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Genetic Guidelines for Mariculture of Invertebrates in Alaska

by

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April 2024

Alaska Department of Fish and Game **Division of Commercial Fisheries**

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Weights and measures (metric)

centimeter	cm
deciliter	dL
gram	g
hectare	ha
kilogram	kg
kilometer	km
liter	L
meter	m
milliliter	mL
millimeter	mm

Weights and measures (English)

cubic feet per second	ft^3/s
foot	ft
gallon	gal
inch	in
mile	mi
nautical mile	nmi
ounce	oz
pound	lb
quart	qt
yard	yd

Time and temperature

day	d
degrees Celsius	°C
degrees Fahrenheit	°F
degrees kelvin	K
hour	h
minute	min
second	s

Physics and chemistry

all atomic symbols	
alternating current	AC
ampere	A
calorie	cal
direct current	DC
hertz	Hz
horsepower	hp
hydrogen ion activity (negative log of)	pH
parts per million	ppm
parts per thousand	ppt, ‰
volts	V
watts	W

General

Alaska Administrative Code	AAC
all commonly accepted abbreviations	e.g., Mr., Mrs., AM, PM, etc.
all commonly accepted professional titles	e.g., Dr., Ph.D., R.N., etc.
at	@
compass directions:	
east	E
north	N
south	S
west	W
copyright	©
corporate suffixes:	
Company	Co.
Corporation	Corp.
Incorporated	Inc.
Limited	Ltd.
District of Columbia	D.C.
et alii (and others)	et al.
et cetera (and so forth)	etc.
exempli gratia (for example)	e.g.
Federal Information Code	FIC
id est (that is)	i.e.
latitude or longitude	lat or long
monetary symbols (U.S.)	$, ¢
months (tables and figures): first three letters	Jan,...,Dec
registered trademark	®
trademark	™
United States (adjective)	U.S.
United States of America (noun)	USA
U.S.C.	United States Code
U.S. state	use two-letter abbreviations (e.g., AK, WA)

Mathematics, statistics

all standard mathematical signs, symbols and abbreviations	
alternate hypothesis	H_A
base of natural logarithm	*e*
catch per unit effort	CPUE
coefficient of variation	CV
common test statistics	(F, t, χ^2, etc.)
confidence interval	CI
correlation coefficient (multiple)	R
correlation coefficient (simple)	r
covariance	cov
degree (angular)	°
degrees of freedom	df
expected value	*E*
greater than	>
greater than or equal to	≥
harvest per unit effort	HPUE
less than	<
less than or equal to	≤
logarithm (natural)	ln
logarithm (base 10)	log
logarithm (specify base)	$\log_{2,}$ etc.
minute (angular)	′
not significant	NS
null hypothesis	H_O
percent	%
probability	P
probability of a type I error (rejection of the null hypothesis when true)	α
probability of a type II error (acceptance of the null hypothesis when false)	β
second (angular)	′
standard deviation	SD
standard error	SE
variance	
population	Var
sample	var

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GENETIC GUIDLELINES FOR MARICULTURE OF INVERTEBRATES IN ALASKA

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ABSTRACT

The "Genetic Guidelines for Mariculture of Invertebrates" (hereafter, the "Guidelines") address the breeding and rearing of aquatic invertebrates in captivity in Alaska and the genetic risk of mariculture activities specific to Alaska's marine invertebrate resources. These Guidelines were developed considering the best available scientific information, as well as state and federal regulations. Although not codified in any Alaska statute or regulation, the protection of genetic resources is connected to the missions of the Alaska Department of Fish and Game (ADF&G) and the ADF&G Commercial Fisheries Gene Conservation Lab and set out in the Constitution of the State of Alaska. The language in the Guidelines is incorporated into relevant permits granted by the State of Alaska, including aquatic farm operation permits, stock acquisition permits (for collection of wild stock to populate a farm or hatchery), and stock transport permits (from a hatchery/farm to another farm). The Guidelines we present here are divided into 3 sections to provide recommendations for stock transport, maintenance of genetic variability, and minimization of domestication. We provide detailed explanations of the concepts and rationale underlying their development. The transport of live animals, seeds, and gametes; the protection of wild stocks; and the maintenance of genetic variability in wild and cultured stocks as related to hatchery production for direct human consumption, stock enhancement, and conservation are addressed. The Guidelines also document proactive steps that address genetic risk under a framework that considers the purposes and systems mariculture operators may use for the captive breeding of invertebrates in the State of Alaska. Finally, these Guidelines are designed to be adaptive and may require periodic modification, based on the best available contemporary scientific information.

Keywords: marine invertebrates, shellfish, crustaceans, permits, regulation, policies, guidelines, genetics, genomics, aquaculture, hatchery, mariculture

INTRODUCTION

The mission of the Alaska Department of Fish and Game (ADF&G) is to protect, maintain, and improve the fish, game, and aquatic invertebrate and plant resources of the state and manage their use and development in the best interests of the economy and the well-being of the people of the state of Alaska. The mission of the Gene Conservation Laboratory is to protect genetic resources and provide genetic information and advice to department staff, policy makers, and the public to support management of resources consistent with the mission of ADF&G. Both missions are, in turn, consistent with the sustained yield principle (Article VIII § 4 of the Constitution of the State of Alaska; see Appendix A) and requires balancing protection of the state's natural resources with the socioeconomic needs of its communities.

The coastal waters of Alaska contain natural populations of numerous marine invertebrate species that support sizeable harvests. Underlying wild productivity is genetic variation within and among populations that enables adaptation to local conditions and resilience to changes in habitat and other environmental characteristics. But there is also interest in captively breeding and rearing individuals for commercial production, stock enhancement, and rehabilitation/restoration activities to diversify the economy; restore, sustain, or increase harvests; and conserve wild populations of various marine invertebrates (referred to as "management of enhanced stocks of shellfish" in Alaska House Bill 41; Alaska Legislature 2022). Captive-bred and -reared individuals and populations, however, have the potential to interact with wild populations and affect wild genetic variation. ADF&G is tasked with developing guidelines that help protect Alaska's wild genetic resources, while simultaneously providing opportunities for resource development.

The "Genetic Guidelines for Mariculture of Invertebrates" (hereafter referred to as the "Guidelines") address breeding and rearing of aquatic invertebrates in captivity in Alaska and were developed considering the best available scientific information as well as existing state and federal regulations. For example, policy guiding Pacific salmon management within Alaska Statute and Administrative Code places priority on conserving the genetic integrity of wild salmon stocks.

Preventing, minimizing, or mitigating the risk to genetic integrity posed by these activities requires managing the potential for negative genetic interactions between captive-bred and wild salmon. Alaska's genetic policy for Pacific salmon thus limits collection of hatchery brood sources to local salmon stocks and requires large broodstock census sizes to help reduce the magnitude of genetic drift (Davis et al. 1985; Davis and Burkett 1989). This genetic policy was used as the framework for the following Guidelines.

These Guidelines cover live animal, seed, and gamete transport; protection of wild stocks; and maintenance of genetic variability in wild and cultured stocks as related to hatchery production for direct human consumption, stock enhancement, and conservation. The Guidelines do not, however, apply to land-based systems, such as Recirculating Aquaculture Systems (RAS); otherwise isolated hatchery systems designed for domestication; or capture-based systems where naturally settled organisms are allowed to grow before harvest (e.g., fattening or raft aquaculture). In all cases, the objectives and measurable goals of the culture program must be documented to allow permit reviewers to assess alignment with the mission of the department. Where stock rehabilitation or restoration is the focus, a proposed timeline to meet the goals of the program must be supplied.

