largely theoretical exercise for populations being considered for protection under the Act. However, information for other populations and/or species may provide some insight into this process.

The level of migration or gene flow occurring among populations can be evaluated in several ways. Approaches that may prove useful for Pacific salmon include:

1) Use of tags to estimate straying rates;
2) Intentional genetic marking of populations;
3) Use of genetic indices (e.g., Wright's (1978) F_{ST} or Slatkin's (1985) private allele method) to estimate levels of gene flow;
4) Observations of recolonization rates; and
5) Identification of physical or geographic features likely to act as barriers to migration.

Since the 1950's, extensive data bases have been developed that provide important information about the oceanic distribution of Pacific salmon (Hartt, 1962).[6] More recently, physical tags have been used to study migrations of fish from individual populations (Johnson, 1990). However, tagging efforts typically focus on hatchery stocks, and much less is known about natural straying rates. Furthermore, tagging studies do not provide direct evidence of gene flow. A salmon

[4] This discussion of reproductive isolation focuses on the degree to which a population is isolated from genetic contact with other natural populations. Straying from hatchery stocks and intentional transfers of fish from one area to another are discussed in the section on "Special Considerations: Hatchery Fish."

[5] Nonanadromous populations or segments of populations are considered in the section on "Special Considerations: Anadromy/Nonanadromy."

[6] See also subsequent International North Pacific Fisheries Commission Bulletins on salmon distribution.

may swim into a nearby stream (and perhaps be counted as a stray) before ultimately making its way to its natal stream to spawn. Reproductive success of strays may also be less than that of fish from the local population.

A direct measurement of gene flow can be obtained by monitoring changes over time in the frequency of genetic markers characteristic of different populations. Because Pacific salmon populations are typically characterized by different frequencies of the same suite of alleles, rather than by qualitative differences in the types of alleles present (Utter et al., 1980, and many more recent references), it often will be difficult to measure gene flow precisely without enhancing the frequencies of different alleles in different populations (intentional genetic marking). Although the few genetic marking studies that have been conducted with Pacific salmon (Seeb et al., 1986; Lane et al., 1990) have provided important information, opportunities for such studies involving populations that are potential candidates for ESA listing are likely to be limited.

An indirect measurement of gene flow is provided by Wright's and Slatkin's methods, which measure some of the genetic consequences of migration. Accuracy of such estimates depends on the degree to which the various assumptions of the models used are satisfied. Notably, both methods assume selective neutrality of the alleles used, and results may be sensitive to the geographic configuration of the study sites included. Both methods are also based on equilibrium models and may overestimate migration rates if isolation has been too recent for a balance to develop between the forces of migration and genetic drift.

The presence of unique alleles (those found in only one population or one geographic region) may also provide insight into the degree of reproductive isolation. A major concern in evaluating such data is sampling error; that is, the failure to find the alleles in other localities may be due to inadequate sampling. Nevertheless, alleles that have been found in only one area and occur there at moderate or high frequency suggest a substantial degree of reproductive isolation. The occurrence of a number of unique alleles at lower frequency may also be indicative of reproductive isolation.

Opportunities to observe recolonization are not common with Pacific salmon, but they do occur. Aspinwall (1974) described one experiment that eradicated an entire run of pink salmon to study straying. Natural events (e.g., the eruption of Mount St. Helens) sporadically occur that cause extinction of populations or allow access to previously blocked habitat. In evaluating the results of such "experiments," it should be recognized that if intraspecific interactions (such as competition) are acting to hinder the success of migrants or strays, recolonization of empty habitat may occur at a higher rate than expected from migration rates among fully-seeded populations.

Because natural straying in Pacific salmon seems to be largely confined to nearby areas (Quinn and Fresh, 1984), geographic proximity of a population from other conspecific populations may provide a useful approximation of the degree of reproductive isolation. A number of studies of Pacific salmon have found genetic clustering of populations to occur largely along geographic lines (see references in Waples, 1991b). However, both types of exceptions to this pattern—pronounced differences between nearby populations and lack of differences between distant populations—have also been found, which emphasize the fact that distance is not the only barrier to gene flow. Consideration should also be given to other factors (e.g., geological history and physical and environmental gradients) that can affect population structuring. It must also be recognized that what appears to be a barrier to a biologist may not be a barrier to fish, and vice versa. Inferring barriers to migration on the basis of geographical or physical features alone can be misleading in the absence of supporting biological information.

As can be seen from this brief discussion, each of the above approaches has limitations. Nevertheless, it is important to consider all available information because the various approaches provide different insights into the question of reproductive isolation. Recolonization rates provide the most direct indication of the likelihood that a population, if eliminated, would become reestablished naturally. Approaches 1, 2, and 4 can provide data on current levels of migration or gene flow (over periods of one or a few generations). It is unlikely, however, that migration rates have been constant over long periods of time. In some cases, significant gene flow may occur only at intervals of decades or centuries. Genetic methods can be very informative in this context because they reflect the cumulative effects of gene flow over evolutionary time scales.

