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**Consanguinidad y dependencia ambiental de la depresión
endogámica en salmón Coho (*Oncorhynchus kisutch*)**

**Tesis entregada a la
UNIVERSIDAD DE CHILE**

**En cumplimiento parcial de los requisitos
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*** Doctor en Ciencias
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Por

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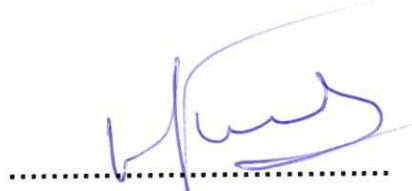
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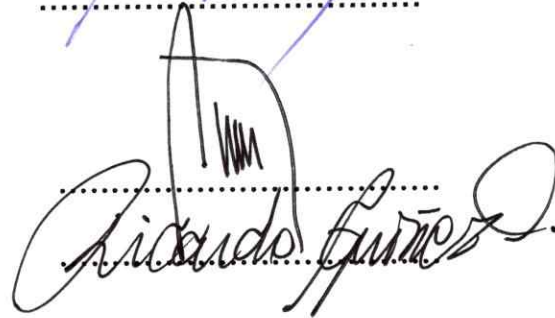
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Dedicatoria

A la memoria de mis abuelos Aliro Gallardo y Santiago Matus

Dedication

To the memory of Aliro Gallardo and Santiago Matus, my grandfathers

Agradecimientos

Este trabajo de tesis no podría haber sido realizado sin la permanente ayuda de mi familia. Estoy tremendamente agradecido de mi hermosa esposa Paula y de mis hijos José Ignacio y Luz Francisca por su comprensión y adorable paciencia. Yo deseo agradecer a mis padres Edith y José quienes me ayudaron permanentemente durante mis estudios de pre y post grado. Deseo agradecer la generosa ayuda de mi abuela Ana Neder y de mi tía Verónica Gallardo, quienes en reiteradas oportunidades cuidaron de mis hijos cuando yo debía trabajar fuera de casa.

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RESUMEN

La consanguinidad se define usualmente como el apareamiento de individuos emparentados, esta incrementa la homocigosidad y reduce la adecuación biológica de los individuos. Este último fenómeno conocido como depresión endogámica (DE) se produce, generalmente, debido a la expresión de genes recesivos perjudiciales. De acuerdo a la teoría genética cuantitativa acerca de la depresión endogámica, rasgos asociados a la adecuación biológica debieran sufrir en una mayor magnitud de las consecuencias perjudiciales de la consanguinidad que rasgos no asociados directamente ella. En salmónidos juveniles varios rasgos conductuales y morfológicos como la agresividad, la conducta de forrajeo y el tamaño del cuerpo han sido asociados fuertemente a la adecuación biológica debido a que determinan las relaciones de dominancia entre individuos competidores. Por esto, se presume que estas características conductuales deberían ser afectadas por la consanguinidad.

En organismos acuáticos varios trabajos han descrito los efectos perjudiciales de la consanguinidad, sin embargo, pocos de ellos han investigado los factores ambientales bióticos que modifican la magnitud de la depresión endogámica. Antecedentes previos en esta última área revelan que: 1) el nivel de agresividad ha sido relacionado positivamente con la diversidad genética, una medida indirecta de consanguinidad; 2) la competencia intraespecífica puede aumentar la magnitud de la depresión endogámica. Este último fenómeno se denomina dependencia ambiental de la depresión endogámica y su base genética parece ser la existencia de alelos perjudiciales condicionales

(Crow, 1997) cuyos efectos son revelados solo en la presencia de un estrés ambiental.

Por otra parte, el cultivo de salmones en Chile utiliza técnicas avanzadas de selección artificial, las cuales tienen la tendencia a incrementar la tasa de consanguinidad. De este modo, limitar el incremento de la consanguinidad en los programas de selección es importante pues mientras más alto es el nivel de consanguinidad aumenta la probabilidad de que se manifieste la depresión endogámica.

Las actividades de esta tesis se realizaron con peces de 2 poblaciones cultivadas de salmón Coho, provenientes del Centro de mejoramiento genético IFOP-Coyhaique, mantenido por el Instituto de Fomento Pesquero y la Universidad de Chile. Estas poblaciones, denominadas clases año par e impar respectivamente, han sido seleccionadas artificialmente por peso a la cosecha durante 4 generaciones mediante la mejor predicción lineal insesgada (best linear unbiased production, BLUP) de los valores de cría. Un análisis preliminar de estas poblaciones permitió establecer: 1) los efectos de la selección artificial sobre la estructura poblacional; 2) los niveles de consanguinidad; 3) y la magnitud de la depresión endogámica, sobre rasgos reproductivos de hembras y sobre la supervivencia de la progenie. La selección artificial produjo que entre un 56 y un 76% de los padres de la población base, de ambas poblaciones, no dejaran descendientes en la cuarta generación. La tasa de consanguinidad fue mayor en la población par (2.45% por generación) que en la impar (1.10% por generación), como consecuencia directa del menor número de individuos

fundadores de la primera generación ($N_e = 61$ y 106 respectivamente). En la última generación el nivel de consanguinidad fue de 9.5 ($DE = 2.7\%$, año 2000) y de 4.3 ($DE = 2.6\%$, año 2001). En la población año 2000 , se estimó un efecto significativo de la consanguinidad para el índice gonadosomático (-5.3% por cada 10% de incremento en consanguinidad), pero no para otros rasgos como peso y longitud del cuerpo al desove, peso de la gónada o número de ova verde. En la población año 2001 , se estimó un efecto significativo de la consanguinidad solo para la longitud del cuerpo al desove (-1.56%). En ambas poblaciones, la supervivencia de las ovas en el estado ova no fue afectada significativamente por la consanguinidad. En resumen, en ambas poblaciones se encontró un incremento de la consanguinidad, y un efecto significativo de ella solo sobre algunos rasgos reproductivos asociados a la adecuación biológica, lo que concuerda con la teoría genética cuantitativa acerca de la consanguinidad.

En esta tesis se investigaron: 1) los efectos de la consanguinidad sobre varios parámetros conductuales relacionados con la dominancia territorial en salmón Coho; 2) los efectos de la dominancia territorial y de la competencia intra-específica sobre la magnitud de la depresión endogámica; 3) el efecto de diferentes métodos de apareamientos no aleatorios para reducir el incremento de la consanguinidad en dos poblaciones de salmón Coho sujetas a selección artificial.

Mediante el estudio de parámetros conductuales se evaluó si el efecto perjudicial de la consanguinidad era consecuencia de una menor habilidad

competitiva de los peces más consanguíneos. Adicionalmente, se determinó si la magnitud de la depresión endogámica varía de acuerdo a las condiciones ambientales de cultivo. Al contrario de la hipótesis planteada no se encontraron diferencias significativas en dos componentes que determinan la habilidad competitiva, la agresividad y la conducta de alimentación, entre peces con dos niveles de consanguinidad distintos evaluados en competición de parejas (consanguinidad baja, LI, $F = 9.5 \pm 0.3\%$; Consanguinidad media, MI, $F = 29.6 \pm 0.9\%$). Las evaluaciones de conducta en parejas no mostraron diferencias significativas en la capacidad de establecer dominancia territorial (Puntaje promedio de interacciones agresivas, LI = 20.0 ± 22 ; MI = 16.7 ± 23 ; intentos de alimentación, LI = 18.3 ± 12 ; MI = 21.1 ± 12). Sin embargo, los peces de consanguinidad baja expresaron casi doble de persecuciones agresivas sobre peces de consanguinidad media y tuvieron una mayor tasa específica de crecimiento (Specific growth rate, SGR promedio del grupo MI = 1.83 ± 0.58 ; SGR promedio del grupo LI = 2.20 ± 0.67) luego de 17.5 ± 3.8 días de cultivo. Por otra parte, dos tipos de evidencias sustentan que la magnitud de la depresión endogámica en salmón Coho se modifica de acuerdo al ambiente social en la cual se evalúa. Primero, en pequeños grupos de peces ($N=20$), peces grandes dominantes de consanguinidad media cultivados junto con peces subordinados pequeños de consanguinidad baja presentaron la misma tasa específica de crecimiento que peces dominantes de consanguinidad baja cultivados con peces subordinados pequeños de consanguinidad media; sin embargo, en uno de nuestros experimentos peces subordinados de

consanguinidad media presentaron una menor tasa específica de crecimiento que peces subordinados de consanguinidad baja. Segundo, para el rasgo supervivencia de juveniles (desde alevín a smolt) bajo condiciones de cultivo, se detectó un efecto significativo de la consanguinidad sólo en ambientes competitivos de alta densidad. Así el número de equivalentes letales fue de 2.70 en alta densidad y de solo 0.24 en el ambiente de baja densidad.

Los resultados de este análisis sugieren que quizás otros factores (Ej. eficiencia de asimilación de alimento) determinan inicialmente la menor SGR de los peces más consanguíneos. Sin embargo, a medida que las diferencias de tamaño aumentan un efecto sinérgico asociado a dominancia territorial debería magnificar los efectos perjudiciales de la consanguinidad en ambientes competitivos. Los resultados de esta tesis indican también que las diferencias de tamaño de los peces, asociadas a la dominancia territorial, pueden enmascarar los efectos perjudiciales de la consanguinidad bajo ciertas condiciones y corroboran que la competencia intra-específica usualmente magnifica los efectos perjudiciales de la consanguinidad.

Por otra parte, el aumento de los niveles de consanguinidad producto de la selección artificial y los efectos perjudiciales detectados en las poblaciones bajo estudio plantearon la necesidad de desarrollar estrategias para limitar el incremento de la consanguinidad. Como parte final de esta tesis se evaluaron tres tipos de cruzamientos no aleatorios para reducir la consanguinidad en el corto plazo (1 generación) utilizando algoritmos de programación lineal mediante el programa Hiper Lindo (Lindo System, 1989). El número de

cruzamientos evaluados simultáneamente (de 45 a 1315 cruzamientos por día de desove) fue menor que el número de cruzamientos teóricos posibles de realizar (19500) debido a que se incluyeron varias restricciones prácticas y reproductivas en el desove. La eficiencia de los distintos esquemas de cruzamientos en reducir la consanguinidad se realizó bajo un mismo nivel de respuesta genética esperada, por medio de fijar la contribución genética de cada progenitor. En la clase año 2001 el esquema de cruzamientos que minimiza la coascendencia promedio del grupo seleccionado (Minimum coancestry, MC) redujo en un 50% el aumento de la consanguinidad de la siguiente generación (F promedio = 2.01 %) en comparación con cruzamientos aleatorios (3.99 %) y en un 46 % en comparación con cruzamientos compensatorios (cruzamientos entre individuos con más altos valores de cría para el peso a la cosecha con los individuos con más bajos valores, F promedio = 3.7 %). En la clase año 2002 el esquema MC reduce el aumento de la consanguinidad (F promedio = 9.7 %) en un 14% versus cruzamientos compensatorios modificados (cruzamiento entre individuos de las familias seleccionadas con valores más altos, según promedio familiar de los valores de cría, con los individuos de las familias de más bajo valor, F promedio = 11.2 %). En ambas clases año (2001 y 2002) el esquema MC reduce además la varianza de la consanguinidad poblacional en un 50 y un 39% respectivamente. Esto se obtuvo por medio de no seleccionar los cruzamientos con más altos valores de coascendencia, lo que es equivalente a no seleccionar aquellos cruzamientos que producirán una progenie con altos valores de consanguinidad. La eficiencia

del esquema de mínima coascendencia se puede aumentar por medio de liberar las restricciones sobre el número de reproductores evaluados simultáneamente, por ejemplo concentrando el período de desove mediante inducción hormonal y/o criopreservando semen.

ABSTRACT

Inbreeding is defined as mating of related individuals, it produce a homocigosity increment and reduce the fitness of individuals. This last phenomenon, known as inbreeding depression (ID), is produced due to the expression of recessive detrimental genes. According to the quantitative genetic theory about inbreeding depression, traits tied to fitness would be more affected by the deleterious consequences of inbreeding than traits not directly tied to fitness. In juvenile salmon, various behavioral and morphological traits such as aggressiveness, foraging behavior, and body size have been strongly associated with fitness, since they determine dominance relations between competing individuals. Thus, these traits must be strongly affected by inbreeding.

There are many works that describe the detrimental effects of inbreeding on aquatic organism. However, little research has been done to evaluate the effects of biotic environmental factors on the magnitude of inbreeding depression. Previous data in this area suggest that: 1) the level of aggressiveness can be positively related to genetic diversity, which is an indirect measure of inbreeding; 2) various data from plants, rats and invertebrates suggested that intra-specific competition may increase the magnitude of inbreeding depression. The latter phenomenon has been termed environmental dependence of inbreeding depression and its genetic basis seems to be the existence of conditional deleterious alleles (Crow, 1997) the effects of which are only demonstrated in the presence of environmental stress.

Chilean salmon culture is based strongly on artificial selection, which has had the tendency to increase the inbreeding. Thus, limiting the inbreeding in selection programs is important, as higher levels of inbreeding increases the probability of inbreeding depression.

The study was based on data from two Coho salmon populations belonging to the genetic improvement center (CMG) maintained by the Institute for Fisheries Development (IFOP) and the University of Chile in Coyhaique (XI Region, Chile). The two populations, termed even year, and odd year were artificially selected by weight at harvest over four generations, using the best linear unbiased prediction (BLUP) of breeding values. In these populations, a previous survey was done to: 1) to determine the effect of artificial selection, following the animal model, on the levels of inbreeding in two populations of Coho salmon; 2) investigate the effects of inbreeding on reproductive traits in females, and 3) evaluate the effects of inbreeding on egg survival. The selection resulted in 56% to 76% of the parents of the base population not contributing with descendents in the fourth generation. The inbreeding rate was greater in the even population (inbreeding rate = 2.45% per generation) than the odd population (inbreeding rate = 1.10% per generation) as a direct consequence of the smaller number of founder individuals in the former population ($N_e = 61$ and 106, respectively). The level of inbreeding in the last generation was 9.5 % (SD=2.7, range 5 to 19%) for year-class 2000 and 4.3 % (SD=2.6, range 1 to 12%) for year-class 2001. Significant inbreeding depression was estimated for the gonadosomatic index (-5.3% per each 10% increase in inbreeding) in

population year 2000, and for body length at spawning (-1.56%) in population year 2001. The inbreeding did not significantly reduce other traits such as weight body at spawning, weight of the gonad, number of green eggs, or relative fecundity. No significant inbreeding depression was observed in either population regarding the survival of eggs in the eyed stage.

In the present thesis work, I researched: 1) the effects of inbreeding on behavioral traits related with territorial dominance in Coho salmon; 2) the effects of territorial dominance and intraespecific competition on the inbreeding depression magnitude; 3) the effects of different non-random matings schemes to reduce the accumulation of inbreeding in artificially selected Coho salmon populations.

Through the study of behavioural parameters I evaluated whether or not the deleterious effect of inbreeding was the consequence of lower competitive ability in more inbred fishes. Additionally, I determined if the magnitude of inbreeding depression changed according to the rearing environment conditions. Contrary to my hypothesis, I did not find significant differences in the two components that determined competitive ability (aggressivity, feeding behaviour) between fish with two different levels of inbreeding in paired contest (Low inbreeding, LI, $F = 9.5 \pm 0.3\%$; medium inbreeding, MI, $F = 29.6 \pm 0.9\%$). Behavior evaluations in paired contest between fishes with two different levels of inbreeding did not show significant differences between their capacities for establishing territorial dominance (Mean aggressiveness score, LI = 20.0 ± 22 ; MI = 16.7 ± 23 ; Feeding attempt, LI = 18.3 ± 12 ; MI = 21.1 ± 12). However, fish

with low inbreeding demonstrated almost twice the aggressive pursuit of fish of medium inbreeding and had a higher specific growth rate (SGR mean of MI group = 1.83, SD = 0.58; SGR mean of LI group = 2.20, SD = 0.67) after 17.5 (SD = 3.8) days in culture. Two types of evidence support the contention that the magnitude of the inbreeding depression in Coho salmon is modified according to the social environment in which the evaluation is made; *First*: In small groups of fish (N=20), large dominant fish having medium inbreeding, cultivated with small subordinate fishes with low inbreeding, showed the same SGR as dominant fishes with low inbreeding cultivated with small subordinate fishes having medium inbreeding. In one of our experiments, however, subordinate fishes with medium inbreeding showed a lower SGR than subordinate fishes of low inbreeding. *Second*: As related to the survival traits in juveniles (from alevins to smolt) in culture conditions, significant inbreeding depression was detected only in high density competitive environments. Thus the number of lethal equivalents was 2.70 at high density, and only 0.24 in a low density environment. Our results suggest that other factors such as the food assimilation efficiency may initially determine the lowest SGR of the more consanguineous fishes. As the size differences increase, however, a synergistic effect associated with territorial dominance should magnify the deleterious effects of the inbreeding in competitive environments. Our results also show that differences in size associated with territorial dominance may mask deleterious effects of inbreeding under certain conditions, and support the concept that intraspecific competition usually magnifies the deleterious effects of inbreeding.