GUIDELINES

I. Stock Transport

   A. Interstate: Live marine invertebrates, including gametes, will not be imported from outside of the state of Alaska.

      1. Exceptions:

         i. Importation of broodstock derived from oysters commercially cultured on the Pacific Coast of North America for 3 or more generations.

         ii. Importation of invertebrate species not listed in I.A.1.i. that cannot reproduce in the wild in Alaska.[1]

         iii. Sterile invertebrates.

   B. Inter-drift zones: The transport and release of non-sterile invertebrates between or among aquatic farms, hatcheries, and broodstock acquisition sites will be limited to locations within larval drift zones (defined below and in Alaska Administrative Code § 5 AAC 41.295 (f), Appendix A).[2]

      1. Southeastern Alaska, from the Canadian border north to Cape St. Elias;

      2. Prince William Sound and Cook Inlet, from Cape St. Elias west and south to Cape Igvak, including Kodiak Island;

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[1] Must provide scientific documentation demonstrating the inability of the proposed species to reproduce in the wild in Alaska (e.g., as documented for Pacific and Kumamoto oysters; see ADF&G 2023).

[2] In some cases, individuals may be transported from one region to another for spawning and culture of progeny, with progeny subsequently transported back to the region of parental origin for release. Culture facilities must state means for preventing the accidental release of nonlocal individuals into the wild, including depurating water flowing out of the hatchery to guard against gamete, larval, and juvenile escape.

        3. Chignik and the Alaska Peninsula-Aleutian Islands, from Cape Igvak west to the tip of Unimak Island;
        4. the Aleutian Islands, including all islands west of Unimak Pass;
        5. the southeast Bering Sea and north Alaska Peninsula, from the westernmost tip of Unimak Island north to the Kuskokwim River, including the Pribilof Islands; and
        6. the northeast Bering Sea, including all coastal islands north of the Kuskokwim River.

    C. Intra-drift zone: Proposals for transport of non-sterile invertebrates over long distances within a drift zone must be accompanied by a justification[3] for the use of a non-local stock.[4,5]

    D. For the purposes of stock enhancement, rehabilitation, and restoration, an area that supports or formerly supported a wild stock must be stocked with progeny from broodstock collected from the nearest available self-sustaining indigenous stock.

    E. For the purposes of stock enhancement, rehabilitation, and restoration and for the purposes of commercial production or research and development (R&D), where hatchery progeny may genetically interact with wild stocks, broodstock originating from different stocks must be kept separate in a hatchery and not allowed to hybridize to help prevent mixing of divergent genetic backgrounds.

II. Maintenance of Genetic Variability

    A. Offspring cohorts at growout must represent a facility-wide effective population size (N_e)[6] ≥ 400 per generation[7] to help prevent loss of genetic diversity.

        1. $N_e \geq 400$ may be achieved as a sum over multiple years or seasons, but outplanting may not occur until $N_e \geq 400$ is achieved.

    B. The broodstock census population size (N_c) required to achieve $N_e \geq 400$ depends on factors like variance in fecundity and reproductive success among individuals and between the sexes.

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[3] For example, justification may include either absence of a local stock or concern for mining the extant local stock. Presence of a justification does not, however, automatically mean it will pass genetic review. It should be noted that presence of a justification does not mean a proposal will pass genetic review; review will be conducted on a case-by-case basis.

[4] In this document, the term "stock" is synonymous with the term "population" in population genetics: "a group of interbreeding individuals that exist together in time and space" (Hedrick 2000). "Local stocks" are thus populations most geographically close to a particular point of reference (e.g., a current or proposed hatchery operation or breeding grounds). The geographic extent of local stocks is variable among species and driven by, for example, life history; geography, including the availability and spatial distribution of suitable habitat; and hydrology.

[5] One or more stocks may exist within a single larval drift zone. Stocks may be genetically isolated from one another, subject to metapopulation dynamics, exhibit gradients consistent with isolation-by-distance, or panmictic (lack of population structure as a result of random mating throughout the range of interest). In the absence of other applicable info, transport within drift zones may be allowed with justification, but if population genetic substructuring has been identified, then transport may be limited based on the best available scientific information.

[6] N_e is often used to conceptually describe the number of individuals contributing offspring to a subsequent generation. In reality, N_e is a theoretical concept that describes the size of an ideal population that experiences the same amount of random genetic drift as a real population (Wright 1931).

[7] The generation time of a species is the average time between 2 consecutive generations in an age-structured species or population in the wild. In humans, for example, the generation time is ~23 years.

1. When a portion of individuals in a broodstock do not spawn[8], a larger broodstock N_c (i.e., >400) may be required to achieve $N_e \geq 400$.
2. A larger broodstock N_c (i.e., >400) may also be required if family sizes vary considerably[9].
    i. Keeping offspring cohorts from pedigree matings separated enables hatchery operators to monitor survival among families.
    ii. A portion of offspring from large families may need to be culled to mitigate variation among family sizes.
3. N_c may be smaller (i.e., ~400) for a broodstock with overlapping generations.
4. When the ratio of males to females diverges substantially from 1:1 in a controlled mating system, a larger broodstock N_c (i.e., >400) may be required to achieve $N_e \geq 400$.[10]

III. Minimizing Domestication Selection

A. Segregated hatchery designed for domestication
    1. No restrictions on selection of traits desired for mariculture.

B. Segregated hatchery systems designed to maintain wild characteristics (commercial production and commercial production R&D)
    1. Broodstock must be selected without regard to phenotype and should represent the full phenotypic diversity of the source population (i.e., no artificial selection).

C. Integrated hatcheries (commercial production and commercial production R&D)
    1. In addition to Criterion 1 under § III.B.:
    2. Broodstock composition must maintain a Proportionate Natural Influence (PNI) ≥ 0.67.

D. Wild-source and capture-based mariculture
    1. Commercial production and commercial production R&D
        i. Criterion 1 under § III.B.
    2. Stock enhancement, rehabilitation, and restoration
        i. In addition to Criterion 1 under § III.B.:
        ii. Stocked progeny must be first filial generation (F_1) offspring of broodstock sourced from the wild.

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[8] The proportional contribution of brood individuals to spawning may depend on several factors, including but not limited to the species of interest, age (too young or too old), maturation of gametes, health, and so on.

[9] It is the responsibility of hatchery operators to monitor survival within and among gamilies.

[10] Acceptable deviations from an equal sex ratio may depend on the presence of differential reproductive characteristics between the sexes and/or the characteristics of the breeding scheme employed (e.g., free or broadcast spawning versus controlled pedigree matings).

BACKGROUND

The introduction connected the concept of protection of genetic resources to the mission of ADF&G as well as the Constitution of the State of Alaska. Genetic risks to wild stocks are predicted by numerous theoretical and modeling studies, and negative genetic impacts have been confirmed by the results of empirical studies in many cases. The best available scientific information was used in the development of the Guidelines to proactively address the genetic risks mariculture practices may pose to wild stocks of aquatic marine invertebrates in Alaska, and the rationale behind their development is described in depth below.

