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Growth and feed utilization in selected outbred families of rainbow trout *(Oncorhynchus mykiss)*

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Abstract

The main objective of this study was to investigate the relationship between the breeding progress and the breeding aims expressed by the growth rate and feed utilization of four families of rainbow trout reared under identical conditions.

The breeding strategy was family breeding: Fifty families were produced by mating individuals of 25 brood stock sires and 25 brood stock dams according to a partly factorial design. The experimental families were selected based on growth data from the parent generation. The families were half siblings two by two.

The four experimental fish families A1, B1, C1 and D1 studied in year 1 were half siblings two by two. The families C1 and D1 showed the significant highest performance in respect of SGR and FCR as well as family C1 had the most efficient conversion of the ingested protein into meat gain. This means that even the overall paternal traits for slow growth individuals from the offspring may be genetically determined for good growth performance.

The four experimental fish families A2, B2, C2 and D2 studied in year 2 were all full siblings. Family B2 demonstrated the lowest growth rate and the lowest utilization of the ingested feed compared to the other 3 families, which amongst them showed similar performances. Following, in this case the heredity for the trait of low growth rate was demonstrated.

The fish families A2 – D2 showed overall higher growth rates compared to the families A1 – D1. And further was the time to achieve the individual fish weight of about 600 g about 1 month shorter with the families of the second experiment (A2 – D2).
These differences in growth performance might be due to differences in rearing temperature. The first year experiment was run at an average temperature of 13.0 ± 1.2 ^{0}C while the second year experiment was run at 16.8 ± 0.8 ^{0}C due to technical circumstances.

However, the overall minor differences in growth performance (SGR and FCR) between the families may indicate that the genetic differences between the four families and in particularly between the genetically distant families were not that large.

Introduction

In Denmark rainbow trout (*Oncorhynchus mykiss*) has been farmed in fresh water for more than 100 years, and Denmark has been among the main producers of rainbow trout in the world (From, 1993). But since 1986 environmental restrictions has been put on the aquaculture industry limiting the annual production to the current level of about 33 000 metric tons (FAO). In contrast, the European production of rainbow trout has been increasing and is currently about 300 000 metric tons per year (FEAP).
However, breeding was acknowledged as a tool for more sustainable farming of rainbow trout. In order to improve the more sporadic breeding experiences made by individual farmers a systematic breeding program was initiated in the year 2000 at Danish Trout Breeding (DTB) – Jokumsen et al., 2001, Jokumsen, 2002.

The success of a breeding program is to a great extent dependent upon the amount of additive genetic variation for the traits in question within family groups of rainbow trout as well as favourable genetic correlations among these traits (Henryon et. al. 2002). Breeding objectives as improved growth and feed utilization expressed by the specific growth rate (SGR) and feed conversion ratio (FCR), respectively, are quantitative measures of productivity and efficiency in development of sustainable aquaculture production (Thodesen et al. 2001, Kolstad et al. 2004). Breeding programs are also valuable tools to control the age at sexual maturation and to improve resistance to specific diseases (Fjalestad et al 2003, Henryon et al., 2005). In farming of salmon breeding progress of 10 % per generation (4 years) in terms of growth rate and feed utilization has been reported (Gjedrem 2000).
The breeding goals of the Danish breeding programme were specific growth rate (SGR) and feed conversion ratio (FCR). Accordingly, the selection of brood stock from the families was related to improved growth and utilization of the feed.

The breeding strategy followed were family breeding crossing the individual males and female fish according to a factorial design. Based on growth and breeding data 8 families were selected for the growth and digestibility experiments and quality assessment.

In two successive years (2002 and 2003) growth studies were carried out with 4 selected families from the breeding station. The 4 selected families from the 2002 and the 2003 generation, respectively, were of similar age and size. The investigations included:

Specific Growth Rate (SGR)
Feed Conversion Ratio (FCR)
Weight/length relationships (condition factor)
Productive Protein Value (PPV)
Mortality

Aim

The primary aim of the study was to investigate the relationship between the breeding aims and the obtained breeding progress expressed in terms of specific growth rate (SGR) and Feed Conversion Ration (FCR) of market size rainbow trout (*Onchorhynchus mykiss*).

Materials and Methods

Facility

The experimental setup at DIFRES was based on recirculation technology with mechanical and biological filtration, and oxygenation of the water. The set-up consisted of 12 square formed fibre glass tanks. Each tank measured 1.2 x 1.2 x 0.9 metres and contained approximately 1,000 litres of water (fig. 1). From the outlet of the tanks the water was carried to a mechanical filter (drum filter) with a cloth width of 60 µ and passed on to a reservoir. From the reservoir the water was pumped to a submerged biofilter (up-flow) and afterwards carried to a trickling filter (aeration and degassing) and ended up in the reservoir. From the reservoir the water was pumped to the tanks in two lines. In one of the lines the water passed an oxygen cone for oxygenation with pure oxygen, while the aerated water in the other line was pumped directly to the tanks (cf. fig. 1). This design secured, that the oxygen content of the water in the tanks was regulated with almost no changes in the amount of water exchanged. The oxygen content of the water in the tanks was monitored by oxygen probes in the tanks and adjusted according to a given set-point to secure stabile oxygen conditions for the fish.

Daily measurements of temperature, pH and oxygen in each tank were carried out. The oxygen content was kept beyond 7 mg $O_2 * l^{-1}$ (70% saturation). The temperature was about 15 ºC and pH was in the range of 7.2-7.6. The temperature in the system was regulated by adjusting the air temperature and the water exchange. pH was regulated by addition of sodiumhydrogencarbonate.

Figure 1. Experimental facility. The fish tanks were covered with net to prevent the fish from escaping/mixing between tanks. Above each tank a pendulum feeding machine was installed. The water treatment/purification was taken place behind the wall in the background (right picture), i.e. mechanical filter (bottom left), biofilter (black tank right side), trickling filter (with cover) and oxygenation cone (bottom right corner).

Measurements of ammonia/ammonium (NH_3/NH_4^+), nitrite (NO^{2-}) and nitrate (NO_3^-) was carried out twice a week.

Light/dark conditions was predetermined by a timer to switch on at 7.50 a.m. and to switch off at 22.30 p.m., i.e. the light was on for 14 hours followed by 10 hours darkness.

An alarm system was connected to key parameters (oxygen, pumps, electricity, water level). In case of alarm an emergency oxygen plant was automatically activated supplying all the tanks with pure oxygen.

Fish

The Danish Trout Breeding (DTB) program has selected rainbow trout *(Onchorhynchus mykiss)* broodstock for several generations for high specific growth rates (SGR) and low feed conversion ratios (FCR, i.e. low feed intake to weight gain ratio). The original brood stock for this program were derived from two Danish trout farms, Mark Mølle Dambrug (Frueled 78, DK-7900 Nykøbing, Denmark) and Fousing Dambrug (Pilgårdsvej 6, Ølby, DK-7600 Struer, Denmark), where the fish had been kept as pure strains for at least 25 years. The breeding strategy was focussed on families, originally selected from 50 families produced by mating 25 sires and 25 dams using a partly factorial design (Berg and Henryon 1998; Henryon et al. 2002).
Each sire was mated to two dams, and each dam was mated to two sires, resulting in 50 full-sib families (i.e. 25 paternal and 25 maternal half-sib families). Specifically, sire 1 was mated to dams 1 and 2, sire 2 was mated to dams 2 and 3, and so on. The final sire, sire 25, was mated to dams 1 and 25.

The four experimental fish families (A1, B1, C1 and D1) for the first year study (2002) were selected based on growth data from the parent generation. The families were half siblings two by two, i.e. family A1 and family B1, respectively, had the same mother but different fathers, and similarly family C1 and D1. The paternal generation of families A1 and B1 showed traits for high specific growth rates, while the paternal generation of families C1 and D1 had performed with lower specific growth rates.

The four experimental fish families (A2, B2, C2 and D2) for the second year study (2003) were as well selected based on growth data from the parent generations. The four fish families in 2003 were all full siblings. However, family B2 was based on parents with low growth performance.

Following, the experiments were carried out with rainbow trout from eight different trout families in 2 successive generations/years (4 families/generation). About 600 randomly selected fish per family were transferred from DTB to the aquaculture facilities at DIFRES at the Department of Marine Ecology and Aquaculture in Hirtshals.

Diet

The experimental diets were a commercial feed type “GEP 576 Export” (Aller Aqua A/S). The feed was stored in cool room. The chemical analyses of the diets are given in table 1.

Table 1. Chemical analyses of the experimental diets "GEP 576 Export – 4 mm pellets" (Aller Aqua A/S). Declared values (prox. analyses) are indicated by *.

Aller Aqua A/S; GEP 576 Export	2002	2003
Raw protein (N*6.25), g/100 g	42,6	42,0
Raw fat (acidhydr.), g/100 g	26,4	27,4
Crude fibre, g/100 g	1,91	0,82
Ash, g/100 g	6,32	7,32
N-free extracts, g/100 g	15,9	18,1
Dry matter, g/100 g	93,1	95,6
Phosphorous, %	0,9*	0,9*
Digest. energy, MJ/kg	19,8*	19,8*
Gross energy, MJ/kg	23,9*	23,9*
Vitamin A, IU/kg	2.500*	2.500*
Vitamin D_3, IU/kg	500*	500*
Vitamin E, IU/kg	150*	150*
Etoxyquine, mg/kg	100*	100*

Feeding

The fish were fed ad libitum by pendulum demand feeders from 8.15 to 15.00 and uneaten pellets were removed and measured to calculate the daily amount of feed ingested.

Growth performance

The Specific Growth Rate (SGR) and the Feed Conversion Ratio (FCR) was calculated based on measurements every third week. SGR were calculated according to the formula: $SGR = (\ln W_2 - \ln W_1) \cdot (t_2 - t_1)^{-1}$, where W_2 and W_1 was the total weight of all the fish at the end (t_2) and at the beginning (t_1) of the growth period. FCR was calculated as the ratio between the amount of feed ingested and the fish weight gain according to the formula: FCR = Feed ingested (kg) · (fish weight gain (kg))$^{-1}$.

The utilization of the dietary protein was expressed by the Productive Protein Value (PPV), which is defined as: $PPV = (B_2 - B_1)/I$, where B_1 and B_2 was the initial and the final protein content of the experimental fish and I was the amount of ingested dietary protein. Accordingly, PPV expressed the fraction of ingested dietary protein, which was converted to body protein in the fish.

At the start and at the end of the growth experiment 100 fish from each family were randomly sampled and weighed and measured for estimation of average individual size and the Condition Factor (CF). CF was calculated according to the formula: $CF = W/L^3 * 100$, where W = fish weight (g) and L = fish length (cm).

Statistics

Data of SGR and FCR from the four families were assessed for homogeneity of variance using Bartlett's test and for normality using Lillierfors test . When conditions for a parametric test was fulfilled, a One-Way ANOVA was used, other wise the non-parametric Kruskal-Wallis test was used. When F values showed significant differences among the mean values, Bonferroni's Multiple Comparison Test was used to detect significant differences among the means (Wilkinson, 1990; Zar, 1984).

Results

Experiment 1

The specific growth rate (SGR) and the feed conversion ratio (FCR) during the first experimental period (15^{td} October – 2002 to 20th February 2003 -128 days) is shown in table 2 and fig. 2. The daily fish growth within the families ranged from 1.64 (families B1 and C1) to 1.78 % · day^{-1} (family D1). The significantly highest SGR was expressed in family D1 (P<0,05). The feed utilization expressed by the FCR ranged from 0.89 (family C1) to 0.96 kg feed · kg^{-1} fish weight gain (family A1). Family C1 had the significantly most effective conversion of feed into growth (P<0.05) compared to the other families. However, splitting up the data for every third week the measurements indicated, that families A1 and B1 showed the highest growth performance during the first 9 weeks, while families C1 and D1 to some extent did catch up during the last 9 weeks.

Table 2. Specific growth rate (SGR) and feed conversion ratio (FCR) measured on the four families of rainbow trout between 15 October 2002 and 20 February 2003. Families A1 and B1 and families C1 and D1 were half siblings. Figures indicated with different sup scripts were significantly different (p<0,05).

	Family			
	A1	B1	C1	D1
SGR [% · day^{-1}]*	1.66^{a}	1.64^{a}	1.64^{a}	1.78^{b}
FCR**	0.96^{a}	0.94^{a}	0.89^{b}	0.93^{a}

* Kruskal-Wallis test
** One-Way ANOVA

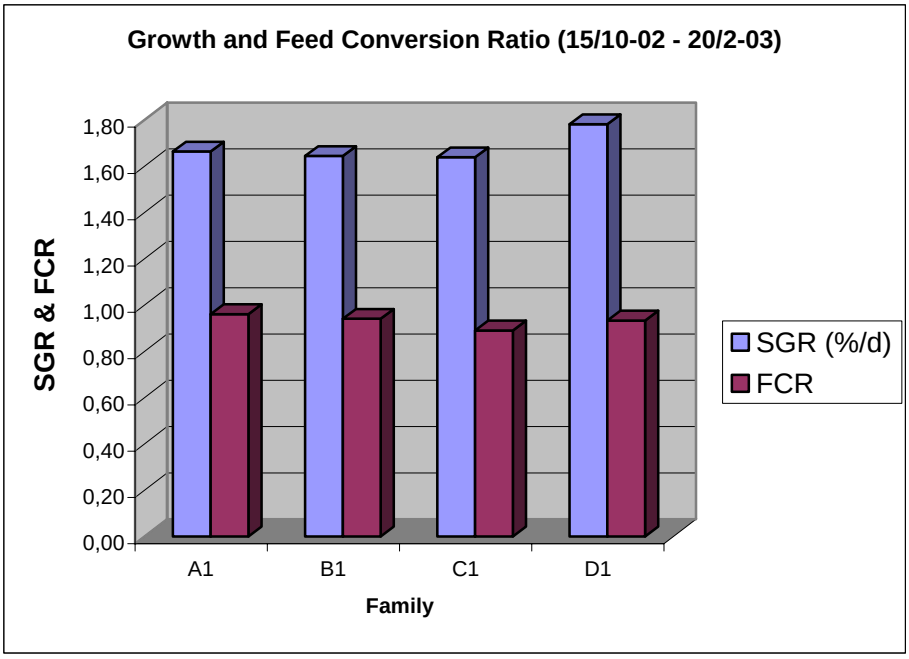

Fig 2. Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) measured on the four families of rainbow trout between 15 October 2002 and 20 February 2003.

There were only minor differences in the utilization of protein between the families as expressed by PPV (cf. table 3). While 40% of the ingested protein was converted into meat in family A1, 44%

was measured as protein gain in family C1 (cf. table 3). This result reflected the finding that family C1 had the significantly most effective conversion of feed into growth compared to the other families (cf. table 2).

Table 3. Productive Protein Value (PPV) ± standard deviation (STD) measured on the four families of rainbow trout between 15 October 2002 and 20 February 2003.

	Family			
	A1	B1	C1	D1
Prod. Prot. Value (PPV)	0.40 ± 0.01	0.41 ± 0.02	0.44 ± 0.01	0.42 ± 0.01

The initial individual weight of the fish was about 55 g each, while the final individual fish weight varied from 590 g to 661 g (cf. table 4).
The average initial Condition Factor (CF) for the four families was 1.4. By the end of the experiment the CF was 1.7 for the families A1, B1 and C1, respectively and 1.9 for family D1 (table 4).

Table 4. Average body weight, body length and Condition Factor ± standard deviation (STD) of about 100 individual fish per family at the start and at the end of the growth experiment.

	Family			
	A1	B1	C1	D1
Init. weight (g/pcs)	55.5 ± 11.0	56.9 ± 11.6	54.6 ± 10.9	55.0 ± 12.4
Init. length (cm)	15.8 ± 1.0	16.1 ± 1.1	15.6 ± 1.0	15.8 ± 4.2
Init. cond. Factor	1.4 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.5 ± 0.2
Final weight (g/pcs)	607.6 ± 110.7	590.8 ± 144.5	606.9 ± 126.3	661.6 ± 142.9
Final length (cm)	32.8 ± 1.8	32.8 ± 2.3	32.8 ± 2.2	32.8 ± 2.3
Final cond. Factor	1.7 ± 0.1	1.7 ± 0.3	1.7 ± 0.1	1.9 ± 0.1

Experiment 2

The specific growth rate (SGR) and the feed conversion ratio (FCR) during the second experimental period (13^{td} October – 2003 to 6th January 2004) is shown in table 5 and fig. 3. The daily fish growth within the families ranged from 2.16 (family B2) to 2.41 % · day^{-1} (family C2). The feed utilization expressed by the FCR ranged from 0.90 (families C2 and D2) to 1.00 kg feed · kg^{-1} fish weight gain (family B2). However, splitting up the data for every third week the measurements indicated, that the differences in growth performance was currently uniform. However, during the last period the differences became less significant between family B2 and the other families. This might be due to fact, that the family B2 fish due to slower specific growth rate were smaller than the fish in the other families.

Table 5. Specific growth rate (SGR) and feed conversion ratio (FCR) measured on the four full sib families of rainbow trout between 13 October 2003 and 6th January 2004.

	Family			
	A2	B2	C2	D2
SGR [% · day^{-1}]	2.31	2.16	2.41	2.39
FCR	0.92	1.00	0.90	0.90

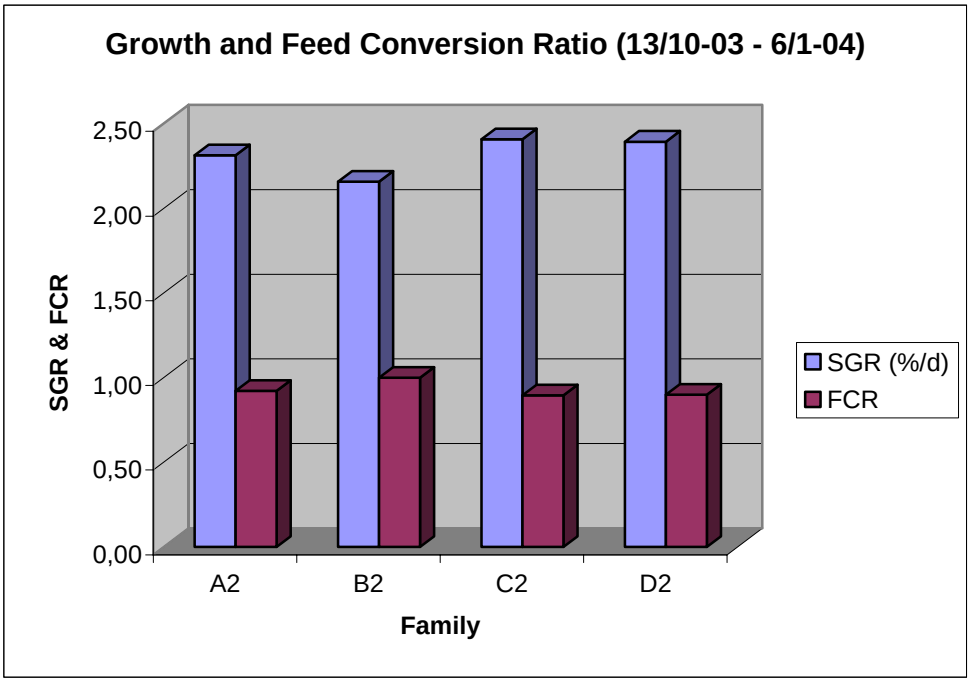

Fig 3. Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) measured on the four families of rainbow trout between 13 October 2003 and 6 January 2004.