Ecological/Genetic Diversity

If available evidence indicates significant reproductive isolation, the next step is to determine whether the population in question is of substantial ecological/genetic importance to the species as a whole. In other words, if the population became extinct, would this event represent a significant loss to the ecological/genetic diversity of the species? In evaluating a population's contribution to ecological/genetic diversity, the following questions are relevant:

1) Is the population genetically distinct from other conspecific populations?
2) Does the population occupy unusual or distinctive habitat?
3) Does the population show evidence of unusual or distinctive adaptation to its environment?

Important factors to consider in addressing these questions include (but are not necessarily limited to) the following:

1) Genetic traits. Examples include presumably neutral characters detected by protein electrophoresis or DNA analyses as well as other genetically-based traits that are more difficult to quantify.
2) Phenotypic traits. Examples include morphological and meristic characters, occurrence of parasites, and disease and parasite resistance.
3) Life-history traits. Examples include time, size, and age at spawning; spawning behavior; fecundity; migration patterns; and timing of emergence and outmigration.
4) Habitat characteristics. This category includes such physical characteristics of the spawning and rearing habitat

as temperature, rainfall, stream flow, and water chemistry, as well as biological attributes of the local ecosystem. Location within a river drainage (e.g., upstream vs. downstream) and elevation can also be important in this regard. In a broader sense, the habitat for a population also includes areas encountered during the entire life cycle. Thus, a waterfall in the migratory route might select for robust fish capable of surmounting it, and a population with distinctive oceanic migration patterns probably utilizes marine habitat differently than do other populations.

Two points are important to consider in evaluating these types of data. First, such data can be properly evaluated only in relation to similar information for the species as a whole. That is, some reference data are necessary before one can determine that a particular population is distinct.

Second, it is unlikely that complete biological information will be available for any given ESA evaluation. This is particularly true for status reviews that must be conducted within a limited time period in response to formal petitions for listing. ESA determinations must be made on the basis of the best scientific information available at the time, and all relevant data should be considered.

Data from protein electrophoresis or DNA analyses permit direct inferences about genetic divergence and thus are particularly applicable to the question of population distinctness under the Act. However, if the common presumption is correct that the genetic characters detected by these methods are largely neutral with respect to natural selection, then it follows that differences among populations in these characters do not by themselves denote evolutionary significance, except in the sense that neutral genes may provide the raw material for future evolution. Rather, these genetic characters are primarily useful as indicators (or proxies) for evolutionary processes that can lead to local adaptation in other parts of the genome more directly related to fitness. In a similar way, although unique alleles do not necessarily reflect adaptation, they may, if numerous or at high frequency, provide an indication of likely adaptive differences elsewhere in the genome.

Several types of genetic analyses can provide information relevant to ESA considerations. Gene diversity analysis (Nei, 1973) can be used to partition the total genetic variance in a species in a variety of ways, including between-population, within-population, and between-years (within population) components. Results can be compared to data for other species (and other salmonids in particular) to provide insight into the degree of genetic distinctness of the population under consideration. Genetic distance indices can be used in a similar fashion, and comparisons of heterozygosity levels may also be informative in some cases.

Phenotypic and life history traits may reflect local adaptations, and for this reason they may be relevant to the evaluation of population distinctness. However, expression of these traits is known to be affected by environmental as well as genetic factors (Barlow, 1961; Clayton, 1981), which complicates their interpretation. Sorting out the genetic and environmental effects on phenotypic and life history characteristics is a challenging task and has been a central focus in evolutionary biology (Endler, 1986).

Analysis of habitat characteristics is important in two ways. First, the existence of unusual or distinctive habitat features allows for the possibility of unique adaptations in the local population. Second, identification of unusual or distinctive habitat is one step toward achieving the broad purpose of the Act—to preserve threatened and endangered "species" and the ecosystems they inhabit. Again, however, caution should be used in drawing inferences based on physical characteristics of the habitat without supporting biological information linking the habitat differences to adaptations. Just as our perception of what constitutes a barrier to fish migration can be faulty, so too our understanding of the importance of various habitat characteristics to organisms is far from complete.

Recommended Approach

The following two-step approach is suggested for making a determination regarding population distinctness under the Act.

1) Evaluate the degree of reproductive isolation. If there is gene flow with other populations, it should be at a level low enough to permit evolutionarily important divergence. If apparent migration rates with adjacent populations are high, the population would not be considered isolated unless there is evidence that the genetically effective migration rate is much lower. Approaches outlined in the previous section on "Reproductive Isolation" should be used to address the questions of migration rate, gene flow, and recolonization rate.

If the population is believed to be reproductively isolated, an evaluation under step 2 (below) should be made; if it is not isolated, the population is not an ESU and should not be considered a separate "species" under the Act (presumably, however, it would be part of a larger unit that is an ESU).

