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Definition of "Species" Under the Endangered Species Act: Application to Pacific Salmon

by
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Summary

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 suggests such an approach and explains in some detail how it can be applied to ESA considerations 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 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 contributed 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, recolonization rates of 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, electrophoretic (or DNA) differences 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 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 making this determination, the following questions are relevant:

- Is the population genetically distinct from other conspecific populations?
- Does the population occupy unique habitat?
- Does the population show evidence of unique adaptation to its environment?

and, more generally,

- If the population became extinct, would this event represent a significant loss to the ecological/genetic diversity of the species?

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. Electrophoretic data provide valuable insight into levels of overall 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 regarding Pacific salmon, including anadromous/non-anadromous population segments, differences in run-timing, supplementation, introduced populations, and the role of hatchery fish.

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 (the Act; ESA). 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, therefore, may merit protection), determination of thresholds for listing as threatened or endangered, and determination of the role (if any) of artificial propagation in development of recovery plans for listed "species."

On receipt of petitions to list several populations of Pacific salmon as "species" under the Act (April-June 1990), NMFS sought public comments on draft Policy Position Paper #1, "Definition of Species" [a summary of a longer document by Utter (1981)]. The present document is a synthesis of ideas outlined in the previous draft species definition paper, public comments on that paper, and concepts developed over the past decade.

I. 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

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[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.

provide a program for the conservation of such endangered species and threatened species." A review of legislative history indicates that a major motivating factor behind the Act was the desire to preserve genetic variability, both between and within 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. No. 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.

Under the 1973 ESA, 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 [Pub. L. 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" (emphasis added).

Congress has provided limited guidance for interpretation of this definition. Congress declined to enact a provision in the 1979 amendments recommended by the General Accounting Office (GAO)[2] that would have removed the authority to list vertebrate populations. The Senate Report to the 1979 amendments, however, stated that "the committee is aware of the great potential for abuse of this authority

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[2]GAO, Endangered Species--A Controversial Issue Needing Resolution, Report to Congress (1979).

and expects the FWS to use the ability to list populations sparingly and only when biological evidence indicates that such action is warranted" (S. Rep 151, 96th Cong., 1st Sess., 1979).

In an attempt to develop guidelines for and consistency in interpreting the language of the Act pertaining to vertebrate populations, FWS and NMFS convened a Vertebrate Population Workshop in June 1990. The goal of the workshop was to recommend a consistent approach for determining whether vertebrate populations are "distinct" under the Act. The framework adopted here reflects concepts discussed at the workshop.

Proposed 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 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."

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[3]The term "evolutionarily significant unit" apparently originated with Ryder (1986). The usefulness of this concept in the context of ESA evaluations was stressed at the Vertebrate Population Biology Workshop by A. Dizon (NMFS, La Jolla, CA). See Dizon et al. 1991 for additional discussion of this and related concepts.

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 unique habitat or in other ways represents an important ecological adaptation for the species may also be an ESU.

This framework 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 species. In this framework, reproductive isolation is a necessary but not a sufficient condition for a population to be considered "distinct." Presumably, given enough time, an isolate will evolve into an ESU, but isolation by itself does not confer distinctness. For example, a population recently isolated as the result of human activity probably does not play an integral role in maintaining ecological or genetic diversity of the species. The same might be true for some natural isolates, particularly those of recent origin.

II. 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.

A. Reproductive isolation[4]

With Pacific salmon, reproductive isolation 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, 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 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 Sewall Wright's work 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 fail to reproduce or do not produce viable offspring.

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,

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[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 Section III-C.

[5]Non-anadromous populations or segments of populations are considered in Section III-A.

the above comments apply to a balance between migration and genetic drift of neutral alleles. If there is local adaptation for different alleles in different populations, the homogenizing effects of migration will be counteracted somewhat by different selective regimes, thus allowing greater differences among populations to be maintained.

Another way to consider the migration problem is to view it as a question of replacement. In this context, the relevant question is, 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 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, some insight can be gained from observations of recolonization rates in other populations and/or species.

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;
5) Identification of physical or geographic features likely to act as barriers to migration.