The initial individual weight of the fish was about 90 g (85.2 – 99.4 g) each, while the final individual fish weight varied from 482.0 g to 672.4 g (cf. table 6).
The average initial Condition Factor (CF) for the four families was 1.4 (1.3 – 1.5). By the end of the experiment the CF was 1.7 (family A2 and D2), 1.8 (family C2) and 1.9 (family B2), respectively – cf. table 6.

Table 6. Average body weight, body Length and Condition Factor ± standard deviation (STD) of about 100 individual fish per family at the start and at the end of the growth experiment.

	Family			
	A2	B2	C2	D2
Init. weight (g/pcs)	90.8 ± 13.3	85.2 ± 20.6	99.4 ± 14.7	90.2 ± 19.8
Init. length (cm)	18.8 ± 1.2	17.6 ± 1.5	19.2 ± 1.0	18.9 ± 1.4
Init. cond. factor	1.4 ± 0.1	1.5 ± 0.09	1.4 ± 0.07	1.3 ± 0.07
Final weight (g/pcs)	584.4 ± 104.9	482.0 ± 99.2	672.4 ± 103.3	635.2 ± 104.9
Final length (cm)	32.5 ± 1.9	29.4 ± 2.0	33.2 ± 1.9	33.3 ± 1.9
Final cond. factor	1.7 ± 0.1	1.9 ± 0.2	1.8 ± 0.1	1.7 ± 0.1

Discussion

The four experimental fish families A1, B1, C1 and D1 were half siblings two by two. The paternal generation of families A1 and B1 had shown relative high growth rates, while the paternal generation of families C1 and D1 had performed with lower specific growth rates.
However, in this study the families C1 and D1 showed the significant highest performance in respect of SGR and FCR as well as family C1 had the most efficient conversion of the ingested protein into meat gain. This means that even the overall paternal traits for slow growth individuals from the offspring may be geneticaly determined for good growth performance.

The four experimental fish families A2, B2, C2 and D2 were all full siblings. The parental generations had shown relative good growth except the parental generation of family B2, which had shown low growth performance.
In this study family B2 demonstrated the lowest growth rate and the lowest utilization of the ingested feed compared to the other 3 families, which amongst them showed similar performances. Following, in this case the heredity for the trait of low growth rate was demonstrated.

The fish families A2 – D2 showed overall higher growth rates compared to the families A1 – D1. And further was the time to achieve the individual fish weight of about 600 g about 1 month shorter with the families of the second experiment (A2 – D2).
These differences in growth performance might be due to differences in rearing temperature. The first year experiment was run at an average temperature of $13.0 \pm 1.2\ ^{0}C$ while the second year experiment was run at $16.8 \pm 0.8\ ^{0}C$ due to technical circumstances.

The four families were reared under identical conditions and although differences between rearing tanks can not be ignored, the differences between the investigated families were most probably due to genetic differences. However, the overall minor differences in growth performance (SGR and FCR) between the families may indicate that the genetic differences between the families and in particularly between the genetically distant families were not that large. However, the genetic variability among farmed salmon is believed to be less compared to wild salmonid populations (Was & Wenne, 2002), and the genetic variation was further found to be less between strains of rainbow trout than between families of rainbow trout within the same strain (Henryon et al., 2002).

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Physiological correlates of diversity in size-at-age and condition factor in rainbow trout (*Oncorhynchus mykiss)* families

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Abstract
Aspects of energetics and cardiorespiratory performance were investigated in two farmed rainbow trout families that differed in size at age (SA) and condition factor (CF), two morphological traits used in breeding programs. Five groups of a family with large SA (LSAF) and six groups of a family with smaller SA (SSAF) were reared in tank respirometers in freshwater at 14^{o}C for 84 days. The LSAF exhibited higher rates of mass gain during the trial, growing from a mean (± SD) mass of 182 ± 6 g to 449 ± 24 g, compared with 77 ± 4 g to 307 ± 22 g in the SSAF, and the LSAF had a higher lifetime specific growth rate (SGR).
When compared over a mean mass interval of approximately 180 g to 300 g, however, the LSAF exhibited lower SGR than the SSAF. This was contrary to expectations and a result of lower daily rates of feed intake coupled with higher metabolic rates in the LSAF during daylight feeding hours, this latter apparently due to increased spontaneous activity. Thus, the higher lifetime SGR in the LSAF presumably reflected rapid growth at earlier life stages, and a large familial SA may bring a tendency to increased aggressive behavioural interactions as fish approach marketable size. Instantaneous fluxes of O2, CO2 and waste nitrogen in the tank respirometers immediately after feeding revealed that lipid fuelled over 50 % of metabolism, protein approximately 40% and carbohydrates less than 10% in the families. When, however, feed had been withheld for 24h, protein fuelled less than 20% of metabolism and carbohydrate increased to over 20%. The LSAF exhibited higher critical swimming speeds, maximum metabolic rates and aerobic metabolic scopes than the SSAF, indicating that selecting broodstock for large SA does not necessarily compromise functional integrity. The SSAF had a more rounded ventricular morphology than the LSAF, and also a higher CF. These results are consistent with other literature reports whereby familial CF in farmed trout is an indicator of ventricular morphology and cardiorespiratory performance.

Introduction
This part of the project investigated physiological correlates of the intrinsic diversities in specific growth rates (SGR) and feed conversion ratios (FCR), comparing two farmed rainbow trout families that differed in size at age (SA) and condition factor (CF), two morphological traits used in breeding programs. In other words how specific growth and feed conversion ratio may be related to nutrient utilisation and physiological performances.

The current study used techniques of respiratory physiology to compare the physiological energetics and functional integrity of a rainbow trout family with a large SA (LSAF) against a family with a smaller SA (SSAF), using families derived from the Danish Trout Breeding (DTB) program. In order to permit direct comparisons of size-matched fish, data were collected upon the LSAF first, and then upon the SSAF when they had grown to achieve the initial mean mass of the LSAF.

Measurements of instantaneous metabolic rate were made on groups of fish in their rearing tanks, using automated intermittent stop-flow respirometry (Steffensen 1989; McKenzie et al. 1995), and then compared with their growth and their feed intake, to provide a measure of relative energetic efficiency in the two families. The utilisation of lipids, proteins and carbohydrates as metabolic fuels was compared in the two families by analysis of instantaneous fluxes of O_2, CO_2 and waste N (Lauff and Wood 1996a,b; Wood 2001) in the growing feeding fish in their rearing tanks. The relative capacities to perform sustained aerobic exercise and to tolerate progressive hypoxia were measured in individuals from each family, as indicators of functional integrity of the cardiorespiratory system (Stevens et al. 1998; McKenzie et al. 2003; Claireaux et al. 2005). Finally, these indicators of cardiorespiratory performance were compared to the CF and ventricular morphology of the two families (Claireaux et al. 2005).

Aim

The aim of the project was to investigate the relationship between specific growth rate and feed conversion ratio, and nutrient utilisation and physiological performances in two families of rainbow trout.

Materials and methods

Facility

The in-vivo respirometry measurements were carried out at DIFRES, Hirtshals, i.e. primarily oxygen consumption, which is stoichiometrically related to metabolic energy expenditure in animals, so such measurements describe metabolic rate. Twelve large tank-respirometers measured the metabolic rate of the growing groups of fish, within a recirculating biofilter system regulated at a fixed water temperature.

The respirometers functioned on the principle of "intermittent stop-flow respirometry" where there was alternation between periods where the respirometers received no water flow (were "closed") and periods where they received a flow of aerated water from a biofilter. When the respirometers were closed, the fish consumed the oxygen, which was recorded by a PC. The oxygen was then replenished with the flow of aerated water. This was all controlled by custom-made software, and allows many repeated measures to be made over time with no interference by experimenters.

Measurements of metabolic rate was used to define energy budgets, comparing energy intake (feed) to energy retention (growth) and energy dissipation (metabolic rate). Measurements of oxygen consumption were also compared to simultaneous analysis of carbon dioxide and ammonia excretion (these latter performed "by hand") to investigate relative utilisation of dietary nutrients as energetic fuels (protein, lipid or carbohydrate). These measures may provide insight into why trout families grow differently.

To obtain more detailed information about physiological traits of metabolism, performance and stress-tolerance in the two trout families respirometry experiments were conducted on individual fish exercised at controlled rates, using similar automated "stop-flow" techniques.

The fish rearing system was a closed-cycle recirculating one, with only limited water replacement. The fish holding tanks were 12 circular 1 metre diameter tanks (water volume approximately 650 litres). The tanks were provided with a flow of aerated freshwater at a temperature of $14 \pm 0.1\ ^{o}C$, within a recirculating biofiltered system (total water volume approximately 13.5 m^3, 10% replacement by volume with fresh Hirtshals tapwater daily). Photoperiod was maintained at 14h light to 10h dark (lights on at 07:00) throughout the rearing trial.

Each of these had two Grundfos pumps. One run continuously circulating and mixing the water in the tank. A smaller pump run intermittently. This small pump drew aerated water from the bottom of the trickle filter into each tank. The intermittent activity of these pumps was the basis for the respirometry system.
Each tank had a central pillar, such that water circulated around this. Below this pillar was a drain, connected to a large tube on the side of the tank. The circular water current drove uneaten feed and faeces into this central drain, and the large tube could be lowered to the ground to flush this out onto the floor.
Each tank had an alarm oxyguard, an alarm oxygen supply and an air-supply.

The biofilter consisted of 2 large circular 2.2 metre diameter biofilter tanks (1 submerged and 1 trickle). Water entered the bottom of the submerged filter, it then flowed into the other filter tank, where it cascaded through the trickle filter. Water levels in the trickle filter were regulated by a floater, which replaced any water lost from the entire recirculating system. The trickle filter also had a low-water alarm controlled by another floater.
Water run continuously between these two biofilters, driven by a large submersible Grundfos pump in a separate tank positioned in front of the biofilters. This tank received water from the bottom of the trickle filter, but also from the fish-holding tanks. The tank also contained a second emergency pump which was controlled by a floater valve.

The water temperature was controlled with a cooling coil in a large cooling tank behind the biofilters. Water was delivered to this tank from the bottom of the trickle filter, by three large submersible Eheim pumps. A temperature probe in the trickle filter was connected to a thermostat, which regulated the activity of the cooler.

An alarm was installed regarding key parameters (water level, electric failure, oxygen, temperature). In case of alarm (except temp.) emergency oxygen was activated to all tanks.

Fish
The original breeding material from DTB provided the broodstock for the current study, maintained at the Trehøje Dambrug trout farm (Godthåbvej 10, DK-8766 Nørre Snede, Denmark), which is a participant in the DTB program. The LSAF (ID no. 2004060DDT) were the progeny of a cross between mother DTB ID no. 2070 (grown up from DTB family 2002041 and father DTB ID no. 2095 (grown up from DTB family 200245.
The SSAF (ID no. 2004100TT) were the progeny of a cross between mother DTB ID no. 3807and father DTB ID no. 3818. All animals were first-time breeders.

The crosses were performed on 02/02/2005 and fertilized eggs were incubated in separate iodophor-disinfected hatching trays supplied with a constant flow of aerated ground water at 9-10 ^{o}C. After 20 days at this temperature (approximately 180 degree-days) the eggs had reached the eyed stage and were then disinfected with Actomar K30 and replaced back into disinfected hatching trays. Hatching started on 16/03/2005 (at day 39 from fertilisation, approximately 350 degree-days). The fry started exogenous feeding at approximately two weeks following hatch, on fine dry feed. One week later, the fry were transferred to 1 m^3 raceways supplied with a constant flow of groundwater. They were fed to satiation daily, with waste feed and dead fish removed each day.

On 14/06/2005, the LSAF had attained a mean mass of approximately 110g per fish whereas the SSAF had a mean mass of approximately 50g per fish. The two families were transported to the

DIFRES facilities at the North Sea Centre and distributed, in groups of 50 to 55 individuals, amongst the twelve circular polythelyene holding tanks such that each family occupied six of the tanks in the system.

Feed

The fish were fed to satiation daily with a commercial extruded 3 mm pellet feed (Ecolife 19, BioMar A/S, Brande, Denmark) on belt-feeders, between 08:00 and 14:30 each day (Table 1). At 15:00 each day, uneaten pellets were collected from a central drain in the bottom of the tank, to allow calculation of daily feed intake. Two batches were used during the course of the experiments, a first batch during the studies upon the LSAF and then a second batch during the studies upon the SSAF. The composition of these two feed batches is given in Table 2, proximate composition was analysed according to Danish Standards by the Technological Institute, Kolding (DK). Feed intake was converted into total energy intake using total energy content per unit mass of feed (Table 2) as measured by bomb calorimetry (IKA C7000 Calorimeter).

Table 1. Declared composition of the experimental diet "Ecoline 19 - 3 mm pellets" (BioMar A/S).

Declaration	**g/100 g (%)**
Fish meal	42
Wheat	14
Fish oil	13
Wheat gluten	12
Soya beans	8
Rap seed oil	6
Soya Prot. Conc.	5

Table 2. Proximate composition and total energy content of the two batches of feed (Biomar Ecolife 19, 3mm extruded pellets) used during the studies of growth and energetics performed sequentially upon the large size at age family (LSAF) followed by the small size at age family (SSAF).

	LSAF	SSAF
Feed batch n.	73038	74196
Water (%)	7.0	8.4
Total protein (%)	48.3	47.0
Total fat (%)	23.8	23.2
Total carbohydrates (%)	10.8	11.2
Fibre (%)	2.4	2.1
Ash (%)	7.8	8.0
Total energy (kJ•g wet weight^{-1})	22.7	21.9

Feeding

The fish were fed ad libitum using clock belt feeders. The feed for each tank was weighed and put into the feeders in the morning (8.30) – and the feeders were adjusted to run out at 14.30. – The tanks were flushed into a net and uneaten pellets were counted to calculate the amount of ingested feed.

Biometrics and specific growth rates

Fish mass was measured over four 21-day intervals, each interval comprising 19 feeding days then 1 day when feed was withheld prior to the day on which the mass of the animals was measured (fish were not fed on this day either). The total biomass of each tank was measured, and then the animals counted to derive a mean mass for the fish. A subsample of 10 fish from each tank were lightly anaesthetised in 50 mg•l^{-1} of tricaine methane sulphonate (MS-222) and their individual mass and forklength recorded. Once the weighing was completed and fish returned to their tanks, current speed was adjusted to reflect the new estimate of mean fish length for each tank. Feeding was resumed the following day at 08:00. Condition factor (CF) at each measurement interval was calculated on the sub-sample from each tank, as:

$$CF = 100 \times (\text{fish mass}/\text{fish length}^3). \quad (1)$$

A mean fish mass for each family was calculated from the individual tank data, and plotted against time (in days) to identify periods during the 84-day rearing trial when the two families had similar masses. These periods were then used to compare specific growth rate (SGR), energetics, exercise performance and hypoxia tolerance in size-matched animals, as described below. Thus, the LSAF was studied first, and the SSAF was then studied when they had grown to achieve the initial mean mass of the LSAF. The SGR for this interval was calculated for each tank, based upon the mean fish mass, as:

$$SGR = 100 \times (\exp((\ln \text{final weight} - \ln \text{initial weight})/\text{number of days})-1) \quad (2)$$

Only feeding days were considered in the calculation of this SGR. The final mean mass at the end of the trial was used to calculate a "lifetime SGR" for each family, where the lifetime was considered to have started on the day the broodstock crosses were performed, and the families both to have started with a theoretical initial mass of zero.

At the end of the trial, 12 individuals from each family were sampled at random, rapidly anaesthetised (200 mg•l^{-1} MS-222), and killed with a blow to the head. Their mass and length were recorded, to calculate their CF, and then their entire hearts, from auricle to bulbus arteriosus, were dissected out and fixed in a solution of 2.5 % glutaraldehyde in phosphate-buffered saline. Forty eight hours later, heart morphology was assessed as the ratio of the height versus the width of the ventricle, as described in Claireaux et al. (2005). Thus, the fixed hearts were oriented in a standard manner and then photographed under a binocular microscope fitted with a digital camera and then the height and length of the ventricle measured to the nearest 0.01 mm with dedicated imaging software.

Metabolic rates of the growing fish

The metabolic rate of the fish in each tank was measured as rates of O_2 uptake. The rearing tanks were designed to measure instantaneous rates of O_2 uptake by each entire tank with techniques of automated stop-flow respirometry. Briefly, the system alternated periods of closed recirculation of the rearing tank with periods when the activation of a second pump flushed the tank with a low-pressure flow of aerated biofiltered water. An O_2 electrode recorded the linear decline in O_2 concentration (mg•l^{-1}) during the period of closed recirculation and the variations in P_{O2} were acquired every one second and stored by a PC and Labtech Notebook software. Water P_{O2} was never allowed to decline below 70% of full saturation during the periods of recirculation. The timing of the stop-flow system was adjusted so that the flushing pump was active for 50 min of every hour and so that the 10 min of closed recirculation, when O_2 uptake by the fish was measured,

fell “on the hour”. Thus, measurements were collected each hour of every day. The software only recorded water P_{O2} during the last 7 min of the 10 min recirculation period, and saved these data as text files.
Oxygen uptake by the fish (M_{O2}) was then calculated, in mmol•kg-1•h-1, from the decline in water O2 concentration and considering the total volume of water and the total biomass of the fish (Steffensen 1989; McKenzie et al. 1995).

Respiratory quotients and instantaneous fuel usage
On the last feeding day of the trial, spot measurements were made of the instantaneous excretion of CO_2 and nitrogenous wastes (ammonia and urea), simultaneous to the automated measures of instantaneous O_2 uptake, to permit the calculation of respiratory quotients and derive patterns of instantaneous fuel usage (Lauff and Wood 1996a,b; Wood 2001).
The respiratory quotient (RQ), was calculated as M_{CO2}/M_{O2} and the nitrogen quotient (NQ) as M_N/M_{O2}, where M_N is total nitrogen excretion (M_{Tamm} plus $M_{urea\text{-}N}$). Instantaneous fuel usage, the relative fraction of total fuels burned arising from protein, carbohydrate and lipid, was then determined exactly as described for rainbow trout in Lauff and Wood (1996a,b) and Kieffer et al. (1998). The known RQs for lipid (0.71) and carbohydrate (1.00) can then be used to factor out the relative contributions of these two fuel sources.

Sustained aerobic exercise performance
Individual trout from each family were starved in darkened plexiglass boxes provided with a flow of water at 14^{o}C for 48h prior to respirometry, to avoid any confounding effects of digestion on metabolic rate or exercise performance. Swimming respirometry was performed with a stainless steel swim-tunnel respirometer (total water vol. 48 l) and designed to exercise individual fish in a non-turbulent water flow with a uniform velocity profile. Water flow was generated by a thermoplastic composite propeller downstream of the swim chamber, attached to a variable speed electric low inertia brushless servo-motor. The respirometer was thermostatted by immersion in a large outer stainless steel tank that received a flow of aerated water. Instantaneous M_{O2} was measured by intermittent stop-flow respirometry with an oxygen electrode, which recorded O_2 partial pressures (pO_2) during periods of closed recirculation and an automated data acquisition system.

Tolerance of hypoxia
Individual trout from each family were starved in darkened plexiglass boxes provided with a flow of water at 14^{o}C for 48h prior to the hypoxia tolerance studies. The chambers were immersed in a large outer tank of normoxic water, and the fish were allowed to recover and acclimate overnight (at least 14 hours). Routine metabolic rate (RMR) was measured in normoxia for at least 1h, then water PO_2 was reduced from normoxia (PO_2 = approximately 18 kPa or 140 mmHg) to less than 2 kPa (15 mmHg) in 8 steps over a 2h period, by bubbling 100% N_2 into the outer tank.
The critical PO_2 below which the trout could no longer regulate routine M_{O2} (P_{crit}), was calculated for each fish by plotting M_{O2} against PO_2, then drawing a line parallel to the abscissa at the routine rates of O_2 uptake measured in normoxia following overnight recovery. A least-squares linear regression was applied to those data points lying below each line, and the resultant equations used to calculate the P_{crit} at the appropriate M_{O2}.

