Title: Increase of Inbreeding by Stocking on Wild Population Assessed by using Individual-based Life History Model

Running Title: Increase of Inbreeding by Stocking

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Abstract:
We assessed the genetic impact of stocking on natural populations by using individual-based life-cycle models. We constructed models that used include density-dependent mortality at the early life stage, natural mortality, fishing mortality, and the number of released fish. We varied these variables by using random numbers. We focused on the genetic impact of the number of released fish, the number of parent fish producing seedlings that were released, the sex ratio of the parent fish, fishing mortality, and the methods to select the parents of the seedlings. When brood stock size in hatchery is 5 male and 5 female, is the coefficient of inbreeding is about 27 times comparing more when 50 male and 50 female at the 50th generation, and the coefficient of inbreeding increases about five times at the 50th generation, when the sex ratio of the parents changes from 50 males and 50 females to 10 males and 90 females. When parents are selected from wild populations at random, extreme fishing mortality may also reduce genetic diversity.

Key words: Individual-based model, stocking, genetic impact, stock enhancement
個体ベース生活史モデルに基づく放流による天然個体群の近交係数増加の推移について

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個体ベース生活史モデルを用いて、種苗放流が天然個体群に与える遺伝的影響を検討した。モデルでは、自然死亡、漁獲死亡、放流個体数等を考慮し、放流個体数、種苗生産時の親魚数、親魚の性比、漁獲死亡及び親魚の由来による遺伝的影響の差違を比較した。親魚数を雄10雌90、雄5雌5としたとき、雄50世代目ににおける近交係数は、親魚数を雄50雌50としたときに比べそれぞれ5倍および27倍高くなった。親魚に天然個体を用いる場合は、過度の漁獲死亡係数が遺伝的多様性に影響を与えることが示された。
INTRODUCTION

Stock enhancement, which releases artificially raised juveniles into wild populations, is widespread.\textsuperscript{1,2} The objectives of stock enhancement are mainly to increase or stabilize living resources. Because of an increased demand on marine products, stock enhancement is expected to increase. The genetic diversity of hatchery fish may differ from that of wild stocks, because the brood stock size in hatchery is much smaller than parents of wild brood stock size.

Many cases have been reported of the reduction of genetic diversity of released fish.\textsuperscript{3-5} When genetic diversity is reduced, the fitness of populations may decrease because of inbreeding depression,\textsuperscript{6-8} and populations may decrease rapidly even with little change in environment.\textsuperscript{9}

To prevent the reduction of genetic diversity, it is important to predict the effect on genetic diversity, and to release as many seedlings as necessary. Practical releasing experiments are rarely possible and so realistic, numerical simulations are necessary and important to predict the risk and the effect on the genetic diversity.

Individual-based life history models are a good way to show the dynamics of population realistically.\textsuperscript{10} This kind of model calculates each virtual individual life cycle, and expresses more realistic assumptions than state variable population models. Virtual individuals have a locus and save two genes, one from male parent and one from female parent. By using individual-based models, we can predict the genetic diversity, the yield, the population size, and their variances even when uncertainties and complex relationships exist between individuals and environments.

In this study, we constructed individual-based life cycle models that include density-dependent mortality at the early life stage, natural mortality, fishing mortality, and the number of released fish, which we varied by using random numbers as the uncertainty. We focused on the genetic impact of the number of released fish, the broodstock size in hatchery, the sex ratio of the parents, fishing mortality, and the methods to select the parents.

METHODS

Model Outline

In our individual-based life cycle model (Fig.1), we first set the sex ratio (the ratio of male population comparing to female population), population of first generation, and numbers of released juveniles in each generation. We devised the individuals that had a locus which records its own genes. We calculated the inbreeding coefficient directly from the proportion of the individuals having identical genes at the locus to the population size.

Fish population size decreases at the probability of a given natural mortality and fishing mortality as described later. In the model, mature individuals mate randomly. Then the mated female spawns at a given fecundity. We set the generation time at 4 years. For simplicity, we did not consider the overlap of generations.