Since the 1950s, extensive data bases have been developed that provide important information about the oceanic distribution of Pacific salmon (e.g., Hartt 1962).[6] More recently, physical tags have been used to study migrations of fish from individual populations. However, tagging efforts typically focus on hatchery stocks, and much less is known about straying in natural populations. Furthermore, such studies do not provide direct evidence of gene flow. A salmon 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, 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 (e.g., Seeb et al. 1986; Lane et al. in press) 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

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[6]See also subsequent INPFC Bulletins on salmon distribution.

overestimate migration rates if not enough time has elapsed to obtain a balance between the forces of migration and genetic drift.

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 (e.g., 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, geographic proximity (e.g., linear stream distance) of a population from other conspecific populations can 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. However, exceptions to this pattern 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.

As can be seen from this brief discussion, each of the above approaches has some 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. Wright's and Slatkin's methods can be very informative in this context because these indices reflect the cumulative effects of gene flow over evolutionary time scales.

B. 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. Because of the emphasis in the Act on conserving native ecosystems, ESA considerations of Pacific salmon should focus on naturally-spawning fish. In determining whether a wild[7] population is an ESU, it is sufficient to demonstrate that the population in question is "distinct" from other wild populations. Relevant questions to ask in this context are

- Is the population genetically distinct from other conspecific populations?
- Does the population occupy unique habitat?
- Does the population show evidence of unique adaptation to its environment?

and, more generally,

- If the population became extinct, would this event represent a significant loss to the ecological/genetic diversity of the species?

Factors that are important to consider in addressing these questions include (but are not necessarily limited to) the following:

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[7]Wild fish in this usage includes all fish that are the progeny of naturally-spawning fish.

1) Genetic traits. Examples include characters detected by protein electrophoresis or DNA analyses.

2) Phenotypic traits. Examples include morphological and meristic characters, occurrence of parasites, and disease 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. 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 distinct marine habitat.

Two points are important to consider in evaluating these 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 essential before one can determine that a particular population is distinct.

Second, it is unlikely that all relevant 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 to list. ESA determinations must be made on the basis of the best available scientific information, and all types of data (including genetic, phenotypic, and life-history traits and habitat characteristics) 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.

Several types of genetic analyses can provide information relevant to ESA considerations. Gene diversity analysis 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 with 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, which complicates their interpretation.

Analysis of habitat characteristics is important in two ways. First, the existence of unique habitat features allows for the possibility of unique adaptations in the local population. Second, identification of unique habitat is one step toward

achieving the broad purpose of the Act--to preserve threatened and endangered "species" and the ecosystems they inhabit.

C. Recommended approach

The following 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 Section A 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 for listing under the Act.

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 electrophoretic or DNA differences from other conspecific populations would strongly suggest that evolutionarily important,

adaptive differences also exist. The failure to find electrophoretic or DNA 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.[8] Data for habitat characteristics should be interpreted in a similar fashion: habitat differences 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).

III. Special Considerations

A. Anadromy/nonanadromy

Some species of Pacific salmon (*Oncorhynchus nerka*, *O. mykiss*, and *O. clarki*) 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

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[8]Although "electrophoretic and DNA" techniques are considered here together for convenience, there are a variety of parts of the nuclear or mitochondrial genome that can be targeted for study. It is important to realize that the sensitivity of the different approaches can vary considerably, and this factor must be considered in interpreting the results of genetic analyses.

evolutionarily important differences to develop in the two forms. Genetic data can be valuable in making this determination, as can observations of general spawning behavior, including time and place of spawning. Information from other populations (and other species) can help provide a context for making the evaluation. However, such studies have shown that a wide 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). 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 of 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 Section II-B. That is, 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 all 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. Therefore, an anadromous/nonanadromous population unit could be listed based on a threat to one of the life-history traits, if loss of that trait would compromise the "distinctness" of the population.

B. 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. 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 distinction between spring- and summer-run (for example) is fairly arbitrary, with fish appearing before a certain date classified as "springs" and those appearing after that date as "summers." In such cases, and in the absence of a discrete distribution of run-times, additional evidence (for example, time and place of spawning) needs to be developed to establish reproductive isolation.

