Guidelines for classification A. salmon, rainbow trout and cod

Kirsti Hjelde, Synnøve Helland, Ingrid Lein and Grete Baeverfjord, Nofima Marin

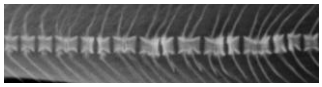
Classification manuals:

- Purpose: To give an overview of the most commonly observed skeletal deformities in farmed fish
- Species handled by our group: Atlantic salmon (S. salar), Rainbow trout (O. mykiss) and Atlantic cod (G. morhua)
- Includes presentations of the diagnostic methods available with protocols for each method (radiography, bone staining)
- Also includes recommended sampling, fixation and sending procedures based on experience from this and other projects
- Deformities are classified, described and illustrated by radiographs and photos of fish and stained skeletal structures

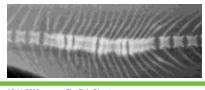
Deformities in Rainbow trout

Vertebral deformities: fusions

- Vertebral deformities in rainbow trout seem to be less differentiated than in other species, and all of them appear more or less like fusions
- The most commonly observed deformity in our materials
- Often complex or multiple

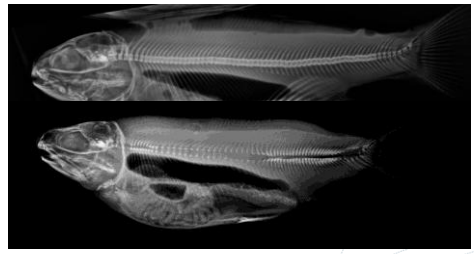


Area with complex and multiple vertebral fusions in rainbow trout (20g)



Complex vertebral fusion in rainbow trout (20g)

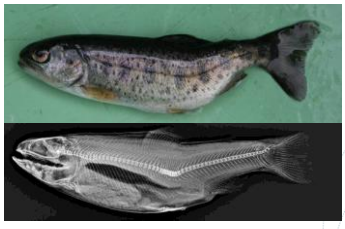
Severe vertebral deformities affecting the entire spine. Rainbow trout, 20g.



”Ghost”-like vertebrae with a non-mineralized centrum

Axial deviations: lordosis, kyphosis, scoliosis

•Axial deviations are more common in rainbow trout than in A. salmon



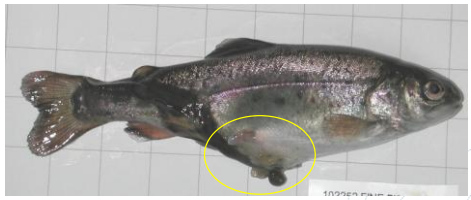
Lordosis with severe vertebral changes in rainbow trout 5g. Photo and x-ray of the same fish.

Severe kyphosis without much vertebral malformation, trout 5g.

Scoliosis in trout 2kg

Developmental malformations:

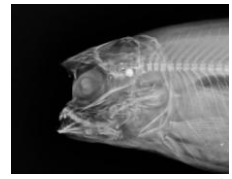
- Missing body parts, twin deformities, some head deformities, strictures
- Commonly observed in some groups of rainbow trout

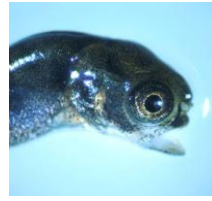


Twin deformity in 20g rainbow trout

Head deformities

Radiograph of severe upper jaw deformity in rainbow trout (20g)





”Pugnose”, severe upper jaw deformity in rainbow trout fry

Egg incubation temperature as a causal factor for malformations in rainbow trout

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The study was done as part of:
FINE FISH - Improving sustainability of European fish aquaculture by control of malformations (EU, COLL-CT-2005-012451)

Background and objective

Previous studies on effects of embryonic temperatures in Atlantic salmon and rainbow trout has shown that temperatures outside the optimum of the species can:

- ✓induce skeletal deformities
  - •fused or compressed vertebrae
  - •deformed skull
- ✓induce soft tissue deformities or deviations
  - •aplasia of septum transversum
  - •inverted abdominal organs
  - •reduced number of pyloric cacea

These studies were done with Norwegian eggs. Are the results valid also for other geographic strains?