We calculated the inbreeding coefficient at each generation. We assumed that mature released fish fully participate in mating.

Population Dynamics

The main factors of population dynamics were variation in number of recruits, in fishing mortality, and in natural mortality. The recruit variation is described later.

We used random numbers to vary fishing mortality and natural mortality;

\[
M_i = M_m + \varepsilon_2 \\
C_i = C_m + \varepsilon_1
\]

\[
\varepsilon_1 \sim N(0, \sigma_1^2) \\
\varepsilon_2 \sim N(0, \sigma_2^2)
\]

where \(i\) represents an individual, \(M_i\) is the probability of death by causes other than by fishing in a generation (natural mortality rate) of each individual \(i\), \(M_m\) is the mean natural mortality rate, \(C_i\) is the probability of death by fishing in a generation (fishing mortality rate) of each individual \(i\), \(C_m\) is
the mean fishing mortality rate, $\varepsilon_1$ and $\varepsilon_2$ are normal random numbers with mean zero and variances $\sigma_1^2$ and $\sigma_2^2$ respectively. Rarely $M_i$ and/or $C_i$ are over one or below zero. In that case, we set them as one or zero.

**Stock recruitment and density dependence**

The density effect is defined as changes in survival rate, growth rate, or the number of spawned eggs, which vary by changes in the population density. Density-dependent factors provide a mechanism for population regulation.11

Our model was assumes that initial mortality depends on the number of spawned eggs and is assumed to be an index of density. We also assumed that the total number of spawned eggs is proportional to the number of spawners, and that the density effect occurs only during the first year.

As shown in Fig. 2, we calculated the number of 1-year-old juveniles $J_{ij}$ produced from one spawner $i$ at generation $j$ as follows,

$$J_{ij} = (g - \tau)(1 - \frac{N}{K}) + \tau + \varepsilon_i + \varepsilon_j$$

where $\tau = \frac{1}{s} \sigma_i^2$ and $\sigma_j^2$ are normal random numbers with mean zero and variances $\sigma_1^2$ and $\sigma_2^2$ respectively, and $s$ is the proportion of female in a population. When only one female exists, it must produce $\tau = \frac{1}{s}$ children to maintain the population balance. Rarely $J_{ij}$ is below zero, but in that case, we set it as zero.

**Stock enhancement**

We used the number of released fish, broodstock size in hatchery, the sex ratio of the parents as input parameters. Juveniles were released at age 1 year.

We assumed two breeding systems: 1) line breeding that produces seedlings successively from several pairs of hatchery-grown parents, 2) one generation breeding that produce seedlings from parents selected from wild populations at random.

**Inbreeding coefficient**

We calculated inbreeding coefficient ($F$) for the index of inbreeding. When the inbreeding coefficient is high, the genetic diversity is small. The inbreeding coefficient is the probability that two genes at any locus in an individual are identical by descent.12 In our model, each gene has its identity number and identical genes are counted directly at a locus. The inbreeding coefficient is calculated as;

$$F = \frac{N_{\text{inb,}j}}{N_j},$$

where $N_{\text{inb,}j}$ is the number of individuals that have identical genes at a locus in generation $j$, $N_j$ is the total number of the wild born and released fish at generation $j$.

**Simulation**

Table 1 shows default and alternative parameter sets that we used in this simulation, referring to
the data of the Japanese flounder (*Paralichthys olivaceus*) in Miyagi prefecture, Japan, which is a typical species used to stock enhancement in Japan.

The natural mortality rate was calculated by using empirical relationship between the mean sea surface temperature, the growth rate and the natural mortality coefficient. We assumed $K$ as 82,000 and the current fishing mortality rate was near to MSY. We set the generation time at 4 years.

In the simulation, we chose the relative hatchery contribution, that is, the proportion of seedlings released into a wild population compared with initial population, from three alternatives 0, 0.1, and 0.2, where italic values indicate default parameters. We also chose the broodstock size in hatchery from 5 males + 5 females, 25+25, and 50+50, to examine the speed of increase in inbreeding. We chose mean fishing mortality rates $C_m$ from 0.4, 0.6, and 0.8, to examine the influence of overexploitation on inbreeding.