The increment of inbreeding levels and the detrimental effects were found in different traits, which drive to necessity of developing strategies to limit its increment. Three types of non-random mating were evaluated to control the short term accumulation of the inbreeding in two BLUP-selected Coho salmon populations using linear programming. These included: compensatory mating on the basis of breeding values (C), modified compensatory mating (C1) on the basis of family mean of the breeding values, and mating which minimise the mean coancestry of the group selected (MC). Practical and reproductive limitations relative to selection programs in fishes were operant, and reduced the number of crosses which could be simultaneously evaluated. In the Odd population the MC scheme reduced to 50% and to 46% the increase in inbreeding of the next generation when compared with random mating of selected individuals with sib mating restricted (R) and with compensatory scheme (C) respectively (MC, F mean = 2.0; R, F mean = 3.9%; C, F mean = 3.7%). In the Even population the MC scheme reduced the increase in inbreeding by 14% (9.7 v/s 11.2) compared with C1. The MC scheme also reduced the variance in the inbreeding by 54-59% when comparing C and R in the Odd population and 39% when compared with the C1 in the Even population. Thus the MC scheme was more efficient in reducing the increase and the variance of the inbreeding thus limiting the expression of inbreeding depression. In practical condition, the MC scheme was more time-consuming than the two mating schemes previously utilised at the CMG (R and C1), however, we recommend this scheme to carry out crosses in each generation.

1. INTRODUCCION

1.1. Teoría genética cuantitativa de la consanguinidad y la depresión endogámica

1.1.1. Consanguinidad

El término consanguinidad se refiere al cruzamiento entre individuos emparentados, y su consecuencia primaria es el incremento de la homocigosis en los individuos consanguíneos. Este fenómeno se puede analizar desde al menos tres puntos de vista que están relacionados con lo que la origina y con el tipo de información disponible para su cálculo o análisis:

a) Desde genealogía.

Un individuo se considera consanguíneo cuando sus padres poseen algún grado de parentesco, ya sea porque comparten algún antecesor común o por ser uno antecesor de otro. Cuando se conocen las relaciones de parentesco, ya sea por registro genealógico directo o por medio de marcadores moleculares (pruebas de paternidad), esta información se puede usar para determinar un coeficiente de consanguinidad individual (F). Malècot (1948) definió el coeficiente de consanguinidad (F_x) de un individuo x , como "la probabilidad de que este individuo posea, para un locus cualquiera, dos genes alelos idénticos por descendencia". Para que dos genes sean idénticos por descendencia estos deben cumplir con dos requisitos: 1) Ambos alelos deben ser iguales en estado,

es decir, genes que producen exactamente el mismo efecto o que codifican para la misma proteína; 2) Ambos alelos deben provenir de un mismo gen, de algún antecesor en común.

b) Como sistema de cruzamiento.

Bajo esta perspectiva, el término consanguinidad se define como “el sistema de cruzamiento cuya progenie se produce por padres que están más estrechamente emparentados que el promedio de la población a la que pertenecen”. Bajo esta definición un individuo es consanguíneo sólo si sus padres son más emparentados que el promedio de la población. Por lo tanto, este tipo de consanguinidad es relativo a una población hipotética con cruzamientos aleatorios del mismo tamaño que la población observada. En este caso la consanguinidad se puede medir con marcadores moleculares a través del índice F_{is} . Este índice corresponde a la desviación de la heterocigosidad observada (H_o) de un individuo relativo a la esperada (H_e) bajo cruzamientos aleatorios ($F_{is} = 1 - H_o/H_e$). $F_{is} > 0$ significa más consanguíneo que lo esperado por azar, mientras que un $F_{is} < 0$ indica que la consanguinidad ocurrió menos que lo esperado por simple azar.

c) Producto de un reducido tamaño poblacional.

Cuando una población se divide en 2 o más subpoblaciones aisladas, la consanguinidad ocurrirá simplemente debido al limitado tamaño poblacional. En este caso la consanguinidad se calcula a partir del tamaño efectivo de la población (N_e), cuyo valor no solo depende del tamaño poblacional en sí, sino que también de la estructura poblacional (proporción de machos y hembras

reproductores) y del número de generaciones transcurridas entre la generación fundadora y la generación bajo estudio.

1.1.2. Depresión endogámica

En genética y biología evolutiva el estudio de la consanguinidad es de alto interés debido a su conocido efecto perjudicial sobre la adecuación biológica de los individuos (Charlesworth and Charlesworth, 1987; Lynch and Walsh, 1998). Este fenómeno conocido como depresión endogámica se define usualmente como la declinación en el fenotipo promedio de un rasgo debido a la consanguinidad (Falconer and Mackay, 1996). Otras definiciones, sin embargo, hacen referencia también al aumento de malformaciones en los animales (Tave, 1996). Dos son las hipótesis que permiten explicar este fenómeno, ambas tienen que ver con el aumento de la homocigosis debido a consanguinidad y con los efectos de dominancia entre alelos de un mismo gen:

- 1) **Hipótesis de dominancia:** Esta hipótesis argumenta que la depresión endogámica se produce por la expresión de genes recesivos perjudiciales en los individuos homocigotos. El término hipótesis de dominancia se mantiene hasta hoy debido a razones históricas pero más apropiadamente debería llamarse hipótesis de recesividad.

2) **Hipótesis de sobredominancia:** Esta hipótesis argumenta que algo especial en los individuos heterocigotos causa un incremento en la adecuación biológica relativo a los individuos homocigotos.

En general, existe consenso de que los rasgos íntimamente asociados a la adecuación biológica (reproductivos) sufren de una mayor depresión endogámica que los rasgos no correlacionados directamente con ella como los rasgos morfológicos (Falconer and Mackay, 1996; DeRose and Roff, 1999). Antecedentes teóricos argumentan que esto se produce porque los rasgos correlacionados a la adecuación biológica presentan una mayor varianza de dominancia direccional que los rasgos morfológicos (Lynch and Walsh, 1998). Básicamente, en rasgos fuertemente ligados a la adecuación biológica, mutaciones favorables, deberían fijarse en las poblaciones independiente de su grado de dominancia. Por otra parte, mutaciones perjudiciales deberían eliminarse rápidamente pero solo si son dominantes, pues si son recesivas deberían acumularse en baja frecuencia por efecto de la mutación (acumulación de dominancia direccional). En cambio para rasgos poco ligados a la adecuación biológica la dominancia direccional debería ser menos pronunciada debido a que mutaciones que aumenten o reduzcan el valor promedio de un carácter serán selectivamente equivalentes.

1.1.3. Dependencia ambiental de la depresión endogámica

Predecir los efectos de la consanguinidad en una población es un asunto bastante complejo, pues la magnitud de la depresión endogámica usualmente varía de acuerdo a las condiciones ambientales bióticas y abióticas sobre las

cuales se evalúa (Keller et al. 1994; Hauser & Loeschke, 1996; Kolewijn, 1998; Bijlsma et al. 1999; Dahlgard & Loeschke 1997, Dahlgard and Hoffmann, 2000). Sin embargo, existe evidencia que sugiere que la magnitud de la depresión endogámica tiende a aumentar en condiciones competitivas o de estrés ambiental (Latter y Mulvey, 1995; Crnokrak and Roff, 1999; Hedrick and Kalinowski, 2000; Haag et al. 2002). La base genética de este fenómeno parece ser la existencia de alelos perjudiciales condicionales (Crow, 1997; Bijlsma et al., 1999) cuyos efectos son revelados solo en condiciones restrictivas y no en condiciones permisivas.

1.1.4. Métodos para reducir la acumulación de la consanguinidad en poblaciones animales

En poblaciones cerradas, la consanguinidad puede llegar a ser una inevitable consecuencia del limitado tamaño poblacional. Si además, estas poblaciones son sujetas a selección artificial la tasa de consanguinidad podría ser bastante alta, producto de que pocos animales son dejados como reproductores en cada generación. Debido a esto, en los últimos años se han desarrollado varios métodos para controlar la tasa de consanguinidad en poblaciones sujetas a selección artificial (Toro y Perez Enciso, 1990; Villanueva *et al.*, 1996; Meuwissen, 1997; Wu y Schaeffer, 2000). Evaluaciones teóricas de estos métodos revelan 2 aspectos importantes al considerar su implementación en los actuales programas de mejoramiento genético: 1.- Independiente de cual

método sea aplicado para seleccionar los reproductores de la siguiente generación, el esquema de apareamientos entre ellos es determinante en reducir la tasa de consanguinidad, siendo los apareamientos aleatorios el menos eficiente (Caballero et al, 1996; Sonesson y Meuwisen, 2000); 2.- Consideraciones prácticas y restricciones reproductivas pueden limitar la eficiencia de cualquiera de estos métodos en reducir la tasa de consanguinidad (Meuwisen, 1997; Fernández y Toro, 1999). Actualmente pocos trabajos han evaluado en la practica los métodos antes descritos (Sanchez et al, 1999; Weiguel y Lin, 2000), en particular ninguno ha sido evaluado tomando en cuenta las características de los programas de selección en peces.

1.2. Consanguinidad, conducta y depresión endogámica en salmones juveniles

En salmónidos juveniles varios rasgos conductuales como la agresividad y la actividad de forrajeo han sido relacionados con la dominancia territorial y con la tasa específica de crecimiento (Damsgård, et al. 1997; Nicieza y Metcalfe, 1999; Lahti, et al. 2001). Ambas características están íntimamente ligadas a la adecuación biológica (Quinn and Peterson, 1996), por lo que deberían ser fuertemente afectadas por la consanguinidad. En condiciones naturales, los salmones juveniles son territoriales y defienden mediante actos agresivos una pequeña porción del río sobre la cual ellos se alimentan (Holtby et al. 1993; Nicieza y Metcalfe, 1999; Healy and Lonzarich, 2000). Estas conductas

agresivas definen jerarquías de dominancia, en las cuales los individuos dominantes usualmente monopolizan los mejores sitios de alimentación (Valdimarsson y Metcalfe, 2001). Esto les permite maximizar su beneficio en términos de ganancia de energía neta (Faush, 1984), aun cuando bajo algunas condiciones de aleatoriedad en el suministro de alimento o de una perturbación ambiental esta situación se puede revertir (Sloman et al. 2001; Vøllestad and Quinn, 2003) revelando un compromiso entre la agresividad y el fitness. En cualquier caso, la jerarquía por dominancia genera un fuerte estrés en los peces subordinados (Ejike y Schreck, 1980; Sloman et al. 2000, 2001) produciendo, entre otras consecuencias a escala fisiológica, una disminución en la resistencia a enfermedades así como un menor crecimiento (Sloman et al. 2000). En condiciones de cultivo, se ha sugerido que la alta densidad debería prevenir el establecimiento de territorios y que la competencia intraespecífica debería ser baja dado que el alimento está disponible en forma permanente. Sin embargo, varios autores han demostrado que algunos peces dominantes pueden defender una parte de los acuarios de cultivo, crear áreas de baja densidad, monopolizar el alimento y crecer más que individuos subordinados (Thorpe et al. 1990; Ryer y Olla, 1996). Por otra parte, la alta densidad de cultivo generalmente aumenta la frecuencia de encuentros entre peces y con esto se incrementan las interacciones agresivas. Estas usualmente producen daños severos en el cuerpo, principalmente en las aletas y en menor grado en los ojos y en el opérculo (Aboot and Dill, 1985; Turnbull et al. 1998). La prevalencia de daño en las aletas puede ser tan alta en poblaciones de cultivo

que su presencia ha sido usada para diferenciar a peces silvestres de los peces de cultivo (Craik et al. 1987).

Existen varios trabajos que describen los efectos perjudiciales de la consanguinidad en peces (Su et al. 1996; Pante et al. 2001; Gallardo et al. 2004a), sin embargo, pocos han evaluado si esto es consecuencia de una menor habilidad competitiva. Recientemente, Tiira et al. (2003) demostró en *Salmo salar* que la agresividad está correlacionada positivamente con la diversidad genética, una medida indirecta del nivel de consanguinidad (Hedrick and Kalinowski, 2000). Investigaciones en otros taxa también sugieren que los individuos consanguíneos poseen una menor habilidad competitiva, por ejemplo en la obtención y defensa de un territorio (Meagher et al. 2000). Adicionalmente, se ha demostrado que un nivel de consanguinidad extremo puede limitar el reconocimiento entre individuos (Ej. similar constitución genética) y alterar los patrones conductuales frente a potenciales competidores (Nevison et al. 2000).

1.3. Observaciones preliminares de la consanguinidad y sus efectos perjudiciales en dos poblaciones de salmón Coho en Chile.

Esta tesis se realizó con peces de cultivo provenientes del centro de mejoramiento genético de salmón Coho IFOP-Coyhaique (Gallardo et al. 2004a, manuscrito 1). Esta población ha permanecido por 5 generaciones (10 años) bajo condiciones de cultivo y en la última generación (clase año 2000) el nivel promedio de consanguinidad fue de $9.4 \pm 2.7\%$. Un efecto significativo de la

consanguinidad se estimó en algunos rasgos relacionado a la adecuación biológica como lo es el índice gonadosomático (GSI, -5.3 % por cada 10% de incremento en consanguinidad). Adicionalmente, existe evidencia que la depresión endogámica sobre el peso del cuerpo cambia de acuerdo a modificaciones en el ambiente de cultivo (dependencia ambiental). Específicamente, se ha descrito que la magnitud de la depresión endogámica sobre el peso del cuerpo incrementa desde alevín (5-10g) a smolt (150g). En este período los peces de familias con distintos niveles de consanguinidad fueron cultivados juntos en balsas jaula en el mar. Básicamente, el análisis muestra que en el estado alevín (antes de que las familias fueran mezcladas) el peso del cuerpo disminuye 1.1 % ($P=0.298$) por cada 10% de incremento en consanguinidad en la clase año 2000, mientras que el peso del cuerpo en el estado smolt (3 meses después de que las familias fueran mezcladas), disminuye significativamente 3.8 % ($P<0.001$).

1.4. Objetivos

1.4.1 Generales

a) Evaluar los efectos perjudiciales de la consanguinidad sobre rasgos conductuales relacionados a la dominancia territorial en salmón Coho (*Oncorhynchus kisutch*).

b) Evaluar métodos para reducir el incremento de los niveles de consanguinidad en dos poblaciones de salmón Coho sujetas a selección artificial.

1.4.2 Específicos

a) Investigar las consecuencias de la consanguinidad sobre la agresividad y la actividad de alimentación.

b) Investigar los efectos del ambiente social (dominancia territorial y competencia intraespecífica) sobre la magnitud de la depresión endogámica tanto bajo condiciones de laboratorio como de cultivo.

c) Investigar el efecto de diferentes métodos de apareamientos no aleatorios para reducir el incremento de la consanguinidad en dos poblaciones de salmón Coho sujetas a selección artificial.

1.4. Hipótesis

Habilidad competitiva

La agresividad y la actividad de alimentación se han asociado fuertemente a la adecuación biológica, de acuerdo con la teoría genética cuantitativa de la depresión endogámica este tipo de rasgos debería sufrir fuertemente los efectos perjudiciales de la consanguinidad. En consideración a esto, individuos con altos niveles de consanguinidad deberían tener una menor habilidad competitiva para establecer dominancia territorial (Ej: menor nivel de agresividad), si esto es así peces consanguíneos deberían ser subordinados frente a peces no consanguíneos.

Dependencia ambiental de la depresión endogámica

Dominancia territorial: El tamaño del cuerpo puede ser determinante en el resultado de una disputa competitiva. Generalmente individuos grandes tienden a ser dominantes frente a individuos pequeños. Si la magnitud de la depresión endogámica se modifica de acuerdo al tamaño del cuerpo, individuos grandes consanguíneos cultivados con peces pequeños no consanguíneos deberían expresar una similar tasa específica de crecimiento que peces grandes no consanguíneos cultivados junto con peces pequeños consanguíneos.

Competencia intraespecífica: La magnitud de la depresión endogámica en la tasa específica de crecimiento debería ser mayor en ambientes restrictivos de alta densidad de cultivo que en ambientes permisivos de baja densidad de cultivo.

2. METODOLOGÍA Y RESULTADOS

La metodología, los resultados y una discusión específica de los antecedentes preliminares y de los objetivos planteados en esta tesis se muestran en los anexos 1, 2 y 3 mediante el formato de manuscritos. La discusión general y las proyecciones de esta tesis se presentan a continuación en la sección 3.