2) Evaluate evidence for ecological/genetic distinctness in the context of similar data from throughout the species range, as well as for other species as appropriate. Often, this process will involve difficult judgments concerning the relative importance to attach to different types of evidence. Although a variety of approaches may prove useful in making this determination, none will provide a completely objective assessment of evolutionary significance. Nevertheless, some general guidelines can be suggested to aid the evaluation process.

The existence of substantial genetic differences from other conspecific populations based on protein electrophoresis or DNA analyses would strongly suggest that evolutionarily important, adaptive differences also exist[7]. The failure to find such differences (or the absence of genetic data) would not rule out the possibility that such adaptive differences exist, but it would place a greater burden of proof on data for other characters. Data for habitat characteristics should be interpreted in a similar fashion: habitat dif-

[7] Although protein electrophoresis and DNA techniques are considered together here for convenience, a variety of parts of the nuclear or mitochondrial genome can be targeted for genetic study. Sensitivity of the different approaches can vary considerably, and this factor must be considered in interpreting the results of genetic analysis.

ferences suggest (but do not prove) the possibility of adaptive differences, whereas the inability to detect habitat differences constrains the scope of possible local adaptations but does not prove they do not exist. In evaluating data for phenotypic and life-history traits, every effort should be made to account for environmental effects that are manifested in periods shorter than one generation (and therefore do not reflect adaptations).

Special Considerations

Anadromy/Nonanadromy

Some species of Pacific salmon, *Oncorhynchus nerka*, *O. mykiss*, *O. clarki*, and perhaps others, have nonanadromous as well as anadromous forms that occur together. A similar phenomenon occurs in some species of the genera *Salmo* and *Salvelinus*. This raises the question whether the two forms should be considered jointly or separately in deciding if a population is "distinct" under the Act. The following general guidelines are suggested.

The two forms should be considered separately if they are reproductively isolated. As noted above, the question of reproductive isolation is likely to be one of degree. Again, the key question is whether isolation is strong enough for evolutionarily important differences to develop in the two forms. Data from protein electrophoresis or DNA analyses can be valuable in making this determination, as can observations of time and place of spawning and behavioral interactions during spawning. Information from other populations (and other species) can help provide a context for making the evaluation. However, such studies suggest that a variety of scenarios probably exists in nature, from substantial reproductive isolation of sympatric anadromous and nonanadromous forms to substantial life-history plasticity within presumably a single gene pool (Foote et al., 1989; Kirpichnikov et al., 1990). Therefore, such studies are unlikely to provide an unequivocal answer for an unstudied anadromous/nonanadromous system.

If substantial gene flow occurs or has recently occurred between the two forms, they represent polymorphisms within a single population and should be considered as a unit for purposes of the Act. In determining whether such a population unit is an ESU, the anadromous and nonanadromous traits should be considered in the same manner as other population characteristics discussed in the section on "Ecological/Genetic Diversity." The important questions are whether the traits have a genetic basis and whether they help to make the population unit "distinct" from other populations. For example, an anadromous/nonanadromous unit might be considered an ESU if other ecologically comparable populations of the species harbored only the nonanadromous form. In this case, if the population unit is considered to be an ESU solely or primarily on the basis of the anadromous trait, then the potential loss of anadromy should be a legitimate ESA concern. A key question would be whether the nonanadromous form was likely to give rise to the anadromous form after the latter had gone locally extinct. Therefore, an anadromous/nonanadromous population unit could be listed based on a threat to one of the life-history traits, if the trait were genetically based and loss of the trait would compromise the "distinctness" of the population.

Differences in Run-time

In several species of Pacific salmon, biologists recognize different run-times, or races, of fish inhabiting the same general area. Generally, run-times are determined on the basis of the time of year at which adults enter fresh water to spawn; in some cases, fish with different run-timing also have different juvenile life history patterns (Healey, 1983; Groot and Margolis, 1991). The question whether such races represent "distinct" populations under the Act can be addressed in the framework developed above.

First, it should be determined whether the different run-times are reproductively isolated. Often, the formal distinction between run-times is rather arbitrary, with (for example) fish appearing before a certain date classified as "springs" and those appearing after that date as "summers." Races that are arbitrarily defined in this fashion may in fact be reproductively isolated, but if so this needs to be demonstrated biologically (for example, by providing evidence for a discrete distribution of run-times or distinct times and/or locations of spawning).

Assuming that fish with different run-times are reproductively isolated, they can be considered distinct populations under the Act if they exhibit evolutionarily important ecological/genetic differences, as outlined in the section on "Ecological/Genetic Diversity." In the absence of substantial isolation between run-times (or if the races are reproductively isolated but do not individually satisfy the ecological/genetic diversity criterion), a "population" unit consisting of two or more recognized run-times could be considered an ESU if it were isolated from and distinct from other populations.

