Genetic consequences of stocking with hatchery strain brown trout: experiences from Denmark

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Agenda

• Brown trout stocking in Denmark

• Introgression of stocked trout into wild populations

• Admixed populations – is there anything left worth conserving?

• Can we identify the genes under selection when hatchery trout are stocked into the wild?

• Future perspectives
Brown trout and stocking

- Brown trout important species for angling
- Habitat destruction for many decades
  - by early 1990s 97% of all rivers in Denmark affected
  - drastic declines of brown trout

- The “solution”: stocking with brown trout from commercial hatchery strains
  - Strains kept in captivity for many generations - up to 120 years
  - Possible fitness loss due to inadvertent domestication selection
  - Are most populations descendants of stocked hatchery strain trout?
• The four quantitatively most important strains share a common history
Obtaining data from the populations prior to stocking

• Analysis of DNA (microsatellites) from old scale samples (Nielsen et al. (1997) Molecular Ecology)
• Samples dating back until the 1910s
Reestablished populations: stocking with hatchery strains or rebound of indigenous trout? (Hansen 2002. Molecular Ecology)

- Karup River – until the 1960s a major sea trout river in Denmark
- Population declines
- Intensive stocking with hatchery strain trout
- At the same time supportive breeding of local spawners caught in the river
- Habitat restoration
- The population increased drastically since the early 1990s.

- Hatchery strain or indigenous trout?????
Stocking with hatchery trout or rebound of indigenous trout?

- Karup River samples:
  - Historical: 1947-1956
  - Contemporary: 1993-1996
- Hatchery strain samples:
  - 1992
- Microsats
- Admixture proportion analysis ("LEA" (Chikhi et al., 2001))

Admixture proportion of hatchery strain:
0.06 (95% CI 0.00 – 0.24)
Stocking with hatchery trout or rebound of indigenous trout?

- Calculation of expected admixture proportion based on
  - estimates of natural reproduction
  - numbers of stocked trout
  - assuming equal survival and reproduction of indigenous and hatchery trout

- Reduced survival and reproduction of stocked hatchery strain trout

**Observed**
- Admixture proportion of hatchery strain: 0.06 (95% CI 0.00 – 0.24)

**Expected**
- Expected admixture proportion of hatchery strain: 0.62
Is selection in the marine phase a major factor in the poor performance of hatchery trout?
(Ruzzante et al. 2004. Molecular Ecology)

- Microsatellite-based mixed-stock analysis (BAYES; Pella & Masuda, 2001) based on all trout populations in the Danish Limfjord as baseline samples (n = 3801)

- Mixed samples from the Limfjord (n = 846).
  1) "Foraging", mostly immature trout (mean length 38 cm, range 20-67 cm)
  2) "Spawning" trout (mean length 66 cm, range 31-90 cm)
Is selection in the marine phase a major factor in the poor performance of hatchery trout?

- Selection against domesticated trout in the marine phase
Is selection in the marine phase a major factor in the poor performance of hatchery trout?

What happens?

Tagging studies of Karup River trout (Stig Pedersen, DIFRES):
• Smolts and postsmolts of indigenous origin exit the Limfjord
• Conduct feeding migrations to the highly productive Kattegat and Baltic Sea
Smolts and postsmolts of hatchery strain origin stocked into the Karup River also exit the Limfjord, but migrate more erratically. Some are swept through the Skagerrak and caught in Norwegian fjords.
Management implications

- Similar conclusions from other studies and populations (Hansen et al. 2000; 2001a,b; 2006; Ruzzante et al. 2001 and others)
- More indigenous trout populations left than expected, but some are strongly admixed
- No examples of population rehabilitation caused by stocked hatchery trout
- Change of management procedures
  - 2003: Decision to abandon stocking with hatchery strains
  - Only supportive breeding of local populations allowed
  - Most emphasis on habitat restoration
- What is the impact of stocking in the cases where populations have been strongly admixed?
Genetic population structure before and after stocking (Hansen et al. (2009) Molecular Ecology)

- Western Jutland brown trout populations, stocked with 100,000s of hatchery trout during the 1970s-90s
- 21 microsatellite loci
What would we like to know?

• How large were EFFECTIVE population sizes ($N_e$) in the past and at present?

• $N_e$ – the number of individuals in an “ideal” population that would give rise to the same amount of inbreeding or random genetic drift as caused by a given assemblage of individuals

• Nearly always much lower than the actual number of individuals – sex ratio, variance in reproductive success, temporal changes of $N_e$

• Correlation of $N_e$ with environmental parameters, such as area of spawning habitat
What would we like to know?

• Gene flow among populations, m

• Does gene flow among populations decrease when geographical distance increases (isolation by distance)?

• How important is the influence of gene flow from stocked hatchery trout on the current genetic population structure?