3. DISCUSIÓN

3.1. Consanguinidad, depresión endogámica y conducta

En esta tesis se demostró que la consanguinidad reduce significativamente la tasa específica de crecimiento de peces juveniles cuando estos son agrupados por tamaño. Otros estudios en peces también han concluido que la consanguinidad reduce significativamente este y otros rasgos correlacionados a la adecuación biológica bajo distintas condiciones de cultivo (Gjerde et al., 1983; Su et al., 1996; Rye and Mao, 1998; Gallardo et al., 2004a).

Mediante el estudio de parámetros conductuales se evaluó si el efecto perjudicial de la consanguinidad era consecuencia de una menor habilidad competitiva de los peces más consanguíneos. Los resultados no apoyan la hipótesis planteada pues no se encontraron diferencias significativas en dos componentes que determinan la habilidad competitiva, la agresividad y la conducta de alimentación, entre peces con dos niveles de consanguinidad distintos evaluados en competición en parejas. Al menos dos antecedentes previos indicaban que la agresividad o la dominancia territorial debieran haber sido afectadas por la consanguinidad. Primero, ambas características conductuales han sido ligadas fuertemente a la adecuación biológica en salmónidos juveniles, por lo que deberían haber expresado un alto nivel de depresión endogámica (Falconer y Mackay, 1996). Segundo, antecedentes previos en salmónidos y otros vertebrados dan cuenta de un menor nivel de

agresividad (Tiira et al. 2003) y de una menor habilidad competitiva en la defensa de un territorio (Meagher et al. 2000) por parte de individuos más consanguíneos. Los resultados de esta tesis contrastan con los de Tiira et al. (2003) quienes demostraron que salmon fry (*Salmo salar*) de baja diversidad genética fueron menos agresivos que fry con un alto nivel de diversidad genética. Sin embargo, dado que las evaluaciones conductuales de Tiira et al. (2003) se realizaron entre individuos de un mismo nivel de diversidad genética (baja y alta), su estudio no permite descartar que peces genéticamente similares (baja diversidad genética) sean menos agresivos entre si. Por otra parte, existe una tendencia en común entre los resultados de esta tesis y los de Tiira (2003), esta es que los individuos menos consanguíneos expresaron un mayor número de conductas altamente agresivas como persecuciones y mordeduras que podría ser determinantes en el establecimiento de territorios en el largo plazo. Por ejemplo, Cutt et al. 2002, demostraron en *Salmo salar*, que en un ambiente nuevo los peces dominantes son más agresivos, pero consumen menos alimento con el objetivo de mantener un territorio en el largo plazo, esta tendencia fue observada en nuestros resultados de agresión y alimentación y quizás sesgó nuestro índice de dominancia. Sin embargo, como el tamaño del cuerpo es un determinante de las relaciones de dominancia entre peces (Holtby et al. 1993), hacer una evaluación de conducta en el medio o al final del experimento no podría haber dado luces de si la conducta fue causa o consecuencia de la mayor SGR.

3.2. Dependencia ambiental de la depresión endogámica

En este estudio se demostró que un cambio en las relaciones de dominancia social puede revertir el efecto perjudicial de la consanguinidad en pequeños grupos de peces (N=20). En este estudio se modificaron las relaciones de tamaño entre dos grupos de peces con distintos niveles de consanguinidad, forzando a los peces consanguíneos a ser dominantes frente a peces menos consanguíneos. En estas circunstancias, la evidencia de dependencia social de la depresión endogámica fue muy consistente en relación con los peces grandes dominantes. En los dos experimentos realizados los peces grandes dominantes expresaron la misma tasa específica de crecimiento independiente del nivel de consanguinidad. Sin embargo, el análisis es un poco más complejo al evaluar el efecto de la consanguinidad sobre los individuos subordinados pues en un experimento (Familia 196-208) ambos grupos de peces subordinados (con consanguinidad baja y media) expresaron la misma SGR pero en el otro experimento no (familias 198-204). Esto pues el grupo subordinado de baja consanguinidad (familia 198) expresó no solo una mayor SGR que los peces subordinados de consanguinidad media, sino que también una mayor SGR que ambos grupos dominantes tanto de consanguinidad baja como media. Estos antecedentes son consistentes con diferencias en la expresión de la depresión endogámica a nivel familiar, lo cual ha sido descrito en otros taxa (Holtsford, 1996) pero sugieren también un efecto de compensación de crecimiento por parte de los peces pequeños.

Se encontraron al menos dos evidencias de dependencia ambiental de la depresión endogámica bajo condiciones de cultivo. Primero, la magnitud de la DE sobre peso del cuerpo y la SGR incrementa desde alevín a smolt cuando los peces son mezclados en jaulas comunes. Esto es coincidente con evaluaciones previas realizadas sobre esta misma población y con datos publicados en trucha arcoiris por Su et al. (1966), quienes muestran que la magnitud de la depresión endogámica (DE) para el peso del cuerpo incrementa con la edad (DE = +1.73%, -0.85% y -2.26% por cada 10% de incremento en consanguinidad en 168, 280 y 364 días, respectivamente, lo cual es coincidente con el marcaje y posterior cultivo de los peces en acuarios comunes (desde los 168 días). Segundo, se expresó una mayor carga genética bajo condiciones de alta densidad de cultivo, de manera similar a lo encontrado en otros taxa (Latter y Mulley, 1995; Haag et al. 2002). Ambos resultados en su conjunto tienen importantes implicancias en acuicultura sobre todo si se consideran los actuales programas de mejoramiento genético en peces. Por ejemplo, una cantidad cada vez mayor del salmón cultivado en Chile proviene de peces mejorados genéticamente mediante selección artificial, la cual tiene la tendencia a incrementar la tasa de consanguinidad. Aunque se han propuesto varios métodos para limitar el incremento de la consanguinidad (Villanueva et al. 1996; Meuwissen 1997; Gallardo et al, 2004b), quizás un cambio en las condiciones de cultivo (Ej. más baja densidad) también podría limitar la expresión de la depresión endogámica. Actualmente existe consenso de que disminuir las densidades de cultivo podría ser beneficioso para los peces pues se reduce el

estrés, sin embargo, esto debería acumular una carga genética para generaciones posteriores (Crow, 1997).

3.3. Métodos para reducir la acumulación de la consanguinidad en poblaciones animales

En esta tesis se demostró que el esquema de mínima coascendencia (MC) fue más eficiente en reducir la consanguinidad promedio de la siguiente generación, en comparación a los otros esquemas de apareamientos aplicados en el CMG de salmón coho. Este método requirió, para cada día de desove, la construcción *in situ* de matrices de coascendencia entre todos los apareamientos posibles, además de matrices necesarias para adecuar la selección de los apareamientos óptimos a un problema de programación lineal. Ambos asuntos se resolvieron exitosamente por medio de aplicar un procedimiento en dos etapas. En la primera, el programa PEDIGREE (Kingham, 1998) fue utilizado para calcular la coascendencia de todos los apareamientos posibles de los reproductores seleccionados (VC). En la segunda, los apareamientos que minimizaron la coascendencia promedio en un día particular fueron obtenidos mediante el programa Hiper Lindo (Lindo System, 1984). Otros autores han utilizado una aproximación similar a la aquí evaluada obteniendo resultados similares en cuanto a la eficiencia del método MC en reducir la consanguinidad, tanto en el corto (Weiguel and Lin, 2000) como en el mediano plazo (Sanchez et al, 1999). Por otra parte, en concordancia con varias predicciones teóricas (Caballero et al, 1996; Sonesson y Meuwisen, 2000; Weiguel and Lin, 2000) el

esquema de apareamientos aleatorios resultó ser el menos eficiente en reducir la consanguinidad aun cuando fueran evitados los apareamientos de FS y HS en cada generación. A pesar de estas evidencias, el esquema de apareamientos aleatorios antes descrito sigue siendo un método comúnmente utilizado en programas de cría selectiva (Su et al, 1996; Pante et al, 2001; Argue et al. 2002; Bolivar and Newkirkb, 2002),

El esquema MC redujo la varianza de consanguinidad entre un 40 y un 60% en relación con los otros esquemas evaluados, generando familias más homogéneas entre si en cuanto al nivel de consanguinidad (Figura 1 y 2). Resultados similares fueron obtenidos por Sanchez *et al.* (1999), quienes encuentran que la varianza de consanguinidad se reduce entre 1 y 7 veces en comparación con apareamientos aleatorios. Esto es una característica deseable en el sentido que no se criaran familias con altos niveles de consanguinidad dentro de cada generación, sobre las cuales es más probable que se exprese la DE (Gjerde et al 1983; Su et al, 1996; Pante et al 2001).

En este trabajo las evaluaciones se realizaron teniendo en cuenta 2 restricciones que limitaron el número de apareamientos probados simultáneamente. La restricción reproductiva limitó el número de reproductores evaluados simultáneamente a aquellos exitosamente desovados en un día particular, mientras que restricciones prácticas en el manejo de los peces impidió que se desovara y evaluara un alto número de reproductores simultáneamente. Bajo estas 2 restricciones sólo entre 45 (5 machos y 9 hembras) y 510 (17 machos y 30 hembras) apareamientos se pudieron evaluar

el año 2002. Esto es inferior al número de apareamientos posibles de realizar en el CMG en un día cualquiera (30 machos y 150 hembras, 4500 apareamientos posibles) y muy inferior al número teórico de apareamientos si todos los peces pudieran haber sido desovados simultáneamente (14630 apareamientos posibles). Al menos dos formas alternativas de manejo del desove se pueden incluir para liberar las restricciones sobre el número de reproductores evaluados simultáneamente y aumentar la eficiencia del esquema de mínima coacendencia: a) concentrar el período de desove en menos días mediante inducción hormonal, b) criopreservar y/o preservar semen de todos los machos utilizados. Ambas técnicas, sobre todo la primera, han sido incorporadas en centros de cultivo en Chile y se pueden utilizar eficientemente en el corto plazo en programas de selección genética aplicado a salmónidos.

En este estudio las comparaciones entre esquemas de apareamientos se realizaron bajo un mismo nivel de respuesta genética esperada, sin embargo, este parámetro se puede mejorar (maximizar) simultáneamente junto con la tasa de consanguinidad como lo muestran Villanueva et al. (1996) y Weiguel y Lin (2000).

4. CONCLUSIONES

1. No se encontraron evidencias de que la menor tasa específica de crecimiento (SGR) de los individuos más consanguíneos sea una consecuencia directa de una menor habilidad competitiva de los individuos consanguíneos en términos de los parámetros conductuales evaluados en este trabajo. Otros factores entonces (Ej. eficiencia de asimilación de alimento) quizás determinan que los individuos consanguíneos expresaran una menor SGR que peces menos consanguíneos. Sin embargo, a medida que las diferencias de tamaño aumentan un efecto sinérgico asociado a dominancia territorial debería magnificar los efectos perjudiciales de la consanguinidad en ambientes competitivos.

2.- Las diferencias de tamaño de los peces, asociadas a la dominancia territorial, pueden enmascarar los efectos perjudiciales de la consanguinidad bajo ciertas condiciones y corroboran que la competencia intra-específica usualmente magnifica los efectos perjudiciales de la consanguinidad.

3.- El esquema MC mostró ser una buena herramienta para retrasar la acumulación de la consanguinidad en el corto plazo. Adicionalmente, este esquema reduce además la varianza de la consanguinidad limitando así la expresión de depresión endogámica. Los efectos antes descritos sobre el promedio y la varianza de la consanguinidad se obtuvieron aun cuando las evaluaciones se realizaron sobre grupos muy pequeños de apareamientos posibles (45 – 1316).

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ANEXOS

ANEXO 1



Inbreeding and inbreeding depression of
female reproductive traits in two populations
of Coho salmon selected using BLUP predictors
of breeding values

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Abstract

Levels of inbreeding and inbreeding depression were studied in two populations of Coho salmon (*Oncorhynchus kisutch*) in Chile. The two populations, termed even year, and odd year were artificially selected by weight at harvest over four generations, using the best linear unbiased prediction (BLUP) of breeding values. Also, general linear models (GLM) were used to analyze the effects of inbreeding on reproductive traits of the females and on survival of the progeny. The selection resulted in 56–76% of the parents of the base population not contributing with descendents in the fourth generation. The inbreeding rate was greater in the even population ($\Delta F=2.45\%$ per generation) than the odd population ($\Delta F=1.10\%$ per generation) as a direct consequence of the smaller number of founder individuals in the former population ($N_e=61$ and 106, respectively). The level of inbreeding in the last generation was 9.5% (S.D.=2.7, range 5–19%) for year-class 2000 and 4.3% (S.D.=2.6, range 1–12%) for year-class 2001. Significant inbreeding depression was estimated for the gonadosomatic index (-5.3% per each 10% increase in inbreeding) in population year 2000, and for body length at spawning (-1.56%) in population year 2001. The inbreeding did not significantly reduce other traits such as weight body at spawning, weight of the gonad, number of green eggs, or relative fecundity. No significant inbreeding depression was observed in either population regarding the survival of eggs in the

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eyed stage. Given the deleterious effects of inbreeding on reproductive traits, salmon selection programs should employ methods which limit the rate of increase of this factor.
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1. Introduction

Genetic improvement of fishes in aquaculture has recently benefited from incorporation of the “best linear unbiased predictor” (BLUP) for breeding values (BV) when using the animal model for genetic evaluation (Gjoen and Gjerde, 1998; Martínez et al., 1999). This procedure maximises the correlation between the true and the predicted BV, using information from all animals recorded in the present generation and of preceding generations, as well as the individual’s own data. This animal estimation produces a more exact estimation for the breeding value, and increases the genetic response compared with other methods such as phenotypic selection (Belonsky and Kennedy, 1988). However, given that the animal model favours selection of related individuals, it also produces an increase in the rate of inbreeding (ΔF) and a reduction in the genetic variance over the long term (Belonsky and Kennedy, 1988; Quinton et al., 1992). There have been few empirical evaluations of the rate of inbreeding in fish populations subject to artificial selection. In rainbow trout populations, Su et al. (1996) estimated a rate of inbreeding (ΔF) between 1.3% and 1.7% per generation, while Pante et al. (2001a) found rates of inbreeding of between 0.53% and 2% per generation.

The control of inbreeding rate is important in selection programs as the higher the level of inbreeding (F), the higher is the probability of occurrence of the phenomenon known as inbreeding depression (ID). Estimations of ID in fishes has consistently shown that consanguineous progeny has lower viability, less growth, and also shows lower resistance to infection by protozoans (Gjerde et al., 1983; Su et al., 1996; Rye and Mao, 1998; Pante et al., 2001b; Arkush et al., 2002). Gjerde et al. (1983) found a strong decrease in survival in various early stages of development in rainbow trout, as well as a low growth rate in adults with high levels of inbreeding ($F=0.25, 0.375, \text{ and } 0.5$). Estimates obtained from artificially selected populations (Su et al., 1996) showed that the magnitude of the inbreeding depression (ID) for body weight increased with age ($ID=+1.73\%, -0.85\%$, and -2.26% for each 10% increment in inbreeding at 168, 280, and 364 days, respectively). Similarly, Pante et al. (2001b) estimated an average decrease of between -1.0 and -2.6 for each 10% increase in inbreeding. Rye and Mao (1998) estimated ID of between -0.6% and -2.6% for each 10% increase in inbreeding for the same trait in Atlantic salmon. Arkush et al. (2002) recently demonstrated that inbreeding could also increase the severity of a protozoan infection in Chinook salmon (*Oncorhynchus tshawytscha*). In spite of the harmful effects of the preceding data, little is known concerning the effects of inbreeding on reproductive traits. These traits are determining factors in the long-term success of selection programs. In rainbow trout, Su et al. (1996) estimated that for each 10% increase in inbreeding the numbers of eggs decreased

significantly by 6.1%. It was also shown that inbreeding of the dam, but not the sire, affected the survival of the progeny (ID=-11.6% and 2.0%, respectively). Data on the effects of inbreeding in reproductive traits in Coho salmon (*Oncorhynchus kisutch*) have not been published.

The objectives of the present study were: (1) to determine the effect of artificial selection, following the animal model, on the levels of inbreeding in two populations of Coho salmon; (2) to investigate the effects of inbreeding on reproductive traits in females; and (3) to evaluate the effects of inbreeding on egg survival.