Assuming that fish with different run-times are reproductively isolated, they can be considered to comprise distinct populations under the Act if they exhibit evolutionarily important ecological/genetic differences, as outlined in Section II-B above. In the absence of substantial isolation between run-times, 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.

C. Effects of supplementation and other human activities

Supplementation (or overplanting) is the release of hatchery-reared fish into a wild area occupied by native fish of the same biological species. The effects of supplementation are 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. However, evidence merely of the release of exogenous fish is not sufficient to disqualify a population from consideration as an ESU. In making that determination, two factors should be considered. It is assumed that the population in question was distinct enough to be an ESU prior to contact with exogenous fish.

First, supplementation (or straying from hatcheries) has 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 (Hindar et al. in press) cited examples in which the native stock had been largely or entirely displaced, examples of hybridization between native and hatchery 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.

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 Section II-B. 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 on the degree of distinctness of the original population. The key question to consider is whether stock mixing has compromised the evolutionarily important adaptations that distinguished the original population.

In evaluating the effects of supplementation on native gene pools, the following types of information should be gathered whenever possible:

1) Genetic, phenotypic, and life history traits and habitat characteristics for the transferred stock;

2) Brood-stock and rearing protocols for the transferred stock;

3) Dates of release, number released, and developmental stage at release;

4) Trends in abundance of the local population for a time span bracketing the time(s) of release;

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

Other human activities (e.g., fishing, pollution, and artificial propagation) can also affect the genetic structure of native populations, and the importance of these factors to ESA considerations can be evaluated in a similar way. Again, 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.

D. Hatchery fish

Hatchery fish may enter into ESA considerations in several ways. For example, the effects of supplementation and straying by hatchery fish should be considered in evaluating whether a wild population is an ESU. Artificial propagation may also play a role in recovery plans for some "species." However, fish hatcheries do not provide a substitute for natural ecosystems that the Act mandates the Department to conserve. The role of artificial propagation under the

Act is to restore populations in natural habitat to the point where they can be removed from formal ESA protection. Therefore, only naturally-spawning populations should be considered in determining whether a population is distinct for the purposes of the Act.

An exception to the rule that an ESU must correspond to a wild stock may be made for a hatchery population that represents the only remaining component of a native gene pool. In that case, the hatchery population could be determined to be an ESU if it met the criteria set out in Section II.

E. 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 ESUs, although a collection of them might be. Therefore, the historic size 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.[9] For a species with 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.[10] 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

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[9]See discussions by Lande and Barrowclough (1987) and Simberloff (1988).

[10]For a discussion of the rate of loss of genetic variability in Pacific salmon and the relationship between N_e and N_b, see Waples (1990).

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. Nevertheless, this concept should prove useful in focusing attention on populations with the greatest probability of representing ESUs.[11]

F. Groups of populations

Geographical considerations often suggest a natural hierarchical structure for Pacific salmon populations: large river systems may contain several drainages, and each drainage may have major tributaries with smaller streams that support spawning aggregations. Determining the appropriate level for consideration as an ESU is a challenging task. Although the strong homing instinct of these species indicates that even small spawning aggregations may in some sense represent 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.

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[11]This concept is related to, but differs from, the concept of minimum viable population size (MVP). 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.

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 Section II, because this allows the greatest flexibility in ensuring the appropriate level of protection for different ESUs within a more comprehensive group. On the other hand, we have seen in Section III-E (above) 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 artificially maintaining units that might naturally undergo episodes of extinction/recolonization on something short of evolutionary time scales?

The following approach is suggested. Identifiable ESUs should not be combined for the sake of convenience. In general, however, ESUs 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 stipulation that NMFS and FWS should use sparingly their authority to list vertebrate populations. 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. 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.

G. 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 ESUs because they do not contribute to maintaining diversity of the species in its native habitats. 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 Section II.

H. Interpreting results of statistical tests

1. 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 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 (e.g., the combination of semelparity with overlapping age classes) provide some additional opportunities for sampling bias. These factors should be kept in mind in designing sampling plans and in evaluating results.

a. 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.[12] 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.

b. 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 than sampling adults. This will be a minor effect if the sample size is small relative to the population size, but this may not always be true for populations under ESA consideration.