Mariculture can provide a direct-to-market source of fish, shellfish, and aquatic plant products; enhance commercial and recreational fishing opportunities; and support conservation and restoration activities. As a consequence of these pursuits, mariculture may threaten the genetic integrity of wild stocks when introduced or escaped cultured individuals interbreed with wild conspecifics. The potential genetic impacts (outcomes) associated with breeding of cultured individuals in the wild include the loss of genetic diversity and fitness, or productivity, within and among wild populations as well as loss of adaptive potential (Grant et al. 2017). The magnitude of the risk to wild stock genetics thus depends on several factors, such as the life history and demographics (e.g., population size, connectivity among populations, survival, recruitment, etc.) of the affected wild stock(s) and the purposes, location, operational systems and practices, and size of mariculture program itself.

MARICULTURE PURPOSES

There are 4 end purposes for the artificial propagation of marine invertebrates in Alaska, including:

1. Commercial production

For *commercial production*, individuals are settled or placed onto privately owned or leased natural substrates or artificial containers to grow out to produce a product for sale. These operations may be sited in marine waters of the state or in land-based facilities.

2. Stock enhancement

Individuals produced for the purposes of *stock enhancement* are released into the environment to bolster wild production above naturally occurring levels, often by circumventing recruitment limitations, to provide sustained or additional opportunity for common property fisheries. This method can be restricted to creating put-and-take fisheries or result in increasing contributions from wild recruitment and may continue in perpetuity.

3. Stock rehabilitation and restoration

Individuals produced for the purposes of *stock rehabilitation and restoration* are released into the environment to recover depleted, functionally extinct, or locally extinct populations for conservation or to rebuild stocks to self-sustaining levels. Implementation of these programs is often driven by a population crash in a stock that provided common property harvest opportunity. These purposes restrict the timeframe of these projects, and activities must cease once the stock is considered rehabilitated or restored (e.g., self-sustaining in the absence of fishing pressure) unless transition to stock enhancement is warranted.

4. Research and development

Individuals used for *research and development* (R&D) of mariculture practices and procedures are usually produced in small quantities from broodstock that often do not meet the minimum effective population size requirements in the Guidelines. Progeny from these efforts may still be released into the wild, if they meet 3 criteria: (1) release is warranted as part of the assessment component of the project, (2) progeny are not purposely genetically divergent from wild populations, and (3) progeny will make up a small proportion of the wild standing stock present at the release location.

MARICULTURE SYSTEMS

Here, mariculture systems are divided into categories that differentially prioritize efficiency and minimize genetic divergence from wild populations. All of these methods can be appropriate for R&D, and progeny from R&D may be released into the wild, if they meet the 3 criteria listed in the R&D description above.

1. Segregated hatchery designed for domestication

A *segregated hatchery designed for domestication* is appropriate for *commercial production*. The objective of this system is to increase farm resource use efficiency and maximize yield; it does not seek to reduce genetic divergence from wild populations. Various genetic improvement methodologies may be used, foremost of which is selective breeding (i.e., controlled breeding to enhance the expression and incidence rate of desirable phenotypic traits). Common selective breeding regimes focus on increased yield or product quality, disease resistance, or better performance under environmental conditions commonly found in hatcheries, such as higher temperatures and densities.

Artificial selection leads to shifts in the nature and magnitude of genetic diversity relative to wild populations. A *segregated hatchery designed for domestication* is therefore required to prevent hatchery-wild interactions by virtually eliminating the potential for escape (e.g., by using land-based recirculating aquaculture systems, or RAS) and/or through development of sterile products (e.g., triploids and species that cannot reproduce in Alaska waters). Because this system should not pose a risk to wild populations, it does not need to adhere to the Guidelines. To date, only Pacific and Kumamoto oyster culture and research-scale programs for other species use *segregated hatcheries designed for domestication*. Animals produced under this system for R&D cannot be released into the wild because they fail to meet the first and second R&D criteria.

2. Segregated hatchery designed to maintain wild characteristics

A *segregated hatchery designed to maintain wild characteristics* is appropriate for *commercial production*. The objective of this system is to produce animals for market, while reducing the genetic divergence from wild populations. A version of this model is used for Pacific salmon aquaculture in Alaska, where cultured individuals are subject to hatchery environmental conditions during early life stages but experience natural selective forces upon release and until harvest (i.e., ocean ranching). In terms of invertebrate broodstock development, the Guidelines limit the geographic origin of source populations to defined larval drift zones, with provision for adjustment of geographic origins, depending on the known population structure of candidate species. Wild individuals may be collected over 1 or more generations to build a hatchery broodstock, which is maintained in captivity for a limited amount of time to produce 1 to a limited number of offspring cohorts.[11] Because cultured progeny are often then used for subsequent generations of broodstock, steps to mitigate domestication and inbreeding must be taken. Genetic and phenotypic deviations from wild populations nevertheless accumulate with each generation in captivity. Animals produced under this system for R&D may be released into the wild, if it can be proven that they meet the R&D criteria.

3. Integrated hatchery

An *integrated hatchery* is appropriate for *commercial production*. The objective of this system is to produce animals for market, while reducing the genetic divergence from wild populations relative to a *segregated hatchery system*. Wild individuals may be collected over 1 or more generations to develop a hatchery broodstock, and although cultured progeny may be used as broodstock in subsequent generations, wild-caught individuals are also periodically introduced to mitigate domestication selection and loss of genetic variation. As with a s*egregated hatchery designed to maintain wild characteristics*, the Guidelines limit the geographic origin of source populations to defined larval drift zones, with provision for adjustment of geographic origins, depending on the known population structure of candidate species. The degree of genetic risk associated with an *integrated hatchery* depends on the extent to which broodstock and cohort hatchery management practices change genetic profiles relative to the wild stock. Several variables may influence the rate of change in a hatchery, such as broodstock source and replacement rates (e.g., proportionate natural influence, or PNI; Paquet et al. 2011), census and effective population sizes, variation in family survival, and the strength and mode of selection associated with the hatchery environment. To date, *integrated hatcheries* are not used in Alaska. Animals produced under this system for R&D may be released into the wild, if it can be demonstrated that they meet the R&D criteria.

4. Wild-source hatchery

A *wild-source hatchery* is appropriate for *commercial production*, *stock enhancement*, and *stock rehabilitation and restoration*. The objective of this system is to produce animals for market, release animals into the wild to increase harvests, or to restore historical levels of stock productivity, while minimizing divergence from wild populations. Only wild-caught individuals are used for broodstock development, and an adequate broodstock census size is required to mitigate loss of genetic variation. The Guidelines again limit the geographic origin of source populations to defined larval drift zones, with provision for adjustment of geographic origins, depending on the known population structure of candidate species. To date, the *wild-source hatchery* is the most common system used in Alaska for invertebrate production. Animals produced under this system for R&D likely meet the R&D criteria.

5. Capture-based mariculture

A *capture-based mariculture system* is most likely used for *commercial production* but may also be appropriate for *stock enhancement* and *stock rehabilitation and restoration*. By capturing and rearing larvae and/or juveniles produced in the wild, divergence from local wild populations is virtually eliminated. For bivalves and other invertebrates, the method may be exemplified by raft culture, where additional (often artificial) surfaces are provided to increase settlement of naturally spawned planktonic larvae, and the physical layout of the culture structure is designed to enhance

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[11] As with all other hatchery and mariculture operations, plans for broodstock acquisition, farm operations, and stock transport will be subject to genetic review on a case-by-case basis.

survival and stimulate growth. Genetic risk may arise if enhanced survival leads to positive selection of or relaxation of selection against certain phenotypes relative to others. We are unaware, however, of literature demonstrating this effect in *capture-based mariculture*. The genetic risk associated with this system is thus likely small, especially when animals are harvested before maturity, and adherence to the Guidelines is not required. To date, *capture-based mariculture* is the second-most common system used in Alaska for invertebrate production. Animals produced under this system for R&D likely meet the R&D criteria.