2. Materials and methods

2.1. Study populations

The study was based on data from two Coho salmon populations from the genetic improvement center (CMG) maintained by the Institute for Fisheries Development (IFOP) and the University of Chile in Coyhaique (XI Region, Chile). The two populations, termed 'even' and 'odd', were produced in 1992 and 1993, respectively, and are managed in a 2-year reproductive cycle. Individuals were ranked using their breeding values for body weight at harvest time and the 25% best individuals (1000 approximately) were kept as breeding stock. Spawning season occurred between April and June each year. The first 300 spawning females and 60 males were randomly mated, but each male was used to fertilize eggs of females spawning at the beginning, at the middle and at the end of the spawning period. Additionally, full siblings (FS) mating were avoided. The eggs of each full-sib family were incubated separately, and at eyed eggs stage 120 families were selected and moved to individual tanks considering the following criterion: (1) keeping a mating design as close as possible to one male to three to four females; and (2) keep families with highest eggs survival (>50%). Then, the progeny of only 100 families were individually PIT tagged in December when the fish averaged about 5–7 g. At this age fish are transferred to estuary water conditions (Ensenada Baja) where each full-sib family was randomly stocked in equal numbers (60–80) into three rearing cages. Smoltification occurred naturally at 8 months post-spawning and weight at harvest time was recorded in February, at 20–21 months of age. For more information about the characters and origins of the populations see Martinez et al. (1999), Winkler et al. (1999), and Gall and Neira (in press).

Artificial selection was practised for four generations in both the even and the odd populations. Characters selected included the weight at harvest, using BLUP predictions of breeding values obtained from the MTDFREML program (Boldman et al., 1995) by means of the animal model, and early spawning by means of phenotypic selection.

2.2. Data

The level and rate of inbreeding in the even and odd populations were determined on the basis of the genealogical records of the animals (Table 1). All together, each generation was composed of between 850 and 4400 individuals in the even population, and between

t1.1 Table 1

t1.2 Numbers of sires, dams, and sexed progeny per generation in two domesticated populations of Coho salmon

t1.3	Year class	Year	Sire	Dam	n
t1.4	Even	1992	22	50	851
t1.5		1994	33	93	951
t1.6		1996	27	103	1796
t1.7		1998	30	100	4458
t1.8		2000	34	99	3796
t1.9		Total	146	445	11852
t1.10	Odd	1993	36	99	1632
t1.11		1995	32	102	1746
t1.12		1997	33	100	4070
t1.13		1999	31	98	2220
t1.14		2001	43	100	2159
t1.15		Total	132	399	9668

1600 and 2200 in the odd population (Table 1). The effective size (N_e) of the base population was 61 for the even population and 106 for the odd population.

Inbreeding depression (ID) of reproductive traits in females was evaluated in the last generation of each population, in 2000 and 2001. The reproductive traits evaluated included weight (SW), and body length at spawning (SL), gonad weight (GW), gonadosomatic index (GSI=GW/SW ratio), number of green eggs (GE=GW×(egg per 10 g)/10) where 'egg per 10 g' is the number of eggs in 10 g of gonad, and finally, the relative fecundity (RF) calculated as the number of green eggs per female divided by the SW. The effect of inbreeding on the survival of eggs was determined on eggs in the eyed stage. Survival was determined as the fraction of eggs remaining alive after shocking, in relation to the total number of eggs successfully fertilized. The survival post-fertilization was evaluated 2–4 h after fertilized eggs were hydrated, as the number of green eggs minus the total number of dead eggs, counted manually, in relation to the total number of green eggs.

2.3. Data analysis

The effect of selection on the long-term contribution of the parents of the base population was studied based on pedigree records in order to determine which had failed to leave descendants in the last generation. The level of inbreeding was calculated for each generation as a mean of the coefficients of inbreeding of all the animals at harvesting time. Individual coefficients of inbreeding were calculated using the PEDIGREE program (Kinghorn and Kinghorn, 1999). Inbreeding was assumed to be zero in both base populations. The rate of inbreeding in each generation (ΔF) was calculated following Falconer and MacKay (1996) as:

$$\Delta F = (F_t - F_{t-1}) / (1 - F_{t-1})$$

where F_t and F_{t-1} are mean coefficients of inbreeding in generations t and $t-1$, respectively. The rate of inbreeding for each population was calculated as the simple mean of the inbreeding rates per generation.

The effects of inbreeding were evaluated from reproductive data and survival of progeny, separately, in 2000 and 2001. The following linear models were used to estimate the magnitude of inbreeding depression on reproductive traits (Eq. (1)) and on survival of eggs (Eq. (2))

$$y_i = u + b_{sd}SD_i + b_f F_i + e_i \quad (1)$$

$$y_i = u + b_{sd}SD + b_D F_D + b_S F_S + b_o F_o + e_i \quad (2)$$

where y_i is the record of each trait in the female or the progeny i ; SD is the spawning date, defined as the correlative number of days (1, 2, 3, ..., m) between the first day of spawning and the day of the respective observation. F is the coefficient of inbreeding of the female; b_{sd} represents the partial regression coefficient of y on SD ; b_f is the partial regression coefficient of y on F . The date of spawning was included as an attempt to increase the precision of the estimations of ID . F_D , F_S , and F_o represent the coefficients of inbreeding of the dam, sire, and progeny (eggs), respectively.

3. Results

3.1. Reproductive performance and survival of eyed eggs

The females from 2001 showed the highest values for all the reproductive traits analyzed, except for relative fecundity, when compared with data from the year 2000 group (Table 2). Similarly, survival of eyed eggs was greater in 2001 (mean=69%,

t2.1 Table 2
t2.2 Average values for different morphological and reproductive traits in females of two domesticated populations of Coho salmon

t2.3	Year	Trait	Mean	Min	Max	S.D.	n
t2.4	2000	SW (g)	4958	3000	6700	641	317
t2.5		SL (cm)	69.0	61.0	78.0	3	318
t2.6		GW (g)	914	531	1339	149	317
t2.7		GE (number)	5695	2982	8834	955	317
t2.8		GSI (%)	18.5	12	24	2	316
t2.9		RF (no. of eggs \times kg $^{-1}$)	1164.9	620	1989	220	316
t2.10	2001	SW (g)	5550	3085	6927	540	221
t2.11		SL (cm)	70.5	64.0	75.5	2	221
t2.12		GW (g)	1065	500	1474	153	221
t2.13		GE (number)	5977	3390	8672	916	221
t2.14		GSI (%)	19.2	12.4	24.1	2	220
t2.15		RF (no. of eggs \times kg $^{-1}$)	1082	715	1923	176	220

t2.16 SW, spawn weight; SL, spawn length; GW, gonad weight; GE, green eggs; GSI, gonadosomatic index; RF, relative fecundity; n, number of individuals measured.

t3.1 Table 3
 t3.2 Number of family selected (sire's and dam's families) by the animal model, and number parents selected from each family full-sib (dam's families) per generation

t3.3	Year class	Year	Families selected			Parents selected per full-sib family			
t3.4			Sires	Dams	% Sires	% Dams	Mean (DS)	Min	Max
t3.5	Even	1992*	20	39	90.9	78.0	3.2 (4.0)	1	24
t3.6		1994	23	41	69.7	44.1	3.1 (2.5)	1	10
t3.7		1996	17	38	63.0	36.9	3.4 (3.7)	1	10
t3.8		1998	21	40	70.0	40.0	3.2 (2.4)	1	2
t3.9		Mean				73.4	49.7		
t3.10	Odd	1993	31	57	86.1	57.6	2.3 (1.8)	1	8
t3.11		1995	25	51	78.1	50.0	2.6 (1.9)	1	8
t3.12		1997	27	46	81.8	46.6	2.8 (1.9)	1	8
t3.13		1999	20	49	64.5	50.0	3.0 (1.9)	1	8
t3.14		Mean			77.6	50.9			

t3.15 * No BLUP selection this year, within family selection was practiced instead

S.D.=19.1, range 15–98%, n=198) than in 2000 (mean=55%, S.D.=23.7, range 5–92%, n=197).

3.2. Effect of BLUP selection on the genetic contribution and on the level of inbreeding

The BLUP selection and the mating design resulted in an average of 75% of sire's families and 50% of dam's families contributing with progeny per generation in both year classes (Table 3). However, in the even year class, the average number of parents selected

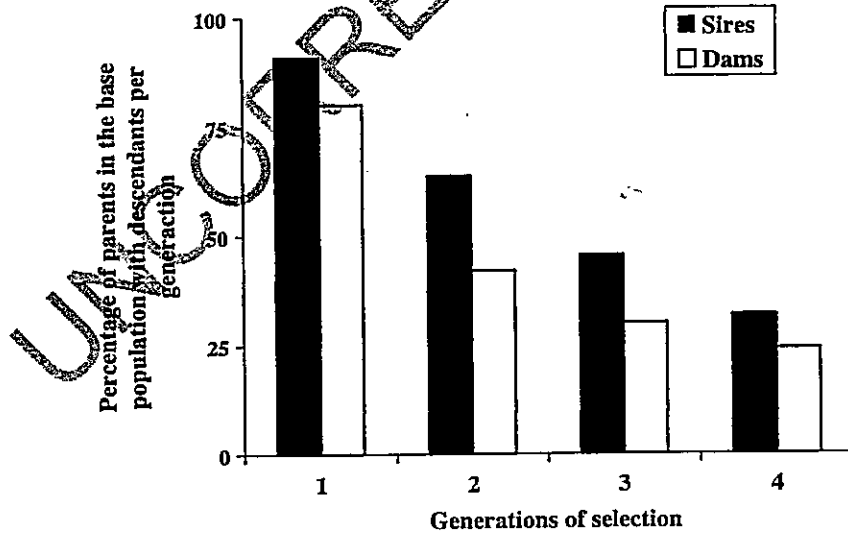


Fig. 1. Genetic contribution of the parents of the base population for the even class year for each generation of selection (n sires=22; n dams=50).

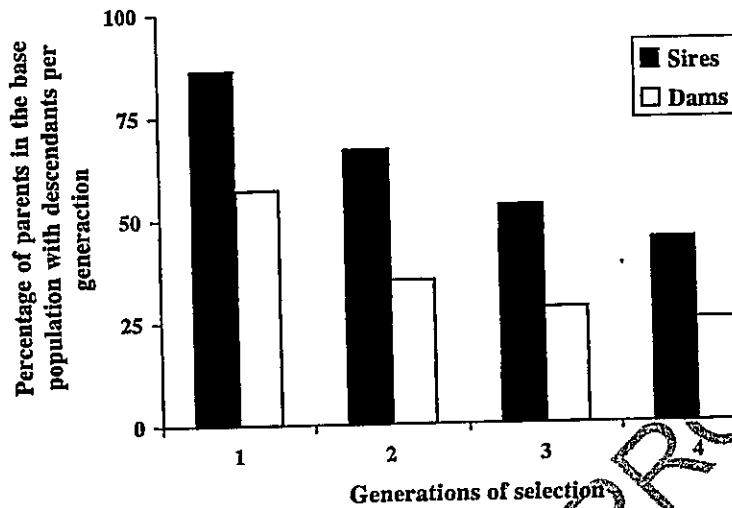


Fig. 2. Genetic contribution of the parents of the base population for the odd class year for each generation of selection (n sires=33; n dams=99).

by full-sib family was greater and more variable than in the odd year class. Consequently, the long-term genetic contribution of the parents of the base population was different between populations and between sexes (Figs. 1 and 2). In the even population, only 31% of the sires and 24% of the dams contributed their genes after four generations of selection. However, in the odd population, although the contribution of the sires was greater than in the even population (44%), the genetic contribution of the dams was similar (25%). Therefore, in both populations, the genetic contribution of the founder females dropped faster than founder males.

The even population showed twofold the rate of inbreeding ($\Delta F=2.45$) of the odd population ($\Delta F=1.10$), result that may be a direct consequence of having a smaller number

t4.1 Table 4
t4.2 Average level of inbreeding and rate of inbreeding (ΔF) for each generation in two populations of domesticated Coho salmon

t4.3	Year class	Year	Mean	S.D.	Min	Max	ΔF
t4.4	Even	1992	0	0	0	0	
t4.5		1994	1.1	4.2	0	25	1.1
t4.6		1996	5.4	3.9	0	18.8	4.3
t4.7		1998	6.9	2.8	2.3	15.6	1.6
t4.8		2000	9.5	2.8	5.1	18.8	2.8
t4.9						Mean	2.5
t4.10	Odd	1993	0	0	0	0	
t4.11		1995	0.2	1.6	0	12.5	0.2
t4.12		1997	1.8	2.5	0	9.4	1.6
t4.13		1999	3.2	2.7	0	10.9	1.4
t4.14		2001	4.3	2.6	0.6	11.7	1.2
t4.15						Mean	1.1

t5.1 Table 5

t5.2 Inbreeding depression (ID) for different reproductive traits in two populations of domesticated Coho salmon

t5.3	Year	Trait	b	S.E.	Mean	ID (%)	P
t5.4	2000	SW (g)	-8.28	13.54	4959	-1.67	0.541
t5.5		SL (cm)	-0.03	0.05	69.2	-0.39	0.615
t5.6		GW (g)	-6.11	3.13	914	-6.68	0.052
t5.7		GSI (%)	-0.10	0.05	18.5	-5.32	0.039
t5.8		GE (number)	-7.87	20.42	5695	-1.38	0.700
t5.9	2001	RF (no. of eggs×kg ⁻¹)	0.74	4.70	1165	0.63	0.876
t5.10		SW (g)	2.02	13.57	5550	0.36	0.880
t5.11		SL (cm)	-0.11	0.05	70.5	-1.61	0.019
t5.12		GW (g)	7.21	3.69	1065	6.77	0.058
t5.13		GSI (%)	0.13	0.05	19.2	6.77	0.013
t5.14		GE (number)	41.81	22.54	5977	6.99	0.067
t5.15	RF (no. of eggs×kg ⁻¹)	-9.16	4.39	1082	-8.47	0.330	

t5.16 ID, inbreeding depression measured in percentage of mean per 10% of inbreeding; SW, spawn weight; SL, spawn
t5.17 length; GW, gonad weight; GE, green eggs; GSI, gonadosomatic index; RF, relative fecundity.

P=level of significance.

of founder individuals ($N_e=61$ and 106 , respectively). Consequently the average level of inbreeding was greater in the even population than in the odd population in equivalent generations (Table 4). For example, in the last generation the average inbreeding for the even population was 9.5% (S.D.= 2.7 , range $5-19\%$) while in the odd population it was only 4.3% (S.D.= 2.6 , range $1-12\%$).

3.3. Inbreeding depression

Significant inbreeding depression (ID) was only observed in a few reproductive traits in the two populations studied (Table 5). In 2000, almost all evaluated traits showed reduction in their values; however, only GSI was significantly associated with inbreeding (-5.3% per each 10% increase of inbreeding). In 2001 the length at spawning was significantly reduced by inbreeding, although by a low value (-1.61% per each 10% increase in inbreeding), while the reverse was observed with GSI, which increased significantly with inbreeding ($+6.77\%$). As shown in Table 6, in neither year class a

t6.1 Table 6

t6.2 Effect of inbreeding of the sire (F_S), dam (F_D), and progeny (F_O) on survival of the progeny at the eyed egg stage

t6.3	F	Year 2000			Year 2001		
t6.4		Mean (S.D.)	ID (%)	P	Mean (S.D.)	ID (%)	P
t6.5	F_D	6.0 (2.6)	-6.3	0.60	3.1 (2.7)	-4.7	0.52
t6.6	F_S	6.9 (2.5)	-12.3	0.32	3.5 (2.9)	4.6	0.51
t6.7	F_O	9.7 (2.7)	-3.3	0.78	4.2 (2.6)	10.9	0.16

t6.8 ID, inbreeding depression measured in percentage of mean per 10% of inbreeding.

t6.9 P=level of significance.

significant ID in egg survival associated with any of the components of inbreeding (of dam, sire, or progeny) was demonstrated. 193
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4. Discussion 195

BLUP selection (animal model) resulted in a high proportion of the parents of the base population not contributing with descendents in the fourth generation. These results were expected according to simulations of BLUP selection in populations with population structures similar to the ones presently studied (Bijma and Woolliams, 2000). Rearing families that do not contribute to the following generations may appear to represent an important loss of resources for the first generations of selection, as suggested by Bijma and Woolliams (2000). However, a selection program must consider the occurrence of this loss, estimate costs (inbreeding) and benefits (genetic gain), and make decisions according to the initial size of the selection program. Particularly, in this study the genetic contribution of the founder females dropped faster than the founder males. This phenomenon has not been reported before and probably occurred as a direct consequence of how the selection was practiced. Commonly, in each generation, 50% of dam's families were represented in the best 25% of individuals selected by the animal model. However, due to the fact that the mating design was nearly one male by three females, a highest percentage of sires of the previous generation (75%) could contribute descendents to the next generation. 196
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In the present study, the calculated rate of inbreeding (1.1% and 2.4%) was similar to other salmon populations subject to artificial selection (Su et al., 1996; Rye and Mao, 1998; Myers et al., 2001; Pante et al., 2001a). All of these studies registered the pedigree accurately, which allowed controlling the increment of inbreeding, for example limiting the possibility of mating closely related individuals. Additionally, several other methods may be applied which may restrain increases in levels of inbreeding in selection programs (Grundy et al., 1994; Caballero et al., 1996; Sanchez et al., 1999; Weigel and Lin, 2000; Sonesson and Meuwissen, 2000). Few of these, however, have been evaluated to be used in practical conditions. Recently, Gallardo et al. (in press) evaluated the use of nonrandom mating schemes for the reduction of the rate of inbreeding on the Coho salmon populations analyzed in this study. Gallardo et al. (in press) showed that the mating scheme that minimized the average coancestry of the group selected was more efficient in reducing the increase in inbreeding, thus limiting the expression of inbreeding depression, when compared with the other mating schemes applied to the Coho salmon program (random mating of selected individuals and compensatory mating schemes). This minimum coancestry mating scheme also reduced the variance of inbreeding, producing more homogeneous families in relation to this parameter. 212
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In this study, variations in the magnitude of the inbreeding depression were found between populations and traits. Similarly, previous authors working with fish have described variations in the magnitude of ID among traits within the same population (Su et al., 1996; Heath et al., 2002). Su et al. (1996) showed that the weight at spawning and the number of eggs were significantly reduced by inbreeding (4–6% per 10% increase in inbreeding), while egg size, age at spawning, and juvenile weight were not affected. 229
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Heath et al. (2002) recently found significant ID in relative fecundity in females of *O. tshawytscha* using a measure correlated with inbreeding as related to multi-locus heterozygosity. However, this was not true for the GSI in males or for egg size. Differences between populations can be explained by differences in the level of inbreeding and in the level of genetic load, while differences among traits within the same population may be attributed to variations in the amount of dominance variance present (Lynch and Walsh, 1998; De Rose and Roff, 1999), and to differences in genetic load too. An explanation of the lower (or lack of) inbreeding depression in the odd population, compared to the even population, may be the lowest level of inbreeding in the odd population. However, we cannot explain differences between traits because we do not have estimates of dominance variance present nor of differences in the initial genetic load. On the other hand, an increment in the gonadosomatic index in the odd population may be explained by effects of genetic drift and by the effect of artificial selection based on the weight at harvest applied to this population, given that there exists a moderate genetic correlation ($r=0.46$) between weight at harvest and gonadal weight (Gall and Neira, in press).