Statistical analysis
Single measured variables (e.g. P_{crit}) were compared between the two families by t-test. Variables that were measured repeatedly in size-matched animals (e.g. daily rates of O_2 uptake in the tanks) were compared between the families by two-way analysis of variance (ANOVA) for repeated

samples, where family was the main factor and the repeated variable (e.g. time interval or increments in mean fish mass) was the interacting factor. Holm-Sidak post-hoc tests were then used to identify where any significant differences in the ANOVA had occurred. In all cases, $p < 0.05$ was taken as the fiducial level for statistical significance.

Results

Mortality rates through "natural" causes were very low during the rearing trial, being less than 1%. However, on day 31 of the trial a breakdown to a flushing pump caused over 50% mortality in one tank of the LSAF, as a result of the ensuing severe hypoxia. This tank was, therefore, removed from the trial, such that data were considered for only five tanks of the LSAF.

Biometrics and Specific Growth Rates

The LSAF gained more mass than the SSAF during the rearing trial, growing from a mean (± SD) initial mass of 182 ± 6 g to a final mass of 449 ± 24 g (n = 5 tanks), compared with 77 ± 4 g to 307 ± 22 g (n = 6 tanks) in the SSAF. This translated into a significantly higher mean (± SE) daily rate of gain in mass, being 3.17 ± 0.12 g•d^{-1} in the LSAF compared with 2.73 ± 0.11 g•d^{-1} in the SSAF. The lifetime daily rate of gain in mass was significantly higher in the LSAF, being 1.73 ± 0.05 g•d^{-1} as compared with 1.19 ± 0.04 g•d^{-1} in the SSAF and, at the end of the trial, the LSAF had a lifetime SGR of 2.38 ± 0.01, significantly higher than the lifetime SGR of 2.23 ± 0.01 measured in the SSAF family.

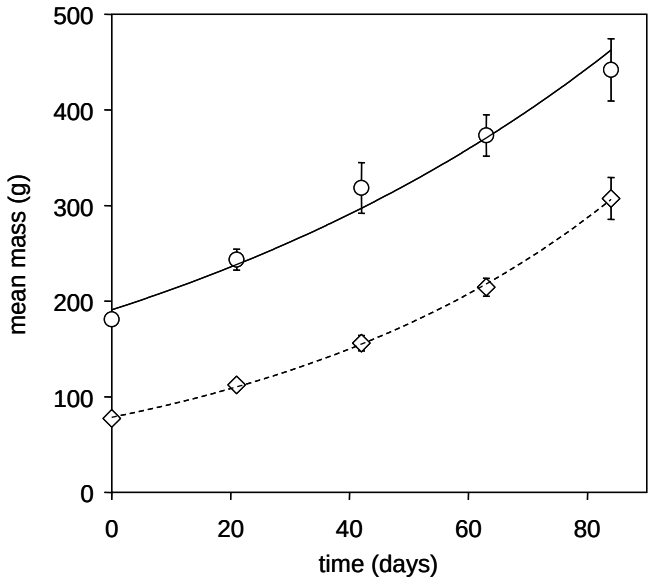

Figure 1. Changes in mean fish mass during the rearing trial. The graph shows the increase in mean (± SD) fish mass in groups of the large size-at-age family (LSAF, circles) and small size-at-age family (SSAF, diamonds) over time during the 84-day rearing trial. The mean values are derived from n = 5 rearing tanks of the LSAF and n = 6 of the SSAF. The solid line is described by an exponential function where LSAF mean fish mass = $191e^{0.0105(days)}$ ($R^2 = 0.979$). The dotted line is described by an exponential function where SSAF mean fish mass = $78e^{0.0162(days)}$ ($R^2 = 0.999$). The shaded area shows where the two families shared a similar mean fish mass, and when comparisons were made of their growth, energetics and cardiorespiratory physiology.

Figure 1 shows the changes in mean mass of the two families over the 84-day rearing period, both exhibited an increase in mean mass that was fitted very well by a standard exponential model. The grey area on Figure 1 shows the periods when the two families had a similar mean mass, this occurred over the first three biometric measurement periods for the LSAF but over the last three measurement periods for the SSAF. The two families exhibited marked differences in their condition factor in these respective measurement periods. Thus, at days 0, 21 and 42, LSAF had mean (± SEM) CFs of 1.31 ± 0.12, 1.40 ± 0.14 and 1.50 ± 0.12 (n = 50 in each case), respectively, whereas the mean CFs measured in SSAF on days 42, 63 and 84 were 1.45 ± 0.10; 1.50 ± 0.11 and 1.61 ± 0.14 (n = 60 in each case), respectively. These CF values cannot be directly compared statistically between the two families because their mean mass was different, but they do indicate that the LSAF had a slimmer overall shape than the SSAF. The analyses of heart morphology performed at the end of the trial also revealed significant differences between the two families, whereby the 12 LSAF subjects had a mean CF of 1.38 ± 0.03, significantly lower than the CF of 1.54 ± 0.03 in the SSAF. This was linked to a significant difference in mean ventricular height:width ratio which at 1.00 ± 0.02 in the LSAF, was significantly lower than that of 1.11 ± 0.03 measured in the SSAF. Thus, the rounder body morphology of the SSAF was linked to a rounder (less triangular) ventricular morphology.

Table 3. Elements of physiological energetics in groups of the large size-at-age family (LSAF) and small size-at-age family (SSAF) as they grew in mean mass from 182g to 300g in their rearing tank-respirometers.

	LSAF (n = 5)	SSAF (n = 6)
Total mass gain (g•fish-1)	118 ± 10	118 ± 6
Total time required (days)	35	30
Specific growth rate (%•d-1)	1.53 ± 0.09*	1.74 ± 0.04
Total feed intake (g•fish-1)	130 ± 2*	120 ± 4
Total E intake (kJ•fish-1)	2959 ± 54*	2623 ± 83
Feed conversion ratio (g feed•g fish-1)	1.12 ± 0.08	1.02 ± 0.02
Gross growth efficiency (mg•kJ-1)	40 ± 3	45 ± 1
Mean daily feed intake rate (mg•g-1•d-1)	15.8 ± 0.2*	17.5 ± 0.1
Mean daily E intake rate (kJ•g-1•d-1)	359 ± 4*	383 ± 3
Mean daily O2 uptake rate (mol•g-1•d-1)	204 ± 4*	179 ± 2
Mean daily E utilisation rate (kJ•g-1•d-1)	89 ± 2*	78 ± 1
Apparent E retention (% of intake)	75.3 ± 0.4*	79.6 ± 0.4
Apparent total E allocated to growth (kJ•fish-1)	2229 ± 50	2125 ± 56

All values are given as mean ± SE. An asterisk denotes a significant difference between the families for that variable (t-test, $p < 0.05$). E, energy. Specific growth rate was calculated as described in the text. Energy intake was calculated as feed intake multiplied by the total energy content of the feed, as reported in Table 2. Gross growth efficiency was calculated as total fish mass gain divided by total E intake. Daily E utilisation rate was calculated as O2 uptake multiplied by an oxycalorific coefficient of 13.6 kJ•g-1 O2. Apparent E retention was calculated as the percentage of daily E intake that remained after daily E utilisation. Apparent total E allocated to growth was calculated from the percentage retention of E and the total E intake.

All of the tanks of the LSAF showed well-defined exponential increases in the mean mass of the fish over the first three measurement periods (days 1, 21 and 42), with a regression coefficient (R^2) for the model which was above 0.99 in all tanks except one, where it was 0.97. The tanks of the SSAF also showed well-defined exponential increases in mean fish mass over the last three measurement periods (days 42, 63 and 84), with R^2's of over 0.99 in all cases. These were the

respective periods when the two families exhibited a similar mean mass, and the good fit of the exponential models meant that mean fish mass could be estimated with a high degree of accuracy for any particular day within the chosen intervals. A comparison of the estimated mean mass (from the model) against the actual measurements of mean mass as taken on days 21 and 42 for the LSAF, or on days 63 and 84 for the SSAF, revealed that the estimates never deviated by more than 5% from the actual measures. Specific growth rate was calculated for the two families when they grew from a mean mass of 182g to 300g, each increasing in mass by 118g (Table 3). For the LSAF, this was between day 1 and day 35 whereas for the SSAF this was between days 52 and 84. Contrary to expectations, the LSAF actually exhibited a lower SGR than the SSAF over this mass interval (Table 3).

Energetics of the growing fish

Table 3 shows elements of an energetic budget for the families as each grew from 182 g to 300 g. For exactly the same weight gain, the LSAF had a significantly higher total feed intake, and hence energy intake than the SSAF, although feed conversion ratio and gross growth efficiency were not significantly different. Analysis of daily feeding rates over the respective growth intervals revealed, however, that the LSAF had a lower mean daily feed intake rate, and hence mean daily rate of energy intake. At the same time, mean daily rates of O_2 uptake were significantly higher in the LSAF, indicating a significantly higher daily rate of energy utilisation as metabolism. This higher rate of energy utilisation meant that a significantly smaller proportion of the daily energy intake was apparently retained for allocation towards somatic growth (feed digestibility was not measured in the two families therefore true energy retention could not be calculated). Nonetheless, the total amount of apparent energy allocation that could have been allocated towards somatic growth was the same in both families over their respective growth intervals, which is consistent with the fact that they both gained the same amount of mass (Table 3).

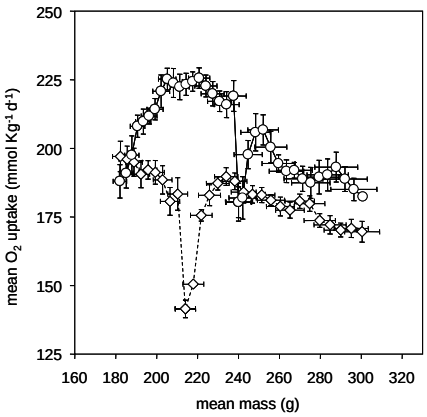

Figure 2. The relationship of metabolic rate to mean fish mass. The graph shows mean (± SE) daily metabolic rate as a function of mean fish mass in groups of the large size-at-age family (LSAF, circles) and small size-at-age family (SSAF, diamonds), as measured in their rearing tanks when they grew from a mean mass of 182 g to 300 g. The mean values are derived from n = 5 rearing tanks of the LSA family and n = 6 of the SSA family. The sudden declines in metabolic rate visible in both families were due to feeding withdrawal and biometric measurements, over a two day period; data for these periods, and for 3 days following them, were not included in any analyses. A two-way analysis of variance with repeated measures revealed a significant interaction of family and fish mass, Holm-Sidak post-hoc tests revealed that both families exhibited a

significant decline in metabolic rate as a function of increasing mass, but the LSAF consistently exhibited significantly higher daily metabolic rates than the SSAF.

Figure 2 shows the daily rates of O_2 uptake as a function of estimated mean fish mass in both families as they grew from 182 g to 300 g. The biometric measurements, with their associated days when feed was withheld, caused large declines in daily rates of O_2 uptake in both families, which then required up to 3 days to recover towards their previous values. Thus, data collected for the biometric measurements and the subsequent three days was not considered in the comparisons of the two families. The two way ANOVA for repeated measures revealed a significant interaction between family and mass for their effects on daily mass-specific metabolic rate. As shown in Figure 2, both families exhibited a significant decline in their metabolic rate as their mean mass increased. However, it is also clear that the LSAF consistently exhibited significantly higher mass-specific rates of O_2 uptake, hence metabolic rate, than did the SSAF.

Figure 3 shows the mean hourly rates of O_2 uptake over a daily cycle in the two families, for four dates when they were estimated, from their growth curves, to have the same mean mass. The two way ANOVA for repeated measures revealed a significant interaction between family and time of day for their effects on O_2 uptake. Thus, in both families, the lowest metabolic rates are observed at 06:00 and, at this time, there was no significant difference in O_2 uptake between them. However, when the lights turned on (at 07:00) this caused a progressive increase in metabolic rate in both families, which peaked during the middle of the feeding period (between 10:00 and 14:00) and then dropped steeply as feeding finished, then more gradually overnight towards the daily minimum rate at 06:00. As is visible in Figure 3, O_2 uptake in the LSAF was significantly higher throughout the feeding period, and also showed periods when it was significantly higher in the evening after feeding. Thus, it was these periods of the day that contributed to the significantly higher mean rates of daily metabolism in the LSAF.

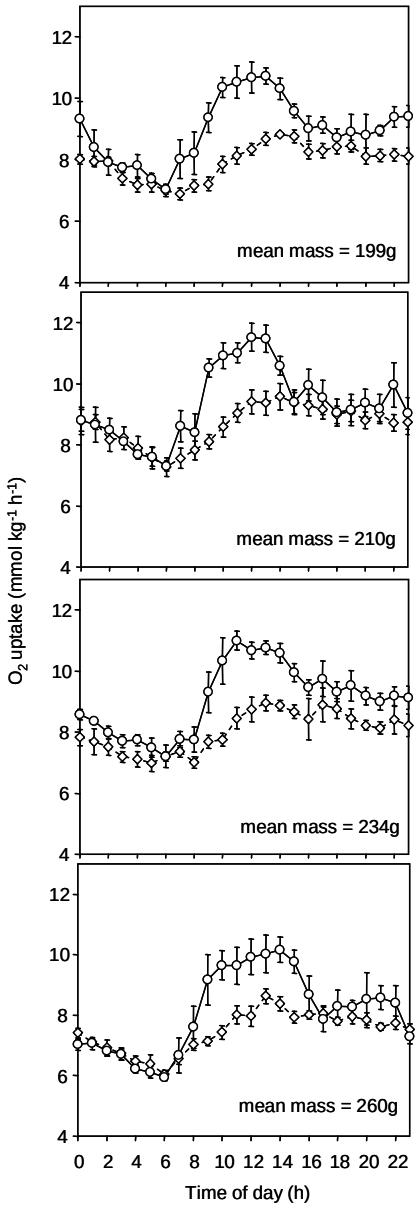

Figure 3. Daily patterns of metabolic rate. Circadian changes in mean (± SE) hourly metabolic rate in groups of the large size-at-age family (LSAF, circles) and small size-at-age family (SSAF, diamonds) as measured in their rearing tanks on four separate days when they were estimated (from the growth data) to have the same mean mass. The mean values are derived from n = 5 rearing tanks of the LSA family and n = 6 of the SSA family. A two-way analysis of variance with repeated measures performed for each day's data revealed a significant interaction of family and time of day. Holm-Sidak post-hoc tests revealed that both families exhibited their lowest metabolic rate at 06.00, and this was similar in both groups. During daylight hours (07.00 to 22.00), metabolic rate increased significantly in both families but the LSAF consistently exhibited significantly higher metabolic rates than the SSAF, and this occurred most often during the feeding period (08.00 to 14.30, shaded area on graph).

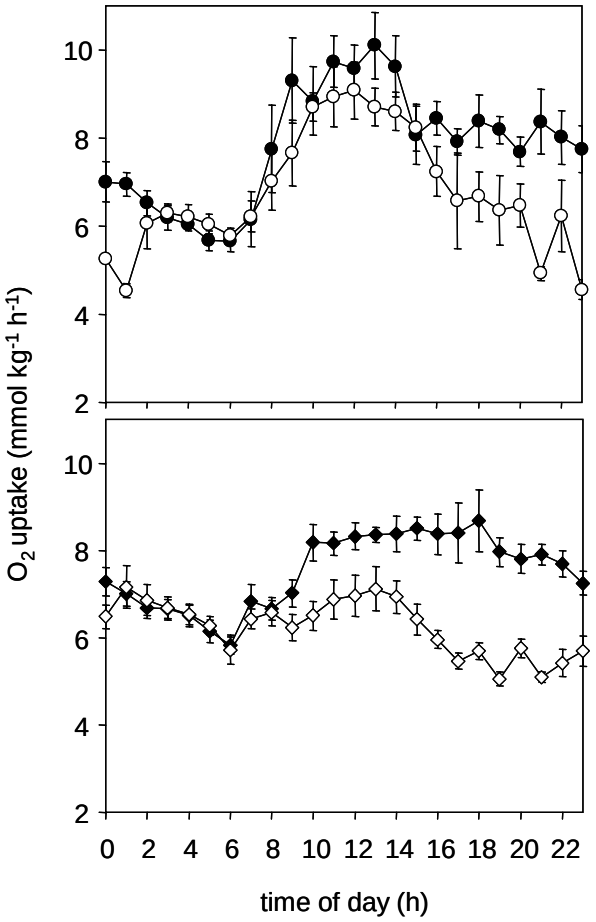

Figure 4. Effects of feed withdrawal on daily patterns of metabolic rate. The graph shows circadian changes in mean (± SE) hourly metabolic rate in groups of the large size-at-age family (LSAF, upper panel, circles) and small size-at-age family (SSAF, lower panel, diamonds) as measured in their rearing tanks on two sequential days where, for the second day, feed was withheld. The mean values are derived from n = 5 rearing tanks of the LSAF and n = 6 of the SSAF. The circadian changes in metabolic rate, with the large increase in rate during daylight hours, were still visible when feeding was withheld, particularly in the LSAF.

Figure 4 shows the effects of 24h starvation on this daily pattern of O_2 uptake, as measured just prior to biometric measurements and when, fortuitously, the families had a similar mean mass (day 41 for the early growers, day 83 for the late growers). This shows that, even when the fish were not fed, they still retained the daily increase in O_2 uptake during the normal feeding hours and that this increase was most pronounced in the LSAF.

Respiratory quotients and instantaneous fuel usage

Table 4 shows the measured values for respiratory quotients and patterns of instantaneous fuel usage in the two families, while feeding and also when feed had been withheld for 24h. At this time, the end of the rearing trial, the mean mass of the LSAF was significantly higher than the mean mass of the SSAF (Table 4). The interaction of family and feeding status (fed versus 24h starvation) were assessed for each variable in Table 4 with a two-way ANOVA for repeated measures.

Just after finishing their feed, both families had an RQ of just above 0.8 (Table 4), with no significant difference between them. However, the SSAF exhibited a significantly higher NQ, hence rate of excretion of nitrogenous wastes relative to rates of O_2 uptake (Table 4). In both families, lipid was the primary fuel, the oxidation of which accounted for approximately 50% of total O_2 consumed (Table 4). Carbohydrate oxidation, on the other hand, accounted for less than 10% of O_2 consumed in each family. The balance was due to protein oxidation and, consistent with their higher NQ, protein oxidation accounted for a significantly higher proportion of total O_2 consumed in the SSAF when compared to the LSAF (Table 4). Indeed, calculation of actual carbon oxidised from these three fuels revealed that the primary fuel in the SSAF was protein whereas it was lipid in the LSAF.

A different picture emerged in the families when they had been denied food for 24h (Table 4). Starvation caused a significant decline in rates of all fluxes, in both families. M_{O2} and M_{CO2} declined by similar proportions and so there was no significant effect of 24h starvation on RQ in either family. In both families, however, M_N dropped by a proportionally much greater extent than M_{O2} and M_{CO2}, leading to a profound decline in their NQ. This, in turn, was associated with significant changes in patterns of fuel use. Lipid remained the primary fuel, the oxidation of which now accounted for over 50% of total O_2 consumed. Carbohydrate oxidation, on the other hand, became the secondary fuel, accounting for up to 32% of O_2 consumed, while protein oxidation accounted for less than 20% in both families, with no differences between the families in the utilisation of any of these fuels. Calculation of actual carbon oxidised revealed the same relative pattern in fuel use (Table 4).