Hatchery Fish

Artificial propagation has been used in one form or another with anadromous Pacific salmon for over a century. Hatcheries have been used both for fisheries enhancement (largely as mitigation for losses of native stocks caused by destruction of habitat or blockage of migratory routes) and in an attempt to boost production of naturally-spawning fish (Lichatowich and McIntyre, 1987). Currently, hatchery operations in the Pacific Northwest are carried out on a large scale, and the majority of adult fish produced in many systems are of hatchery origin (Washington, 1985; Vreeland, 1986). It is important, therefore, to consider the role that hatchery fish play in ESA considerations for Pacific salmon.

The Act (Sec. 3(3)) identifies "propagation" as one method that may be used to conserve threatened or endangered species, and both NMFS and FWS have used captive breeding or other artificial propagation techniques with listed "species," including several fishes. Artificial propagation may thus be an appropriate tool for use in recovery plans for some "species" of Pacific salmon. However, the Act also mandates conservation of native ecosystems "upon which endangered species and threatened species depend." The key here is the link between threatened and endangered species and their native ecosystems; the link may be jeopardized if either component (the species or the ecosystem) is emphasized to the exclusion of the other. Because a

fish hatchery is not a substitute for a natural ecosystem, maintaining a "species" in a hatchery while allowing degradation of its native habitat is not consistent with the stated purposes of the Act. For this reason, attention in ESA evaluations of Pacific salmon should focus on fish that spend their entire life cycle in their native habitat—i.e., those that are progeny of naturally spawning parents. Such fish will be referred to as "natural" in the ESA context[8]. Threshold determinations also will focus on natural fish, on the premise that an ESU is not healthy unless a viable population exists in the natural habitat.

Once the natural component of a population has been identified, the next step is to determine whether this population component is "distinct" for purposes of the Act. In making this determination, the twofold criteria for defining an ESU should be used. If it is thought that artificial propagation may have affected the genetic composition of the natural population (either directly through supplementation or indirectly through straying of hatchery fish), factors outlined in the following section should be considered. Fish are not excluded from ESA consideration simply because some of their direct ancestors may have spent time in a fish hatchery. However, there are a number of potential genetic consequences of artificial propagation that should be considered in this context. Thus, fish meeting the definition of "natural" adopted here may subsequently be excluded from ESA consideration for other reasons.

In developing recovery plans for "species" listed as threatened or endangered, the use of artificial propagation may be considered. If a hatchery is associated with the listed "species," an important question to address in formulating a recovery plan is whether the hatchery population is similar enough to the natural population that it can be considered part of the ESU defined on the basis of that natural population. Factors to consider in this regard are discussed in the next section. Given various uncertainties, it should generally be presumed that hatchery fish are not part of an ESU unless there is a compelling reason for including them.

Effects of Artificial Propagation and Other Human Activities

Two possible effects of artificial propagation are of particular concern in ESA evaluations: 1) Genetic changes within a population and 2) mixtures of genetically distinct populations. These effects should be evaluated from the perspectives of both the hatchery population(s) involved and any natural population that may have been affected. That is, an evaluation of the nature and extent of these effects will help to determine 1) whether a natural population is an ESU and 2) whether a hatchery population(s) should be included in an ESU defined on the basis of a distinct natural population.

Supplementation (the release of hatchery-reared fish into habitat occupied by fish of the same biological species), transfer of eggs or fish among hatcheries, and unintentional straying are all aspects of artificial propagation that can lead to population mixing. Such mixing is relevant to the question of population distinctness because a population that has been overplanted with fish of different origin may not be an ESU even if it once was distinct. Similarly, a hatchery stock that has resulted from extensive transfers of exogenous fish is unlikely to be part of an ESU.

Apart from the effects of stock mixtures, artificial propagation can also lead to either random or directional genetic change within cultured populations. Random genetic changes, if of sufficient magnitude, may lead to the erosion of genetic variability and may overwhelm selection for locally adapted genotypes, thus reducing fitness. Directional genetic change can occur at the time of founding a hatchery population (or between generations in a hatchery) through choice of which individuals will be allowed to reproduce. In addition, fish hatcheries differ from the natural environment in a number of ways that may affect selective pressures experienced by the population.

Determinations regarding the distinctness of populations that may have been affected by artificial propagation should be consistent with the twofold criteria that define an ESU. In making this determination, it may be useful to consider whether the population was likely to have been an ESU in the past and to ask whether stock mixing (or other aspects of artificial propagation) has compromised the evolutionarily important adaptations that distinguished the original population. Several factors should be considered in this context. (It is assumed that the population in question was distinct enough to be an ESU prior to the effects of human activities.)