Aim of the study

The present study aimed to investigate temperature tolerance of rainbow trout eggs of different geographic origins (north-south).

The temperature tolerance of triploids versus diploids was also addressed.

Effect parameters:
- •Survival
- •Growth
- •Skeletal deformities
- •Soft tissue deformities

Experimental setup

Pooled egg groups originating from 3 different genetic strains (1, 2 and 3) were fertilized and transported to Nofima, Norway in March 2007.

After fertilisation, half of the eggs originating from strain 2 were pressure treated to produce 3n individuals

The eggs were incubated in triplicates in small, insulated units equipped with individual inlets and outlets at 6, 10 and 14ºC from fertilization to first feeding

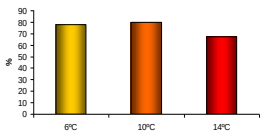
At first feeding two out of the three replicates were transferred to duplicate150 l tanks and reared at 12ºC

The experiment was terminated at approx. 40 g fish size

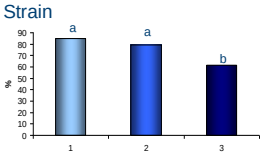
Experimental units for egg incubation

Survival from hatching to first feeding

Temperature

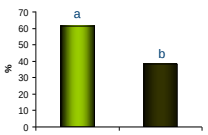


Strain



Ploidity

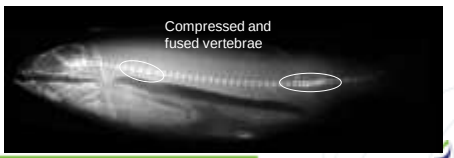
Diploid vs. Triploid, Strain 3



Examples of deformed vertebrae

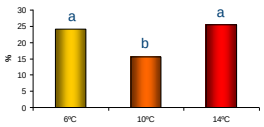
a. Rainbow trout with a normal vertebral column

b. Severely compression of the vertebral column in the caudal area



Fish with vertebral deformities

Temperature



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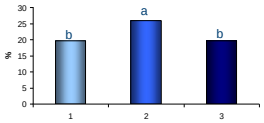

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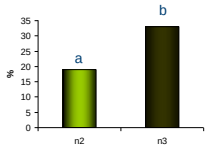
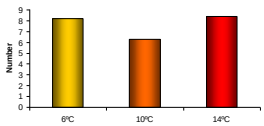
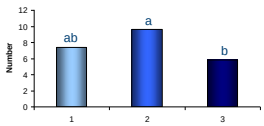


Number of deviant vertebrae per affected fish

Temperature



Strain



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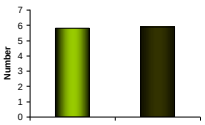
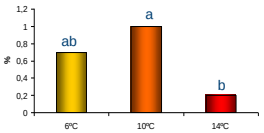


Fish with ”ghost” vertebrae

Temperature



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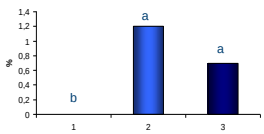

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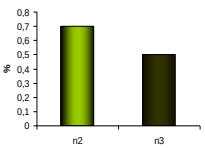


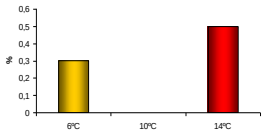
Examples of malformed skull or snoute

Short snote

Missing forehead

Fish with malformed snout/forehead

Temperature



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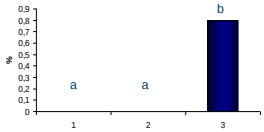

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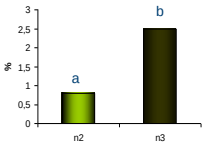
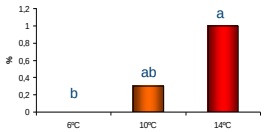