Table 4. Respiratory gas exchange and fuel usage in groups of the large size-at-age family (LSAF) and small size-at-age family (SSAF) when feeding or following 24h starvation in their rearing tank-respirometers. Mean (± SE) instantaneous fluxes of O_2, CO_2 and nitrogenous wastes, derived respiratory quotients, nitrogen quotients, percentage of oxidative metabolism fuelled by lipids, proteins or carbohydrates, and metabolised carbon provided by lipids, proteins or carbohydrates. Mean ± SD fish mass is also provided.

	LSAF (n = 5)		SSAF (n = 6)	
	Feeding	24h starved	Feeding	24h starved
M_{O2} (mmol kg^{-1} h^{-1})	8.10 ± 0.52	6.40 ± 0.37*	8.64 ±0.35	5.98 ± 0.18*
M_{CO2} (mmol kg^{-1} h^{-1})	6.58 ± 0.36	5.35 ± 0.37*	7.20 ± 0.38	4.88 ± 0.16*
M_{Tamm} (mmol kg^{-1} h^{-1})	0.689 ± 0.029	0.249 ± 0.024*	0.937 ± 0.050†	0.256 ± 0.017*
$M_{urea\text{-}N}$ (mmol kg^{-1} h^{-1})	0.064 ± 0.024	0.006 ± 0.002*	0.074 ± 0.019	0.023 ± 0.007*
M_N (mmol kg^{-1} h^{-1})	0.753 ± 0.026	0.256 ± 0.025*	1.011 ± 0.063†	0.280 ± 0.017*
RQ	0.815 ±0.010	0.837 ± 0.027	0.832 ± 0.011	0.816 ± 0.024
NQ	0.094 ± 0.003	0.040 ± 0.003*	0.118 ± 0.008†	0.047 ± 0.003*
% lipids	56.7 ± 3.2	53.1 ± 8.4	48.9 ± 3.9	59.8 ± 8.1
% protein	34.6 ± 1.1	14.8 ± 1.3*	43.6 ± 0.3†	17.3 ± 1.1*
% carbohydrates	8.6 ± 2.5	32.0 ± 7.7*	7.5 ± 4.9	22.9 ± 8.9*
C_{lipid} (mmol kg^{-1} h^{-1})	3.29 ± 0.36	2.42 ± 0.41*	2.97 ± 0.17	2.56 ± 0.38
$C_{protein}$ (mmol kg^{-1} h^{-1})	2.68 ± 0.08	0.91 ± 0.08*	3.61 ± 0.22†	1.00 ± 0.06*
$C_{carbohydr}$ (mmol kg^{-1} h^{-1})	0.67 ± 0.19	2.04 ± 0.51*	0.72 ± 0.49	1.35 ± 0.51*
Mean mass (g)	449 ± 24		307 ± 22	

An asterisk denotes a significant effect of 24h starvation on that variable (Holm-Sidak post-hoc to two-way ANOVA for repeated measures). A dagger denotes a significant difference between the LSA and SSA families when feeding (Holm-Sidak post-hoc to two-way ANOVA for repeated measures). $P < 0.05$ in all cases. M_{O2}, O_2 uptake; M_{CO2}, CO_2 excretion; M_{Tamm}, total ammonia excretion; $M_{Urea\text{-}N}$, urea-N excretion, M_N, excretion of T_{amm} plus Urea-N, RQ, respiratory quotient;

NQ, nitrogen quotient; C with subscript lipid, protein or carbohydr, CO_2 flux due to oxidation of these three fuels.

Sustained aerobic exercise performance

These studies were performed upon 10 subjects from each family and, as shown in Table 5, although the families did not differ significantly for mean mass or length, the SSAF had a significantly higher CF than the LSAF, a result which confirms the data collected during the biometric measurements. Both of the families performed exercise well, achieving relative U_{crit}'s in excess of 4 $BL \bullet s^{-1}$, hence absolute speeds of around one $m \bullet s^{-1}$. The LSAF, however, achieved a significantly higher absolute and relative U_{crit} than the SSAF (Table 5). This was linked to significant differences in their respiratory metabolism. That is, although the values derived for IMR did not differ between the two families, the LSAF exhibited a significantly higher MMR and, therefore, also net aerobic metabolic scope than the SSAF did (Table 5).

Table 5. Exercise performance and metabolism in size-matched individuals from large size-at-age family (LSAF) and small size-at-age family (SSAF). Mean (± SD) mass, length and condition factor, and mean (± SE) maximum sustainable swimming speed, immobile metabolic rate, active metabolic rate, and resultant aerobic scope.

	LSAF (n = 10)	SSAF (n = 10)
Mass (g)	205 ± 11	211 ± 12
Forklength (cm)	24.6 ± 0.5	23.9 ± 0.6
CF 100 x(mass x length^3)	1.38 ± 0.03	1.54 ± 0.03*
Absolute U_{crit} ($cm \bullet s^{-1}$)	110 ± 1	100 ± 2*
Relative U_{crit} ($BL \bullet s^{-1}$)	4.50 ± 0.07	4.20 ± 0.10*
IMR (mmol $O_2 \bullet kg^{-1} \bullet h^{-1}$)	4.30 ± 0.24	4.27 ± 0.13
MMR (mmol $O_2 \bullet kg^{-1} \bullet h^{-1}$)	21.39 ± 0.53	19.41 ± 0.45
AS (mmol $O_2 \bullet kg^{-1} \bullet h^{-1}$)	17.09 ± 0.57	15.14 ± 0.42

An asterisk denotes a significant difference from the LSA family for that variable. CF, condition factor; U_{crit}, maximum sustainable swimming speed; IMR, immobile metabolic rate; MMR, maximum metabolic rate; AS, aerobic metabolic scope.

Tolerance of hypoxia

Tolerance of hypoxia was investigated in 10 individuals from each family, with a mean (± SD) mass of 252 ± 23 g in the LSAF and 236 ± 54 g in the SSAF. As shown in Figure 5, the families did not differ in their RMR in normoxia, and both showed a similar regulation of metabolic rate during progressive hypoxia. Therefore, both families exhibited a similar tolerance of hypoxia with no significant difference in mean (± SE) P_{crit}, which was 6.62 ± 0.60 kPa in the LSAF as compared with 7.05 ± 0.48 kPa in the SSAF.

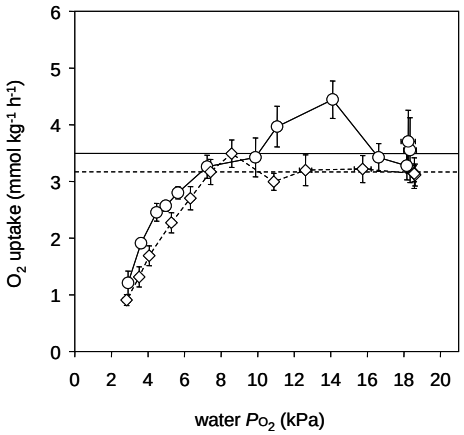

Figure 5. Tolerance of progressive hypoxia in size-matched individual trout from the large size-at-age family (LSAF, circles) and small size-at-age family (SSAF, diamonds). The graph shows mean (± SE) rates of oxygen uptake (metabolic rate) as a function of water PO_2, n = 10 in both cases. The solid line shows the mean routine metabolic rate of the LSAF in normoxia, the dotted line shows that of the SSAF. There were no statistical differences between families in their responses to hypoxia.

Discussion

The results demonstrate that rainbow trout families with different SA and lifetime growth rates exhibited significant differences in their physiological energetics, notably in their rates of energy intake and metabolic energy dissipation, and in their utilisation of nutrients as metabolic fuels. The energetic picture that emerged as the families grew to marketable size was, however, complex and contrary to expectations. It did not demonstrate that large SA was associated with increased energy intake or greater efficiency, and this was apparently because of different familial behaviour patterns. The families also differed in their functional integrity, revealed as differences in maximum aerobic performance. This was directly related to differences in their CF and cardiac morphology.

Growth and energetics

The LSAF would seem the most desirable as broodstock for a breeding program, in that they have a greater lifetime SGR and so would achieve a marketable size more rapidly. It was unexpected, therefore, that the SSAF should actually exhibit a higher SGR when it had grown to a mean mass that could be compared with the LSAF. Although this was contrary to expectations, the daily feeding and O_2 uptake data provide a cogent energetic explanation. That is, the LSAF were feeding at a lower rate and also metabolising more energy during daylight feeding hours, such that the allocation of energy towards somatic growth occurred at a slower rate.

The differences in daily energy dissipation did not appear to be due to differences in intrinsic metabolic biochemistry between the two families. That is, the swimming respirometry on individual fish from each family did not reveal any differences in IMR, which is supposed to provide an estimate of the minimum metabolic costs for maintenance in fish (Brett 1964; Beamish 1978), and the static respirometry did not reveal any differences in normoxic RMR. Tilapia (*Oreochromis* sp.) that were transgenic for growth hormone and grew more rapidly than their wild-type conspecifics exhibited significantly higher IMR and RMR than wild-types, and this was associated with significantly increased activities of the metabolic enzymes phosphofructokinase and citrate synthase in their muscle and liver (Martinez et al. 1999; McKenzie et al. 2003). Coho salmon (*Oncorhynchus kisutch*) transgenic for growth hormone and with accelerated growth rates also exhibit higher activities of metabolic enzymes such as cytochrome *c*-oxidase in their red muscle (Hill et al. 2001). In the current study, the circadian patterns of M_{O2} in the rearing tanks

also revealed that metabolic rates were very similar in the two families during the night, but that the increased energy dissipation in the LSAF was a result of elevated metabolic rates during the day, when the lights were on and they were feeding. The fact that this increased metabolic rate during the daytime persisted even when feeding was withheld indicates that it was behavioural rather than linked to any energetic costs of feeding *per se*.
The increased metabolic rate of the LSAF during the daytime was presumably a consequence of increased rates of spontaneous activity. The LSAF showed some increases in metabolic rate immediately when the lights came on at 07:00, one hour prior to actual feeding, which may be evidence of increased feeding anticipatory activity (Sanchez-Vasquez and Madrid 2001), but the majority of their increased daily metabolic rate was clearly due to differences in behaviour while feeding. One factor that is known to increase spontaneous activity and metabolic rates in cultured salmonids is the development of dominance hierarchies, with increased aggression and competition for food (Christiansen et al. 1991; Jobling et al. 1993). Visual observation of the two families revealed different patterns of space use in the tanks. The SSAF tended to occupy the whole tank quite uniformly whereas the LSAF frequently had a single fish that occupied the upper region of the tank, with the remaining animals swimming close to the bottom. The LSAF were also more active, particularly the single fish, which harried the other fish during the daily feeding interval. This behaviour in the LSAF occurred despite the fact that both families were exposed to a low level of sustained exercise in their tanks specifically because this is known to inhibit the development of aggressive dominance hierarchies (Davison 1997). Rapidly-growing strains of farmed salmonids have been shown to have an increased tendency towards competitive aggression and this behaviour can intensify as the fish increase in size (Huntingford 2004). This behaviour pattern in the LSAF could also explain the reduced feed intake by the fish, if the dominant aggressive fish interfered with the ability of the other animals to feed to satiation (Huntingford 2004). There may, therefore, be a trade-off whereby a large SA and high lifetime SGR can bring a risk of increased aggressive behaviours and a decline in feed intake and growth performance as the fish approach marketable size.

Patterns of fuel usage
These are the first reports of respiratory exchange quotients and instantaneous fuel usage in growing feeding fish (Wood 2001). It has been known for some time that when salmonids feed they exhibit large increases in their rates of ammonia excretion and, although coupled with parallel increases in rates of oxygen uptake, there is also a large increase in NQ (Brett and Zala 1975; Beamish and Thomas 1984; Alsop and Wood 1997). This has, historically, been the basis for the assumption that protein is a major metabolic fuel in fish (Wood 2001), despite the evidence that oxidation of lipids and carbohydrates predominates in starved individual rainbow trout (Lauff and Wood 1996a,b; Wood 2001). The current data confirm that protein is indeed a major metabolic fuel in feeding growing trout, second only to lipid in terms of the proportion of aerobic metabolism that it supports and providing similar overall amounts of carbon for oxidation. Nonetheless, the data also reveal that 24h starvation is sufficient to cause a very significant drop in the NQ and degree of protein oxidation, which is spared in favour of an increased reliance upon carbohydrates (Lauff and Wood 1996a). Indeed, the current data for instantaneous fuel usage in the tanks of fish at 24h starvation are very similar to the patterns described previously on starved individual rainbow trout (Lauff and Wood 1996a).

It is very interesting that significant differences in relative rates of protein utilisation were observed between the two families when feeding, whereby the LSAF excreted less ammonia and therefore burned less protein as a fuel than did the SSAF. This reduced oxidation of protein in the LSAF could be indicative of an increased capacity for allocating proteins towards somatic growth (Wood

2001) and, therefore, could be a contributing factor to their larger SA. This conclusion would not, however, appear to be consistent with the fact that each family allocated a similar amount of total apparent energy towards growth when they increased in mass from 182 to 300g. Thus, this possibility would need to be confirmed in future studies upon size-matched trout from each family. Indeed, it is also possible that the difference in protein oxidation between the two families was a result of their difference in mean body mass when the measurements were made. The LSAF, being larger, may have started processes of gonadal development which might, conceivably, also cause changes in the metabolism of different nutrient groups, with proteins being spared for allocation towards gamete production. Whatever the mechanism, this interesting difference between the trout families in their instantaneous rates of nutrient utilisation is worthy of further study.

Functional integrity: aerobic exercise performance and hypoxia tolerance

The mean U_{crit} in excess of 1 $m \bullet s^{-1}$ observed in both families in the current study compares favourably with published reports for farmed rainbow trout (Gallaugher et al. 1995; Shingles et al. 2003; Claireaux et al. 2005) and indicates, therefore, that neither family was in any way physiologically "compromised". Nonetheless, the higher U_{crit} and greater aerobic scope of the LSAF reveals that they were physiologically "fitter" than their SSAF conspecifics. This, in turn, indicates that rapid early growth, and rapid growth rates *per se*, do not necessarily engender compromised functional integrity in trout (Claireaux et al. 2005). Claireaux et al. (2005) showed that when rainbow trout exercise at their maximum sustainable aerobic swimming speed, the heart is also working at its maximal sustainable pumping output, and that trout with the highest maximum pumping capacity during exercise have the best swimming performance. Thus, the higher U_{crit} of the LSAF in the current study should be indicative of an increased underlying cardiac performance. Claireaux et al. (2005) also found that low swimming and cardiac performance were directly linked to a more rounded (less triangular) ventricular morphology, and suggested that this difference in morphology was the basis for the reduced pumping capacity of the heart. Furthermore, there was an intriguing relationship between fish external morphology and the shape of the heart, whereby fish with higher CF (i.e. a rounder body shape) also possessed significantly rounder ventricles (Claireaux et al. 2005). The current study confirmed this unusual morphological relationship as a general pattern in farmed rainbow trout. The relatively poorer swimming performance of the SSAF was linked to a significantly higher CF than the LSAF and this, in turn, was linked to a significantly rounder ventricular morphology. This indicates that breeding programs aimed at improving SGR in rainbow trout should select families with large SA but low CF.

The P_{crit} of around 7 kPa during progressive hypoxia in the current study is coherent with previous observations in rainbow trout at a similar water temperature (Marvin and Heath 1968). Despite the evidence that differences in cardiac morphology might have caused differences in maximum aerobic performance between the two trout families, there was no difference in their ability to regulate aerobic metabolism at routine levels in hypoxia. Cardiac responses to hypoxia in rainbow trout comprise a bradycardia but no change in cardiac output (Randall 1982), the current results indicate that the effectiveness of these adaptive reflexes was not influenced by the observed differences in cardiac morphology between the two families.

Conclusions

A large SA and high lifetime SGR are desirable genetic traits in farmed rainbow trout but there may be a trade-off whereby they bring a greater tendency towards aggressive competition. In the current experiment, poor growth performance in the LSAF as they approached marketable size was a result of decreased rates of feed intake and increased rates of energy expenditure, which may have reflected the development of dominance hierarchies. Thus, the behaviour of trout broodstock should be considered carefully in breeding programs (Huntingford 2004) to avoid declines in

growth performance that might accompany increased aggression as the fish approach marketable size.
The results indicate that selecting broodstock on the basis of a large SA does not, however, necessarily bring a risk of selecting animals with compromised functional integrity. Instead, it appears that a high CF is a trait that is indicative of reduced aerobic scope and maximum aerobic performance, because it is indicative of a rounded cardiac morphology. An increasingly rounded ventricle is one of the morphological correlates of domestication in salmonids (Poppe et al. 2003; Claireaux et al. 2005) and associated cardiac malfunctions are a growing problem for the fishes' welfare (Poppe et al. 2000, 2002). This is most significant for fish that are grown to large sizes in which cardiac malfunctions can cause mortalities due, for example, to the stresses associated with high summer temperatures (Mercier et al. 2000), and so lead to significant losses of investment.

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Digestibility, Biological Value (D_a) and Productive Protein Value (PPV) in 8 families of rainbow trout *(Oncorhynchus mykiss)*

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Introduction

Farmed fish requires protein with a well balanced content of essential amino acids in their diet to obtain high efficiency and productivity. Protein is the most expensive single component in fish feed and the quality of the dietary protein is decisive for the productivity of fish farming.

Accordingly improved utilization of the feed is included in the breeding goals in the Danish breeding programme in terms of Feed Conversion Ratio (FCR) and Specific Growth Rate (SGR).

In two successive years (2002 and 2003) the digestibility of one feed type fed to 4 selected families was investigated. The 4 selected families from the 2002 and the 2003 generation, respectively, were of similar age and size. The investigations included relationships between growth, feed intake, feed conversion and fish digestibility as well as Biological Value (BV) and Productive Protein Value (PPV) in the experimental diets.

The digestibility was expressed as the apparent digestibility, D_a, as the contribution from the metabolism was excluded:

$$D_a = (I - F)/I, \text{ where} \qquad (1)$$

I = Amount of ingested protein
F = Protein content in faeces

Followingly, the digestibility expresses the fraction of the ingested feed, that can be taken up in the intestine and used for growth and energy expenditure. Differences in protein digestibility may be due to chemical and physical properties, content of nutritional inhibitors, feed processing conditions etc.

The relationship between the amount of ingested protein and the protein gain is expressed by the Productive Protein Value (PPV):

$$PPV = (B_2 - B_1)/I, \text{ where} \qquad (2)$$

B1 = Protein content in the fish at the start of the exp.
B2 = Protein content in the fish at the end of the exp.

However, a high protein digestibility does not necessarily mean a high growth rate of the fish. The growth is evaluated by the apparent Biological Value (BV_a), which express the fraction of the digested protein, that is converted into fish gain, i.e. fish meat:

$$BV_a = (B_2 - B_1)/(I-F) \qquad (3)$$

The linkage between D_a, PPV and BV_a can be expressed by combining equation 1-3:

$$BV_a = PPV/D_a$$

This means that the protein digestibility as well as the protein gain are integrated elements in the biological value of the specific protein.

Aim

The aim of this study was to investigate the genetic related effects on the apparent digestibility, the Productive Protein Value and the Biological Value in rainbow trout from eight different trout families in 2 successive generations/years (4 families/generation).