DISCUSSION OF GENETIC IMPACTS

Genetic interactions (interbreeding) between wild and cultured individuals may lead to loss of genetic diversity and fitness (productivity) within and among wild populations, as well as loss of adaptive potential. The Guidelines were designed to help protect wild stock genetics by proactively mitigating losses of diversity and fitness due to interbreeding between cultured and wild marine invertebrates. To accomplish this goal, the Guidelines are arranged such that they address 3 potential negative genetic outcomes: (1) erosion of among-population genetic variation, (2) erosion of within-population genetic variation, and (3) domestication.

Application of the Guidelines, however, will depend on the type and purpose of the hatchery program and system employed. All 4 purposes of mariculture covered in this document – *commercial production*, *stock enhancement*, *stock rehabilitation and restoration*, and *research and development* – pose genetic risk to wild populations resulting from inadvertent introduction of cultured individuals into wild populations through escape from commercial operations or intentional introductions by stock enhancement, stock rehabilitation/restoration, and research and development programs. Of the 5 mariculture systems described in this document, the 3 that carry the most genetic risk to wild populations in Alaska are *segregated hatcheries designed to maintain wild characteristics*, *integrated hatcheries*, and *wild-source hatcheries*. These 3 systems are covered in depth in this document. The remaining 2 systems do not typically pose significant risk because they eliminate hatchery-wild interactions as an operational requirement (*segregated hatcheries designed for domestication*) or minimize divergence from local wild populations because artificially reared individuals are naturally recruited from the wild (*capture-based mariculture*). These 2 systems are not covered further here.

The Guidelines also do not apply to cultured invertebrates that cannot naturally reproduce in Alaska waters, which alleviates genetic interaction concerns for the state. These species can neither interbreed with wild populations nor establish nonnative feral populations. As of 2023, 2 species meet this criterion: the Pacific oyster (*Crossostrea gigas*) and the Kumamoto oyster (*Crossostrea sikamea*). Although introduced oyster spat can survive and grow in some Alaska coastal waters, water temperatures here are below the critical threshold required for Pacific and Kumamoto oysters to spawn. Additional species or product types, such as sterile triploids, may be added to this list in the future.

EROSION OF AMONG-POPULATION GENETIC VARIATION

Among-population genetic variation helps provide resilience to environmental changes (e.g., in a portfolio effect; Schindler et al. 2010). Conserving genetic variation among populations derived from differential selective forces provides raw material for future adaptation within species and hedges against declines in productivity associated with changes over short and long time periods.

Erosion of among-population genetic variation is a potential consequence of both inadvertent escapes from commercial production and purposeful introductions by stock enhancement, rehabilitation/restoration, and R&D programs based on the assumptions that (1) interbreeding among genetically divergent stocks compromises the genetic integrity of a local wild stock and (2) adaptation of wild populations to local environmental conditions has occurred. This genetic impact results from hybridization between cultured and local wild-origin individuals and introgression of (possibly maladapted) hatchery alleles into wild genetic backgrounds (e.g., Anane-Taabeah et al. 2019). Depending on the rate and magnitude of migration of cultured individuals into the wild, genetic swamping can occur if hatchery-origin individuals contribute to a disproportionate number of offspring (Ryman and Laikre 1991). Swamping may be of particular concern in species with high fecundities like most marine invertebrates (e.g., due to sweepstakes recruitment; Hedgecock 1994), despite their high early life-stage mortality (i.e., Type III survivorship).

Hybridization between cultured and wild individuals may also disrupt co-adapted gene complexes. These adaptations may be phenotypically cryptic due to genetic-environmental interactions (review in Sparks et al. 2022). Introgression of nonlocal or maladapted genes into the genomes of wild populations can disrupt the function of these suites of interacting genes associated with particular phenotypes (Burton et al. 2013), which can lead to severe loss of fitness (Rhymer and Simberloff 1996). First-generation hybrids may not be affected or may even experience heterosis (i.e., hybrid vigor), but genetic recombination during reproduction or incompatible mitochondrial and nuclear source backgrounds can break down co-adaptation and reduce fitness in the F_2 generation and beyond (i.e., outbreeding depression; systematic review in Whitlock et al. 2013). The probability of outbreeding depression in crosses between populations is elevated when the populations are distinct species (including cryptic species), have fixed chromosomal differences, experience reduced gene flow, or inhabit different environments (Frankham et al. 2011).

Section I of the Guidelines addresses erosion of among-population genetic variation:

I. Stock Transport
    A. Interstate: Live marine invertebrates, including gametes, will not be imported from outside of the state of Alaska.
        1. Exceptions:
            i. Importation of broodstock derived from oysters commercially cultured on the Pacific Coast of North America for 3 or more generations.
            ii. Importation of invertebrate species not listed in I.A.1.i. that cannot reproduce in the wild in Alaska.
            iii. Sterile invertebrates.
    B. Inter-drift zone: The transport and release of non-sterile invertebrates between or among aquatic farms, hatcheries, and broodstock acquisition sites will be limited to locations within larval drift zones (defined below and in Alaska Administrative Code § 5 AAC 41.295 (f)).
        1. Southeastern Alaska, from the Canadian border north to Cape St. Elias;
        2. Prince William Sound and Cook Inlet, from Cape St. Elias west and south to Cape Igvak, including Kodiak Island;
        3. Chignik and the Alaska Peninsula-Aleutian Islands, from Cape Igvak west to the tip of Unimak Island;
        4. the Aleutian Islands, including all islands west of Unimak Pass;
        5. the southeast Bering Sea and north Alaska Peninsula, from the westernmost tip of Unimak Island north to the Kuskokwim River, including the Pribilof Islands; and
        6. the northeast Bering Sea, including all coastal islands north of the Kuskokwim River.

C. Intra-drift zone: Proposals for transport of non-sterile invertebrates over long distances within a drift zone must be accompanied by a justification for the use of a nonlocal stock.

D. For the purposes of stock enhancement, rehabilitation, and restoration, an area that supports or formerly supported a wild stock must be stocked with progeny from broodstock collected from the nearest available self-sustaining indigenous stock.

E. For the purposes of stock enhancement, rehabilitation, and restoration and for the purposes of commercial production or research and development (R&D), where hatchery progeny may genetically interact with wild stocks, broodstock originating from different stocks must be kept separate in a hatchery and not allowed to hybridize to help prevent mixing of divergent genetic backgrounds.

Further description of Sections I.A. through E. are as follows:

A. Interstate transport

No invertebrates may be transferred into Alaska from out-of-state for culture except species that cannot reproduce naturally in Alaska waters. As of 2023, 2 species meet this criterion: the Pacific oyster (*Crossostrea gigas*) and the Kumamoto oyster (*Crossostrea sikamea*).

B. Inter-drift zone

Transport of individuals into or among hatcheries and the release of cultured individuals in the wild may result in the inadvertent mixing of individuals from genetically distinct stocks. A common method of detecting population structure is to survey genetic variability among locations with genetic markers, such as microsatellites, mitochondrial DNA, or single nucleotide polymorphisms (SNPs). These markers are typically situated in neutral regions of the genome, where allele frequency distributions in sampled populations are primarily determined by gene flow (mediated by pelagic larval dispersal or adult migration) and random genetic drift (mediated by effective population size, a concept discussed in depth in Appendix B) rather than selection.