Generally, eggs from many females are fertilized by sperm from a small number of males. We examined the influence of the increase in inbreeding by changing the proportion of female in the parent fish as 0.1, 0.3, and 0.5.

Our model was run for 50 generations with different parameter sets. To calculate the inbreeding coefficient, each simulation was run 100 times. All these simulations were done by using FORTRAN on a personal computer.

**RESULTS**

**Dynamics of population**
Fig. 3 shows two examples of dynamics of population size. The population fluctuate widely because the generation is not overlapped.

**Breeding systems**
Fig. 4 shows the change in inbreeding coefficient in two breeding systems that have default parameters. The inbreeding coefficients differed significantly between the two breeding systems. In the early generations, line breeding fish had lower inbreeding coefficients than one generation breeding fish; because line breeding does not need to catch wild parents and the number of the wild parents is larger using line breeding than using one generation breeding. However, the inbreeding coefficient of the line breeding fish rapidly increased. It rose above the inbreeding coefficient of fish having wild parents at the 7th generation, and it was about 2.5 times larger than fish having wild parents at the 50th generation in the default setting.

**Relative hatchery contribution**
Fig. 5(a) shows the change in inbreeding coefficient at the seedling relative hatchery contribution 0.1 and 0.2. In the early generations, the higher was the hatchery contribution, the higher was the inbreeding coefficient increased in the two breeding systems. In later generations, the inbreeding coefficient levelled asymptotically to the inbreeding coefficient of released fish. This is because the gene of wild fish is replaced to the gene of released fish. The inbreeding coefficient increased for more generations in fish having wild parents than in line breeding fish.

**Broodstock size in hatchery**
Fig. 5(b) shows the change in inbreeding coefficient when the broodstock size in hatchery was 10, 50 and 100. The broodstock size in hatchery had a large effect on inbreeding. At the 50th generation, when the broodstock size was 10, the coefficient of inbreeding was about 27 times larger than when the broodstock size was 100 by using line breeding fish and about 10 times larger by using wild parents.

**Sex ratio of parent fish**
Fig. 5(c) shows the change in inbreeding coefficient when the sex ratio of the parents was 10%,
30% and 50%. The higher the sex ratio of the parents, the more the coefficient of inbreeding increased. When the sex ratio of the parents changed from 50 males and 50 females to 10 males and 90 females, the coefficient of inbreeding increased about five times at the 50th generation by using line breeding fish, and over twice by using fish having wild parents.

**Fishing mortality rate**

Fig. 3(d) shows the coefficient of inbreeding at fishing mortality rate 0.4, 0.6 and 0.8 by using fish having wild parents and line breeding fish. In line breeding fish, the influence of the fishing mortality rate was moderate, but in fish having wild parents, the coefficient of inbreeding increased as the fishing mortality rate increased.

When the mean fishing mortality rate changed from 0.6 to 0.8, the coefficient of inbreeding increased by about five times at the 50th generation.

**DISCUSSION AND CONCLUSIONS**

We assessed the impact of stocking on the fish genetics of wild populations. We showed that the impact of the broodstock size in hatchery and the sex ratio of the parents had a large influence in the line breeding system. We also showed that when parents are selected from wild populations at random, fishing mortality rate also had a large influence.

When the broodstock size in hatchery is small in line breeding, the inbreeding coefficient of seedlings was increases dramatically, and because of continued stocking, the inbreeding coefficient of the populations increases asymptotically to the inbreeding coefficient of seedlings. In the breeding system that uses wild parents, the inbreeding coefficient less increases less than in line breeding. Seedlings from a fewer parents reared with great care seedlings from many will be worse effect on gene diversity.

The sex ratio of parents has a large effect on inbreeding. When the proportion of females is 10-30%, the inbreeding coefficient increases, but at 40~50% females small differences are in the inbreeding coefficient. Generally, the volume of sperm is enough to fertilize eggs of several females. If seedlings are produced many in a limited space, the sex ratio may have a higher proportion of females intentionally, and will have a bad effect on conserving genetic diversity.