Materials and Methods

Facility

The test was carried out in DIFRES experimental digestibility setup consisting of 12 cylindrical-conical acrylglass tanks. Each tank had a diameter of 44 cm and the height was about 130 cm, i.e. the tank volume was about 150 l. The bottom of each tank was conical i.e. the lowerst 50 cm was narrowing with a ball-valve at the outlet. At this outlet a container for collecting faeces was mounted. I order to keep the faeces cool the collectors were submerged in ice-water, 0 ^{o}C in white polystyrene containers (cf. fig. 1).

The facility was a flow through system without water purification. The water passed from a high level reservoir to the tanks via a 70 cm vertical pipe along the inside of each tank. As the pipe had Ø3 mm holes in the longitudinal direction a circular current was created in the tank even at low water flow ensuring a good mixing of the water. The water flow to each tank was regulated by a ball-valve.

At the transition between the tank wall and the cone each tank was equipped with a bottom grate with 1x1 cm holes, so that uneaten feed pellets and faeces could pass through to the collector.

The outlet water passed through an Ø25 mm cross pipe placed below the bottom grate. This pipe was equipped with holes, Ø8 mm, on the ventral side so that feed pellets and faeces neither were sucked out nor disturbed the flow of the water. Further an upturned V-formed superstructure was fitted on the pipe to prevent feed loss and faeces etc. to settle on top of the pipe. In order to reduce the water flow, pure oxygen was added to the incoming water through a diffuser in the high level reservoir tank. The content of oxygen in the tanks was regulated to a minimum of 70% saturation.

Figure 1. Experimental digestibility facility. 12 tanks for feeding and collection of faeces kept cool i.e. collectors submerged in ice-water, 0 ^{o}C in white polystyrene containers.

Above each tank an electrical bulb was placed (soft tone 40 W), which was predetermined by a timer to switch on at 8 a.m. and off at 8 p.m., i.e. the light was on for 12 hours. The intensity of the light could be regulated as to minimize stress behaviour of the fish.

Fish

The 8 experimental fish families for this study were selected based on growth data from the parent generations. The families for the first year experiments (2002) were half siblings two by two, i.e. family A1 and family B1, respectively, had the same mother but different fathers, and similarly family C1 and D1. The paternal generation of families A1 and B1 showed traits for high specific growth rates, while the paternal generation of families C1 and D1 had performed with lower specific growth rates.
However, the experimental fish families (A2, B2, C2 and D2) in 2003 were all full siblings.

Fish (about 200 g/pcs.) from each selected family were transferred to separate tanks in the digestibility facility. The fish were getting used to the facility and the feed for one week before the experiment was started. The experiments were run with 10 fish (2002) and 15 fish (2003) in each tank. The experiments were run in triplicate, i.e. 3 tanks with each family.

At the start and at the end of the experiment individual weights of the fish and the total biomass in each tank were measured. The temperature in the fish tanks was about 10 ^{0}C and the oxygen saturation was always at least 70%, measured by Oxyguard hand meter every day (cf. app. 3 and 6).

Diet

The experimental diets were a commercial feed type "GEP 576 Export – 4 mm pellets" (Aller Aqua A/S). The feed was stored in cool room. The chemical analyses of the diets are given in table 1.

Table 1. Chemical analyses of the experimental diets "GEP 576 Export – 4 mm pellets" (Aller Aqua A/S). Declared values (prox. analyses) are indicated by *.

Aller Aqua A/S; GEP 576 Export – 4 mm	**2002**	**2003**
Raw protein (N*6.25), g/100 g	42.6	42.0
Raw fat (acidhydr.), g/100 g	26.4	27.4
Crude fibre, g/100 g	1.91	0.82
Ash, g/100 g	6.32	7.32
N-free extracts, g/100 g	15.9	18.1
Dry matter, g/100 g	93.1	95.6
Phosphorous, %	0.9*	0.9*
Digest. energy, MJ/kg	19.8*	19.8*
Gross energy, MJ/kg	23.9*	23.9*
Vitamin A, IU/kg	2.500*	2.500*
Vitamin D_3, IU/kg	500*	500*
Vitamin E, IU/kg	150*	150*
Etoxyquine, mg/kg	100*	100*

Feeding

During the experiments the daily feeding rate was 1,2% (2002) and 1,5% (2003) of the biomass. The fish were fed according to a feeding table prepared on account of the feeding rate and expected feed conversion ratio.

Half of the daily feed amount was fed to the fish at 10 am. and the rest at 2 pm.

To prevent mixing of feed and faeces the ball valve to the faeces collector was closed prior to feeding, and the collector removed. After each feeding a fine mesh was held below the outlet – the ball valve opened – and uneaten pellets were collected in the mesh. The feed pellets were counted and converted to grams based on estimated weights of individual pellets (weighing/counting of 3 portions of pellets). The faeces collector was mounted again immediately after pellet collection and the ball valve opened.

The experiments were run in 3 successive periods of 3 days each.

Faeces collection

Every morning at 9.30 the collected faeces from each tank was transferred to a seperate container and immediately frozen (- 20 ^{o}C) for later analysis for protein (nitrogen), fat, dry matter and ash. The faeces from each of the 3 periods was kept separate in each container and each tank.

Data handling

The experiments were carried out in triplicate 3 * 3 day series.

The following parameters were investigated in the diets:

- Apparent Protein digestibility
- Apparent Oil digestibility
- Apparent Dry Matter digestibility
- Apparent Biological Value
- Productive Protein Value
- Specific Growth Rate
- Feed Conversion Ratio

Based on the chemical analysis and the amount of consumed feed the apparent digestibility for protein (nitrogen, N), oil, carbohydrate and dry matter was calculated according to the equation (ex. N):

D_a (N) = ((Ingested N – N in faeces)/Ingested N))*100, cf. equation (1)

Calculations of the Productive Protein Value (PPV) and the apparent Biological Value (BV_a) were done according to equation (2) and (3), respectively.

For calculation of growth SGR) and feed conversion ratio (FCR) the following formulas were used:

Specific growth rate (SGR) = (exp (ln W_1/W_o) t-1 * 100)), where

W_1 = Weight at the end of the trial
W_2 = Weight at the start of the trial
t = Feeding days

Net feed conversion ratio (FCR) = Net intake of feed (kg)/growth (kg)

Results

2002

The experiment was carried out from 3rd to 13th December 2002. The individual weight of each experimental fish and the total biomass in each tank is shown in appendix 1. The average initial weight of the fish was 208-229 g/pcs and the final weight was 241-265 g/pcs.

The feeding scheme including the amount of feed administered, collected amount of not eaten pellets and calculation of ingested feed is given in appendix 2. The amounts of pellets per gram feed were estimated by weighing and counting the pellets in 5 random samples of feed. The calculated average pellet weight (0,0707 g) was used to convert the counted number of uneaten pellets into grams (cf. appendix 2).

The oxygen saturation and the temperature in the fish tanks and of the inlet water (high level tank) during the experiment is given in appendix 3.

The specific growth rate (SGR - %/day) and the feed conversion ratio (FCR) given in table 2 and in figure 2.

Table 2. Specifik growth rate (SGR - %/day) and feed conversion ratio (FCR) during the digestibility experiment - 3/12 – 13/12 – 2002 (9 feeding days).

CODE	A1	A1	A1	B1	B1	B1	C1	C1	C1	D1	D1	D1
SGR (%/dag)	1.55	1.80	1.66	1.42	1.45	1.48	1.61	1.14	1.56	1.45	1.52	1.59
Average SGR (%/day)	1.67			1.45			1.44			1.52		
FQ	0.76	0.65	0.67	0.79	0.81	0.65	0.80	0.74	0.73	0.79	0.75	0.75
Average (FQ)	0.69			0.75			0.76			0.76		

The SGR between the families ranged from 1.44 to 1.67 %/day and the FCR ranged from 0.69 to 0.76 and were in the same normal range of magnitude as in the growth studies with the same families. This means that the fish were well performing during the experiment.

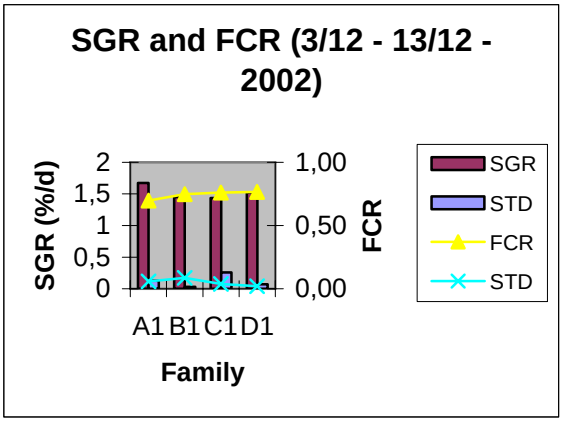


Figure 2. Specific Growth Rate (SGR - %/day) ± standard deviation (STD) – columns – and feed conversion ratio (FCR) – curves – in digestibility experiments - 3/12 – 13/12 – 2002 (9 feeding days).

The digestibility of nitrogen (protein), oil (fat), carbohydrate and dry matter is shown in table 3 and in figure 3.

Table 3. Digestibility (%) ± standard deviation (STD) of nitrogen, oil, carbohydrate and dry matter in the four families (3/12 – 13/12 – 2002).

CODE	A1	B1	C1	D1
N-digest. ± STD.	92.0 ± 0.26	91.8 ± 0.80	92.3 ± 0.25	92.7 ± 0.06
Oil-digest. ± STD.	92.6 ± 0.50	92.6 ± 0.92	93.0 ± 0.32	91.0 ± 0.42
Carbo.-digest. ± STD.	68.5 ± 0.36	70.1 ± 2.55	71.4 ± 2.79	75.3 ± 0.41
D.M.-digest. ± STD.	84.5± 0.27	85.1 ± 0.51	85.4 ± 0.42	85.6 ± 0.42

The highest digestibility of nitrogen (protein) was 92.7% in family D1, compared to91,8% in family B1.The protein digestibilities in family A1 and C1 was 92,0 and 92,3 %, respectively.

The highest digestibility of oil was 93,0% in family C1, compared to 91,0% in family D1.The oil digestibilities in family A1 and B1 were both 92,6 %.

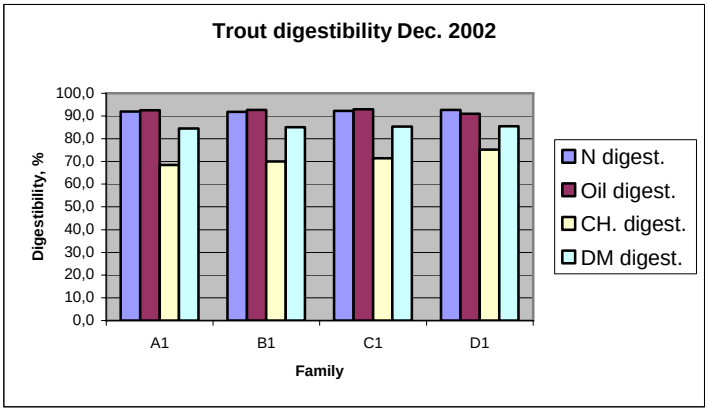


Figure 3. Digestibility (%) of nitrogen (protein), oil (fat), carbohydrate CH.) and dry matter (DM) in the experimental diet using four selected families of rainbow trout from 2002 generation from Danish Trout Breeding. 10 fish in each tank (initial weight 218.8 ± 14.8 g/pcs and final weight 250.7 ± 20.5 g/pcs).

The calculated carbohydrate digestibilities varied from 68,5 to 75,3%. The observed variations may be due to the fact, that the content of carbohydrate and faeces was calculated as a difference.

The digestibilities of dry matter was about 85% in all four families.

The apparent Biological Values (BV_a) and the Prod. Prot. Values (PPV) are given in table 4. BV_a ranged from 0.41 (family D1) to 0.46 (family A1), i.e. 41 to 46% of the digested protein was converted to meat.
However, calculations of PPV showed, that 38 to 42% of the ingested protein was converted to fish protein. Family A1 showed the highest BV_a and the highest SGR elucidating the linkage between growth performance and BV_a (cf. tables 2 and 4).

Table 4. Apparent Biological Value (BV_a) and Productive Protein Value (PPV) ± standard deviation (STD) measured in the four families of rainbow trout in the digestibility experiments - 3/12 – 13/12 – 2002.

	Family			
	A1	**B1**	**C1**	**D1**
Biological Value (BV_a)	0.46 ± 0.04	0.44 ± 0.05	0.45 ± 0.02	0.41± 0.04
Prod. Prot. Value (PPV)	0.42 ± 0.04	0.40 ± 0.05	0.41 ± 0.02	0.38 ± 0.04

2003

The experiment was carried out from 11th to 21st November 2003. The individual weight of each experimental fish and the total biomass in each tank is shown in appendix 4. The average initial weight of the fish was 168-181 g/pcs and the final weight was 199-222 g/pcs.

The feeding scheme including the amount of feed administered, collected amount of not eaten pellets and calculation of ingested feed is given in appendix 5. The amounts of pellets per gram feed were estimated by weighing and counting the pellets in 5 random samples of feed. The calculated average pellet weight (0,0688 g) was used to convert the counted number of uneaten pellets into grams (cf. appendix 5).

The oxygen saturation and the temperature in the fish tanks and of the inlet water (high level tank) during the experiment is given in appendix 6.

The specific growth rate (SGR - %/day) of the fish during the experiment is given in table 5 and in figure 4.

Table 5. Specifik growth rate (SGR - %/day) and feed conversion ratio (FCR) during the digestibility experiment - 11/11 – 21/11 – 2003 (9 feeding days).

CODE	**A2**	**A2**	**A2**	**B2**	**B2**	**B2**	**C2**	**C2**	**C2**	**D2**	**D2**	**D2**
SGR (%/dag)	**2.14**	**2.19**	**2.28**	**1.88**	**1.54**	**1.83**	**2.28**	**1.76**	**2.16**	**1.98**	**2.02**	**1.78**
Average SGR (%/day)	**2.20**			**1.75**			**2.07**			**1.93**		
FQ	**0.68**	**0.66**	**0.63**	**0.74**	**0.77**	**0.72**	**0.64**	**0.73**	**0.66**	**0.68**	**0.68**	**0.73**
Average (FQ)	**0.66**			**0.75**			**0.68**			**0.70**		

The specific growth rates ranged from 1.75 to 2.20 %/day and the feed conversion ratios between the families ranged from 0.66 to 0.75 and were in the same normal range of magnitude as in the growth studies with the same families. Accordingly, this means that the fish were well performing during the experiment.

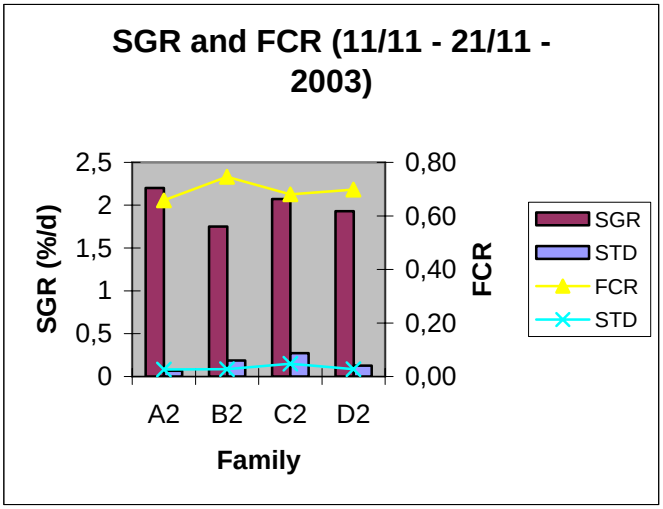


Figure 4. Specific growth rate (SGR - %/day) ± standard deviation (STDEV) of rainbow trout in digestibility experiments - 11/11 – 21/11 – 2003 (9 feeding days).

The digestibility of nitrogen (protein), oil (fat), carbohydrate and dry matter is shown in table 6 and in figure 5.

Table 6. Digestibility (%) ± standard deviation (STD) of nitrogen, oil, carbohydrate and dry matter in the four families (11/11 – 21/11 – 2003).

CODE	A2	B2	C2	D2
N-digest. ± STD.	91.1 ± 0.33	90.2 ± 0.34	92.2 ± 0.55	92.0 ± 0.75
Oil-digest. ± STD.	91.4 ± 0.68	91.9 ± 1.05	93.7 ± 0.75	92.5 ± 0.20
Carbo.-digest. ± STD.	78.0 ± 1.00	77.0 ± 0.55	78.8 ± 1.85	77.8 ± 2.28
D.M.-digest. ± STD.	85.1 ± 0.55	84.5 ± 0.52	86.6 ± 0.81	85.8 ± 0.94

There were only minor differences in the registered digestibilities – Nitrogen: 90,2 – 92,2%; oil: 91,4 – 93,7 %; carbohydrate: 77,0 – 78,8 % and dry matter: 84,5 – 86,6 %.

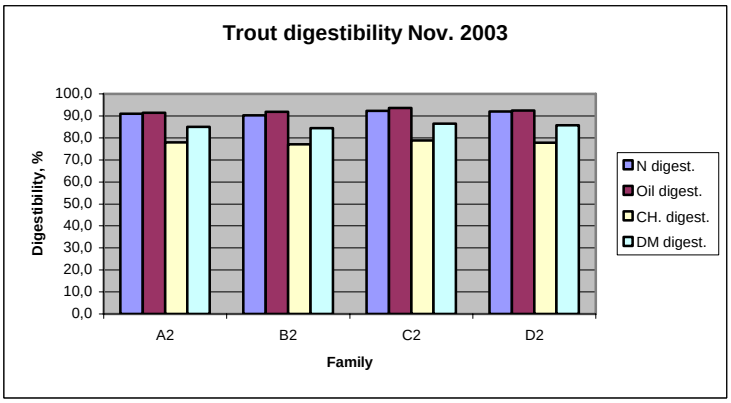


Figure 5. Digestibility (%) of nitrogen (protein), oil (fat), carbohydrate and dry matter in the experimental diet using four selected families of rainbow trout from 2003 generation from Danish Trout Breeding. 15 fish in each tank (initial weight 174,8 ± 11,0 g/pcs and final weight 208,7 ± 14,3 g/pcs).

The apparent biological values (BV_a) and the Prod. Prot. Values (PPV) are given in table 7. BV_a ranged from 0.33 (family C2) to 0.54 (family D2), i.e. 33 to 54% of the digested protein was converted to meat.

However, calculations of PPV showed, that 30 (family C2) to 49% (family D2) of the ingested protein was converted to fish protein. Family C2 appeared to have a significantly lower utilization of protein compared to the other families even the protein digestibility was high (cf. table 6).

Table 7. Apparent Biological Value (BV_a) and Productive Protein Value (PPV) ± standard deviation (STD) measured in the four families of rainbow trout in the digestibility experiments -11/11 - 21/11 – 2003.

	Family			
	A2	**B2**	**C2**	**D2**
Biological Value (BV_a)	0.49 ± 0.02	0.49 ± 0.10	0.33 ± 0.05	0.54 ± 0.04
Prod. Prot. Value (PPV)	0.45 ± 0.02	0.44 ± 0.09	0.30 ± 0.05	0.49 ± 0.04

Discussion

The fish had performed well as the specific growth rate and the feed conversion ratios have been very good during the experiments.

In respect of the obtained digestibilities of nutrients in the experimental diets there seems to be genetic related differences in the digestion of nitrogen (protein) and oil between the experimental families, while the digestibilities of carbohydrate and dry matter seemed to be similar across the family relationships.