Although neutral markers may provide insight into the demographic features underlying genetic structure, they do not detect genetic differences among stocks associated with adaptation to local environments or structural genomic variation. Moreover, the spatiotemporal scales of adaptive variation are often smaller than the scales of neutral genetic differentiation (Conover et al. 2006). Historically, most evidence for selective differentiation was detected with laboratory or common garden experiments, which can be difficult to design and expensive to conduct (F. Evans et al. 2004). However, newer sequencing technologies available today are enabling evaluation of structural and adaptive variation across genomes with unprecedented resolution (e.g., Hornick and Plough 2022).

The protection of both neutral and adaptive variation is essential to protecting the genetic integrity of wild stocks. Unfortunately, population structuring is unknown for many marine invertebrate species in Alaska, including those that are candidates for mariculture. In the absence of applicable genetic data, ADF&G defined six larval drift zones (Alaska Administrative Code Section 5 AAC 41.295 (f), see Appendix A; stock transport permits 2005), which focus primarily on preservation of neutral variation on broad spatial scales, based on a review of the statewide hydrographic literature by RaLonde (1993). Meanwhile, preservation of adaptive variation remains largely unaddressed due to the paucity of relevant scientific information within and among species in Alaska.

C. Intra-drift zone

The larval drift zone hypothesis stipulates that the "transport of stock between aquatic farm, hatchery, or stock acquisition sites will be limited to waters within an approved larval drift zone of the state." This requirement assumes that stocks within a drift zone are genetically homogeneous, a presupposition that, as noted, remains largely untested for most marine invertebrates in Alaska. For those species for which population genetic information is available, stock transport should be limited according to empirically derived putative stock boundaries. Within the Southeast Alaska (SEAK) larval drift zone, for example, red king crab (*Paralithodes camtschaticus*) in Seymour Canal are genetically divergent from other SEAK populations, indicating that reproductive isolation may be affected by shoreline configurations and mesoscale eddying (Grant and Cheng 2012). Transport of red king crab into and out of Seymour Canal should therefore be restricted to prevent mixing of genetically divergent stocks, despite sub-drift zone scaling. It must be noted, however, that the spatial scale of population genetic structuring may be unique to each species and geographic region.

D. and E. Broodstock management

Hatcheries are permitted to culture invertebrates from multiple drift zones with the objective of providing seed stock back to ancestral drift zones. To prevent mixing of divergent neutral genetic backgrounds, broodstock originating from different sources must be kept separate and not allowed to hybridize.

EROSION OF WITHIN-POPULATION GENETIC VARIATION

Loss of within-population genetic variation has the potential to contribute to reductions in fitness due to inbreeding (e.g., Christie et al. 2013). Loss of genetic variability may also compromise a stock's ability to respond to environmental perturbations like disease, suboptimal water quality or temperatures, and habitat degradation in the wild or, alternatively, physical stressors common in culture scenarios, such as pelleted food, vitamin deficiencies, cannibalism, and high densities. Genetic variability also influences the expression of many morphological, life-history, and behavioral traits of a population, and its loss can lead to reduced productivity.

Broodstock and cohort management is key to maintenance of genetic variability in hatchery populations. Sourcing an "adequate" number of individuals for broodstock is important in combating loss of diversity. Using too few individuals can produce the same effect as a classic bottleneck in population size (Nei et al. 1975). Population bottlenecks may purge alleles from a population, and the loss of alleles can be severe even with little to no reduction in heterozygosity (in diploid species, heterozygous individuals have 2 different alleles at a particular locus; Ryman et al. 1994). The effect may be pronounced in species with large wild stock sizes (Ryman et al. 1994), including many invertebrates, where a fraction of the total census size is collected for broodstock (Dimond et al. 2022). Losses of allelic richness and heterozygosity have been documented in commercial broodstock as well as supplementation programs for several invertebrate species (Gaffney et al. 1996; F. Evans et al. 2004; B. Evans et al. 2004).

Section II of the Guidelines addresses erosion of within-population genetic variation:

> II. Maintenance of Genetic Variability
>
> A. Offspring cohorts at growout must represent a facility-wide effective population size (N_e) ≥ 400 per generation to help prevent loss of genetic diversity.

    1. $N_e \geq 400$ may be achieved as a sum over multiple years or seasons, but outplanting may not occur until $N_e \geq 400$ is achieved.

B. The broodstock census population size (N_c) required to achieve $N_e \geq 400$ depends on factors like variance in fecundity and reproductive success among individuals and between the sexes.
    1. When a portion of individuals in a broodstock do not spawn, a larger broodstock N_c (i.e., >400) may be required to achieve $N_e \geq 400$.
    2. A larger broodstock N_c (i.e., >400) may also be required if family sizes vary considerably.
        i. Keeping offspring cohorts from pedigree matings separated enables hatchery operators to monitor survival among families.
        ii. A portion of offspring from large families may need to be culled to mitigate variation among family sizes.
    3. N_c may be smaller (i.e., ~400) for a broodstock with overlapping generations.
    4. When the ratio of males to females diverges substantially from 1:1 in a controlled mating system, a larger broodstock N_c (i.e., >400) may be required to achieve $N_e \geq 400$.

Further discussion of Sections II.A. and B. are as follows:

A. Effective population size requirements

The ADF&G Genetic Policy for Pacific salmon requires using a minimum 400 individuals in a broodstock composed of equal numbers of males and females. Although this number describes a broodstock census size (N_c), it nevertheless represents a practical requirement arising from consideration of the rate of loss of genetic diversity through random genetic drift and assuming contribution of all 400 breeding individuals to the offspring pool. Here, genetic diversity is measured using observed heterozygosity, or the empirically determined proportion of heterozygous individuals in a population at a particular suite of loci (H_o). Random genetic drift is the shift in allele frequencies resulting from the finite sampling of gene variants during reproduction. Drift tends toward reducing heterozygosity (i.e., increasing homozygosity) at a rate of $1/2N_e$ per generation, where N_e is the effective population size, or the size of an ideal population that loses heterozygosity at the same rate as a real population.[12]

Drift occurs in all populations, regardless of size, but the proportional loss of genetic diversity due to drift is greater in small versus large populations. A population with $N_e = 40$ experiences an expected decrease in heterozygosity of 1.25% per generation, whereas $N_e = 400$ experiences a decrease of 0.013% per generation. Over several generations, compounded losses in genetic diversity can be substantial, particularly in small populations.

B. Census population size considerations

In a hatchery, several variables influence the N_e relative to N_c. Diversity may be lost through inbreeding (i.e., mating between relatives), for example, which is not uncommon in selective breeding programs. Selective breeding seeks to enhance the incidence and expression of desirable phenotypic traits. Because traits can be heritable, their expression may manifest as more similar among relatives than among nonrelatives. Relatives with similar desired trait values may then be interbred, leading over time to inbreeding. Inbreeding in captivity reduces genetic diversity and exposes deleterious recessive alleles, negatively affecting adaptive potential and increasing morbidity and mortality. Although breeders today are aware of the consequences of inbreeding in

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[12] See Appendix B for a more detailed discussion of N_e and its central role in the mathematics of genetic variability.

terms of animal welfare and the economic performance of their operations, combating inbreeding depression can be difficult (Lozada-Soto et al. 2021). Strategies to slow the rate of inbreeding include using large numbers of brood individuals, periodic introduction of new brood, incorporating marker-assisted and genomic selection in breeding value estimation, or intentional crossbreeding of selected strains (e.g., Weigel 2001; D'Ambrosio et al. 2019).