First, although stock transfers of anadromous Pacific salmon have been widespread in the past (Withler, 1982), evidence merely of the release of exogenous fish is not sufficient to disqualify a population from consideration as an ESU. Stock transfers (or straying) have a direct genetic effect only if the transplanted fish successfully reproduce and contribute to subsequent generations of the native stock. Results of supplementation efforts with Pacific salmon have been quite variable (Ricker, 1972) and at present are largely unpredictable. For example, a recent review of salmonid studies (Hindar et al., 1991) cited examples in which the native stock had been largely or entirely displaced, examples of hybridization between native and introduced fish, and examples in which repeated hatchery releases had no detectable genetic effect on the native population. Therefore, it should not automatically be assumed that transplantation efforts have permanently altered the genetic structure of native populations; similarly, some stock transfers among hatcheries may not have permanent genetic effects.

Second, a population that does not represent a completely pure native gene pool may still qualify as an ESU if it is adapted to its local environment and is "distinct" by the criteria outlined in the section "Application to Pacific Salmon." This point is important for Pacific salmon, because there are relatively few populations for which the possibility of some genetic influence from transplants or hatchery strays can be completely excluded. How much introgression from other gene pools must occur before a population no longer merits consideration as an ESU depends to some extent on

[8] This corresponds to the usage suggested by Bjornn and Steward (1990); some other authors have used the term in a different way.

the degree of distinctness of the original population.

In evaluating the effects of population mixture, the following types of information should be gathered whenever possible:

1) Genetic, phenotypic, and life history traits and habitat characteristics for all stocks involved;

2) Broodstock and rearing protocols for all relevant stocks;

3) Dates of release, number released, and developmental stage at release for transplanted stocks (or those involved in straying);

4) Trends in abundance of the local population for a time span bracketing the periods(s) of release (or straying events); and

5) Evidence for reproductive success of transplanted (or stray) fish, including evidence for incorporation of foreign genes into the local population.

Ideally, information will be available for the transferred stock as well as for the local stock both before and after the mixture event(s). This type of data would allow an evaluation of whether changes in the local stock are in the direction predicted under the assumption that the mixing has had a permanent effect. In practice, such extensive data will not always be available, and in this event the evaluation can be much more difficult. Nevertheless, there are some approaches (Waples and Smouse, 1990) that have reasonable power to detect population mixtures under certain conditions.

Finally, although genetic changes within cultured populations are a legitimate ESA concern, the effects of such changes on the viability of natural populations of anadromous salmonids are largely unknown. Random changes occur in all populations, at a rate inversely proportional to the effective population size. Unless the number of spawners is severely limited, random changes can generally be minimized in cultured populations by following appropriate broodstock practices (Meffe, 1986; Simon et al., 1986; Allendorf and Ryman, 1987). This has not always been the case with fish hatcheries, however. Allendorf and Ryman (1987) reviewed a number of studies of cultured populations of trout and Atlantic salmon that provide evidence for severe inbreeding depression and/or substantial loss of genetic variation. Such dramatic effects have not been documented with Pacific salmon, but there is indirect evidence (Waples and Teel, 1990) that effective population size in some hatcheries is small enough that such problems are a potential concern.

For populations under ESA consideration, the importance of random changes attributable to a history of artificial propagation can be evaluated by examining the number and sex of spawners each year, methods of fertilization, and rearing protocols. Exports of eggs or progeny offsite should also be considered; if (as has often occurred in the past) the entire production of certain families is shipped to another hatchery, those families do not contribute to the effective size of the local population (Simon et al., 1986). In addition, the variability among individuals in reproductive success is a key factor in determining effective population size, but this parameter is very difficult to measure for Pacific salmon. For this reason, a monitoring program that uses indirect genetic methods (Waples, 1990b) can provide useful insights into the magnitude of random genetic changes.

Directional genetic changes can occur from a variety of factors. The practice of culling fish according to age, time of return, size, or appearance was formerly widespread in Pacific salmon hatcheries (Donaldson and Menasveta, 1961). More recent awareness of the drawbacks to this approach can help to minimize such effects, but they cannot be eliminated entirely. In addition, anadromous fish hatcheries, if successful in their goal of ensuring that a large proportion of progeny survive to time of release, also dramatically alter the mortality pattern for the population. In general, this can be expected to lead to genetic change relative to a population that spawns naturally (Waples, 1991b). Furthermore, a number of characteristics of the hatchery environment—both physical (e.g., substrate type, water temperature and flow, and the variability of same) and biological (e.g., density, food type and source, behavioral interactions, incidence of predators)—differ so markedly from the natural environment that selective changes are likely.

Unfortunately, it is easier to identify the potential genetic risks posed by artificial propagation than to evaluate their actual impact on a given population. As a general principle, it is probably fair to say that genetic changes in a population that result from adaptation to hatchery conditions are unlikely to increase the fitness of the population in the natural environment. How rapidly such effects occur, however, and whether they are reversible are open questions at present.