Thus, the nitrogen digestibility was highest in the closely related families C1, D1 and C2, D2 (92 to 92,7%) compared to the other groups of closely related fish families A1 and B1, and A2 and B2 (90,2 to 92%). However, the high protein digestibility in D1 was counteracted by a lower oil digestibility compared to families A1, B1 and C1 (cf. table 3).

A relationship between specific growth rate and the apparent biological value was indicated in family A1 having the highest SGR and BV_a compared to families B1, C1 and D1.

Improvements in growth performance in the selective breeding programme was also to some extent revealed by BV_a and PPV. Accordingly about 50% of the digested protein was converted into meat (protein gain) in the second year generation (except family C2) compared to about 45% in the first year generation (cf. tables 4 and 7). Similar figures were obtained for the utilization of ingested protein, i.e. PPV (cf. tables 4 and 7).

The observed protein gain in the fish families are determined by as well the content of as the distribution between available/metabolically essential amino acids in the digestible fraction of the protein. Following, the nutritional value of the protein depends basically on the amino acid composition, but also on the bioavailability of the individual amino acids. In case that just one single essential amino acid is deficient or it has a low availability, this amino acid will be the first limiting for fish protein growth. This is due to the fact, that all other essential amino acids will only be utilized for growth to a certain degree that corresponds to the first limiting amino acid. That part of the amino acids that are not used for growth will be burnt off as energy resulting in loss of valuable protein gain and outlet of nitrogen to the environment.

This means that BV_a is an unequivocal expression of protein quality as it takes as well the digestibility of the protein (D_a) as the protein gain (PPV) in the fish into account.

Appendix 1. The initial and final individual fish weight and the total biomass in the digestibility study (3/12 – 13/12 – 2002).

Initial weight (g) of rainbow trout 3rd December 2002												
Tank no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Family code	**D1**	**B1**	**C1**	**D1**	**A1**	**A1**	**C1**	**B1**	**A1**	**D1**	**B1**	**C1**
Fish no. 1	212,2	211,1	245,4	226,5	197,6	222,4	245,4	202,5	222,6	212,6	231,1	215,5
2	218,9	216,4	247,2	218,6	233,7	212,2	215,4	219,6	196,3	203,0	235,9	241,3
3	222,8	218,4	244,2	230,4	208,8	217,9	196,2	200,2	228,8	227,7	233,0	200,2
4	212,1	196,3	226,8	219,2	191,8	200,6	208,4	250,0	215,1	217,6	236,6	244,1
5	207,5	197,3	225,0	241,1	237,5	215,2	234,1	197,7	223,0	239,5	225,6	233,3
6	218,4	247,1	247,8	228,1	198,6	205,3	224,7	238,2	178,2	209,7	223,9	218,1
7	217,1	202,5	207,6	206,2	243,7	232	228,1	226,8	214,9	231,1	229,4	233,4
8	228,5	223,6	204,4	243,8	218,5	220,3	231,7	208,0	190,0	221,0	206,6	206,3
9	199,0	234,9	235,2	200,8	230,7	218,5	212,4	216,3	204,9	211,3	239,2	206,7
10	214,6	194,8	214,5	218,1	226,7	226,5	205,2	199,2	204,7	200,2	184,8	219,1
SUM	2151,1	2142,4	2298,1	2232,8	2187,6	2170,9	2201,6	2158,5	2078,5	2173,7	2246,1	2218,0
AVG	215,1	214,2	229,8	223,3	218,8	217,1	220,2	215,9	207,9	217,4	224,6	221,8
SDS	8,2	17,5	16,7	13,7	18,5	9,4	15,2	17,9	16,1	12,6	16,8	15,4

Final weight (g) of rainbow trout 13th December 2002												
Tank no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Family code	**D1**	**B1**	**C1**	**D1**	**A1**	**A1**	**C1**	**B1**	**A1**	**D1**	**B1**	**C1**
Fish no. 1	250,5	271,1	242,5	270,8	250,4	260,8	214,0	244,1	249,1	253,1	208,9	249,9
2	228,2	194,1	271,1	289,5	242,2	267,9	255,1	228,9	261,8	230,2	270,4	267,2
3	245,3	260,6	285,5	239,3	272,2	245,7	252,4	237,5	205,3	252,9	230,1	225,8
4	247,0	216,2	253,7	256,2	263,6	265,6	274,6	247,3	229,1	273,7	266,2	277,9
5	252,6	276,8	260,7	269,1	269,5	254,4	233,2	220,8	228,3	251,0	262,0	239,0
6	232,3	228,0	280,8	269,5	285,1	238,7	205,2	244,0	253,9	228,5	259,7	245,6
7	253,7	240,6	257,0	228,4	267,6	267,5	213,8	274,8	225,2	253,5	271,4	286,2
8	264,3	255,1	286,2	257,7	256,5	249,2	280,9	264,5	239,8	225,2	254,6	231,4
9	253,3	257,8	236,1	242,3	216,9	254,7	220,1	199,3	262,6	239,5	267,1	252,6
10	237,3	238,2	280,5	250,8	188,7	245,2	288,5	288,5	254,8	267,7	272,1	274,7
SUM	2464,5	2438,5	2654,1	2573,6	2512,7	2549,7	2437,8	2449,7	2409,9	2475,3	2562,5	2550,3
AVG	246,5	243,9	265,4	257,4	251,3	255,0	243,8	245,0	241,0	247,5	256,3	255,0
SDS	11,0	25,7	18,1	18,1	28,9	10,3	30,7	26,2	18,7	16,4	20,7	20,6

Appendix 2. Feed administered, collected amount of not eaten pellets and calculation of ingested feed during the digestibility study (3/12 – 13/12 – 2002).

DECEMBER 2002												
Tank	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Code	**D1**	**B1**	**C1**	**D1**	**A1**	**A1**	**C1**	**B1**	**A1**	**D1**	**B1**	**C1**
Init. biomass (g)	2151,1	2142,4	2298,1	2232,8	2187,6	2170,9	2201,6	2158,5	2078,5	2173,7	2246,1	2218,0
Feed %=	**1,2**	**FQ=**	**0,85**									
Feed in gram:	**Fodertype: Aller GEP 576 - 4 mm**											
Tank	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Code	**D1**	**B1**	**C1**	**D1**	**A1**	**A1**	**C1**	**B1**	**A1**	**D1**	**B1**	**C1**
Administered (g)												
04-dec	25,8	25,7	27,6	27,0	26,3	26,1	26,4	25,9	24,9	26,1	27,0	26,6
05-dec	26,2	26,1	28,0	27,4	26,6	26,4	26,8	26,3	25,3	26,5	27,3	27,0
06-dec	26,5	26,4	28,4	27,8	27,0	26,8	27,2	26,6	25,7	26,8	27,7	27,4
Period 1	78,5	78,2	83,9	82,2	79,9	79,3	80,4	78,8	75,9	79,4	82,0	81,0
07-dec	26,9	26,8	28,8	28,2	27,4	27,2	27,6	27,0	26,0	27,2	28,1	27,8
08-dec	27,3	27,2	29,2	28,6	27,8	27,6	27,9	27,4	26,4	27,6	28,5	28,2
09-dec	27,7	27,6	29,6	29,0	28,2	27,9	28,3	27,8	26,8	28,0	28,9	28,5
Period 2	81,9	81,6	87,5	85,8	83,3	82,7	83,8	82,2	79,1	82,8	85,5	84,5
10-dec	28,1	28,0	30,0	29,4	28,6	28,3	28,7	28,2	27,1	28,4	29,3	29,0
11-dec	28,5	28,4	30,4	29,8	29,0	28,7	29,1	28,6	27,5	28,8	29,7	29,4
12-dec	28,9	28,8	30,9	30,2	29,4	29,1	29,6	29,0	27,9	29,2	30,2	29,8
Period 3	85,4	85,1	91,3	89,4	86,9	86,2	87,4	85,7	82,5	86,3	89,2	88,1
Total (g)	245,9	244,9	262,7	257,4	250,1	248,1	251,7	246,7	237,6	248,5		253,5
Tank	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Uneaten pellets	**D1**	**B1**	**C1**	**D1**	**A1**	**A1**	**C1**	**B1**	**A1**	**D1**	**B1**	**C1**
04-dec	3	70	1	1	3	1	5	4	22	3	2	0
05-dec	2	209	3	0	5	1	100	2	22	0	5	3
06-dec	12	83	3	4	3	3	101	4	49	3	0	0
Period 1	17	362	7	5	11	0	206	10	93	6	7	3
07-dec	7	101	3	1	4	0	90	10	49	15	6	6
08-dec	32	84	4	6	4	3	221	6	40	78	0	2
09-dec	14	35	1	0	1	1	79	15	23	22	1	0
Period 2	53	220	8	7	9	4	390	31	112	115	7	8
10-dec	19	43	1	0	0	1	48	57	1	16	0	6
11-dec	13	42	2	3	1	0	136	130	9	9	0	63
12-dec	38	72	1	0	3	0	95	28	0	1	0	28
Period 3	70	157	4	3	4	1	279	215	10	26	0	97
Total number	140	739	19	15	24	5	875	256	215	147	14	108
Pellet weight (g)	0,0714	0,0707	0,0700	0,0711	0,0704	***AVG. 0,0707***						
g uneaten pellets P. I	1,20	25,59	0,49	0,35	0,78	0,00	14,56	0,71	6,57	0,42	0,49	0,21
g uneaten pellets P. II	3,75	15,55	0,57	0,49	0,64	0,28	27,57	2,19	7,92	8,13	0,49	0,57
g uneaten pellets P. III	4,95	11,10	0,28	0,21	0,28	0,07	19,72	15,20	0,71	1,84	0,00	6,86
g consumed feed P. I	77,34	52,63	83,41	81,85	79,09	79,26	65,82	78,10	69,31	78,94	81,51	80,77
g consumed feed P. II	78,17	66,03	86,94	85,31	82,67	82,38	56,27	80,00	71,23	74,64	85,03	83,89
g consumed feed P. III	80,48	73,99	90,99	89,19	86,60	86,15	67,71	70,53	81,84	84,49	89,20	81,23
Total g consumed feed	235,98	192,65	261,34	256,34	248,35	247,79	189,80	228,63	222,38	238,07	255,75	245,89

Appendix 3. Oxygen saturation and temperature in the fish tanks and of the inlet water (high level tank) during the digestibility experiment (3/12 – 13/12 - 2002).

Oxygen (%-sat.)															
Tank no. /date	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	Oxygen (%-sat)- High level tank	Temp. (High level tank	Temp. tank
03-dec	106	89	92	94	103	98	87	95	91	93	87	93	141	8,3	9,2
04-dec	113	87	96	94	89	96	93	114	96	101	85	86	140	8,3	9,3
05-dec	97	88	93	94	101	93	111	107	105	103	87	88	148	8,3	9,3
06-dec	108	90	102	95	110	103	114	116	102	92	83	101	152	8,3	9,2
07-dec	94	75	98	85	98	91	109	104	93	74	72	90	137	8,2	9,1
08-dec	89	74	101	85	84	84	103	95	83	80	68	84	133	8,2	9,4
09-dec	89	69	93	76	93	83	95	96	78	78	62	80	133	8,1	9,4
10-dec	92	67	93	78	94	87	96	95	84	78	59	83	135	8,3	9,5
11-dec	87	70	94	78	87	89	98	101	77	83	63	85	139	8,1	9,3
12-dec	90	67	98	77	92	76	92	106	78	77	84	82	141	8,1	9,3

Appendix 4. The initial and final individual fish weight and total biomass in the digestibility study (1/11 – 23/11 – 2003).

Initial weight (g) of rainbow trout 11 November 2003												
Tank no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Family code	**A2**	**A2**	**B2**	**C2**	**A2**	**B2**	**C2**	**B2**	**D2**	**C2**	**D2**	**D2**
Fish no. 1	178,1	182,6	167,7	163,2	164,2	164,8	168,3	177,1	173,4	190,5	147,8	193,0
2	168,1	168,7	167,0	181,7	169,0	165,1	164,5	173,4	195,1	179,3	167,9	153,7
3	187,0	184,5	175,6	178,6	174,1	168,6	171,2	155,2	168,2	177,5	157,0	179,0
4	184,3	163,1	171,5	184,9	172,9	169,1	194,8	189,3	162,5	155,1	192,1	184,0
5	166,6	171,9	173,6	176,7	173,3	192,9	186,5	168,1	181,6	187,1	171,6	176,7
6	182,7	187,6	178,4	183,1	167,1	176,9	168,3	172,4	197,0	173,0	175,4	149,2
7	160,4	189,1	166,0	182,5	183,8	160,0	176,0	172,1	167,0	184,3	156,8	162,2
8	167,5	159,9	165,1	180,7	163,9	166,8	164,0	182,9	181,7	192,1	185,8	181,4
9	178,2	177,9	183,6	184,7	198,9	176,1	185,8	167,5	208,0	169,1	173,4	164,4
10	187,4	182,3	176,0	194,8	184,2	153,6	167,6	168,1	150,2	179,5	156,3	167,7
11	181,6	166,7	170,9	182,2	165,5	187,3	164,0	166,3	143,5	179,0	165,0	176,0
12	171,2	187,2	163,4	200,2	187,0	170,8	167,2	178,3	197,3	173,7	159,8	154,0
13	178,5	177,6	172,0	187,5	173,1	175,0	181,7	174,5	178,4	184,1	182,7	170,6
14	164,0	191,7	171,7	171,4	170,2	183,9	197,2	189,9	173,3	178,9	157,8	148,2
15	168,9	161,2	184,0	176,3	160,1	191,7	189,8	184,9	174,5	167,4	179,9	187,0
SUM	2624,5	2652,0	2586,5	2728,5	2607,3	2602,6	2646,9	2620,0	2651,7	2670,6	2529,3	2547,1
AVG	175,0	176,8	172,4	181,9	173,8	173,5	176,5	174,7	176,8	178,0	168,6	169,8
STD	8,78	10,79	6,27	8,79	10,49	11,49	11,77	9,40	17,74	9,56	12,87	14,22

Final weight (g) of rainbow trout 21 November 2003												
Tank no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Family code	**A2**	**A2**	**B2**	**C2**	**A2**	**B2**	**C2**	**B2**	**D2**	**C2**	**D2**	**D2**
Fish no. 1	223,1	179,2	215,7	214,0	212,6	214,5	185,0	207,1	210,9	244,5	211,3	202,2
2	200,7	219,6	205,4	230,6	199,0	191,4	203,8	185,6	220,0	199,7	197,6	165,9
3	200,8	220,2	202,1	230,7	199,5	196,7	200,4	209,1	228,9	188,3	180,6	183,3
4	204,5	229,1	205,9	220,1	208,1	165,6	195,5	198,1	190,5	215,3	206,7	214,7
5	205,3	206,5	223,7	222,0	220,9	200,5	193,3	219,4	201,6	219,5	175,3	189,0
6	207,8	189,8	197,8	229,3	205,7	197,4	230,1	196,4	229,5	205,5	195,8	203,4
7	196,4	192,6	213,7	213,2	207,3	190,1	194,6	200,3	238,7	224,1	210,8	185,2
8	225,0	224,7	199,1	226,1	211,5	222,0	219,8	218,3	181,9	222,4	233,3	213,2
9	222,1	225,8	191,8	212,5	209,7	188,4	198,5	213,2	215,0	220,0	220,8	220,2
10	210,2	234,5	200,7	235,2	240,2	194,8	186,7	198,6	225,2	225,7	176,9	223,0
11	221,0	222,3	198,6	245,1	227,1	190,9	227,3	209,0	188,2	229,6	195,5	210,9
12	201,2	222,6	204,9	220,9	220,9	225,2	228,5	224,5	182,3	218,7	193,8	180,6
13	224,6	223,4	193,0	222,1	216,9	215,9	215,7	192,9	179,4	208,0	212,3	178,2
14	204,4	208,7	203,5	222,9	195,0	200,3	200,0	206,3	221,0	201,1	214,0	229,2
15	228,1	224,0	202,0	197,0	218,0	192,0	218,0	206,5	250,0	215,5	203,6	187,4
SUM	3175,2	3223,0	3057,9	3341,7	3192,4	2985,7	3097,2	3085,3	3163,1	3237,9	3028,3	2986,4
AVG	211,7	214,9	203,9	222,8	212,8	199,0	206,5	205,7	210,9	215,9	201,9	199,1
STD	11,0	16,1	8,5	11,3	11,7	15,2	15,4	10,6	22,4	13,8	16,3	19,1

Appendix 5. Feed administered, collected amount of not eaten pellets and calculation of ingested feed during the digestibility study (11/11 – 21/11 – 2003).

NOVEMBER 2003												
Tank	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Code	**A2**	**A2**	**B2**	**C2**	**A2**	**B2**	**C2**	**B2**	**D2**	**C2**	**D2**	**D2**
Int. biomass (g)	2624,5	2652,0	2586,5	2728,5	2607,3	2602,6	2646,9	2620,0	2651,7	2670,6	2529,3	2547,1
Feed % =	**1,5**	**FQ=**	**0,85**									
Feed in gram:	**Feed type: Aller 576 - S (4 mm)**											
Tank	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Code	**A2**	**A2**	**B2**	**C2**	**A2**	**B2**	**C2**	**B2**	**D2**	**C2**	**D2**	**D2**
Administered												
12-nov	39,4	39,8	38,8	40,9	39,1	39,0	39,7	39,3	39,8	40,1	37,9	38,2
13-nov	40,1	40,5	39,5	41,6	39,8	39,7	40,4	40,0	40,5	40,8	38,6	38,9
14-nov	40,8	41,2	40,2	42,4	40,5	40,4	41,1	40,7	41,2	41,5	39,3	39,6
Period 1	120,2	121,5	118,5	125,0	119,4	119,2	121,2	120,0	121,4	122,3	115,8	116,7
15-nov	41,5	41,9	40,9	43,1	41,2	41,1	41,8	41,4	41,9	42,2	40,0	40,3
16-nov	41,5	41,9	40,9	43,1	41,2	41,1	41,8	41,4	41,9	42,2	40,0	40,3
17-nov	43,0	43,4	42,3	44,7	42,7	42,6	43,3	42,9	43,4	43,7	41,4	41,7
Period 2	125,9	127,3	124,1	130,9	125,1	124,9	127,0	125,7	127,2	128,2	121,4	122,2
18-nov	43,7	44,2	43,1	45,5	43,4	43,4	44,1	43,6	44,2	44,5	42,1	42,4
19-nov	44,5	45,0	43,9	46,3	44,2	44,1	44,9	44,4	45,0	45,3	42,9	43,2
20-nov	45,3	45,8	44,6	47,1	45,0	44,9	45,7	45,2	45,8	46,1	43,6	43,9
Period 3	133,5	134,9	131,6	138,8	132,6	132,4	134,6	133,3	134,9	135,8	128,7	129,6
Sum	379,6	383,6	374,1	394,7	377,2	376,5	382,9	379,0	383,6	386,3	365,9	368,4
Tank	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Uneaten pellets	**A2**	**A2**	**B2**	**C2**	**A2**	**B2**	**C2**	**B2**	**D2**	**C2**	**D2**	**D2**
12-nov	1	0	4	0	2	102	1	3	1	0	17	10
13-nov	0	0	15	0	0	236	53	75	24	12	96	125
14-nov	0	4	17	2	0	42	266	85	60	9	56	140
Period 1	1	4	36	2	2	380	320	163	85	21	169	275
15-nov	16	13	119	4	17	123	126	115	328	24	114	234
16-nov	2	3	36	1	8	190	130	52	62	78	77	135
17-nov	0	4	12	0	0	60	24	9	8	3	5	25
Period 2	18	20	167	5	25	373	280	176	398	105	196	394
18-nov	5	13	25	0	0	102	47	80	6	1	5	15
19-nov	5	15	52	0	46	130	52	108	6	8	0	3
20-nov	8	75	69	1	36	173	65	113	7	5	0	3
Period 3	18	103	146	1	82	405	164	301	19	14	5	21
Sum	37,0	127,0	349,0	8,0	109,0	1158,0	764,0	640,0	502,0	140,0	370,0	690,0
Pellet weight (g)	0,0689	0,0687	0,0685	0,069	**AVG. 0,0688**							
g uneaten pellets P. I	0,07	0,28	2,48	0,14	0,14	26,14	22,01	11,21	5,85	1,44	11,63	18,92
g uneaten pellets P. II	1,24	1,38	11,49	0,34	1,72	25,66	19,26	12,11	27,38	7,22	13,48	27,10
g uneaten pellets P. III	1,24	7,09	10,04	0,07	5,64	27,86	11,28	20,71	1,31	0,96	0,34	1,44
g consumed feed P. I	120,13	121,18	115,98	124,82	119,27	93,06	99,21	108,78	115,60	120,87	104,21	97,74
g consumed feed P. II	124,71	125,89	112,63	130,59	123,40	99,23	107,76	113,62	99,87	120,93	107,89	95,13
g consumed feed P. III	132,26	127,81	121,52	138,72	126,99	104,53	123,36	112,57	133,58	134,88	128,31	128,12
Total g consumed feed	377,10	374,88	350,14	394,14	369,66	296,82	330,33	334,97	349,05	376,68	340,42	320,98

Appendix 6. Oxygen saturation and temperature in the fish tanks and of the inlet water (high level tank) during the digestibility experiment (11/11 – 21/11 - 2003).