Some authors have shown that traits in the progeny may be affected by the inbreeding of their parents (Su et al., 1996; Margulis, 1998; Keller, 1998). Su et al. (1996) showed that survival of eggs from fertilization to hatching in rainbow trout (*Oncorhynchus mykiss*) was significantly reduced by inbreeding of the dam, and slightly reduced by inbreeding of the progeny, but not affected by the inbreeding of the sire. We did not find such effects, probably due to the low level of inbreeding of parents (3–6%) in this study. Similarly, Heath et al. (2002) did not find any association between egg survival from eyed egg to hatching, and the genetic variation measured as heterozygosity in microsatellites.

5. Conclusions

In agreement with theoretical predictions, BLUP selection (animal model) resulted in a high proportion of the parents of the base population (56–76%) not contributing descendants in the fourth generation. However, the levels of inbreeding in these populations were similar to those previously described in other fish populations, using other methods of genetic evaluation (individual or family selection). The prejudicial effects of the inbreeding, although significant with regard to some reproductive traits such as the GSI or body length at spawning, may not limit the viability of a genetic improvement program in the medium term. However, methods that limit inbreeding should be employed as a precautionary measure.

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UNCORRECTED PROOF

ANEXO 2

1 ENVIRONMENTAL DEPENDENCE OF INBREEDING DEPRESSION IN
2 CULTURED COHO SALMON (*Oncorhynchus kisutch*): AGGRESSIVENESS,
3 DOMINANCE AND INTRASPECIFIC COMPETITION.

4

5 **Short title:** Inbreeding and social environment in Coho salmon

6

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1 **Abstract**

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3 We evaluated the effects of inbreeding on traits related to territorial dominance
4 and search if the magnitude of the inbreeding depression was modified according to
5 the social environment in Coho salmon. Evaluations of behaviour in paired contest
6 between juvenile salmon with different inbreeding (Low, LI = 9.5%; medium, MI, =
7 29.6%), did not show significant differences between their capacities for establishing
8 territorial dominance (mean aggressiveness score, LI = 20.0 ± 22 ; MI = 16.7 ± 23 ;
9 Feeding attempt, LI = 18.3 ± 12 ; MI = 21.1 ± 12). However, fish with low inbreeding
10 showed almost twice the aggressive pursuit of fish of medium inbreeding and had a
11 higher specific growth rate (SGR) in culture ($SGR_{MI} = 1.83 \pm 0.58$; $SGR_{LI} = 2.20 \pm 0.67$).
12 Additionally, we found evidences that the magnitude of inbreeding depression was
13 modified according to social environment: 1. *Masking*: In small groups of fishes (N=20),
14 large dominant fishes of medium inbreeding, cultivated with small subordinate fishes of
15 low inbreeding, showed the same SGR as dominant fishes of low inbreeding cultivated
16 with small subordinate fishes of medium inbreeding. 2. *Magnifying*: A significant
17 inbreeding depression on juvenile survival was detected only in high density
18 competitive environments. Thus the number of lethal equivalents was 2.70 at high
19 density, and only 0.24 in a low density environment. Our results show that differences
20 in size associated with territorial dominance may mask deleterious effects of
21 inbreeding under certain conditions, and support the concept that intraspecific
22 competition usually magnifies the deleterious effects of inbreeding.

23

24 **Keywords:** salmon culture, dominance, inbreeding depression, behavioural traits,
25 Chile.

1 **Introduction**

2

3 The deleterious effect of inbreeding on fitness has been broadly researched in
4 genetics and evolutionary biology (Lynch and Walsh, 1998). This phenomenon, known
5 as inbreeding depression is usually defined as the declination in the average
6 phenotype of a given trait due to inbreeding (Falconer and Mackay, 1996). There is a
7 general consensus that traits linked to fitness undergo greater inbreeding depression
8 than traits not directly linked with adaptation, such as morphological traits (Falconer
9 and Mackay, 1996; DeRose and Roff, 1999). Theoretical considerations argue that this
10 is produced because the traits linked to fitness have greater variance in directional
11 dominance than the morphological traits (Lynch and Walsh, 1998).

12 In juvenile salmon, behavioural traits such as aggressiveness and foraging
13 behaviour have been related to territorial dominance and to the specific growth rate
14 (Damsgård, *et al*, 1997; Nicieza and Metcalfe, 1999; Lahti, *et al*, 2001). Both
15 characteristics have been tied to fitness (Quinn and Peterson, 1996) and should
16 therefore be strongly influenced by inbreeding. Under natural conditions juvenile
17 salmon are territorial, and defend small portions of the rivers in which they feed by
18 aggressive acts (Holtby *et al*, 1993; Nicieza and Metcalfe, 1999; Healy and Lonzarich,
19 2000). These aggressive behaviours produce hierarchies of dominance in which the
20 dominant individuals usually monopolize the best feeding areas (Valdimarsson y
21 Metcalfe, 2001). This allows them to maximize their benefits in terms of net energy
22 gain (Faush, 1984), although under some conditions of unpredictability of the food
23 supply or of environmental disturbance, this situation may be reversed (Sloman *et al*,
24 2001; Vøllestad and Quinn, 2003), revealing a trade-off between aggressiveness and
25 fitness. In any case the dominance hierarchy generates a high degree of stress in
26 subordinate fishes (Ejike and Schreck, 1980; Sloman *et al*, 2000, 2001) producing,
27 among other physiological consequences, a decrease in disease resistance as well as
28 depressed growth (Sloman *et al*, 2000). In culture conditions it has been suggested
29 that the high densities employed should prevent the establishment of territories and
30 that intraspecific competition should be low, given that food is always available.

1 Several authors have, however, demonstrated that some dominant fishes may defend
2 portions of culture tanks, create areas of low density, monopolize the food, and grow
3 better than subordinate individuals (Thorpe *et al*, 1990; Ryer and Olla, 1996). Also,
4 high culture densities generally increase the frequency of encounters between fishes,
5 thus increasing aggressive interactions. These usually produce severe body damage,
6 principally on the fins and to a lesser degree on the eyes and operculum (Abbott and
7 Dill, 1985; Turnbull *et al*, 1998). The prevalence of damage on fins may be so high in
8 cultured populations that it has been used to differentiate cultured fishes from wild
9 fishes (Craik *et al*, 1987).

10 There are several studies which describe the deleterious effects of inbreeding in
11 fishes (Su *et al*, 1996; Gallardo *et al*, 2004a), few have determined whether or not this
12 is a consequence of lower competitive ability of inbred individuals. Tiira *et al*, (2003)
13 recently demonstrated that aggressiveness in *Salmo salar* was positively correlated
14 with genetic diversity, which is an indirect measure of inbreeding (Hedrick and
15 Kalinowski, 2000). Researchers of other taxa also suggest that consanguineous
16 individuals have lower competitive abilities, for example, in obtaining and defending
17 territory (Maegher *et al*, 2000). Also, it has been demonstrated that the level of
18 extreme inbreeding may limit recognition among individuals (ie. those of similar genetic
19 constitution) and alter behavioural patterns in the presence of potential competitors
20 (Nevison *et al*, 2000).

21 From another perspective, prediction of effects of inbreeding in a population has
22 become a highly complex undertaking, since the magnitude of inbreeding depression
23 usually varies according to the conditions of the biotic and abiotic environment in which
24 it is evaluated (Hauser and Loeschke, 1996; Bijlsma *et al*, 1999; Dahlgard &
25 Loeschke 1997, Dahlgard and Hoffmann, 2000). There is a tendency, however, for
26 the magnitude of inbreeding depression to increase under competitive conditions or
27 environmental stress (Latter and Mulvey, 1995; Crnokrak and Roff, 1999; Hedrick and
28 Kalinowski, 2000; Haag *et al*, 2002). The genetic basis of this phenomenon seems to
29 be the existence of conditionally deleterious alleles (Crow, 1997; Bilisma *et al*, 1999),
30 the effects of which are revealed only under restrictive and not under permissive

1 conditions.

2 In this study we evaluated the effects of inbreeding on behavioural traits related
3 to territorial dominance in the Coho salmon (*Oncorhynchus kisutch*). We specifically
4 investigated the consequences of inbreeding on aggressiveness and feeding activity.
5 Both these traits have been strongly linked to territorial dominance and to fitness, for
6 which we expected to encounter a high degree of inbreeding depression on these
7 traits. This could be expressed, for example in that more inbred individuals might tend
8 to be more subordinate than fishes which were not inbred. We also studied the
9 effects of the social environment (territorial dominance and intraspecific competition)
10 on the magnitude of the inbreeding depression on body weight, both under laboratory
11 and culture conditions.

12 The study was carried out with cultured fishes from the Center for Genetic
13 Improvement of the Coho Salmon: IFOP-Coyhaique (Gallardo *et al.*, 2004a). This
14 population has remained in culture conditions for five generations (10 years), with the
15 last generation (year-class 2000) having a mean inbreeding of $9.4 \pm 2.7\%$. Significant
16 inbreeding depression has been estimated for some traits related to fitness, such as
17 the gonadosomatic index (GSI, -5.3% per each 10% increase of inbreeding).
18 Additionally, we have some evidence that the effect of inbreeding depression on body
19 weight varies according to the environment (*environmental dependence*). Our results
20 showed that the magnitude of inbreeding depression on body weight increased from
21 the alevins (5-10 g) to the smolt size (150 g) during a period in which fishes from
22 families having different levels of inbreeding were cultivated together in floating cages
23 in the sea. The overall results of our analyses for the 2000 year-class showed
24 decreases of 1.1% in weight for each 10% increase in inbreeding at the alevin stage
25 (before families were mixed); body weight in the smolt stage three months later, and
26 after the families were mixed, showed a 3.8% decrease for each 10% increase in
27 inbreeding.

28

29 **Materials and Methods**

30

1 *Inbreed families*

2

3 Coho salmon broodstock from the 2002 year-class maintained at the Center for
4 Genetic Improvement, IFOP-Coyhaique, were bred to produce six full-sib families, with
5 different levels of inbreeding. One group of three families had a low level of inbreeding
6 ($F = 9.5 \pm 0.3\%$), while a second group of three families had an intermediate level of
7 inbreeding ($F = 29.6 \pm 0.9\%$). The six families were cultivated in separate tanks, with
8 about 1000 fishes per tank for seven months, until the fishes had a mean weight of
9 seven grams. Subsequently, 300 fishes from each family were randomly placed in
10 systems for observation as follows.

11

12 *Behavior in paired contest and analysis of inbreeding depression*

13

14 Behavior related to territorial dominance was evaluated with 21 pairs of fishes,
15 testing medium inbreeding (MI) v/s low inbreeding (LI) as indicated below. Fishes
16 obtained from four families of the previously mentioned groups were paired by size
17 (body weight difference for medium-low inbreeding (MI-LI) fishes = 0.013 g and fork
18 length difference = -0.2 mm) and cultivated in independent 30 x 30 x 30 cm aquaria for
19 17.5 days (SD=3.8). The fishes were fed twice a day with artificial food at 6% of the
20 initial body weight. At the end of the culture period, body length and weight were
21 determined to obtain the specific growth rate $SGR = 100 (\ln W_2 - \ln W_1) \cdot t^{-1}$, where W_1
22 and W_2 represented the final and initial weights (g) over the culture period t (days).
23 Each fish in the aquarium was labeled with a subcutaneous unique dye mark which
24 allowed its identification (Kelly, 1967). Other studies have shown that this marking
25 technique had no adverse effects on the fishes, nor on their competitive behavior
26 (Sloman *et al.*, 2001), which we verified in small-scale pilot testing. A 10 cm length of
27 PVC tubing, 6 cm diameter, was placed in each test aquarium to provide a refuge to
28 protect subordinate individuals from chronic stress, and to favor establishment of
29 territories.

30 The behaviour of the fishes was recorded by direct observation, and using video

1 recording beginning at one day after placing the fishes in the aquariums. During this
2 period the fishes were isolated from one another by a solid barrier which prevented
3 visual contact between them, and the barrier was taken away just prior to beginning
4 the first behavioural observation in each aquarium. Various types of aggressive and
5 feeding behaviours have previously been described in salmonids (Metcalf *et al.*, 1989;
6 Sloman *et al.*, 2000, 2001; Griffiths and Armstrong, 2002). Table 1 lists the different
7 aggressive behaviours presently evaluated, with the point weight assigned to each of
8 these (1-4) following dominance behaviours: Lateral display < Charge < Chase <
9 Biting. An aggressiveness score was calculated for each fish, assigning point values
10 for each behaviour displayed by the aggressor. A totally subordinate fish was scored
11 as zero. In this way, if one fish attacked another with a bite, and received a charge in
12 return, the first fish scored four points while the second only two points. On the other
13 hand, if the two fishes demonstrated the same behavior at the same time, each fish
14 received the same score. The amount of food consumed was employed as an
15 additional measure of dominance, observing the numbers of pellets consumed by each
16 fish during each observational period. For this, feeding times were made to coincide
17 with observational periods, usually once in the morning and once in the afternoon. The
18 feeding behaviour was termed "attempt to feed" since video recordings and direct
19 observations failed to provide an exacting record of how many pellets were ingested
20 daily. The dominance behaviour was observed 1-2 times a day for two days, for 25-30
21 minute periods each. All observations were made from behind a darkened screen
22 located at one meter distance from the aquariums so as not to interfere with the
23 behaviour of the fishes.

24 An analysis of principal components (APC) was carried out to determine which
25 fish of each pair was dominant. This was done using the total aggressiveness score
26 and on the mean value of the score obtained on "attempt to feed". The fish with the
27 highest score within each pair was termed "dominant" as long as the difference in
28 dominance score between the fishes was greater than one.

29 An analysis of variance (ANOVA) was used to detect the effect of inbreeding on
30 aggressiveness and feeding using the GLM procedure (SAS, Statistical Analysis

1 Systems Institute Inc. 1996). The analysis was carried out using a mixed linear model
2 which included inbreeding as a fixed effect, and the family within the inbreeding as a
3 random effect. Since the aggressiveness score deviated slightly from normality, the
4 analysis was carried out by applying a square root transformation to the variable. Also,
5 an analysis of covariance (ANCOVA) was used to estimate the effect of dominance
6 and inbreeding on the SGR. As above, a mixed linear model was used which included
7 the dominance score as the covariable.

8

9 *Social environment and inbreeding depression*

10

11 *Territorial dominance*

12

13 Apart from previously described behavior, body size has a strong effect on
14 whether one fish will be dominant over another in competitive interactions between
15 juvenile salmonids (Huntingford, *et al*, 1990; Holtby *et al*, 1993). Generally the larger
16 fishes tend to be dominant over the smaller ones. The effect of social environment
17 (territorial dominance) on inbreeding depression was determined by modifying the
18 body size relation among inbreed and non-inbreed fishes. Two modal size groups of
19 10 fishes each were established by body size from the four Coho families described
20 above, with one group consisting of large, dominant individuals, and the other of small,
21 subordinate individuals (Table 2). The modal group of large fishes having low level of
22 inbreeding was cultured with the modal group of small fishes having medium level of
23 inbreeding. A parallel experiment was carried out with this arrangement reversed.
24 Weights and lengths of all fishes were obtained after 14 days in culture, and the
25 condition factor, SGR for given body size, and magnitudes of the inbreeding
26 depression were determined. The differences between members of the same modal
27 group but of different levels of inbreeding were analyzed for each pair of families using
28 a *t* test. In this way we were able to compare the performance of the more inbreed
29 fishes under two different environmental conditions presented by dominance and
30 subordination.