Some idea of the likely magnitude of selective changes due to artificial propagation can be gained by considering past hatchery practices for the stock in question and the number of generations in culture. If possible, baseline data from the original (prehatchery influence) population should be compared to data from the current population. Focus should be on 1) possible reductions in the ability of the population to survive and reproduce in the natural environment, and 2) possible changes in characteristics that help to make the population distinct. Again, genetic changes within cultured populations are important to consider from the perspective both of the hatchery population (and its relation to an ESU) and any natural populations that may have been affected by the cultured stock.

Other human activities (e.g., fishing, habitat degradation) can also alter the genetic structure of native populations, and the importance of these factors to ESA considerations can be evaluated in a similar way. For example, fishing pressure can selectively affect certain size or age groups (Ricker, 1981; Nelson and Soule, 1987), and these characteristics may be heritable. The relevant question is whether the activities have changed the population so much that it no longer represents an evolutionarily significant component of the biological species.

Introduced Populations

In general, populations resulting from the introduction of fish into a local area not occupied by the biological species (particularly if the area is outside the historic range of the species) are probably not ESU's because they do not contrib-

ute to maintaining diversity of the species in its native habitats. Again, the key is the link between a "species" and its native habitat, and this link is broken when fish are moved from one ecosystem to another. Some introduced populations should not be excluded from ESA consideration, and these include populations occupying habitat that is ecologically similar and geographically proximate to the source population, and those that represent the only remaining component of a native gene pool. In the former case, the introduced population may be determined to be part of the same ESU as the parent population; in the latter case, the population could be determined to be an ESU if it met the criteria outlined in the section "Application to Pacific Salmon."

Historic Population Size

For a population that once was abundant but since has declined in numbers, there should be no minimum size for ESA consideration. However, populations may also be small because of limiting physical or biological factors. For Pacific salmon, suitable habitat may severely limit the potential number of spawners and hence the carrying capacity of small streams. Given the large temporal fluctuations in abundance documented for every species (and many populations) of Pacific salmon, and given the likelihood that even greater fluctuations have occurred over evolutionary time, there must be some size below which a spawning population is unlikely to persist in isolation for a long period of time. The fact that small spawning aggregations are regularly observed may reflect a dynamic process of extinction, straying, and recolonization. Such small populations are unlikely to be ESU's, although a collection of them might be. Therefore, the historic size[9] of a population may be useful in evaluating whether it is an ESU.

Both genetic and demographic factors should be considered in making this evaluation. Although there is no consensus among geneticists regarding the minimum effective population size per generation (N_e) necessary to avoid long-term problems of inbreeding and loss of genetic variability, most estimates are in the range of several hundred (see discussions by Lande and Barrowclough (1987) and Simberloff (1988)). For a species with overlapping age classes and an average age at spawning of 3-5 years (typical of many populations of steelhead and chinook, chum, and sockeye salmon), this would correspond to an effective number of breeders per year (N_b) of perhaps 50-100. (Waples (1990a) provides a discussion of the rate of loss of genetic variability in Pacific salmon and the relationship between N_e and N_b.) Because not all individuals successfully spawn, and because the variance among individuals in reproductive success may be high, the total number of adults must generally be somewhat more than this (perhaps several times as many). The long-term persistence of an isolated population also depends on its ability to withstand inevitable (and often large) fluctuations in abundance caused by the interplay of population dynamics, changing environmental conditions, and chance events. The importance of these factors varies among species and among populations within species and must be evaluated on a case-by-case basis. In general, however, such fluctuations may place greater constraints on the long-term survival of small populations than do genetic factors associated with inbreeding.

A Pacific salmon population should not be considered an ESU if the historic size (or historic carrying capacity) is too small for it to be plausible to assume the population has remained isolated over an evolutionarily important time period. In making this evaluation, the possibility should be considered that small populations observed at present are still in existence precisely because they have evolved mechanisms for persisting at low abundance. Population genetics theory indicates that gradual inbreeding over a period of time may purge deleterious, recessive alleles from a population, lessening the effects of inbreeding depression and allowing a smaller effective size than would ordinarily be the case. It is possible that, in some populations, a similar process—evolution of demographic parameters, for example—may have occurred that modulates the effects of environmental variability. Because such populations would contain adaptations that might truly be considered to be of evolutionary significance to the species, and because small populations in general are a likely source of evolutionary innovation, it is prudent to exercise caution in eliminating a population from ESA consideration simply on the basis of historic size. In particular, theoretical considerations about the likely persistence time of small populations should not override strong evidence for long-term reproductive isolation. Nevertheless, this concept should prove useful in focusing attention on population units with the greatest probability of representing ESU's.