Example of twins

Incidence of fish with twins

Temperature



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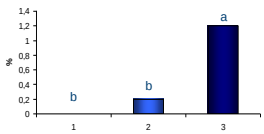

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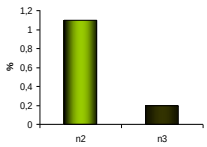
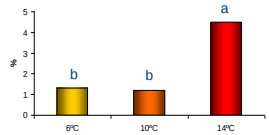


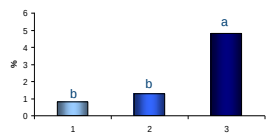
Example of swim bladder torsion

Fish with malformed swimbladder

Temperature



Strain



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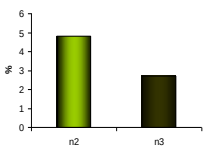
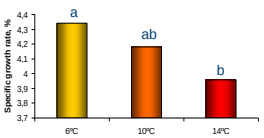
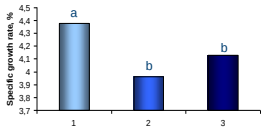


Specific growth rate, SGR (%)

Temperature



Strain



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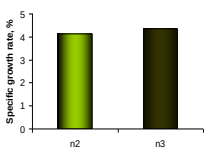
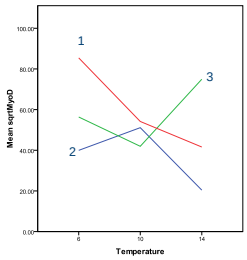


MyoD expression

The RVC (Prof. Neil Stickland) investigated the levels of MyoD gene expression in rainbow trout at first feeding.

It was hypothesised that each incubation temperature would result in a different optimal expression of MyoD from each strain dependant on the latitude of the strain's origin.

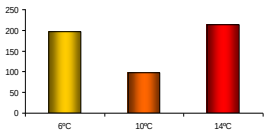
MyoD expression at first feeding



Severity index

(%fish with malformed vertebrae * # affected vertebrae)

Temperature



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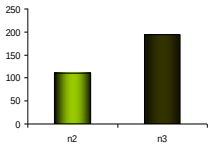
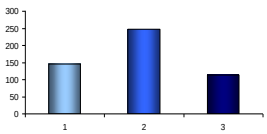


Strain



Conclusions/recommedations

- Main results:
  - 10ºC is the optimal egg incubation temperature
  - 14ºC is too high and
  - 6ºC is too low for rainbow trout eggs
- Relatively moderate effects on malformation rate between 8ºC and 12ºC (results from previous study)
- Results were basically the same for all strains, with some variation between strains for specific malformations
- There were differences between geographic strains in temperature induced malformations (NB: possible effects of egg group/spawning season)
- There were generally more malformations in the triploid groups compared to diploid groups

Impact of some dietary components on rainbow trout development

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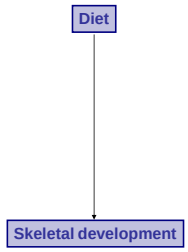
Impact of some dietary components on rainbow trout development

- **FINE FISH project**
  Improving sustainability of European fish aquaculture by control of malformations

- **Malformations**
  - **Skeletal deformities (spinal column, caudal fin, skull and operculae) ⇒ most important in terms of economy**
  - **Malformations of heart/other internal organs ⇒ less well recognized**
- **Numerous potential causes of skeletal deformities:**
  - **Three focus areas of research: rearing temperature, tank environment and nutrition**

Impact of some dietary components on skeletal development in fish



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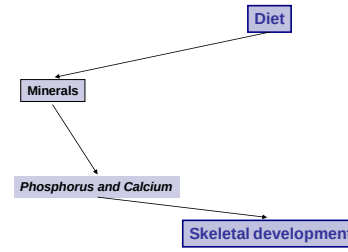