N_e and N_c may also differ because not all individuals mate and not all offspring survive to maturity. In fact, N_e in the wild has been measured at as many as 3 orders of magnitude smaller than N_c (Hauser et al. 2002; Turner et al. 2002). N_e in a hatchery setting may be reduced by unequal broodstock sex ratios, but non-destructive identification of sex prior to breeding may be difficult to impossible for many invertebrate species. However, even when a broodstock does consist of equal numbers of males and females, the effective sex ratio may be altered by natural breeding dynamics or hatchery breeding protocols. The common practices of pooling sperm from one to few males to fertilize eggs from several females or mass mixing of gametes from multiple individuals, for instance, may lead to variation in fertilization rates due to variable gamete quality, gamete compatibility issues, sperm competition, or other intrinsic factors (Campton 2004).

N_e is also influenced by breeding individuals from different generations. Invertebrates tend to be iteroparous and have overlapping generations in the wild, and those sourced from the wild for broodstock development often represent multiple generations because accurate aging of live individuals, particularly soft-bodied invertebrates, is often impossible. In contrast to other variables, however, the effect of including individuals of different generations in a broodstock is an increase in N_e for a given number of breeders.

In addition, invertebrate species are often highly fecund and naturally susceptible to high variance in reproductive success (Hedgecock 1994; Boudry et al. 2002). Larger variances in individual and family success tend to suppress N_e. Wild populations of invertebrates typically broadcast spawn, for example, and spawn timing, distance among spawning individuals, and local hydrographics all influence fertilization rates. Although these variables can be controlled in culture, other characteristics may still influence reproductive success. Maturity of sperm, swimming velocity, sperm concentration, sperm competition, and genetic differences among males—as well as egg maturation, egg quality, and genetic differences among females—all influence fertilization rates and offspring viability (Campton 2004; Wedekind et al. 2007).

Variance in contribution among brood individuals to offspring cohorts may be reduced by facilitating 1-to-1 mate pairings, but if one female releases 30 million eggs and another 3 females release 4 million eggs each, 26 million eggs from the first female may need to be discarded to normalize female contribution. During rearing and depending on hatchery operational capacity, mitigation for unequal family sizes may need to include isolating offspring from different parental pairings, monitoring grow-out among families, and culling progeny from highly successful matings to avoid differential representation among families. For *stock enhancement* and *stock rehabilitation and restoration*, in particular, outplanting equal numbers of progeny from a diverse number of families helps ensure that the genetic diversity available in the broodstock is captured and individual contributions are well represented. If few parents or parental pairings are represented, outplants may represent a fraction or skewed sampling of the genetic diversity of the wild stocks into which they are released (e.g., Gaffney et al. 1996).

GENETIC MODIFICATION AND DOMESTICATION SELECTION

Shellfish and other invertebrates that are genetically modified organisms (GMOs) will not be permitted for use for mariculture in Alaska. Under AS Chapter 12. Shellfish Enhancement Projects. § 16.12.030. Conditions of a permit. "The department shall require, in a permit issued under this chapter, that the permit holder […] not procure genetically modified shellfish or place genetically modified shellfish into the water of the state". Under AS Chapter 12. Shellfish Enhancement Projects. § 16.12.199. Definitions. "In this chapter […] 'genetically modified shellfish' means shellfish whose genetic structure has been altered at the molecular level by recombinant DNA and RNA techniques, cell fusion, gene deletion or doubling, introduction of exogenous genetic material, alteration of the position of a gene, or other similar procedure using artificial processes." Under AS Article 2. Aquatic Farming. § 16.40.199. Definitions. […] "'shellfish' means a species of crustacean, mollusk, or other invertebrate, in any stage of its life cycle, that is indigenous to state water or that is authorized to be imported into the state under a permit issued by the commissioner." (See Appendix A.)

Selection is the process by which individuals and populations that are better adapted to, or have phenotypes better suited to, their environment survive longer and/or at a higher rate, resulting in a higher lifetime reproductive success (fitness). Whereas natural selection occurs in the wild due to natural processes, domestication selection is artificial, or human-mediated. Domestication implies an amenability to human control of care and reproduction and is often an intentional end goal of selective breeding (e.g., in *segregated hatchery systems designed for domestication*). However, domestication can also occur unintentionally, which is of interest for the hatchery systems focused on in this document (i.e., *segregated hatcheries designed to maintain wild characteristics*, *integrated hatcheries*, and *wild-source hatcheries*). Unintentional domestication selection is a byproduct of rearing in a hatchery environment, where temperatures, food, water quality, and densities are controlled and typically dissimilar to that found in the wild. It can be both directional, if selective pressures result in differential survival among individuals and families, as well as relaxed, if phenotypes that would experience high mortality in the wild survive and even thrive in a hatchery.

If either intentionally or unintentionally domesticated individuals escape or are introduced into the wild for conservation purposes, and they survive to reproduce, their domesticated genotypes and phenotypes may be passed on to wild-born progeny. Moreover, hybridization between domesticated and wild individuals implies that hatchery alleles and phenotypes may then introgress into wild populations, changing allele frequencies and potentially affecting fitness.

Section III of the Guidelines addresses ways to minimize signatures of domestication in hatchery and wild populations:

III. Minimizing Domestication Selection
  A. Segregated hatchery designed for domestication
    1. No restrictions on selection of traits desired for mariculture.
  B. Segregated hatchery systems designed to maintain wild characteristics (commercial production and commercial production R&D)
    1. Broodstock must be selected without regard to phenotype and should represent the full phenotypic diversity of the source population (i.e., no artificial selection).
  C. Integrated hatcheries (commercial production and commercial production R&D)
    1. In addition to Criterion 1 under § III.B.:

        2. Broodstock composition must maintain a Proportionate Natural Influence (PNI) ≥ 0.67.

D. Wild-source and capture-based mariculture
    1. Commercial production and commercial production R&D
        i. Criterion 1 under § III.B.
    2. Stock enhancement, rehabilitation, and restoration
        i. In addition to Criterion 1 under § III.B.:
        ii. Stocked progeny must be first filial generation (F_1) offspring of broodstock sourced from the wild.

Further discussion of Sections III. B., C., and D. are as follows:

B. Artificial selection

Maintaining genetic and phenotypic variation in a hatchery across all life stages is key to minimizing unintentional domestication selection. As with addressing erosion of genetic diversity, domestication selection can be mitigated in part by maintaining large hatchery N_e. Moreover, no intentional selection according to phenotype should be performed. Another consideration is minimizing the time over which domestication selection can act. In stock enhancement and stock rehabilitation and restoration, a balance must therefore be struck between releasing early life stages (larvae and spat) that are theoretically less domesticated and later life stages (feeding juveniles and adults) that experience higher proportional survival.