Groups of Populations

As anadromous species, Pacific salmon spawn in a freshwater environment that is often naturally organized in a hierarchical fashion: Major river systems may contain several large tributaries, each with numerous streams fed by smaller creeks, etc. Other areas may be characterized by numerous smaller streams, each entering directly into a tidewater area. In both cases, geographic, environmental, or other factors may naturally lead to genetic structuring of the various spawning aggregations into more or less discrete units. The first step in determining the appropriate hierarchical level for consideration as an ESU is to identify units within which levels of gene flow are high relative to the rate of exchange between units. Often, however, there will be more than one hierarchical level for which this is true. Therefore, it is important to identify reproductively isolated units that also contribute substantially to ecological/genetic diversity of the species as a whole.

Determining the appropriate level for consideration as an ESU is a challenging task with Pacific salmon. Although the strong homing instinct of these species indicates that even small spawning aggregations may potentially represent

[9] This concept is related to, but differs from, the concept of minimum viable population size (MVP) (Shaffer, 1981; Soule, 1987). The MVP concept considers the future and asks how large a population must be to have an acceptably high probability of surviving a specified period of time. The historic population size concept considers the past and asks how small a population must be before it becomes unreasonable to assume it has persisted in isolation long enough for important adaptations to evolve.

biological populations, such populations may not meet the criteria to be considered "distinct" under the Act. A group of populations, however, might be distinct from, and isolated from, other groups of populations. Such a group of populations can constitute an ESU and, if determined to be threatened or endangered, can be afforded protection under the Act.

In evaluating the appropriate grouping level, a balance must be struck between two opposing concerns. On the one hand, it is important to identify the smallest units that meet the criteria set out in the section on "Application to Pacific Salmon," because this allows the greatest flexibility in ensuring the appropriate level of protection for different ESU's within a more comprehensive group. On the other hand, we have seen in the previous section, "Historic Population Size," that the smallest units supporting local populations of salmon may not be evolutionarily independent from other nearby populations. A key question is: How can evolutionarily important units be protected without running the risk of attempting to artificially maintain units that might naturally undergo episodes of extinction/recolonization on something short of evolutionary time scales?

The following approach is suggested. Identifiable ESU's should not be combined for the sake of convenience. In general, however, ESU's should correspond to more comprehensive units unless there is clear evidence that evolutionarily important differences exist between smaller population segments. This approach is consistent with the recommendation that NMFS and FWS should use sparingly their authority to list vertebrate populations, and only if biological evidence clearly warrants it. In addition, this approach reflects 1) the view that population "distinctness" should be supported by positive scientific evidence and 2) the concern that fragmenting groups of populations into multiple ESU's on the basis of insufficient data may create artificial units without a biological basis.

Nevertheless, it is recognized that the long-term viability of a larger unit may also depend on the continued existence of multiple, semi-independent units it comprises. Fragmentation or gradual loss of habitat can pose a threat to larger population units. The underlying concern should be whether important genetic resources of the biological species are at risk because of the fragmentation. If so, then the appropriate action may be to protect the larger population as a whole, rather than the individual fragments. In this context, NMFS recognizes that thresholds for threatened and endangered status must be flexible enough to deal with threats to groups of populations (metapopulations) and clinal populations as well as more discrete population units. Just as there is no simple formula for determining evolutionary significance, there is no universally applicable numerical threshold for a listing determination; in both types of evaluation, a variety of factors must be considered. Recovery plans for listed "species" could take this into account by ensuring protection for smaller units within a more comprehensive ESU. This might be appropriate, for example, if the smaller units differ in various characteristics but it is uncertain how these differences relate to evolutionary significance.

Interpreting Results of Statistical Tests

Sampling Considerations

Rigorous analyses of data used in ESA considerations will include testing hypotheses whenever possible, and sampling protocols are important to consider in this context. In general, regardless of the characters being considered, the appropriate null hypothesis to test is that no differences exist between the populations being compared. Sampling from the populations introduces a source of random error with magnitude inversely proportional to sample size. In many statistical tests, the implicit assumption behind the null hypothesis is that the samples being compared were randomly drawn from the same population. There are several ways in which this basic assumption might be violated by the method of sampling, and the effects of violating the assumption are often magnified in small populations (as may frequently be encountered in ESA evaluations). Furthermore, the unusual life history features of Pacific salmon (in particular, the combination of overlapping age classes with one-time reproduction) provide some additional opportunities for sampling bias. These factors should be kept in mind in designing sampling plans and in evaluating results.

Temporal changes within populations. Although most Pacific salmon spend the majority of their life at sea, they exist in recognizably discrete populations only during rearing as juveniles and spawning as adults. By necessity, samples are generally taken from local spawning populations, and often only a single brood year is sampled. It must be realized that the population as a whole includes several brood years, and values for a given character will show year-to-year variation around the mean for the population as a whole. The effects of temporal variation within a population must be considered in comparing single-brood-year samples from different populations; in general, this factor will inflate the observed level of difference above that predicted by the null hypothesis, even if the overall population means do not differ (see discussion of a similar point in Waples and Teel (1990)). A study plan that involves temporally spaced samples within sites as well as samples from geographically distinct localities is the best way to evaluate the significance and stability of between-population differences.