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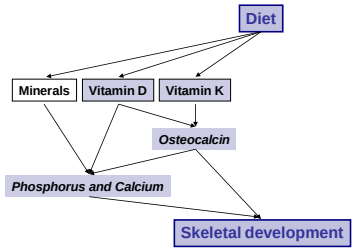

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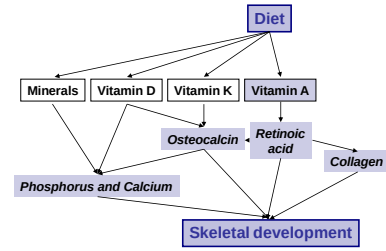

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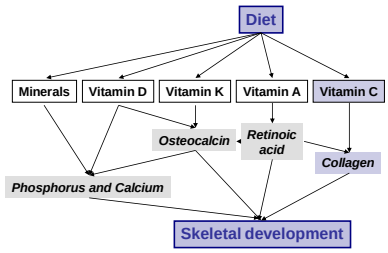

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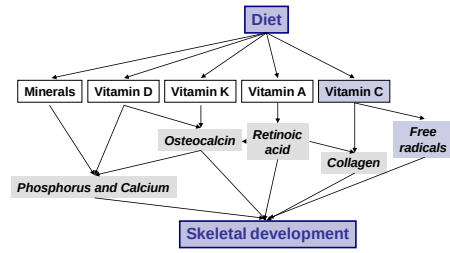

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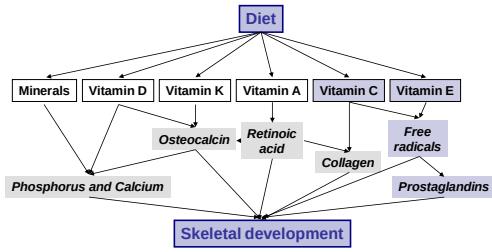

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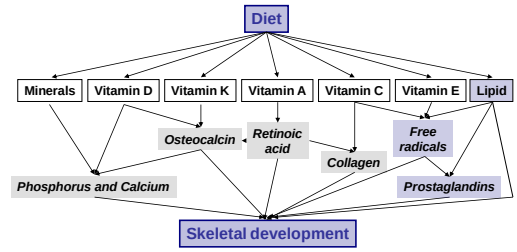

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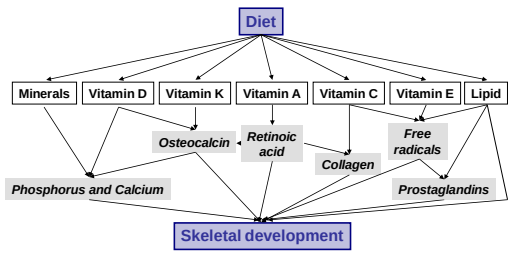
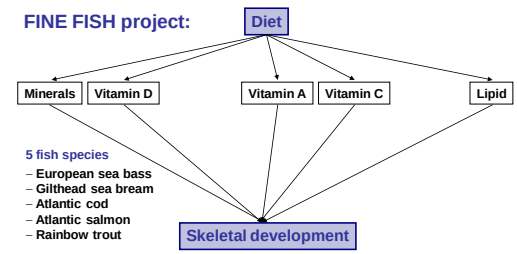


Impact of some dietary components on skeletal development in fish

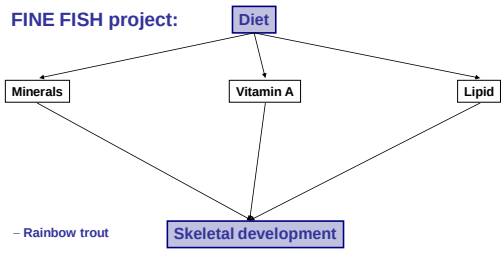
FINE FISH project:



5 fish species

- European sea bass
- Gilthead sea bream
- Atlantic cod
- Atlantic salmon
- Rainbow trout