Although hatchery-bred broodstock may be used sparingly in later generations, integrated hatcheries rely heavily on local wild-caught individuals for broodstock development and replacement. In a segregated hatchery stock, guarding against domestication and reductions in genetic diversity can be more challenging, even if all individuals in the founding broodstock are unrelated. One way to boost N_e is by periodic introduction of new wild individuals into a broodstock. For example, toward reducing genetic risk to wild populations, Straus et al. (2008) suggested collecting larger numbers of adult wild geoduck annually to replace previously-spawned broodstock. Depending on the timeframe (seasonally, annually, or per generation) of introduction and/or replacement, N_e then depends on the total number of individuals breeding within that timeframe and not on N_c at any given moment.

C. Proportionate natural influence

An additional practical measure for mitigating domestication selection and erosion of diversity employs the concept of proportionate natural influence (PNI; Paquet et al. 2011). Paquet et al. (2011) based PNI on a quantitative model of fitness effects built by Ford (2002) and applicable to any species, where PNI is defined as a ratio of the proportion of natural-origin individuals in a broodstock (pNOB) divided by the sum of pNOB plus the proportion of hatchery-origin individuals spawning in the wild (pHOS; i.e., PNI = pNOB / (pNOB + pHOS)). A minimum PNI = 0.67 was recommended in the Hatchery Scientific Review Group quantitative standards for integrated salmon hatchery systems in the Pacific Northwest (Mobrand et al. 2005; Paquet et al. 2011), implying that two-thirds of naturally spawning individuals at any given moment must be wild-origin. To maintain PNI ≥ 0.67 requires regular oversight to effectively manage the balance between pNOB and pHOS; sourcing prospective broodstock without hatchery ancestry becomes increasingly difficult when subsequent broodstocks are drawn from an enhanced stock, and the use of individuals with hatchery ancestry accelerates the Ryman-Laikre effect because hatchery-origin alleles may displace wild alleles over time (Ryman and Laikre 1994).

D. Considerations for stock enhancement and stock rehabilitation and restoration

Höfner et al. (2021) determined that "[r]egionally sourced seeds can produce genetically diverse populations at natural levels of genetic differentiation." In support of this intended result in Alaska, the Guidelines require in sum that (1) F_1 progeny outplanted for conservation purposes must have parents (broodstock) sourced from the nearest available self-sustaining indigenous stock (Section I.), (2) the outplants and contributing broodstock must be genetically diverse (Section II.A.), and (3) no more than 1 generation of separation from the donor site to stocking of progeny is allowed (Section III.D.2.ii.), which reduces the potential for drift and domestication in the hatchery environment. Under the last criterion, gametes or larvae may be collected and used for fertilization and/or reared in a hatchery, with the progeny subsequently returned to the donor system at the appropriate life history stage; gametes or larvae harvested in a given year must be used in culture only to produce offspring for release in the wild.

CONCLUSIONS

Artificial propagation of marine organisms has the potential to impact the genetic integrity of wild stocks when introduced or escaped cultured individuals encounter and genetically interact (interbreed) with wild conspecifics. Genetic impacts associated with cultured individuals in the wild include the loss of genetic diversity and fitness within and among wild populations and the loss of adaptive potential. The magnitude of the risk posed by a hatchery-wild system depends on factors like the life history and demographics of the potentially affected wild stock(s), the corresponding characteristics of the hatchery population, and the siting, operational structure, and magnitude of mariculture program.

The "Genetic Guidelines for Mariculture of Invertebrates" outlines proactive steps that address genetic risk under a framework that considers the purposes and systems mariculture operations may use for the captive breeding of invertebrates in the State of Alaska. The Guidelines are divided into 3 sections, providing recommendations for stock transport, maintenance of genetic variability, and minimization of domestication, that help direct permit application and decision-making for the state. This document also provides an explanation of the concepts and rationale underlying development of the Guidelines. Finally, the Guidelines are designed to be adaptive and may require periodic modification, based on the best available scientific information.

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APPENDICES

Appendix A.–Excerpts from the State of Alaska Constitution, Alaska Administrative Code (AAC), and Alaska Statutes (AS) referenced in this text.

State of Alaska Constitution

Article 8 Natural Resources,

Section 4 Sustained Yield

> Fish, forests, wildlife, grasslands, and all other replenishable resources belonging to the State shall be utilized, developed, and maintained on the sustained yield principle, subject to preferences among beneficial uses.

Merriam-Webster definition of sustained yield: *production of a biological resource (such as timber or fish) under management procedures which ensure replacement of the part harvested by regrowth or reproduction before another harvest occurs*

Alaska administrative Code (AAC): Title 5 Fish and Game.

Part 1. Commercial and Subsistence Fishing and Private Nonprofit Salmon Hatcheries. (5 AAC 1 - 5 AAC 41)

Chapter 41 Collection, Transportation, Possession, Propagation, or Release of Aquatic Organisms; Aquatic Farming

Article 4 Aquatic Farming

5 AAC 41.295. Stock transport permits

(a) A transfer of stock to, from, or between an aquatic farm, hatchery, or stock acquisition site may not occur without a stock transport permit issued by the commissioner. An applicant shall apply on a stock transport permit application form provided by the department and submit the application form to the department at least 45 days before date of transport.

(b) Before an applicant submits a stock transport permit application to the department under this section, the supplier of the stock must contact the department to arrange to send samples of the stock intended for transport. The department will conduct a health inspection of the samples. Within 120 days after receipt of the supplier's samples of stock, the department will provide a written disease history report to the supplier of the stock to notify the supplier that the

(1) current disease history report is acceptable and that no further inspection is required;

(2) health inspection detected the presence of pathogens or parasites of a type that make transport

(A) acceptable under specified conditions; or

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(B) unacceptable.

(c) Transport of stock between aquatic farm, hatchery, or stock acquisition sites will be approved, without an inspection and report required in (b) of this section, if the commissioner determines that either

(1) the disease history for the stock on site is acceptable based on previous laboratory examination of samples;

(2) the risk of disease transmission between sites is minimal; or

(3) the sites are within the larval drift zone for wild stock of the species.

(d) Transport of stock, other than geoduck, between aquatic farm, hatchery, or stock acquisition sites will be limited to waters within an approved larval drift zone of the state.

(e) This section does not apply to aquatic farm products that are

(1) sold or transferred to commercial markets or consumers; and

(2) not intended for additional exposure to waters of the state.

(f) For the purposes of this section, "larval drift zone" includes all coastal and island areas in

(1) Southeastern Alaska, from the Canadian border north to Cape St. Elias;

(2) Prince William Sound and Cook Inlet, from Cape St. Elias west and south to Cape Igvak, including Kodiak Island;

(3) Chignik and the Alaska Peninsula-Aleutian Islands, from Cape Igvak west to the tip of Unimak Island;

(4) the Aleutian Islands, including all islands west of Unimak Pass;

(5) the southeast Bering Sea and north Alaska Peninsula, from the westernmost tip of Unimak Island north to the Kuskokwim River, including the Pribilof Islands; and

(6) the northeast Bering Sea, including all coastal islands north of the Kuskokwim River.

Alaska Statutes (AS): Title 16 Fish and Game.