Oxygen (%-sat.)															
Tank no. /date	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	Oxygen (%-sat)- High level tank	Temp. (High level tank	Temp. tank
11-Nov	92	89	98	97	78	77	93	72	89	97	84	97	143	9,3	10,8
12-Nov	90	88	97	95	71	71	92	72	88	95	80	96	141	9,3	10,8
13-Nov	91	93	94	103	105	87	83	75	81	84	93	81	144	9,2	10,8
14-Nov	90	92	93	102	107	94	87	76	88	95	94	80	146	9,1	10,7
15-Nov	105	102	104	101	117	105	99	97	60	101	102	104	155	9,2	10,5
16-Nov	104	103	105	109	110	103	108	107	110	108	104	108	162	9,3	10,8
17-Nov	94	85	99	110	107	97	107	87	102	94	91	98	146	9	10,7
18-Nov	86	90	93	100	103	84	102	73	94	88	86	88	145	9,1	10,9
19-Nov	94	88	96	101	106	88	105	76	97	90	85	90	150	9,1	10,9
20-Nov	88	86	91	97	103	85	101	85	90	85	79	98	147	9,1	10,8

Casestudie

Samfundsøkonomisk potentiale i avslarbejde på regnbueørred

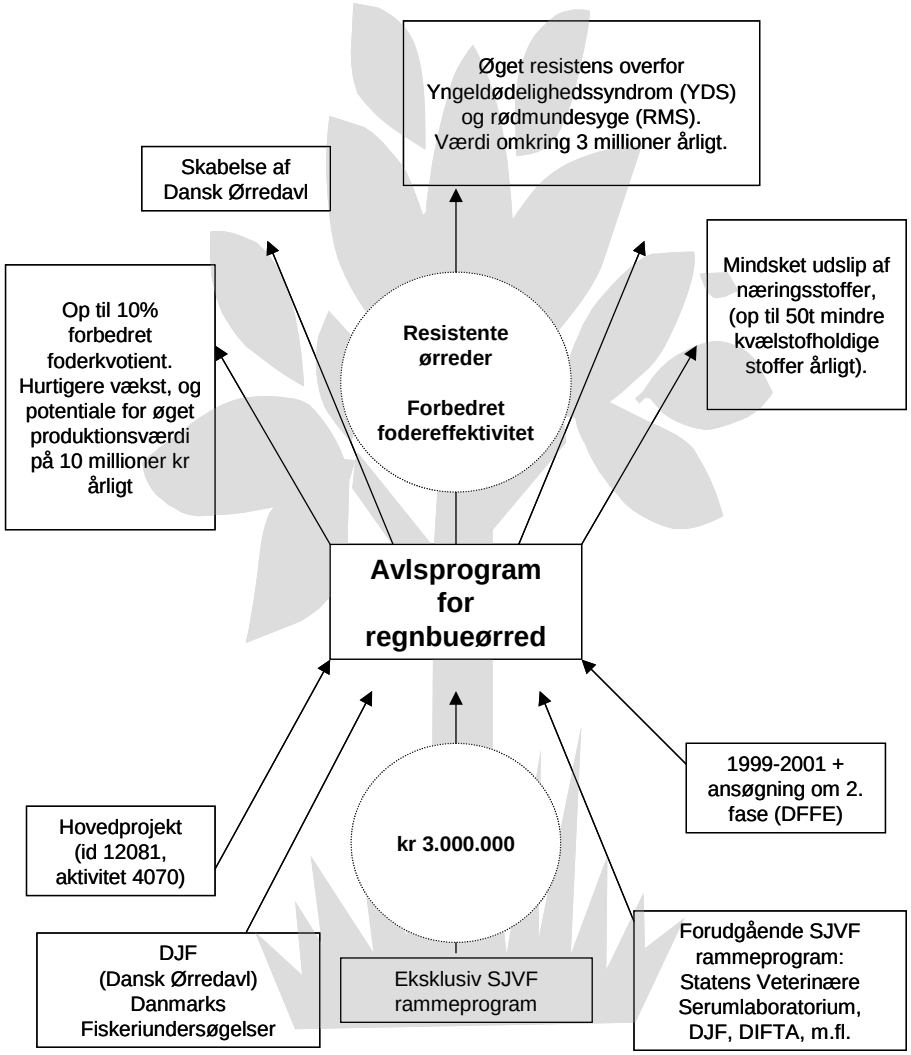
Husdyravl og Genetik

Indholdsfortegnelse *Side*

Avlsprogram for regnbueørred
DJF etablerede og implementerede i perioden 1999 – 2001 et program for avl af ørreder, hvor målet var øget vækst, øget foderudnyttelse og øget sygdomsresistens. Projektet finansieredes af Direktoratet for FødevareErhverv /DFFE). DJF's forskning i ørredavl har allerede været medvirkende til etableringen af den fondsejede virksomhed Dansk Ørredavl, som driver avlsstationen for ørreder. Også Danmarks FiskeriUndersøgelser (Hirtshals) har deltaget i projektet/virksomhedsdannelsen.

Formål

DJFs avlsarbejde giver mulighed for at selektere for såvel foderudnyttelse, som for øget resistens overfor sygdomme som yngeldødelighedssyndrom (YDS) og rødmundssyge (RMS). Danske dambrug er underlagt foderkvota, og forbedret foderudnyttelse vil derfor give dambrugerne mulighed for at forøge deres produktion. Samtidig vil udvaskningen af miljøskadelige næringsstoffer fra dambrugene falde, da en større del vil blive optaget i fiskene. Den samfundsmæssige nytte af forskningen er illustreret i nedenstående figur, der også viser hvilke aktiviteter og investeringer, avlsprogrammets resultater er vokset ud af.



Historik

Det seneste ørredprojekt blev gennemført i perioden fra 1999 til 2001 med deltagelse af DJF og Danmarks FiskeriUndersøgelser. Efter sin etablering undervejs i projektet deltog også Dansk Ørredavl. Firmaet arbejder stadig på den gren af projektet, der drejer sig om at forbedre fiskenes foderudnyttelse.

Forud for projektet lå undersøgelser gennemført under SJVF i perioden fra 1993 til 1998. Det drejede sig om et rammeprogram med et samlet budget på omkring 10 millioner kr., hvoraf det anslås, at omkring 3 millioner blev anvendt til aktiviteter, der siden hen ledte til ørredprojektet (kilde: Peer Berg, DJF). I dette program deltog Statens Veterinære Serumlaboratorium, DJF og DIFTA.

DJFs forskning i ørredavl har som anført været medvirkende til etableringen af den fondsejede virksomhed Dansk Ørredavl. Dermed har projektet haft en klassisk spin-off effekt i form af vidensbaseret økonomisk vækst.

Projektøkonomi

Tabel 1 og 2 neden for viser hvilket forbrug af midler, der har været i forbindelse med ørredprogrammet samt det forudgående SJVF program. I forhold til ørredprogrammet har Danmarks Fiskeriundersøgelser haft det største forbrug, mens DJF tegner sig for det største forbrug samlet set. Forskningsresultaterne er således i høj grad opnået i kraft af samspillet mellem flere organisationers udviklingsarbejde.

Tabel 1. Avlsprogram for regnbueørreder (finansiering fra DFFE)

Organisation	*Forbrug, kr*
Dansk Ørredavl	539.000
DJF	767.000
Danmarks Fiskeriundersøgelser	1.711.000
I alt	**3.017.000**

kilde: Peer Berg, DJF

Tabel 2. SJVF rammeprogram

Organisation	*Forbrug, kr*
Statens Veterinære Serumlaboratorium	500.000
DJF	2.274.000
DIFTA[1]	210.000
I alt	**2.984.000**

1 Udover deltagelse i SJVF foretog DIFTA associerede undersøgelser, der var finansieret ad anden vej. Imidlertid har det ikke været muligt at finde omkostningerne ved dette arbejde.
kilde: Peer Berg, DJF

Rammeprogrammet under SJVF har som nævnt haft et budget på omkring 10 mio., hvoraf de 3 mio. er gået til aktiviteter, der er ført videre i selve ørredprojektet. Dansk Ørredavl har bidraget med 539.000 kr. til selve forskningsarbejdet, hvilket er det eneste bidrag til forskningen, der kan betegnes som privat. Som en følge af firmaets oprettelse er der imidlertid også foretaget anlægsinvesteringer i størrelsesordenen 5-6 millioner kroner, og der planlægges fortsatte investeringer for et tilsvarende beløb. Disse omkostninger er naturligvis ikke en del af de direkte projektomkostninger, men er dog væsentlige i forhold til at bringe de opavlede ørreder fra forskningsstadiet til produktstadiet. Udviklingsforløbet i dette case er således et godt eksempel på, hvordan offentlige investeringer baner vejen for efterfølgende private investeringer, der forbereder produktet til at blive fremstillet og solgt på almindelige markedsvilkår.

Markedet

Der er i Danmark 433 dambrug, hvori ørred er den altdominerende produktionsfisk. Omsætningen i primærerhvervet beløber sig til ca. 500 mio. kr. (Statistisk årbog 2000), mens den samlede årsomsætning inklusiv følgeerhverv er ca. 1 mia. kr. Indtægterne i dambrugserhvervet er sagens natur tæt forbundet med salgsprisen på ørred, som er omkring 15 kr. per kg. opdrættet fisk.

Dansk dambrug er underlagt en foderkvota. Dette betyder, at en oplagt mulighed for at øge produktiviteten, er at øge fiskenes foderudnyttelse via avl. Fordelen ved forbedret foderudnyttelse er, at produktions-input i form af vand, arbejdsløn og foder er uændret samtidig med et forøget output. Vand, arbejdsløn og foder er de dominerende løbende udgifter. Samtidig falder udledningen af miljøskadelige næringsstoffer, da en større del bliver optaget i fisken. Ses der på kvælstof og fosfor, var de årlige udledninger i 1997 henholdsvis 1.227 ton og 92 ton (NMPR, 1999). Til sammenligning var de samlede direkte og indirekte udledninger til farvandene fra samtlige punktkilder på 10.070 ton kvælstof og 1.394 ton fosfor.

En undersøgelse udført Dansk Dambrugerforening og Fiskepatologisk Laboratorium anslår, at de direkte årlige omkostninger forbundet ved YDS er ca. 18 mio. kr. Dette er beregnet udfra prisen på yngel, som er 20 øre pr. stk. sammenholdt med en yngel dødelighed på ca. 34 %, som svarer til ca. 88 mio. stk. døde yngel – i alt 18 mio. kr. (Jensen et al., 2001).

Der er ikke foretaget tilsvarende overslag med hensyn til rødmundsyge (ERM), men det antages at ligge i samme område. Antagelsen bygger på, at væsentligt færre fisk rammes af ERM, men at sygdommen indtræder på et senere trin i vækstfasen og derfor giver væsentligt større omkostninger pr. fisk. Et konservativt bud er at de direkte årlige omkostninger ved YDS og ERM samlet set er ca. 30 mio. kr.

Produkt

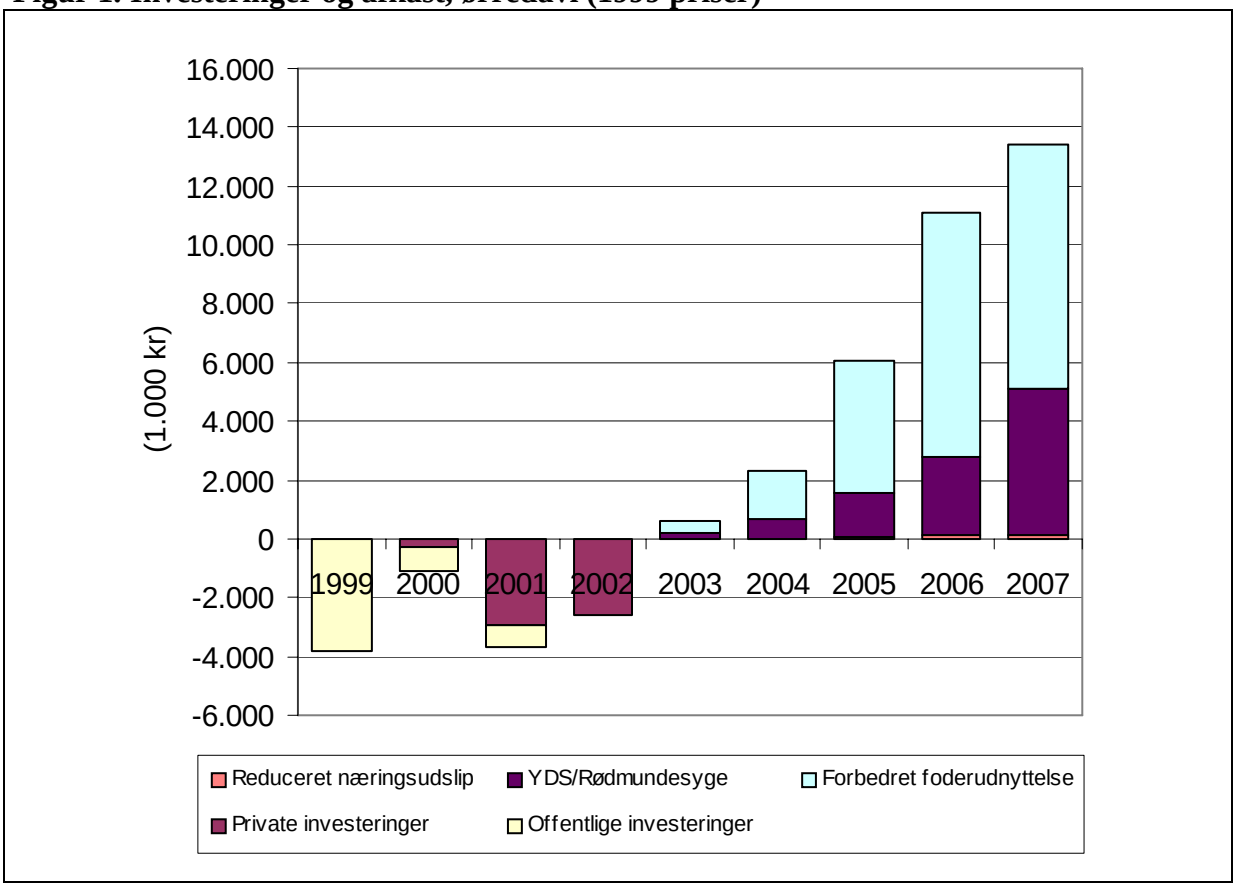
Den samfundsmæssige nytte af avlsprojektet kan kun vurderes økonomisk, hvis der gøres en række antagelser om det fremtidige potentiale for produktet[1] på markedet. Mest centralt drejer det sig om at få belyst, hvor stor

[1] Med "produktet" menes de ørredæg, som er fremavlet med forbedrede egenskaber enten i forhold til vækst eller resistens overfor sygdomme (begge dele samtidigt er længere ude i horisonten).

udbredelse produktet forventes at blive, samt hvor store besparelserne vil være ved anvendelse af produktet. Samtidig er det klart, at det vil blive tale om en gradvis udvikling, hvor både udbredelse og potentialet for besparelser vil stige over årene.

I samarbejde med blandt andet Dansk Ørredavl er det lykkedes at få sat tal på en række af disse forhold. Disse tal findes i bilag, som udgør grundlaget for figuren nedenfor. Figuren viser dels hvilke investeringer, der har været foretaget i projektet, samt hvilke afkast der kan forventes indtil år 2007. Investeringerne er opdelt efter om de er offentlige og private; de private er i dette tilfælde Dansk Ørredavls, og inkluderer både investeringer i forskning og anlæg. Afkastet stammer fra de 4 nævnte kilder, nemlig forbedret foderudnyttelse/vækst, resistens overfor YDS og RMS, samt reduceret udledning af næringsstoffer.

Figur 1. Investeringer og afkast, ørredavl (1999 priser)



Note: Omkostningerne under SJVF programmet fra 1993-98 er diskonteret til 1999-tal

I denne case er det meget tydeligt, at midlerne til forskning er givet godt ud, da afkast efter ganske få år er store nok til at betale forskningsomkostninger tilbage. I dette tilfælde er tilbagebetalingstiden udregnet til omkring 4½ år målt fra programmets afslutning i 2001. For hele perioden er det interne afkast fundet til 24 pct., hvilket må betegnes som meget højt.

- Internt afkast: 24 %
- Nutidsværdi: 13 mio. kr.
- Tilbagebetalingstid: 4½ år fra 2001

Foderudnyttelse
Figuren viser, at det er via forbedret foderudnyttelse størstedelen af det forventede afkast findes. Fremskrivningen viser, at afkastet i år 2007 vil nærme sig knap 10 millioner kroner. Dette bygger på en

antagelse om, at man kan nå en forbedret foderudnyttelse på 10 pct., og at markedspenetrationen når 15 pct. Disse tal er estimeret på basis af oplysninger fra Dansk Ørredavl, og må betegnes som relativt konservative estimater. Forbedringen på 10 pct. svarer til en årlig forbedring på omkring 2 procent, hvilket kan opnås såfremt fodereffektivitet er den eneste parameter i avlsarbejdet.

Foderudnyttelse er et område, hvor der meget hurtigt kan opnås meget store gevinster. Bare en forbedring med 5 pct., vil alt andet lige give et 5 pct. større output og en 5 pct. større omsætning. Var markedspenetrationen 100 pct., ville det på landsplan være en årlig forøget omsætning på omkring 25 millioner kroner (1999-priser).