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[12]See discussion of a similar point in Waples (1989) and Waples and Teel (1990).

c. Non-representative 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 only during 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.

2. 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 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.

IV. 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.[13] 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 artificially isolated as a result of human activities (e.g., by the construction of a dam) probably also does not meet the second criterion, although it might eventually become an ESU if the isolation were to persist for a long enough period of time.

It will have been noted by many 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

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[13]GAO, Endangered Species--A Controversial Issue Needing Resolution, Report to Congress (1979).

not seem consistent with the stipulation of the Act [Sec. 4(b)(1)(A)] 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. This does not necessarily mean that more definitive guidelines for making species determinations under the ESA are not possible. It is hoped that public comment and discussion of ideas presented here will help to focus attention on those characteristics thought to be most important in defining "distinct population segments" of Pacific salmon.

Acknowledgments

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Citations

Aspinwall, N.

1974. Genetic analysis of North American populations of the pink salmon (*Oncorhynchus gorbuscha*): Possible evidence for the neutral mutation-random drift hypothesis. Evolution 28:295-305.

Dizon, A. E., C. Lockyer, W. F. Perrin, D. P. Demaster, and J. Sisson.

1991. Rethinking the stock concept: A phylogeographic approach. Southwest Fish. Ctr. Administrative Report LJ-91-01. Southwest Fish. Ctr., Natl. Mar. Fish. Serv., NOAA, P.O Box 271, La Jolla, CA 92038.

Hartt, A. C.

1962. Movement of salmon in the North Pacific Ocean and Bering Sea as determined by tagging, 1956-1958. Int. North Pac. Fish. Comm. Bull. 6, 157 p.

Hindar, K., N. Ryman, and F. Utter.

In press. Genetic effects of cultured fish on natural fish populations. Can. J. Fish. Aquat. Sci.

Lande, R., and G. F. Barrowclough.

1987. Effective population size, genetic variability, and their use in population management. *In* Soule, M. E. (editor), Viable Populations for Conservation, p. 87-123. Cambridge University Press, Cambridge, UK.

Lane, S., A. J. McGregor, S. G. Taylor, and A. J. Gharrett.

In press. Genetic marking of an Alaskan pink salmon population, with an evaluation of the mark and the marking process. Am. Fish. Soc. Symp. 7:395-406.

Ricker, W. E.

1972. Hereditary and environmental factors affecting certain salmonid populations. *In* The stock concept of Pacific Salmon, p. 19-160. H. R. MacMillan Lectures in Fisheries. University of British Columbia, Vancouver.

Ryder, O. A.

1986. Species conservation and systematics: The dilemma of subspecies. Trends Ecol. Evol. 1(1):9-10.

Seeb, J. E., L. W. Seeb, and F. M. Utter.

1986. Use of genetic marks to assess stock dynamics and management programs for chum salmon. Trans. Am. Fish. Soc. 115:448-454.

Simberloff, D.

1988. The contribution of population and community biology to conservation science. Ann. Rev. Ecol. Syst. 19:473-511.

Slatkin, M.

1985. Rare alleles as indicators of gene flow. Evolution 39:53-65.

Utter, F.

1981. Biological criteria for definition of species and distinct intraspecific populations of anadromous salmonids under the U.S. Endangered Species Act of 1973. Can. J. Fish. Aquat. Sci. 38:1626-1635.

Waples, R. S.

1989. Temporal stability of allele frequencies: Testing the right hypothesis. Evolution 43:1236-1251.

Waples, R. S.

1990. Conservation genetics of Pacific salmon. II. Effective population size and the rate of loss of genetic variability. J. Heredity 81:267-276.

Waples, R. S., and D. J. Teel.

1990. Conservation genetics of Pacific salmon. I. Temporal changes in allele frequency. Conserv. Biol. 4:144-156.

Wright, S.

1978. Evolution and the Genetics of Populations, vol. 4. Variability Within and Among Natural Populations. Univ. of Chicago Press, Chicago. 580 p.