1

2 *Competitive conditions in sea cages*

3

4 In order to evaluate the magnitude of inbreeding depression under culture
5 conditions, a group of 60 fishes randomly drawn from each family were individually
6 marked using PIT tags, and then individually measured for weight and length. These
7 fishes were intermixed in January 2002 and placed in Ensenada Baja Bay (XI Region,
8 Chile) in floating cages measuring 5 x 5 x 4 m (100 m³) to give N= 180 fishes per cage
9 and 30 fishes per family. Each cage contained an additional different number of fishes
10 which allowed evaluation of the effect of density on the magnitude of inbreeding
11 depression. The initial number of fishes in cages 1 and 2 were 2020 and 3738
12 individuals, respectively, producing an initial density of 20 fishes per m³ in cage 1 and
13 38 fishes per m³ in cage 2. Both groups were fed with pellets using standard culture
14 procedure. After 78 days in culture the body weights, SGR, and survival were
15 measured for all the fishes in each group and environment.

16 An analysis of variance was carried out (GLM procedure, above) to determine
17 the effect of inbreeding, environment, and interactions on body weight and SGR. For
18 this we used a mixed linear model which included the inbreeding and the density as
19 fixed effects, and family within inbreeding as the random effect. The effect of
20 inbreeding on survival was evaluated using a *t* test following arcsine transformation of
21 the data. To allow comparisons with other studies, the genetic load was also
22 determined, by means of calculating the number of lethal equivalents (Morton *et al.*,
23 1956). When there are two groups having different levels of inbreeding, Lynch and
24 Walsh (1998) recommend using the following equation for estimation of the genetic
25 load:

26

$$B = -\ln (S_F/S_O) / \delta_F$$

27

28 where S_O and S_F are the probabilities of survival of the groups with low and medium
29 inbreeding, respectively, and δ_F is the difference in inbreeding between the groups; B

1 gives an estimation of the reduction in biological adaptation due to inbreeding, and
2 gives the number of lethal equivalents per gamete.

3

4 RESULTS

5

6 *Analysis of inbreeding depression in behavior traits in paired contest*

7

8 A total of 319 aggressive acts (TA) and 2852 "feeding attempts" (FA) were
9 recorded for 21 pairs of fishes, during 2190 min. of observation (mean = 104 min per
10 couple; SD = 18 min). The total number of aggressive acts and feeding attempts were
11 highly variable at the individual level for the two levels of inbreeding evaluated (Table
12 3), however, the individual aggression score, was positively correlated with the
13 average level of feeding attempts per fish ($r = 0.55$ Pearsons linear correlation
14 coefficient, $P < 0.001$; Fig 1).

15 Inbreeding did not significantly affected the level of aggressiveness ($F_{1,38} = 0.19$;
16 $P = 0.62$), nor did it affect the average number of feeding attempts ($F_{1,38} = 0.43$; $P =$
17 0.51 , Table 3). There was however, a tendency for fishes having a low level of
18 inbreeding to demonstrate almost double the aggressive chases against fishes of
19 medium inbreeding, when compared with those of fishes of medium inbreeding against
20 those of low inbreeding (Fig. 2). The analysis APC on the two behavioural variables
21 allowed obtaining a territorial dominance score in the first principal component that
22 explained 77% of the observed variance. Based on this score the territorial dominance
23 could be clearly established in 14 pairs of fishes of which one was dominant, while in
24 the remaining 7 pairs, no dominance relation could be shown (ie. difference in score of
25 less than one point). The analysis of the dominance score allowed the deduction that
26 in eight cases the more Inbreed fish was dominant, while in only six cases the fish of
27 low inbreeding was dominant. By this method, there was no evidence that that
28 inbreeding modified the ability to establish short-term (1-2 day) territorial dominance in
29 fishes paired by size. In contrast, the dominance score, and the inbreeding significantly
30 affected the specific growth rate (Table 4), with the fishes of medium inbreeding having

1 a lower SGR than those of low inbreeding (SGR mean of MI group = 1.83, SD = 0.58;
2 SGR mean of LI group = 2.20, SD = 0.67).

3

4 *Analysis of social environment on inbreeding depression*

5

6 *Territorial dominance*

7

8 Table 5 shows the effects of territorial dominance on inbreeding depression in
9 two independent experiment (families 196 vs 208 and 204 vs 198; MI vs LI,
10 respectively). In both experiment, large dominant fishes of medium inbreeding,
11 cultivated with small subordinate fishes of low inbreeding, showed the same BW, FL,
12 CF and SGR as dominant fishes of low inbreeding cultivated with small subordinate
13 fishes of medium inbreeding. This suggested that the social dominance status
14 (dominant) masked the deleterious effects of the inbreeding. This effect was not
15 consistently showed in small fish as in only one of our experiment both small fish (LI
16 and MI) showed the same morphological parameters (Families 196-208). In the other
17 experiment (families 204-198), small subordinate fishes of low inbreeding had a
18 greater CF (t test = -1.96, P = 0.03) and a greater SGR than subordinate fishes of
19 medium inbreeding (t test = -2.58, P = 0.01), revealing an inbreeding depression of low
20 magnitude ($\delta_{FC} = 0.07$; $\delta_{SGR} = 0.14$). Surprisingly, the mean SGR for the subordinate
21 of low inbreeding was also greater than the SGR of the large, dominant fishes of
22 medium inbreeding (t test = 2.50, P = 0.01) with those sharing the culture aquarium,
23 and greater than their large, dominant, complete siblings of low inbreeding (t test 1.90,
24 P = 0.03). Thus, the dominance status (subordination) did not consistently modify the
25 deleterious effect of inbreeding on the SGR.

26 Inbreeding significantly reduced the body weights of juvenile salmon (alevin stage),
27 although at a low magnitude, when fishes of low and medium inbreeding were
28 cultivated in separate aquariums (Table 6). This same group of fishes demonstrated
29 significant inbreeding depression of greater magnitude when the fishes were cultured

1 under competitive conditions between families from the alevin to the smolt stages
2 (Table 7). In this experiment there was a tendency for increase in the magnitude of
3 inbreeding depression on body weight and SGR with increase in the culture density.
4 No significant interaction between inbreeding and density was detected on these traits,
5 however (Table 8). To the contrary, survival from alevin to smolt was significantly
6 reduced by inbreeding (t test = 3.76, $P < 0.01$), but only in the high density group,
7 suggesting a strong interaction between inbreeding and density on viability (Fig. 3).
8 Thus the number of lethal equivalents expressed in the higher density culture was
9 2.70, while in the lower density culture it was only 0.24.

10

11 DISCUSSION

12

13 *Inbreeding and behaviour*

14

15 It was shown in the present study that inbreeding significantly reduced the
16 growth of juvenile fishes under different environmental conditions. Other studies on
17 fishes have also concluded that inbreeding significantly reduces this and other traits
18 related to fitness under different culture conditions too (Su *et al.*, 1996; Gallardo *et al.*,
19 2004a).

20 Through the study of behavioural parameters we evaluated whether or not the
21 deleterious effect of inbreeding was the consequence of lower competitive ability in
22 more inbred fishes. Contrary to our hypothesis, we did not find significant differences
23 in the two components that determined competitive ability (aggressivity, feeding
24 behaviour) between fishes with two different levels of inbreeding in paired contest. At
25 least two previous results indicated that aggressiveness or territorial dominance should
26 have been affected by the inbreeding. First, both behavioral characteristics have been
27 strongly linked to fitness in juvenile salmonids, for which they should have expressed a
28 high level of inbreeding depression (Falconer and Mackay, 1996). Second, previous
29 data on salmonids and other vertebrates have shown comparatively lower levels of
30 aggressiveness (Tiira *et al.*, 2003) and lower degrees of competitive ability in the

1 defense of territory (Meagher *et al*, 2000) in individuals having higher relative degrees
2 of inbreeding. Our study contrasted with the results of Tiira *et al*, (2003) who recently
3 showed that the fry of *Salmo salar* having low genetic diversity were less aggressive
4 than fry having a high degree of genetic diversity. However, since the behavioral
5 evaluations of Tiira *et al*, (2003) were carried out between individuals having the same
6 degree of genetic diversity, their study did not permit dismissal of the possibility that
7 genetically similar fishes might be less aggressive among themselves. Although our
8 results may not agree with Tiira *et al*, (2003) there is a clear and common tendency
9 that less consanguinous individuals express a greater number of highly aggressive
10 behaviours such as chases and biting which may be determinant in the establishment
11 of territories over the long term (Cutt *et al*, 2002). The latter authors showed in *Salmo*
12 *salar* that in a new environment, the dominant fishes are the most aggressive, but
13 consume less food with the objective of maintaining a territory over a long term. This
14 tendency was observed in our results on aggression and feeding, and may have
15 skewed our dominance index. However, since body size is the determining factor in
16 dominance relations among fishes, the making of a behavioural evaluation in the
17 middle or at the end of an experiment may not have shown if the behaviour was the
18 cause or the consequence of the greater SGR. In summary, our data do not permit us
19 to conclude that the lower SGR of the more consanguinous individuals was the direct
20 consequence of lower initial competitive ability in terms of the behavioral parameters
21 evaluated in this study. Thus other factors (i.e. the assimilation efficiency) perhaps
22 determined that the consanguinous individuals expressed a lower SGR than less
23 inbred fishes and remains a hypothesis to be evaluated in future research. In any
24 case, as size differences increase, a synergic effect associated with territorial
25 dominance should magnify the deleterious effects of inbreeding in competitive
26 environments.

27

28 *Environmental dependence of inbreeding depression*

29

30 In this study we have shown that a change in social dominance relations may

1 reverse the deleterious effect of inbreeding in small groups of fishes (N=20). In order to
2 do this, we modified the size relations between groups of fishes with different levels of
3 inbreeding, forcing the inbred fishes to be dominant over the less inbred fishes.
4 Under these circumstances the evidence of social dependence of inbreeding
5 depression was very consistent in relation to the dominant fishes. In the two
6 experiments carried out, the dominant fishes expressed the same specific growth rate,
7 independently of the level of inbreeding. The analysis is more complex, however, when
8 evaluating the effect of inbreeding on subordinate individuals since in one experiment
9 both groups of subordinate fishes (MI and LI) expressed the same SGR, but did not in
10 another experiment. This occurred in spite of the fact that the subordinate group (LI) of
11 family 198 not only expressed a greater SGR than the subordinate fishes MI, but also
12 a higher SGR than both MI and LI dominant groups. These data are consistent with
13 differences in the expression of inbreeding depression at the family level which has
14 been described for other taxa (Holtsford, 1996), but also may suggest a compensatory
15 effect as shown in the growth of the small fishes.

16 We found at least two areas of evidence of environmental dependence of
17 inbreeding depression under culture conditions. *First*, the magnitude of the ID over the
18 body weight and the SGR increase from the alevin to the smolt stage when the fishes
19 are mixed in common cages. This coincides with previous observations carried out on
20 this same population (unpublished data), as well as on published data on rainbow trout
21 (Su *et al*, 1966). They showed that the magnitude of the inbreeding depression (ID) for
22 body weight increased with age: ID = +1.73%. - 0.85% and -2.26% for each 10%
23 increment in inbreeding at 168, 280, and 364 days, respectively. *Second*; the greatest
24 genetic load was expressed under high-density conditions in a similar manner to that
25 observed for other taxa (Latter and Mulley, 1995; Haag *et al*, 2002). Both results
26 together have important implications for aquaculture, especially when present
27 programs for genetic improvement of fishes are taken into consideration. For instance,
28 Chilean salmon culture is based on a high degree of artificial selection (BLUP, best
29 linear unbiased prediction) which has had a tendency to increase the rate of
30 inbreeding. Although various methods have been devised to reduce this tendency

1 (Villanueva *et al*, 1996; Gallardo *et al*, 2004b), it may be that a change in rearing
2 conditions (*ie.* lower densities) could also limit the expression of inbreeding
3 depression. At present there is a consensus that lowering culture densities could be
4 beneficial for the fishes, since stress becomes reduced, although this may produce an
5 increase in genetic loading for subsequent generations (Crow,1997).

6

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8

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32

LEGENDS

Table 1 Scoring system used to rate aggressiveness interaction in paired Coho salmon juveniles, one with low inbreeding and the other with a medium level of inbreeding. When the two fish showed the same behavior, each received the same score, and it was assumed that the higher the score, the higher was the degree of dominance.

Behaviour		Score in each observation
Agonistic interactions	Biting another fish	4
	Chasing another fish (with or without biting)	3
	Charge another fish	2
	Lateral display	1
	Receiving or avoiding an aggressive behaviour of another fish.	0

Table 2 Body weight (g), fork length (mm), and condition factor for upper and lower modal group juvenile Coho salmon with low (F mean =10%) and medium (F mean = 29.5%) levels of inbreeding per couple of family reared in the same aquarium. Values \pm one standard error.

Trait	Family	F	Modal group		N
			Upper	Lower	
BW (g)	204	Medium	6.66 \pm 0.16	4.78 \pm 0.16	20
	198	Low	6.79 \pm 0.12	4.82 \pm 0.09	20
	196	Medium	6.45 \pm 0.12	4.59 \pm 0.08	18
	208	Low	6.37 \pm 0.06	4.64 \pm 0.07	18
FL (mm)	204	Medium	83.2 \pm 0.5	75.1 \pm 0.8	20
	198	Low	83.7 \pm 0.6	74.4 \pm 0.8	20
	196	Medium	82.10 \pm 0.43	73.10 \pm 0.82	18
	208	Low	83.10 \pm 0.46	74.30 \pm 0.56	18
CF	204	Medium	1.14 \pm 0.02	1.11 \pm 0.03	20
	198	Low	1.14 \pm 0.01	1.15 \pm 0.02	20
	196	Medium	1.15 \pm 0.01	1.16 \pm 0.03	18
	208	Low	1.09 \pm 0.01	1.11 \pm 0.02	18

BW = Body Weight; FL = Fork Length; CF = condition factor = $BW \times FL^{-3}$; F = inbreeding level.

Table 3 Means and standard deviations of feeding traits, aggression score and dominance score (first principal component) by inbreeding level in juvenile Coho salmon.

Level of inbreeding	<i>N</i>	TA	AS (SD)	TE	FE (SD)
LI	21	177	20.0 (22)	1328	18.3 (12)
MI	21	142	16.7 (23)	1524	21.1 (12)

LI, low inbreeding (*F* mean =9.5 %); MI, medium inbreeding (*F* mean =29.6 %); TA, total agonistic act; AS, average aggressiveness score; TE, Total feeding intent; FE, average feeding intent (*N* / 30 min).

Table 4 ANCOVA of the effects of inbreeding, family within inbreeding and dominance score (covariate) on specific growth rate (%/g per day) using type III Sum of squares (d.f. degrees of freedom; MS, mean squares; P, Probability). Data satisfied parametric requirement without transformation.

Source of variation	d.f	MS	P
Inbreeding	1	1.58	0.037
Family within inbreeding	2	0.89	0.084
Dominance score (covariate)	1	1.80	0.027
Residual	37	0.34	

Table 5 Body weight (g), fork length (mm), condition factor and specific growth rate (% body weight day⁻¹) for upper and lower modal group juvenile coho salmon with low (F mean = 9.8) and medium (F mean = 29.3) level of inbreeding. Values \pm one standard error.