• Has the genetic population structure broken down after stocking with hatchery trout?
Genetic population structure prior to stocking

- Genetic population structure 1920s-50s
- Migrate (Beerli & Felsenstein, 2001)
- Parameters scaled by mutation rate, $\mu$
- Migration rate, $M = m/\mu$
- Historical effective population size (= over the past many generations), $\Theta = 4N_e\mu$

- Positive correlation between historical areas of spawning habitat and historical effective population sizes

![Graph showing the correlation between historical effective population size ($\Theta$) and area of historical spawning habitat ($m^2$). The correlation coefficient is $r^2 = 0.72$ and the p-value is $P = 0.033$.](image-url)
Genetic population structure prior to stocking

• Historical gene flow primarily between neighbouring populations (MIGRATE)

• Isolation by distance

• Patterns as expected for a natural, undisturbed system

\[ R^2 = 0.301 \]
\[ P = 0.005 \]
Genetic contribution by stocked hatchery trout in contemporary populations

- Admixture proportions estimated using STRUCTURE (Falush et al. 2003)

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<td>% hatchery trout in gene pool</td>
<td>22%</td>
<td>64%</td>
<td>45%</td>
<td>31%</td>
<td>19%</td>
<td>14%</td>
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- Very strong admixture in particularly two populations (Skjern R., Varde R.)

- Surprisingly little admixture in Ribe R. and Kongeaa R.
Genetic population structure after stocking


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<td>Effective population size (95% CI)</td>
<td>299 (189–500)</td>
<td>133 (91–193)</td>
<td>224 (55–452)</td>
<td>417 (175–1188)</td>
<td>423 (241–725)</td>
<td>587 (298–1980)</td>
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- **No** correlation between contemporary effective population size and areas of spawning habitat
Genetic population structure after stocking

- No positive correlation between historical effective population size, \( \Theta \), and contemporary effective population size

- The two smallest contemporary populations (Skjern and Varde R.) are also most admixed with hatchery trout (64% and 45%)

- Habitat degradation or stocking?
- Admixture \( \rightarrow \) smaller population sizes?
- Small population sizes \( \rightarrow \) higher likelihood of admixture?
Genetic population structure after stocking

- Still isolation by distance

...but also significant association between genetic differentiation and difference in hatchery trout admixture between populations ($r^2 = 0.45$, $P = 0.015$) (also significant after Partial Mantel tests)

- Contemporary genetic structure has changed considerably due to
  - Habitat degradation leading population declines
  - Stocking leading to admixture
  - Interactions between the two
Has the original genetic structure broken down completely?

- No, it is to a large extent retained despite strong admixture
  - STRUCTURE (Falush et al. 2003): \( k = 3 \) groups of populations
  - Individual admixture proportions
  - Northern populations yellow cluster
  - Southern populations purple cluster
  - Hatchery strains red cluster
  - Distribution of yellow and purple clusters retained in contemporary samples despite strong admixture
Do non-admixed trout still exist even in strongly admixed populations? Hansen & Mensberg 2009. Biology Letters

- Skjern River strongly (64%) admixed with hatchery strain trout
- Admixture has taken place over 20 years ≈ 6 generations
- Expectation: no remaining non-admixed trout

50 microsat loci chosen from the brown trout linkage map (Gharbi et al. 2005), covering 36 linkage groups

• STRUCTURE (Falush et al. 2003)
Non-admixed trout in strongly admixed populations?

- 7 individuals with > 90% admixture proportion of indigenous population
- Ripe in January-February, vs. early November-December for the majority of individuals
- Genetic basis of spawning time differences in salmonids
- Selection for early spawning in hatchery strain trout
- Spawning time differences has "rescued" a part of the indigenous population from admixture
Is selection against hatchery strain trout evident at the molecular level? (Hansen et al. (2010) Molecular Ecology

- **Principle**: genes under directional selection show higher differentiation ($F_{ST}$) among populations than neutral genes
- 61 microsatellite loci
- 9 EST-linked or QTLs (i.e. tightly linked to functional genes)
- Historical and contemporary samples from western Jutland populations
- Hatchery strain trout
- Hitch-hiking selection
Are footprints of selection evident at some of the loci?

- Hierarchical Island Model outlier test (Excoffier et al., 2009) – all samples
- 5 loci may be under diversifying hitch-hiking selection
- 2 (Ssa19NVH, CA060208 [EST-linked]) outliers in hatchery-wild comparisons
- CA060208 also outlier between Atlantic salmon populations from freshwater vs. salt water (Vasemägi et al. 2005)
Footprints of selection?

- $F_{ST}$ for outlier loci
- $F_{ST}$ and 99% confidence intervals for other loci

- Loci presumably under diversifying selection in historical wild trout vs. hatchery strain trout
Footprints of selection?

- $F_{ST}$ for outlier loci
- $F_{ST}$ and 99% confidence intervals for other loci

...and in contemporary wild trout vs. hatchery strain trout
Footprints of selection?

- $F_{ST}$ for outlier loci
- $F_{ST}$ and 99% confidence intervals for other loci

- .....but not in the strongly admixed Skjern R.
- In contrast to other trout populations, supportive breeding in SKJ uses permanent gene bank → trout are kept in hatchery for several generations

Ongoing selection to hatchery environment?
Insights into the genetic effects of stocking

• Stocking with hatchery strain trout must be considered detrimental and with limited effect in terms of rehabilitating populations.

• Indigenous populations show some resilience towards introgression by stocked trout.
  
  • *Number of stocked trout relative to the size of the wild population*

• Even in strongly admixed populations there may still be components of the original populations worth conserving.

• Selection against genetic material introduced by hatchery strain trout is evident at the DNA level.
Future perspectives - brown trout SNPs

- With Sigbjørn Lien & Matthew Kent, CIGENE, Norwegian University of Life Sciences and DTU Aqua

- Sequencing of ca. 80% of the genome of the brown trout

- 4,152 good single nucleotide polymorphisms - SNPs

- Linkage map constructed

- Identifying genes under selection among wild populations and between wild and hatchery trout
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