The effect of the relative hatchery contribution rate also has as large as the effect of the broodstock size in hatchery and sex ratio. By using wild parents, however, the inbreeding coefficient increases proportionally to the relative hatchery contribution. If hatchlings used for stocking have genes different from the wild population, and if stocking continues, the wild gene could be displaced by the seedling genes by the 20 generations.14 The speed of displacement is strongly related to the seedling addition rate. As the cost of producing hatchlings increases proportionally to the number of seedlings, the number of seedlings for stocking must be the minimum not only to conserve the genetic diversity, but also to minimize the costs.

Fishing mortality affects the inbreeding coefficient especially by using wild parents to produce seedlings, because the broodstock size in a wild population decreases because of fishing. When the broodstock size is sufficient to breed in a hatchery, the genetic diversity is conserved by line breeding, and is one alternative for endangered populations. Stock management is necessary not only to sustain stocks for commercial catches, but also to conserve genetic diversity.

In our model, we did not use the mutation rate, because of lack of concrete information. Thus the values of the inbreeding coefficient were over-estimated because of the neglecting the mutation rate. However, the mutation rate would be small and thus would be negligible. We also did not consider population structure and age composition. These issues are open for the further study.

We chose the parameters values referring to the data of Japanese flounder for make this simulation more realistic. We believe the parameters covers the situation of real stock enhancement, but for making policy of the stock enhancement, we recommend to use reliable parameters, and to monitor the genetic diversity for long time.
Individual based life history model approach is quite flexible and can describe various situations in stocking. Especially, this kind of model can include various error terms, and has a large potential to expand. This is an advantage comparing to the state variable or the deterministic population model.  

We have to mention that this simulation needs rather long calculation time and need considerable computer power, but these disadvantage will soon be diminished by the innovation of computer. Sometimes the implementation of the result may be difficult because of the complexity of the model. Sensitivity analysis will be useful for examining the model behaviour. From now on, stock enhancement plans must be made by considering the effect of stocking both on commercial catch yields and on the conservation of genetic diversity.

REFERENCES
Fig. 1. Outline of the individual-based life history model used in this study: The number of juvenile from wild parents is calculated from number of eggs produced by the spawning stock of previous generation. The juveniles from hatchery are also added to the population. Line breeding or one generation breeding is used as the breeding system. The numbers of individuals decrease from juvenile to mature individuals with natural mortality and fishing mortality. When mature, it mate randomly and produce eggs.

Fig. 2. The relation between density dependence to the number of juveniles
Fig. 3. Two examples of the population size dynamics

![Population size dynamics graph](image)

Fig. 4. Inbreeding coefficient of two breeding systems: crosses are a natural population, closed circles are line breeding, and open diamonds are one generation breeding

![Inbreeding coefficient graph](image)

Fig. 5. Inbreeding coefficients of a population: closed circles are line breeding, open diamonds are one generation breeding. (a) Inbreeding coefficient at the relative hatchery contribution: 0.1(solid lines), 0.2(dotted lines). (b) Inbreeding coefficient at three numbers of parent fish that produce seedlings: 10 parents (dotted lines), 50 parents (broken lines), and 100 parents (solid lines). (c) The sex ratio of the parent fish: 10% (dotted lines), 30% (broken lines), and 50% (solid lines). (d) The fishing mortality rate in a generation: fishing mortality rate at 0.4(dotted lines), 0.6(solid lines), 0.8(broken lines).
Table 1 Parameters used in this model.

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<th>Alternatives</th>
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<td>Sex ratio of juvenile</td>
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<tr>
<td>Relative hatchery contribution</td>
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<tr>
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<td>10,50</td>
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<td>Sex ratio of parents</td>
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<td>Recruitment ( g=22.5 ) ( \tau=2 ) ( K=82000 )</td>
<td>( \varepsilon_i \sim N(0,0.52) )</td>
<td>( \varepsilon_j \sim N(0,0.22) )</td>
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