Life history stage sampled. Waples and Teel (1990) showed that, in comparing two (or more) samples, the probability of a statistically significant result may depend on the life history stage sampled. In general, sampling juveniles will tend to produce larger differences (and a higher probability of a significant test result) than sampling adults. This will be a minor effect if the sample size is small relative to the population size, but this will not always be true for populations under ESA consideration.

Nonrepresentative sampling. Most statistical tests assume random sampling, which means that every individual in the population(s) has an equal opportunity to appear in the sample. There are several ways in which this condition might not be met with Pacific salmon. For example,

adults may be sampled during only part of the spawning run, or only in limited areas of a stream. Under certain circumstances, samples of juveniles may include large numbers of individuals from the same family. Methods for sampling either life history stage may select for certain types of individuals.

Significance of Results

It is important to realize that "statistical significance" is a different concept than "evolutionary significance" as it relates to the Act. In the present context, a statistically significant result indicates that the means for a pair or group of samples differ by more than would reasonably be expected if a single population were sampled repeatedly. The conclusion, then, would be that the population means are different for the character under consideration. Being "different," however, is not the same as being "distinct" under the Act. For a population to be considered an ESU, it must differ from other populations in an evolutionarily important way. Statistical tests can be useful in making this determination but do not in themselves provide direct evidence regarding evolutionary significance. Similarly, failure to find a statistically significant difference does not disprove the existence of population differences. Power to detect true differences in population means is a function of sample size, so this factor should also be considered in evaluating results of statistical tests.

General Comments

This paper presents a simple, flexible framework for interpreting language in the Endangered Species Act pertaining to vertebrate populations: Simple because a pair of criteria can be applied to determine whether a population segment is distinct and hence a "species" for purposes of the Act, and flexible because the two criteria can be used to address a variety of issues of particular concern for Pacific salmon.

By focusing on evolutionary significance, the ESU concept also provides a means of dealing with several recurring problems posed by the term "distinct population segment." For example, in 1979 the GAO pointed out potential abuses in the authority to list vertebrate populations, suggesting that this could lead to absurdities such as the listing of squirrels in a specific city park[2]. Such a result is unlikely under the present framework. Although squirrels in a park might at present be effectively isolated from squirrels in other parks or natural habitat, such a population would be unlikely to meet the second criterion for an ESU (evolutionary significance). Similarly, a population unit recently isolated as a result of human activity (by the construction of a dam, for example) probably also does not meet the second criterion, because most of the diversity presumably would be retained in the larger source population. The same might be true for some natural isolates, particularly those of recent origin.

Many will have noted that although this paper establishes a framework for considering populations of Pacific salmon under the Act and provides guidance for its application, it does not provide a simple formula for determining whether the unit under consideration is a "species." To the extent that the process would be simpler and more objective with such a formula, the approach adopted here is a disadvantage. However, use of a simple (or even a complex) formula does not seem consistent with the stipulation of the Act to make decisions "solely on the basis of the best scientific and commercial data available." The process of evolution and differentiation within and between species is manifest in so many different ways that no simple yardstick will be universally applicable. Ryder (1986:10) came to essentially the same conclusion regarding the difficulties in identifying important conservation units within mammalian species:

"Identification of ESU's within a species was recognized as a difficult task, requiring the use of natural history information, morphometrics, range and distribution data, as well as protein electrophoresis, cytogenetic analysis, and restriction mapping of nuclear and mitochondrial DNA."

Acknowledgments

This paper has drawn heavily on several sources. First, the paper by Utter (1981) laid a solid foundation to build upon, and many of the ideas expressed in that earlier paper appear here in similar form. Second, NMFS solicited public comments on a summary of the 1981 paper, and a number of those suggestions, including ideas discussed at ESA Technical Committee meetings, have been incorporated into the present document. Third, many of the concepts outlined here were discussed in one form or another at the FWS-NMFS Vertebrate Population Workshop held in Washington, D.C., in June 1990. The goal of the workshop was to develop a consistent approach for determining whether vertebrate populations are "distinct" under the Act. In particular, I want to acknowledge Andrew Dizon (NMFS, La Jolla, Calif.) for introducing the ESU concept and Mark Shaffer (FWS, Washington, D.C.) for stressing the importance of considering ecological and habitat characteristics. Fourth, public comments on the NMFS Interim Policy and accompanying technical paper were useful in preparing this revised document. Finally, a number of people within NMFS and FWS and the NMFS Peer Review Group critically reviewed drafts of the manuscript, and Pat Montanio (NMFS, Silver Spring, Md.) and Karl Gleaves (Office of NOAA General Counsel, Silver Spring, Md.) provided valuable guidance relating to the legislative and legal history of the Endangered Species Act.

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