Impact of some dietary components on skeletal development in fish



Impact of some dietary components on rainbow trout development

FINE FISH project:

– **Minerals (phosphorus and calcium)**
- Involved in the development/maintenance of skeletal system
- In fish feeds, availability can vary (plant/marine ingredients)

⇒ important to define their impact on the conformation of fish

– **Vitamin A**
- Vitamin required for reproduction but its metabolite, retinoic acid, is highly teratogenic

⇒ important to define the right level in broodstock diets

– **Lipid**
- Significant amounts in skeletal tissues including collagen which are particularly susceptible to lipid peroxidation

⇒ important to define their impact on the conformation of fish

Impact of dietary minerals on rainbow trout development

- **FineFish project: identify critical components of dietary minerals in juvenile rainbow trout**

➡ Assess the influence of dietary P and Ca levels on bone mineralization

- **FineFish experimental design**

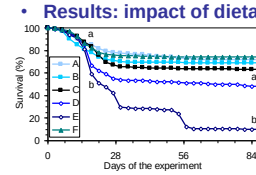
6 diets:

Diet	A	B	C	D	E	F
Available P	-	0.4	0.8	1.2	1.6	0.8
Available Ca	1	1	1	1	1	-

From first-feeding onwards, 12-week growth trial at 17 1 C

Impact of dietary minerals on rainbow trout development

- **Results: impact of dietary phosphorus level**



Lower survival of rainbow trout fry fed the high-P diet E

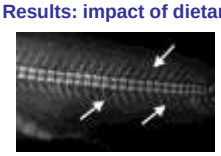
Impact of dietary minerals on rainbow trout development

- **Results: impact of dietary phosphorus level**

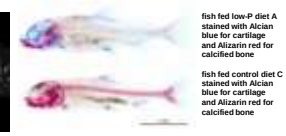
Lower skeletal mineralization in fish fed low-P diet A

Impact of dietary minerals on rainbow trout development

- **Results: impact of dietary phosphorus level**



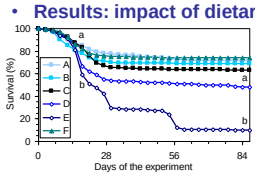
Higher incidence of abnormalities with irregular placement of vertebrae and twisted arches in fish fed low-P diet A



Lower skeletal mineralization in fish fed low-P diet A

Impact of dietary minerals on rainbow trout development

- **Results: impact of dietary phosphorus level**



fry fed low-P diet A stained with Alcian blue for cartilage and Alizarin red for calcified bone

fry fed control diet C stained with Alcian blue for cartilage and Alizarin red for calcified bone

Lower survival of rainbow trout fry fed the high-P diet E

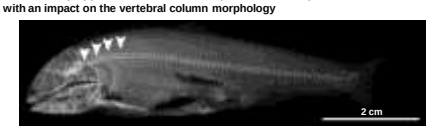
Lower skeletal mineralization in fry fed low-P diet A

➧ **Both dietary deficiency and excess of P are detrimental to rainbow trout fry development**

Impact of dietary minerals on rainbow trout development

- **Results: impact of dietary calcium level**
  - **No effect on growth performance and whole body composition**
  - **Effect on bone mineralization was less clear:**

Ca deficiency appears to exert some delay in ossification processes with an impact on the vertebral column morphology



Kyphosis in the cranial region with smaller vertebrae in fish fed low-Ca diet F

Impact of dietary minerals on rainbow trout development

- **Conclusion**

Importance of dietary mineral supply for adequate skeletal mineralization

Special attention should be paid to the level and availability of phosphorus in the formulation of diets for normal vertebral development during early ontogeny of rainbow trout (~1% available P)

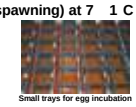
Impact of dietary vitamin A on rainbow trout development

- **FineFish project: assess the influence of vitamin A level in broodstock diets on trout fry development**
- **FineFish experimental design**