Chapter 12. Shellfish Enhancement Projects

Conditions of a Permit (Sec. 16.12.030)

The department shall require, in a permit issued under this chapter, that the permit holder

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(1) procure shellfish from the department or a source approved by the department;

(2) place shellfish only in water of the state specifically designated in the permit;

(3) not procure genetically modified shellfish or place genetically modified shellfish into the water of the state;

(4) not resell or transfer shellfish sold to a permit holder by the state or by another party approved by the department;

(5) not release shellfish before approval by the department, and, for purposes of pathological examination and approval, that the permit holder notify the department at least 15 days before the date of the proposed release of shellfish;

(6) destroy diseased shellfish in a specific manner and location designated by the department;

(7) harvest shellfish only at specific locations and under specific conditions as designated by the department;

(8) make surplus shellfish available for sale first to the department and then, after inspection and approval by the department, to other permit holders operating under this chapter;

(9) provide a copy of the sales transaction to the department if surplus shellfish are sold by a permit holder to another permit holder;

(10) release shellfish in an area where the shellfish will be available to traditional fisheries, subject to the provisions of this chapter and regulations adopted under this chapter.

Alaska Statutes (AS): Title 16 Fish and Game.

Chapter 12. Shellfish Enhancement Projects

Definitions (Sec. 16.12.199)

In this chapter,

(1) “facility” means a hatchery as defined in AS 16.40.199, a facility for the release of shellfish into natural water of the state, or a facility for a project under AS 16.12.010;

(2) “genetically modified shellfish” means shellfish whose genetic structure has been altered at the molecular level by recombinant DNA and RNA techniques, cell fusion, gene deletion or doubling, introduction of exogenous genetic material, alteration of the position of a gene, or other similar procedure using artificial processes;

(3) “shellfish” has the meaning given in AS 16.40.199.

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Alaska Statutes (AS): Title 16 Fish and Game.

Chapter 40. Commercial use of Fish and Game

Article 02 Aquatic Farming

Definitions (Sec. 16.40.199)

In AS 16.40.100 — 16.40.199,

(1) “aquatic farm” means a facility that grows, farms, or cultivates aquatic farm products in captivity or under positive control;

(2) “aquatic farm product” means an aquatic plant or shellfish, or part of an aquatic plant or shellfish, that is propagated, farmed, or cultivated in an aquatic farm and sold or offered for sale;

(3) “aquatic plant” means a plant indigenous to state water or that is authorized to be imported into the state under a permit issued by the commissioner;

(4) “commissioner” means the commissioner of fish and game;

(5) “hatchery” means a facility for the artificial propagation of stock, including rearing of juvenile aquatic plants or shellfish;

(6) “insignificant population” means a population of shellfish that, in the determination of the commissioner, would not attract and support a commercial fishery for that species of shellfish and the harvest and sale of the shellfish would not result in significant alteration in traditional fisheries or other existing uses of fish and wildlife resources if the population were included within an aquatic farm site;

(7) “positive control” means, for mobile species, enclosed within a natural or artificial escape-proof barrier; for species with limited or no mobility, such as a bivalve or an aquatic plant, “positive control” also includes managed cultivation in unenclosed water;

(8) “shellfish” means a species of crustacean, mollusk, or other invertebrate, in any stage of its life cycle, that is indigenous to state water or that is authorized to be imported into the state under a permit issued by the commissioner;

(9) “stock” means live aquatic plants or shellfish acquired, collected, possessed, or intended for use by a hatchery or aquatic farm for the purpose of further growth or propagation.

The effective population size (N_e) is a key concept in broodstock management. N_e is the size of an ideal population that loses heterozygosity at the same rate as a real population. An ideal population experiences no mutation or selection and has an equal number of males and females that mate randomly. The concept is useful because of its mathematical simplicity, although natural populations are unlikely to meet all of the assumptions. Broodstock management in the hatchery is a key component of hatchery N_e. Certain breeding schemes can potentially reduce hatchery N_e, even if a broodstock consists of a large census size (N).

Random genetic drift and inbreeding

Genetic drift is the shift in gene frequencies resulting from the finite sampling of gametes during reproduction. Selection can also lead to gene frequency changes in a hatchery stock, but selection is not considered in the following formulation. Random genetic drift leads to the loss of the genetic diversity at a rate of $1/2N_e$ per generation and produces a tendency toward homozygosity. Genetic diversity can be measured by observed heterozygosity, or the empirically observed proportion of heterozygous individuals in a population for a particular locus (h_o). Heterozygosities for a sample of loci can be averaged to produce an estimate of average heterozygosity (H). Generally, heterozygosity is calculated as expected heterozygosity, which assumes Hardy-Weinberg genotypic proportions and is estimated from allele frequencies (p) for a locus:

$$h = \Sigma\ 1\text{-}p_i^2.$$

The amount of heterozygosity lost each generation is inversely proportional to twice N_e for a diploid species:

$$h_1 = h_0\ (1\ /\ 2N_e).$$

Random genetic drift occurs in all populations, regardless of their size. However, the proportional loss of genetic diversity in small populations is greater per generation than in large populations. A population with N_e = 40 experiences an expected decrease in heterozygosity of 1.25% each generation, while a population with N_e = 400 experiences a decrease of 0.013% each generation.

Maintenance of hatchery N_e

N and N_e can be quite different because not all individuals mate and not all families produce offspring that survive. In fact, N_e can be as much as 3 or 4 orders of magnitude smaller than N (Hauser et al. 2002; Turner et al. 2002). In a hatchery setting, several variables influence the N_e of a broodstock:

Fluctuating broodstock size

Variability in the number of individuals from year to year also influences broodstock N_e. N_e across generations (t) is approximately the harmonic mean of the number of breeders (N_i) each generation:

$$1\ /\ N_e = (1\ /\ t)\ (1\ /\ N_1 + 1\ /\ N_2 + \ldots\ 1\ /\ N_t).$$

-continued-

The harmonic mean is most influenced by the smallest number of breeders in a given year. Small numbers of breeders can result from a small number of founders or from a bottleneck in broodstock size. Either factor may lead to the loss of genetic diversity.

Sex ratio

Multigenerational N_e can also be reduced by unequal numbers of breeding males and females each generation, depending on pedigree development. The equations above involving N_e assume that a broodstock consists of a 1:1 ratio of males to females, and departures from this ratio will decrease N_e as estimated. The N_e when the sexes of breeding individuals are known is approximately (Wright 1938)

$$N_e = (4N_fN_m) / (N_f + N_m),$$

where N_f and N_m are the numbers of breeding females and males, respectively.

Overlapping generations

N_e is also influenced by the use of breeders from different generations. When the numbers of breeding males and females are equal but breeders are from different generations, N_e is

$$N_e = gN_b,$$

where N_b is the number of breeders and g is the average age at sexual maturity (Waples 1990). This equation is an approximation for salmon, which die after spawning and hence applies to invertebrates only if an individual is used once for gamete production. The effect of including individuals of different generations in a broodstock is to increase N_e for a given number of breeders, when other variables remain constant.

Variation in offspring survival among families

Invertebrates that produce large numbers of eggs are especially susceptible to large variances in family success. Variance in family size (V) influences N_e and is related to the breeding effective size (N_b) in the following way

$$N_e = 4N_b / (V + 2)$$

according to Wright (1938). Maximal N_e for a given number of breeders ($N_e = N_b$) is achieved when V equals 2. The ratio of males to females also affects the variance in family success and hence N_e as

$$N_e = 8N_b\ (V_m + V_f + 4).$$

Larger variances in family success tend to reduce N_e and can negate the increase in N_e gained by including multiple generations in a broodstock. In this case,

$$N_e = 4N_cg / (V + 2),$$

where N_c is the number of breeders from a cohort of individuals born in the same year and g is generation length, which is estimated as the average time to sexual maturity (Hill 1979; Nunney 1993) or the average age of breeding individuals in a population (Charlesworth 1994).