Family	Trait	F	Modal group		Trait	Modal group	
			Upper	Lower		Upper	Lower
196 208	BW (g)	Medium	9.24 \pm 0.27	6.53 \pm 0.12	FL (mm)	89.0 \pm 0.70	79.9 \pm 1.05
		Low	9.04 \pm 0.17	6.65 \pm 0.13		89.2 \pm 0.39	80.9 \pm 0.59
		ID	-0.02	0.02		0.00	0.01
		P (t test)	0.28	0.25		0.40	0.21
	CF	Medium	1.29 \pm 0.02	1.27 \pm 0.04	SGR	2.55 \pm 0.11	2.53 \pm 0.10
		Low	1.26 \pm 0.01	1.24 \pm 0.01		2.50 \pm 0.10	2.57 \pm 0.09
		ID	-0.02	-0.02		-0.02	0.02
		P (t test)	0.08	0.23		0.36	0.37
204 198	BW (g)	Medium	9.66 \pm 0.27	6.88 \pm 0.25	FL (mm)	91.11 \pm 0.56	81.89 \pm 0.84
		Low	9.87 \pm 0.17	7.40 \pm 0.12		91.00 \pm 0.47	82.22 \pm 1.09
		ID	0.02	0.07		-0.00	0.00
		P (t test)	0.26	0.06		0.44	0.41
	CF	Medium	1.26 \pm 0.03	1.23 \pm 0.02	SGR	2.65 \pm 0.10	2.60 \pm 0.13
		Low	1.29 \pm 0.01	1.32 \pm 0.02		2.73 \pm 0.11	3.05 \pm 0.12
		ID	0.02	0.07		0.03	0.14
		P (t test)	0.14	0.03		0.30	0.01

BW, Body Weight; FL, Fork Length; CF, condition factor = $BW \times FL^{-3}$; F, inbreeding level; SGR, specific growth rate; ID, inbreeding depression measure as $\delta = 1 - (X_{MI}/X_{LI})$, where X_{MI} y X_{LI} is the average of each trait in the group with medium and low inbreeding respectively. *= $P < 0.05$. P = probability.

Table 6 Inbreeding depression (ID) for body weight (g) and fork length (mm). Each group is a random sample of three families (N=60 fish per family) reared in independently tanks.

Mean Inbreeding (SD)	N	Fork length (SD)	Body weight (SD)
9.5 (0.3)	180	78.1 (4.0)	5.60 (0.95)
29.6 (0.9)	180	75.1 (6.3)	5.09 (1.30)
ID		0.04*	0.09*

ID, inbreeding depression measure as $\delta = 1 - (X_{MI}/X_{LI})$, where X_{MI} y X_{LI} is the average of each trait in the group with medium and low inbreeding respectively. *=P<0.05.

Table 7 Inbreeding depression (ID) for body weight (g) and for specific growth rate (SGR, % day⁻¹) on juvenile coho salmon reared in two initial density conditions. Values \pm one standard error.

Initial density (fish x m ⁻³)	Mean inbreeding (SD)	N	Body weight	SGR
20	9.4 (0.9)	65	92.71 \pm 6.13	3.36 \pm 0.11
	29.6 (0.3)	62	66.25 \pm 6.17	3.01 \pm 0.12
	ID		0.29***	0.10***
38	9.4 (0.9)	62	94.82 \pm 7.20	3.38 \pm 0.12
	29.6 (0.3)	36	63.47 \pm 8.69	2.89 \pm 0.11
	ID		0.33***	0.14***

ID, inbreeding depression measure as $\delta = 1 - (X_{MI}/X_{LI})$, where X_{MI} y X_{LI} is the average of each trait in the group with medium and low inbreeding respectively. ***P<0.001

Table 8 ANOVA of the effects of inbreeding, family within inbreeding, density and their interactions on smolt body weight and specific growth rate (%/g per day) using type III sum of squares (d.f. degrees of freedom; MS, mean squares; P, Probability). Data satisfied parametric requirement without transformation.

Source of variation	Body weight			SGR	
	d.f.	F-Value	P-value	F-Value	P-value
Inbreeding	1	26.48	< 0.0001	22.14	< 0.0001
Family (inbreeding)	4	24.93	< 0.0001	27.15	< 0.0001
Density	1	0.08	0.775	0.34	0.558
Inbreeding x density	1	0.46	0.496	0.70	0.405

1 **FIGURES**

2

3 **Figure 1** Relationship between aggression score and average feeding attempts of low
4 inbred (open circles) and medium inbred (solid circles) Coho salmon in paired contest.

5

6 **Figure 2** Distribution of agonistic behaviours in paired contest between juvenile Coho
7 salmon with low (open bars) and medium (solid bars) inbreeding.

8

9 **Figure 3** Inbreeding depression in survival (mean \pm SD from three families) of salmon
10 reared from alevin to smolt as function of density (one sea cage per density). Open
11 and solid bars indicate low and medium inbreeding respectively.

Fig. 1.

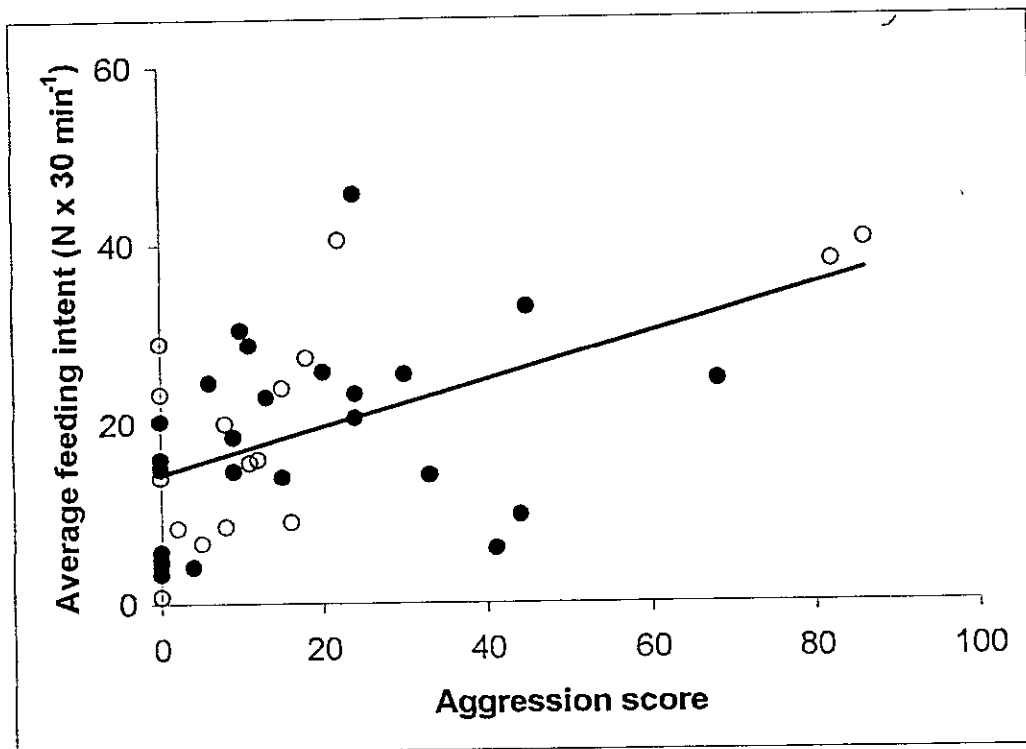


Fig. 2

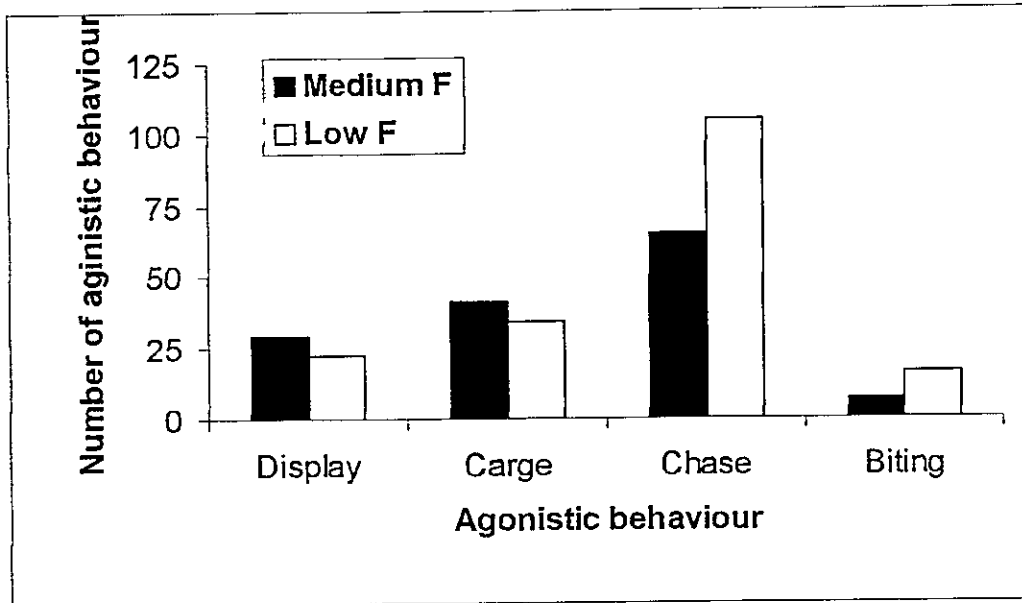
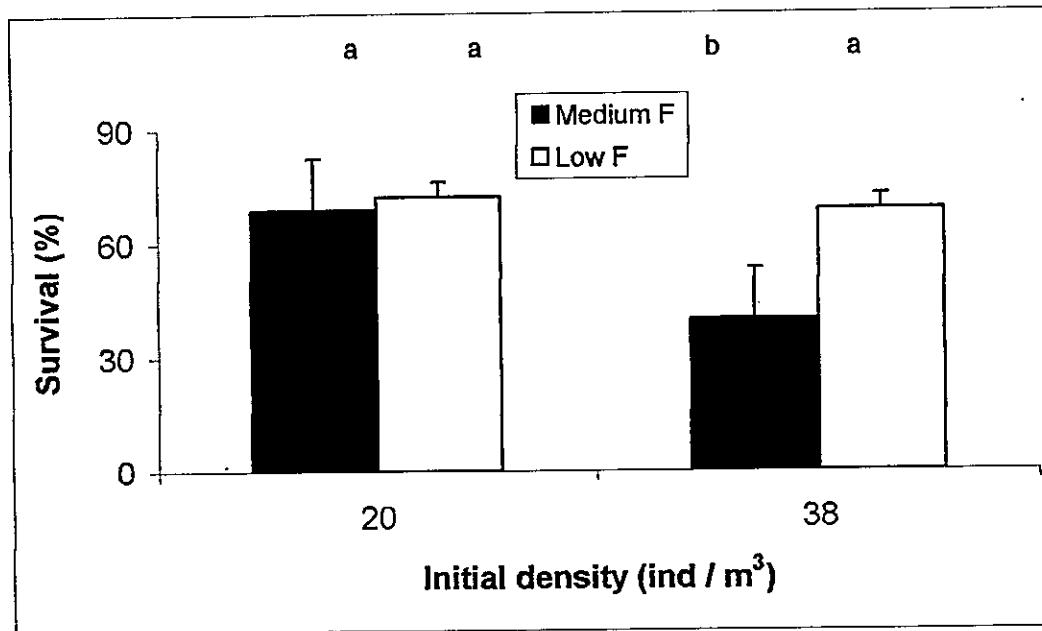


Fig. 3



ANEXO 3

Effects of nonrandom mating schemes to delay the inbreeding accumulation in cultured populations of coho salmon (*Oncorhynchus kisutch*)

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Abstract: Chilean salmon culture is based on a high degree of artificial selection, which has had the tendency to increase the inbreeding (F). Three types of nonrandom mating were evaluated to control the inbreeding in two best linear unbiased prediction selected coho salmon (*Oncorhynchus kisutch*) populations (even and odd). These included compensatory mating on the basis of breeding values (C), modified compensatory mating (C1) based on the family mean of breeding values, and mating that minimized the mean co-ancestry of the group selected (MC scheme). In the odd population, the MC scheme ($F = 2.0\%$) reduced the increase in inbreeding of the next generation by 50% and 46% when compared with random mating of selected individuals with sib mating restricted ($F = 3.9\%$) and with C ($F = 3.7\%$), respectively. In the even population, the MC scheme reduced the increase in inbreeding by 14% compared with C1 (9.7 versus 11.2). In both populations, the MC scheme also reduced variance in inbreeding (even, 59%; odd, 39%). Thus, the MC scheme was more efficient in reducing the increase and variance of inbreeding, thus limiting the expression of inbreeding depression. Although the MC scheme was more time consuming, we recommend this scheme to carry out crosses in each generation.

Résumé :

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Introduction

Genetic improvement of fishes in aquaculture has recently benefited by incorporation of the "best linear unbiased predictor" (BLUP) (Henderson 1975) for breeding values when using the animal model for genetic evaluation (Gjoen and Gjerde 1998; Martínez et al. 1999). This procedure maximizes the correlation between the true and the predicted breeding values using information from all parents in the

present generation and of preceding generations, as well as the individual's own data. This animal estimation produces a more exact estimation for the breeding value and increases the genetic response compared with other methods, e.g., phenotypic selection (Belonsky and Kennedy 1988). However, given that the animal model favours selection of related individuals, it also produces an increase in the rate of inbreeding and a long-term reduction in the genetic variance (Belonsky and Kennedy 1988; Quinton et al. 1992). There

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have been few empirical evaluations of the rate of inbreeding in fish populations subject to artificial selection. In rainbow trout (*Oncorhynchus mykiss*) populations, Su et al. (1996) estimated a rate of inbreeding of 1.3–1.7% per generation, whereas Pante et al. (2001a) for other populations found rates of inbreeding of 0.53–2% per generation. J.A. Gallardo et al. (unpublished data) recently found that two populations of coho salmon (*Oncorhynchus kisutch*) selected following an animal model gave inbreeding rates of between 1.05 and 2.37% per generation.

Limiting inbreeding in selection programs is important, as higher levels of inbreeding increase the probability of inbreeding depression. Estimation of inbreeding depression in fishes has consistently shown that consanguineous progeny have lower viability and less growth and also show lower resistance to infection by protozoans (Su et al. 1996; Pante et al. 2001b; Arkush et al. 2002).

Various methods have been devised to reduce tendencies towards an increase in the rate of inbreeding produced by artificial selection (i.e., BLUP or mass selection) while at the same time maintaining a high genetic gain (Villanueva et al. 1996; Meuwissen 1997; Wu and Schaeffer 2000). These include (i) BLUP estimations of breeding values with incremented heritabilities (Grundy et al. 1994; Wu and Schaeffer 2000), (ii) estimations using a suboptimal selection criterion, for example, giving a lower weighting to family information (Verrier et al. 1993; Wu and Schaeffer 2000), (iii) selection methods that manipulate the genetic contribution to the next generation of each candidate selected (Toro and Nieto 1984; Meuwissen 1997), (d) nonrandom mating schemes such as compensatory mating or those that minimize the average co-ancestry of the group selected (Toro and Perez-Enciso 1990; Caballero et al. 1996), and (e) mixed methods that used some preceding approaches simultaneously (Grundy et al. 1994; Sonesson and Meuwissen 2000). Regardless of which method is to be applied for selecting the reproducers for the next generation, the mating scheme among selected candidates is a determining factor in reducing the inbreeding rate, with random mating of selected individuals the least efficient (Grundy et al. 1994; Caballero et al. 1996; Sonesson and Meuwissen 2000). Practical considerations and reproductive restrictions may also limit the efficiency of these methods in reducing the rate of inbreeding (Meuwissen 1997; Fernández and Toro 1999). To date, few studies have evaluated the methods cited above (Sanchez et al. 1999; Weigel and Lin 2000), and none has been evaluated taking into account the characteristics of programs used in genetic selection in fishes.

The objective of the present study was to evaluate the effects of different nonrandom mating schemes in reducing inbreeding accumulation over a short term (one generation) in two populations of coho salmon selected by means of an animal model. This evaluation takes into account a series of practical and reproductive limitations relevant to various artificial selection programs in salmonids.

Materials and methods

Study populations

The study was based on data from two coho salmon populations belonging to a genetic improvement center (Centro

de mejoramiento genético (CMG)) maintained by the Institute for Fisheries Development and the University of Chile in Coyhaique (XI Region, Chile). The two populations, termed “even” and “odd”, were produced in 1992 and 1993, respectively, and are managed in a 2-year productive cycle. Each year class was produced using a hierarchical mating system by means of mating of 30–35 males with approximately 100 females, producing a population of 30–35 families of half-siblings (HS) and 100 families of full siblings (FS). Both populations were submitted to artificial selection: five generations for the even population and four generations for the odd population. Characters selected included the weight at harvest using the BLUP prediction of the breeding value and early spawning by means of phenotypic selection. More details on characters and origins of the populations are given in Martínez et al. (1999) and Winkler et al. (1999). The genealogy has been recorded from the beginning of the program to the present, giving calculated mean rates of inbreeding of 2.37% for the even population and 1.05% for the odd population. The mean inbreeding calculated in the previous year class investigated in this study was $3.16 \pm 2.65\%$ (year class 1999) and $9.4 \pm 2.7\%$ (year class 2000). The inbreeding rate was greater in the even population than in the odd population as a direct consequence of the smaller number of founder individuals in the former population ($N_e = 61$ and 106, respectively).