3 different dietary supplementations of vitamin A as retinyl acetate

Dietary supplementation ➡	Dietary level
0 IU/g diet in A1 ➡	20 IU/g in A1
20 IU/g diet in A2 ➡	40 IU/g in A2
200 IU/g diet in A3 ➡	200 IU/g in A3

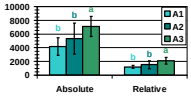
6-month trial (prior to spawning) at 7 1 C

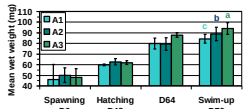


Small trays for egg incubation

Impact of dietary vitamin A on rainbow trout development

- **Results**





⇒ **Best fecundity, early growth and potential of muscle development with the highest level of vitamin A**

⇒ **Very low level of external malformations (<0.5%) in all groups of rainbow trout fry (approximately 5g)**

⇒ **Low impact of broodstock diets on skeletal development of trout fry (small delay in ossification processes in A3 group)**

Impact of dietary vitamin A on rainbow trout development

- **Conclusion**

High dietary level of vitamin A is recommended for rainbow trout broodstock (~ 200 IU/g)

⇒ **beneficial for reproduction and larval growth (no effect on skeletal development)**

Level of retinoic acid, the active metabolite of vitamin A, appears to be well controlled in eggs of rainbow trout (no difference between dietary groups contrary to other retinoids)

Impact of dietary lipids on rainbow trout development

Previous studies have highlighted the influence of dietary lipids on the incidence and development of malformations

- FineFish project: assess the impact of dietary lipids (phospholipid content and level of peroxidation) on rainbow trout fry development
- FineFish experimental design

6 diets:

Diet	Diet	R1	R2	R3	R4	R5	R6
Phospholipid source	Soybean lecithin	6%	6%	-	-	-	-
	Egg lecithin	-	-	6%	6%	-	-
	Soybean oil	-	-	-	-	6%	6%
Level of peroxidation	Fresh fish oil	12%	-	12%	-	12%	-
	Oxidised fish oil	-	12%	-	12%	-	12%

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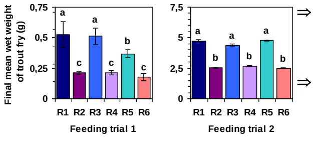
Impact of dietary lipids on rainbow trout development

- FineFish experimental design

From first-feeding onwards or 8 weeks after this stage
Two 4-week feeding trials at 17 1 C

- Results

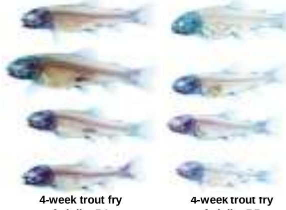


⇒ Decreased growth with diets R2, R4 and R6 containing oxidised lipid

⇒ Improved early growth with dietary phospholipid supplementation (R1-R3 vs. R5)

Impact of dietary lipids on rainbow trout development

- Results



4-week trout fry fed diet R1 with phospholipids

4-week trout fry fed diet R5 without phospholipids

⇒ Very low level of external malformations

⇒ Skeletal development of fish was not affected by dietary oxidised lipid

⇒ Lower mineralization in trout fry fed diet R5 without phospholipids

Impact of dietary lipids on rainbow trout development

- **Conclusion**

Special attention should be paid to the control of lipid peroxidation in fish feeds for normal growth of trout fry

⚠ **if feeds contain large amounts of polyunsaturated fatty acids**
⇒ correct supply of antioxidants

Importance of dietary phospholipid supply for early growth and adequate skeletal mineralization

phospholipid requirement for lipid absorption in early developmental stages (ratio dietary phospholipid/neutral lipid = 1/2)

Impact of some dietary components on rainbow trout development

- **Conclusion**

➞ nutritional components affect skeletal development

Compared to other fish species, in rainbow trout fry the impact of nutritional components on the development of external malformations is quite low

➞ "plasticity" of rainbow trout to different nutritional conditions

➞ at first-feeding, rainbow trout is well developed