Mating schemes

The following mating schemes were compared in terms of the level of predicted next-generation inbreeding. (i) For random mating of selected individuals (R), crosses between selected males and females were carried out randomly, avoiding crosses between HS and FS. This scheme was routinely used at the CMG until 2001. (ii) In compensatory mating (C), a ranking was obtained following the breeding values for harvest weight of all individuals, grouped per spawning day. Based on this ranking, a mating system was simulated in which the male with the highest breeding value was assigned to cross with the female with the lowest breeding value, followed by assignment of the second-ranked male with the next-to-last ranked female and so forth until all individuals had been assigned. If the female was HS or FS with the assigned male, the assignment was replaced with a female that had the next highest breeding value, assigning the next male to this female. (iii) For modified compensatory mating (C1), a ranking was arranged of breeding values for harvest weight based on the average of paternal HS. A total of 19 families of half-brothers and 34 families of half-sisters were separately classified. The number of individuals per HS family varied between 1 and 59 for the males and between 3 and 86 for the females. Compensatory matings were carried out in a similar manner to the preceding scheme based on the family average. The male or female breeder with the highest family breeding value was crossed with the female or male with the lowest value for a particular spawning day and so forth. Crosses between HS and FS were avoided. (iv) The minimum co-ancestry (MC) scheme took into account the use of all genealogical information to assign the crosses and consisted of selecting a group for crosses of males and females having the minimum average co-ancestry. Co-ancestry between two individuals is the

Table 1. Number of sires (S), dams (D), and crosses (S × D) evaluated per spawning day in two year classes of coho salmon.

2001 year class				2002 year class			
Spawning day	S	D	S × D	Spawning day	S	D	S × D
26 April	8	12	96	27 April	6	12	72
27 April	11	22	242	29 April	5	9	45
30 April	15	28	420	3 May	17	30	510
1 May	17	24	408	7 May	17	29	493
4 May	13	25	325	7 May ^a	14	28	392
8 May	20	40	800	9 May	15	33	495
9 May	24	38	912	9 May ^a	7	13	91
12 May	28	47	1316	11 May	10	18	180
16 May	14	21	294	14 May	16	24	384
22 May	8	12	96	17 May	8	9	72
				20 May	7	8	56
				24 May	5	9	45

same as the probability that two alleles for a locus, each one taken from two different individuals, would be equal through descendency (Malecot 1948). This is equivalent to the inbreeding of the hypothetical progeny of these individuals if they are crossed (Falconer and Mackay 1996). Thus, minimization of the co-ancestry of a group of breeders is equivalent to reducing the inbreeding of their progeny. Co-ancestry coefficients were determined using PEDIGREE VIEWER program version 4.9 (B. Kinghorn and S. Kinghorn, University of New England, Armidale, New South Wales, Australia). Determination of which group of crosses minimizes the average co-ancestry has been generally treated as a problem in linear programming (Jansen and Wilton 1985; Sanchez et al. 1999; Weigel and Lin 2000), and this is the strategy used in this study. The following objective function was minimized in the present study:

$$(1) \quad \frac{\sum \sum x_{ij}(f_{ij})}{H}$$

This equation represents the average co-ancestry of the group selected, where x_{ij} is a decision variable that indicates whether the sire and dam are paired ($x_{ij} = 1$) or not ($x_{ij} = 0$), f_{ij} is the coefficient of co-ancestry between the sire i and the dam j , and H is the number of females spawned on a given day (usually between 10 and 40). The objective function must comply with the following conditions:

$$(2) \quad \sum \sum x_{ij} = H$$

$$(3) \quad \sum \sum x_{ij} = 1 \quad (\text{per each mother})$$

$$(4) \quad \sum \sum x_{ij} = 1, 2, 3 \quad (\text{per each father})$$

Equation 2 indicates the number of crosses carried out for a given day, eq. 3 indicates that one dam was crossed with only one sire, and eq. 4 indicates that one sire could cross with one, two, or three females on a particular day. The vector of crosses was obtained using the Hiper Lindo program (Lindo Systems, Inc. 1985).

Practical and reproductive restrictions

The two main limitations in this study were related to the number of crosses that could be evaluated simultaneously. The number of crosses evaluated was limited by the number of breeders successfully spawned each day (Table 1) and not by the entire group of breeders selected for the animal model. This resulted in 58 sires and 279 dams in 2001 (15 660 possible crosses) and 55 sires and 266 dams in 2002 (14 630 possible crosses). Management of the spawning at the CMG was carried out following natural maturation in the fishes, which occurred in periods of 5–6 weeks, following a normal distribution. Gametes were obtained day by day from mature breeders, and after the quantity and quality of the gametes were determined, it was possible to obtain “optimum” crosses using methods cited above for each particular day. In relation to the preceding, a practical limitation was also included as follows. Because of limitations on personnel and space at the CMG, two groups of mature fishes were spawned separately some days, one in the morning and the other in the afternoon. Crosses of each group were optimized separately in 2002.

Evaluation of the mating schemes

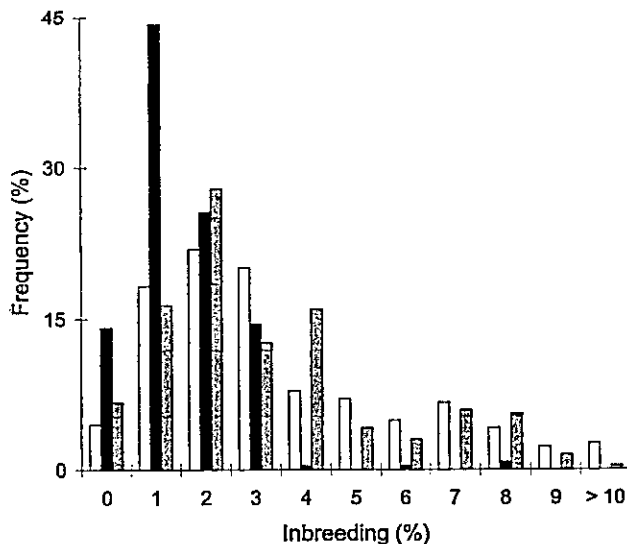
Evaluation of the different mating schemes was carried out in two steps, each one involving the year class of each population (2001–2002). Only the reproductive limitation was applied to year class 2001. Here, the efficiency in reducing inbreeding in the next generation was compared between two nonrandom mating schemes, including the C mating and MC mating versus the R mating schemes of selected individuals carried out in this year. In year class 2002, the CMG placed into practice the C1 mating scheme instead of the R mating schemes used until this time. In this stage, both reproductive and practical restrictions were taken into account. The efficiency of the C1 scheme was compared with that of the MC scheme, which had been found to be the better scheme from the previous stage. Following the system of hierarchical crosses used in this program, and with the objective that the comparisons between the different schemes should be carried out under the same expected genetic response level, each breeder was utilized the same number of

Table 2. Mean inbreeding (*F*) and inbreeding ratios between random mating of selected individuals (R), minimum co-ancestry (MC), and compensatory (C) mating of the coho salmon 2001 year class by spawning day.

Spawning day	Mean <i>F</i>			Inbreeding ratio					
	R	MC	C	MC/R	<i>P</i>	MC/C	<i>P</i>	C/R	<i>P</i>
26 April	4.77	1.92	5.16	0.40	*	0.37	*	1.08	ns
27 April	3.04	1.81	3.07	0.60	**	0.59	ns	1.01	ns
30 April	3.48	1.76	3.47	0.51	**	0.51	**	1.00	ns
1 May	4.57	1.99	3.33	0.44	**	0.60	**	0.73	ns
4 May	3.67	1.91	2.95	0.52	*	0.65	**	0.80	ns
8 May	4.21	1.91	3.47	0.45	**	0.55	**	0.82	ns
9 May	3.86	1.94	3.19	0.50	**	0.61	**	0.83	ns
12 May	4.13	1.82	4.17	0.44	**	0.44	**	1.01	ns
16 May	4.15	2.61	4.20	0.63	*	0.62	**	1.01	ns
22 May	4.57	3.65	5.91	0.80	ns	0.62	ns	1.29	ns
Mean	3.99	2.01	3.70	0.50	***	0.54	***	0.93	ns

Note: *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001; ns, nonsignificant.

Fig. 1. Frequency distribution of inbreeding in hypothetical progeny of the coho salmon 2001 year class for random mating of selected individuals (open bars), compensatory mating (shaded bars), and minimum co-ancestry mating (solid bars) (*N* = 269 families).



times in each mating scheme (identical genetic contribution of each parent between mating schemes).

Statistical analyses

In both year classes, the efficacy of each scheme in reducing the inbreeding rate was evaluated by day and by spawning season, at the population level. The total number of crosses evaluated per spawning day was between 96 and 1316 for the 2001 year class and between 45 and 510 for the 2002 year class (Table 1). To compare mean inbreeding per spawning day among schemes, a nonparametric Mann-Whitney test was used, whereas the variances of inbreeding were compared using an *F* test (Zar 1996). A Kolmogorov-Smirnov analysis (Zar 1996) was used at the population level to compare the distribution of frequencies of inbreeding arising from all of the crosses selected in each year class by the mating schemes.

Results

Evaluation of mating schemes for the 2001 year class

The MC scheme was more efficient in reducing the average inbreeding and the variance of the inbreeding in the next generation in comparison with the other mating schemes evaluated. The MC scheme reduced the inbreeding (*P* < 0.001) 50% compared with the R mating schemes of selected individuals and 46% compared with the C mating scheme (Table 2). In relation to spawning day, this reduction in average inbreeding ranged between 20% and 60%. In comparison, the C scheme was only slightly more effective (7%) at reducing inbreeding in comparison with the R scheme, although none of the daily differences in the tests was significant (Table 2). Also, the MC scheme significantly reduced (*P* < 0.001) the variance in the inbreeding by 59% compared with the R scheme and 54% compared with the C scheme (Fig. 1). Conversely, the C scheme reduced (*P* < 0.05) the variance of the inbreeding only 12% when compared with the R scheme (Fig. 1). Consequently, the frequency distribution of inbreeding was significantly different between MC and R (Kolmogorov-Smirnov test, *D* = 0.42, *P* < 0.001) and between MC and C (Kolmogorov-Smirnov test, *D* = 0.37, *P* < 0.001) but not between C and R (Kolmogorov-Smirnov test, *D* = 0.081, *P* not significant).

Evaluation of mating schemes for the 2002 year class

The MC scheme was more efficient in reducing the inbreeding in all of the parameters evaluated in comparison with the C1 scheme. The MC scheme reduced the inbreeding by an average of 14% in comparison with C1 (Table 3), whereas on a per spawning day basis, this reduction ranged between 5% and 17%. As with year class 2001, the MC scheme also significantly reduced (*P* < 0.001) the variance of the inbreeding, with an average reduction of 39% (Table 3). Consequently, the frequency distribution of the inbreeding was significantly different (Kolmogorov-Smirnov test, *D* = 0.33, *P* < 0.001) between the two nonrandom mating schemes (data not shown).

The practical and reproductive restrictions evaluated in this year class limited the efficiency of the MC scheme over the C1 scheme in inbreeding reduction when less than

Table 3. Mean inbreeding (*F*) and standard deviation of the inbreeding and the inbreeding ratio between minimum co-ascendancy (MC) and modified compensatory (C1) mating in the coho salmon 2002 year class per number of crosses evaluated (*N*).

N	Mean <i>F</i>				Standard deviation			
	C1	MC	Inbreeding ratio		C1	MC	Inbreeding ratio	
			MC/C1	<i>P</i>			MC/C1	<i>P</i>
45	10.6	10.0	0.95	ns	1.5	0.9	0.60	ns
45	10.8	10.0	0.93	ns	1.5	1.9	1.27	ns
56	11.3	10.1	0.89	ns	1.2	1.1	0.92	ns
72	11.0	9.6	0.87	ns	2.0	1.0	0.50	*
72	10.6	9.9	0.94	ns	2.0	0.9	0.45	*
91	11.0	9.9	0.90	ns	2.2	1.3	0.59	*
180	12.0	9.9	0.83	*	2.9	1.8	0.62	*
384	11.3	9.6	0.85	**	1.9	1.4	0.74	ns
392	11.3	9.7	0.86	**	2.0	1.1	0.55	**
493	11.1	9.2	0.83	***	2.0	0.7	0.35	***
495	11.2	9.4	0.84	***	2.0	1.2	0.60	**
510	11.1	9.7	0.88	*	2.5	1.4	0.56	**
Total	11.2	9.7	0.86	***	2.1	1.3	0.61	***

Note: *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001; ns, nonsignificant.

100 crosses were evaluated simultaneously (Table 3). The reduction in inbreeding was not significant when the number of crosses dropped from 91 to 45; however, when 180–510 crosses were evaluated simultaneously, the reduction in inbreeding was highest and significant.

Discussion

This study showed that the MC scheme was the most efficient in reducing mean inbreeding of the next generation when compared with the other mating schemes applied to coho salmon at the CMG. Other authors have obtained similar results on the efficiency of the MC method for reducing inbreeding both over short (Weigel and Lin 2000) and medium (Sanchez et al. 1999) terms. Also, in agreement with various theoretical predictions (Grundy et al. 1994; Caballero et al. 1996; Sonesson and Meuwissen 2000), the random mating schemes of selected individuals were less efficient in reducing inbreeding even when avoiding mating FS and HS individuals in each generation. In spite of this evidence, previously described random mating schemes are still commonly used in selective breeding programs (Pante et al. 2001a; Argue et al. 2002; Bolivar and Newkirk 2002).

The MC scheme reduced the variance of inbreeding between 40% and 60% in relation to the other schemes studied, producing families that were more homogeneous among themselves with regard to inbreeding. Similar results were obtained by Sanchez et al. (1999), who found that the variance of inbreeding was reduced by one to seven times in comparison with random mating of selected individuals. This is a desirable situation in the sense that families having high levels of inbreeding within each generation will not be produced, reducing the probability of inbreeding depression being expressed (Gjerde et al. 1983; Su et al. 1996; Pante et al. 2001b).

In the present study, the evaluations were carried out under the conditions of two restrictions that limited the number of crosses tested simultaneously. The reproductive restriction

limited the number of breeders evaluated simultaneously to those successfully spawned on a given day, and the practical restrictions in the handling of the fishes prevented a large number of them from being spawned and evaluated simultaneously. Limited by these two restrictions, only between 45 (5 males and 9 females) and 510 (17 males and 30 females) crosses could be evaluated in 2002. This was lower than the number of crosses potentially carried out at CMG on any given day (30 males and 150 females, 4500 possible crosses) and much lower than the total of theoretically possible crosses if all of the available fishes could have been spawned simultaneously (14 630 possible crosses). At least two alternative forms of spawning management could be adopted to avoid the limitations on the number of breeders simultaneously evaluated and to increase the efficiency of the minimal co-ancestry scheme. These include a reduction of the spawning period to a few days by the use of hormonal induction and the preservation of semen from early-spawning males to be used later in the fertilization process. Both techniques, especially the first one, have been incorporated in Chilean culture farms and may be efficiently utilized in the short term in genetic selection programs applied to salmonids.

Practical application of MC scheme was more time consuming than the two mating schemes previously utilized at the CMG. The MC method requires in situ (each day of spawning) construction of matrices of co-ancestry among all of the possible crosses of the breeders selected, as well as matrices necessary to adapt the selection of optimal crosses for each spawning day to a linear programming context (optimization by Hiper Lindo program, Lindo Systems Inc. 1985). In the first step, PEDIGREE VIEWER program version 4.9 (B. Kinghorn and S. Kinghorn, University of New England, Armidale, New South Wales, Australia) was used to calculate the co-ancestry of all possible crosses, but the second step was made manually. In fact, this procedure delayed the fertilization of green eggs by 2 or 3 h in comparison with R or C1 schemes. In the last step, however, the use

of a computer program to adapt the selection of optimal crosses could reduce the time consumed in this period.

In this study, the comparisons among mating schemes were made after selection had been carried out and on the same level of expected genetic response; therefore, the selection and mating decisions were made independently. Under these conditions, the MC mating scheme can delay the inbreeding accumulation in the short term but does not reduce it permanently because the genetic contribution of each parent remains constant between mating schemes and the effective population size is not modified. Thus, in the long term, two populations with similar effective population size but different mating scheme should reach the same inbreeding level. Nevertheless, the MC scheme can be improved (e.g., reducing inbreeding permanently) if selection and mating decisions are simultaneously made. In the context of linear programming, an objective function should be constructed to maximize the genetic response, with a restricted inbreeding rate (Sanchez et al. 1999). However, in this case, it is expected that a compromise between the genetic response and the inbreeding rate exists where looking for a low rate of inbreeding will produce a smaller genetic response (Villanueva et al. 1996).

The MC scheme, shown to be a useful tool in delaying the accumulation of inbreeding in the short term, also reduced the variance of the inbreeding, thus limiting the early expression of inbreeding depression. These results were obtained even when the tests were carried out on very small groups of possible crosses (45–1316) because of reproductive and practical limitations. Although the MC scheme was more time consuming than the two mating schemes previously used at the CMG (R mating of selected individuals and C1 mating), we recommend this scheme to carry out crosses in each generation.

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