Resistens overfor YDS og ERM
Et andet område hvor ørred produktionen i dambrug kan optimeres indenfor de nuværende rammer, er ved at mindske sygdomstilfælde blandt fiskene. De to dominerende sygdomme er Yngel Dødeligheds Syndrom (YDS) og Rødmundesyge (ERM). YDS er en bakteriel sygdom som indtræffer blandt yngelen, og som er skyld i, at dødeligheden blandt yngelen i de første uger er gennemsnitligt 34%. ERM er ligeledes en bakteriel sygdom som især rammer ørreder. ERM forårsager manglende vækst og resulterer ofte i at fisken dør.

Der foreligger ikke konkrete tal for, hvor meget man kan forøge ørredernes resistens, men fra lignende forsøg i svinesektoren har en resistensforbedring på 50 pct. via avl vist sig at være indenfor rækkevidde! En så stor forbedring er dog næppe realistisk indenfor ørredavl, men kilder peger på at forbedring på 30 pct. bør være indenfor rækkevidde. Disse opgørelse er meget usikre og blandt andet mangler vi viden om hvordan resistens overfor forskellige sygdomme er relateret. Dette undersøges i et igangværende projekt. Sygdomsresistens indgår derfor på nuværende tidspunkt endnu ikke i avlsmålene på avlsstationen.

Imidlertid kan denne forbedring ikke komme hele erhvervet til gode, da der kan være forhold, der forhindrer den enkelte dambruger i at bruge denne type fisk. Dels er det usikkert, hvor stort udbudet af resistente fisk bliver, og dels kan dambrugeren blive nødt til at vælge mellem avl, hvor der er selekteret for resistens, og avl, hvor der er selekteret for foderudnyttelse. Alligevel er det forventningen, at markedspenetrationen når omkring 50 pct., ikke mindst i lyset af, at særligt YDS opfattes som et relativt stort problem i branchen.

Beregningerne viser, at en markedspenetration på 50 pct. og en forbedret resistensen på 30 pct. vil udmønte sig i en årlig økonomisk gevinst på 5 millioner kroner (1999-priser), hvoraf YDS- og ERM-besparelser hver udgør omkring halvdelen.

Reduceret næringsudslip
Forbedret foderudnyttelse giver mindre næringsudslip fra ørredproduktionen eftersom en større del af næring bliver i fisken og dermed i produktionscyklen. Der vil udover de økonomiske gevinster også være nogle miljømæssige gevinster ved forbedret foderudnyttelse blandt ørreder. Som det ses af figuren, er også disse gevinster blevet værdisat, om end de betydeligt mindre end de øvrige gevinster.

Værdisætningen er baseret på den antagelse, at når foderudnyttelsen stiger med et vist antal procent, så falder næringsstofudledningen med det halve. En forbedret foderudnyttelse på 10 pct. svarer dermed til reduktion af udledningerne med 5 pct. I forhold til de tidligere omtalte udledninger på 1.227 t kvælstof og 92 t fosfor, og en markedspenetrationen på 15 pct., bliver reduktionerne på henholdsvis 9 t kvælstof og knap 1 t fosfor. Udfra enhedsomkostninger ved rensning på 5 kr/kg N og 80 kr/kg P[2], bliver den samlede værdi af reduktionen dermed omkring 100.000 kroner (1999-priser). Den miljømæssige gevinst er således meget lav i forhold til de øvrige gevinster.

Effekt på udenrigshandelen
Dansk Ørredavl planlægger ikke at sælge æg til udenlandske dambrug, da man ikke ønsker at forringe den eksisterende danske eksport. Denne politik forventes fastholdt så længe avlsstationen er finansieret af danske investorer. Leverandørleddet vil dermed ikke bidrage til øget eksport (men muligvis til svagt øget import). Derimod forventes de danske dambrugere at kunne øge deres eksport, da de vil blive i stand til at producere flere fisk med lavere stykomkostninger. Erhvervet forventer dog ikke en egentlig ændring af eksportandelen. Værdien

[2] Kilde: Tidskrift for landøkonomi 4/2001

af eksporten fra ferskvandsdambrug i 1998 var 267 millioner kroner (DS, 2000), hvilket skal ses i forhold til en omsætning i hele erhvervet på 1.050 millioner kroner (inklusiv følgeerhverv) –altså en eksportandel på godt 25 pct. Dermed er det også givet, at 25 procent af den værdi, som forbedret foderudnyttelse skaber, og som er illustreret i figur 1 stammer fra eksport. I kroner svarer dette til 25 pct. af 8,3 millioner, dvs. omkring 2,1 million (1999-priser).

Konklusion

Samlet set viser casen, at der er et vist samfundsmæssigt potentiale i avlsprogrammet for regnbueørreder i form af økonomiske gevinster for erhvervet samt miljøeffekter som følge af mindre næringsudslip og mindre medicinanvendelse. Selv om de økonomiske beregninger skal tages med visse forbehold, synes det sandsynliggjort, at forskningen i ørredavl kan medføre relativt substantielle samfundsmæssige gevinster.

Referencer og kontaktpersoner

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MEM, Natur og Miljøpolitisk redegørelse, 1999

Bilag A

Ørreder (alt er i 1000)

Inflation	**2%**								
Kalkulationsrente/tidspræference	**5%**								
År	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**
Prisindeks	1,00	1,02	1,04	1,06	1,08	1,10	1,13	1,15	1,17
Gevinst 1: Forbedret foder									
Markedsandel, ny avl	0%	0%	0%	0%	2%	5%	10%	15%	15%
Forbedret foderkvotient	0%	0%	0%	2%	4%	6%	8%	10%	10%
Foderkvotient (pr. kg fisk)	**0,94**	0,94	0,94	0,92	0,90	0,89	0,87	0,85	0,85
Baseline produktion (kg)	33.333	33.333	33.333	33.333	33.333	33.333	33.333	33.333	33.333
Foderkvote (kg)	31.433	31.433	31.433	31.433	31.433	31.433	31.433	31.433	31.433
Potentiel produktion	33.333	33.333	33.333	33.333	33.361	33.442	33.633	33.889	33.889
Merproduktion (kg)	0	0	0	0	28	109	299	556	556
Merværdi (kr) v 15kr/kg	0	0	0	0	456	1.800	5.059	9.572	9.764
Gevinst 2: YDS									
Markedsandel	0%	0%	0%	0%	10%	20%	30%	40%	50%
Forbedret yngeldødelighed	0%	0%	0%	2%	5%	10%	15%	20%	30%
Yngeldødelighed	34%	34%	34%	33%	32%	31%	29%	27%	24%
Yngel (startantal)	258.824	258.824	258.824	258.824	258.824	258.824	258.824	258.824	258.824
Baseline døde (styk)	88.000	88.000	88.000	88.000	88.000	88.000	88.000	88.000	88.000
Forbedret antal døde (styk)	88.000	88.000	88.000	88.000	87.560	86.240	84.040	80.960	74.800
Merværdi (kr) v 5øre/stk	0	0	0	0	95	389	892	1.617	3.093
Gevinst 3: RMS									
Markedsandel	0%	0%	0%	0%	10%	20%	30%	40%	50%
Resistensforbedring	0%	0%	0%	2%	5%	10%	15%	20%	30%
Sygdomstilfælde (dødelighed)	3%	3%	3%	3%	3%	3%	3%	2%	2%
Fisk (kg)	35.000	35.000	35.000	35.000	35.000	35.000	35.000	35.000	35.000
Baseline døde (kg)	1.050	1.050	1.050	1.050	1.050	1.050	1.050	1.050	1.050
Forbedret antal døde (styk)	1.050	1.050	1.050	1.050	1.045	1.029	1.003	966	893
Merværdi (kr) v 15kr/kg	0	0	0	0	85	348	798	1.447	2.768
Gevinst 4: Mindsket næringsudslip									
Markedsandel, svarende til foder	0%	0%	0%	0%	2%	5%	10%	15%	15%
Baseline kvælstofudledning (kg)	1.227	1.227	1.227	1.227	1.227	1.227	1.227	1.227	1.227
Baseline fosforudledning (kg)	92	92	92	92	92	92	92	92	92
Reduceret N-udledning (kg)	0	0	0	0	0	2	5	9	9
Reduceret P-udledning (kg)	0	0	0	0	0	0	0	1	1
Værdi af reduceret N (kr) v 5kr/kg	0	0	0	0	3	10	28	53	54
Værdi af reduceret P (kr) v 80kr/kg	0	0	0	0	3	12	34	63	65
Udgift 1: Ørredprojekt									
DJF	-256	-256	-256						
Danmarks Fiskeriundersøgelser	-570	-570	-570						
Dansk Ørredavl (forskning)		-269,5	-269,5						
Dansk Ørredavl (faste investeringer)			-2.750	-2.750					
Udgift 2: Forprojekter									
SJVF (DJF)	-2.274								

SJVF (Statens Veterinære Seruminst.)	-500								
SJVF (DIFTA)	-210								
Total, løbende priser	**(3.810)**	**(1.096)**	**(3.846)**	**(2.750)**	**639**	**2.546**	**6.777**	**12.690**	**15.679**
Total, faste priser (1999)	**(3.810)**	**(1.074)**	**(3.696)**	**(2.591)**	**590**	**2.306**	**6.018**	**11.047**	**13.382**
Nutidsværdi (1999)	**(3.810)**	**(4.833)**	**(8.185)**	**(10.424)**	**(9.938)**	**(8.131)**	**(3.641)**	**4.211**	**13.268**
Løbende priser, opdelt på kildetype									
Private investeringer	0	-270	-3.020	-2.750	0	0	0	0	0
Offentlige investeringer	-3.810	-826	-826	0	0	0	0	0	0
Forbedret foderudnyttelse	0	0	0	0	456	1.800	5.059	9.572	9.764
YDS/Rødmundesyge	0	0	0	0	180	736	1.690	3.065	5.861
Reduceret næringsudslip	0	0	0	0	6	23	63	116	119
Faste priser (1999), opdelt på kildetype									
Private investeringer	0	-264	-2.902	-2.591	0	0	0	0	0
Offentlige investeringer	-3.810	-810	-794	0	0	0	0	0	0
Forbedret foderudnyttelse	0	0	0	0	421	1.630	4.492	8.333	8.333
YDS/Rødmundesyge	0	0	0	0	167	667	1.501	2.668	5.003
Reduceret næringsudslip	0	0	0	0	5	21	56	101	101

Tilbagebetalingstid	4½fra 2001
Internt aFQast (løbende priser)	24%årligt, perioden fra 1999 til 2007

EVA

Version 1.5

EVolutionary Algorithm for Mate Selection

User's Guide

DRAFT
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Content

Introduction

This document is a guide to the use of the two programs in EVA. The two programs and their use are

EVA_inbred : Computes inbreeding coefficients, generation coefficients, pedigree completeness indeces, genetic contributions and additive genetic relationships between animals.

EVA : Computes optimal genetic contributions given a cost of inbreeding and proposes a mating list based on minimum coancestry matings.

With EVA_inbred, a population can be thoroughly described in terms of inbreeding, Pedigree completeness, generation coefficients, genetic contributions and relationships.

With EVA proposals for new matings can be obtained, which maximize a function

$$F = w_{ebv} \cdot c'\hat{a} + w_{xAx} \cdot c'Ac$$

c : *genetic contributions* (*to be optimised*)

$\hat{a}$: *estimated breeding values*

A : *numerator relationship matrix*

w_{ebv} : *scalar weight on ebv* (*input*)

w_{xAx} : *scalar weight on average relationship* (*input*)

EVA_inbred algorithms

EVA_Inbred is a program to calculate inbreeding coefficients and generation coefficients for a pedigree of any size. The program is based on the algorithm described in :

MEUWISSEN, THE & LUO, Z.; 1992. COMPUTING INBREEDING COEFFICIENTS IN LARGE POPULATIONS.
GENET. SEL. EVOL. 24:305-313.

The program renumber the animal identification to consecutive numbers using a bit-tree. This will run quicker if the input file is sorted by half- and fullsibs. Animals with both parents unknown are the base population. Generation coefficients are 1 in the base population.

Pedigree completeness indeces are computed as described by

Sigurdsson, A. & Jonmundsson, J.V. 1994
Proceedings of the 5th WCGALP, vol 17 : 140-143
and
MacCluer J.W.; Boyce A.J.; Dyke B.; Weitkamp L.R.; Pfennig D.W. & Parsons C.J: 1983.

Journal of Heredity 74:394-399

In version 1.3 added computing genetic contributions of base animals to the last generation and the possibility of computing genetic contributions and additive relationship between selected animals as given in the input file inbred.prm (see below)

In testing for the next version : Average relationships between groups of animals computed with the algorithm proposed by

Colleau, J.-J. 2002. An indirect approach to the extensive calculation of relationship coefficients. Genet.Sel.Evol. 34:409-421

EVA Algorithms

Evolutionary algorithm

Evolutionary algorithms is a class of algorithms based on biological evolution. For a thorough description of evolutionary algorithms see Michalewicz (1998)

For details on algorithms used in EVA, see appendix A and B

Input to EVA and EVA_Inbred

Input to EVA is provided in two files, one file with data and a parameter file. In the following sections the content of these files are described.

Data file

The data file is an ASCII file with the following columns

Column	**Description**
Animal ID	Integer ID for animal
Sire ID	Integer ID for sire of animal
Dam ID	Integer ID for dam of animal
Sex	Sex of animal (1 if male, 2 if female). Integer
Birth time	Time when animal is born. In units of time period. Integer
Max. matings	Maximum number of matings for an animal, 0 if the animal is not available as a candidate. Integer.
Breeding value	Predicted breeding value, e.g. BLUP of animals breeding value. Real.
Text	**Optional** field with a text string of up to 15 characters to be printed in the mating list

If maxage is the maximal age (in time periods) of a parent the data file should have complete records of parent contributions in the previous maxage+1 time periods. This

means that there should be records for all animals born in the previous maxage+1 time periods.
Unit of time is chosen such that birth times can be given as integers. The program assumes that animals fro matings to be planned will be born at a time period advanced by 1 relative to the maximal value observed in the data.

For EVA_inbred only the first 5 columns are needed.

EVA_Inbred Parameter files

Filenames for input and output files are supplied in a file given in the command line, e.g.
eva_inbred *[filename]*
where filename is optional and defaults to *eva_inbred_filenames.prm*
In this file, filenames are given in a namelist format, e.g.
&files [[file=filename]] /
The following filenames can be given with default values also given

File description	file	Default filename
Data file	datafile	Eva.dat
Log file	logfile	Eva_inbred.txt
File with inbreeding coefficients	Fcoeff_file	F_coeff.txt
Summary file	Fsummary_file	F_summary.txt
Genetic contributions	Gc_file	Gencont.txt
Largest genetic contributions	Maxgc_file	Max_gc.txt
Parameters to compute genetic contributions and relationships	Gcparm_file	Inbred.prm
Generations to compute Pedigree Completeness Index	Pci_ngen	3

Example
&files datafile="rdm1970_a.dat", fcoeff_file="rdm_f.txt", pci_ngen=5 /
Filenames or parameters not given has default values

To compute additive relationships between animals and genetic contributions from an ancestor to a descendant, supply a parameter file named by gcparm_file above, containing in each line

&contrib ancestor=id1 descendant=id2 group=idg /
where at most two of the three arguments need to be specified
id1 is the id in the pedigree of an ancestor (0 if all base animals)
id2 is the id in the pedigree of a descendant
idg is the id of a group of animals in the pedigree file

if id1 and id2 are both specified then both additive relationships

and genetic contribution are computed
if id1 and idg is supplied then the average contribution of id1
to animals in group idg is computed
if id1 is not specified the contribution of all base animals
to id2 or animals in idg is computed
if both id2 and idg are specified idg is ignored

Example :
&contrib ancestor=0 group=2002 /

computes average genetic contributions of all ancestors (animals with unknown parents) to all animals in year-group 2002.

EVA Parameter files

EVA can be called with an optional parameter, specifying a file with parameters.
***Eva** [parameter]*

If a parameter is specified in the command line, this should be a valid filename, referring to a file containing parameters as Fortran90 namelists. If a parameter is not specified parameters are read from the default parameter file, **eva.prm**.

The file with parameters for running EVA (default **eva.prm)**, has to be located in the current directory (the directory where EVA is running).
Eva.prm/parameter file contains two sections, one with parameters for the problem to be solved and another with parameters for the evolutionary algorithm.

Data Parameters
These parameters are given as a Fortran90 namelist.

&mselparm [Parameter=x [,parameter=x]] /

where parameter is one of the parameters specified below and x is the value given. If a parameter is not given it takes a default value also given below.

Mselparm parameter	Description	Default value
Data_file	Text string with path and filename for file with data (input)	'eva.dat'
Log_file	Text string with path and filename for log file (output)	'eva.log'
Lst_file	Text string with path and filename for file with optimal matelist (output).	'eva_mate.lst'
Male_list	List of males and number of matings per male	Male_list.txt
CNV1_file	Text string with path and filename	'eva_best.txt'

	for file to hold convergence of best solution	
CNV2_file	Text string with path and filename for file to hold convergence of evolutionary algorithm	'eva_cnv.txt'
Oldsol_file	File with solutions from previous run, in the same format as the output file lst_file (see below)	'_null_'
Nmatings	Number of matings to be selected	10
W_merit	W_{ebv} as given in the introduction	0.0
W_relationship	W_{xAx} as given in the introduction	-1.0
Repeated_matings	.True. if repeated matings are allowed (females can be mated more than once) .false. otherwise	.true.

Example :

```
&mselparm nmatings=5,
        w_merit=1.0,
        w_relationship=-100.0,
        repeated_matings=.false.    /
```

Algorithm Parameters

These parameters are given as a Fortran90 namelist.

&evaparm [Parameter=x [,parameter=x]] /

where parameter is one of the parameters specified below and x is the value given. If a parameter is not given it takes a default value also given below.

Algorithm parameter	Description	Default value
Popsize	population size in the evolutionary algorithm	100
n_offspring	Number of offspring per generation in evolutionary algorithm	10
Generations	Number of generation to run algorithm	1000
restart_interval	Number of generations without improvements before mutate_probability is increased to 0.5 and a new population created from the present best solution	1000
exchange_algorithm	Interval in generations between running the exchange algorithm	100
mutate_probability	mutation probability in evolutionary algorithm	1/nmatings
directed_mutation_probability	Probability of exchanging parent with "best" alternative.	

crossover_probability	crossover probability in evolutionary algorithm	1/nmatings
Seed_rng	Seed for random number generator. If 0 the number of seconds since midnight is taken as seed.	0

Example :

```
&evaparm  popsize=10,
          generations=100,
          n_offspring=10,
          restart_interval=1000,
          exchange_algorithm=10
          /
```

Output from EVA

All output files are in ASCII format and can be imported into Excel.

LOG files

EVA prints a log file in EVA.LOG. This gives information on the parameters, a description of the population and the iteration history.
The iteration history it only written to
Eva_conv.txt Gives convergence of the population of solutions
Eva_best.cnv Gives information on the evolution of the best solution

Mating list

The mating list written to the file specified by the parameter lst_file (by default EVA_MATE.LST) has 1 line with the name of the variables and in the rest of the lines in the ASCII file :
'Male','Female ','N mating','Nm','Nf(m)','EBV','F','vF','Male txt','Female txt'

Variable	Description	Format
Sire	Sire id	Integer
Dam	Dam id	Integer
N Mating	Number of mating, numbered from 1 to number of matings	Integer
Nm	Mating number for sire	Integer
Nf(m)	Mating number of dam within sire	Integer
EBV	Estimated Breeding value	Real
F	Coefficient of inbreeding of	Real

	offspring from mating between sire and dam	
vF	Mendelian sampling variance factor for offspring, that is mendelian variance is vf*Va, where Va is genetic variance. VF is between 0 and ½	Real
Sire Text	field with an optional text string from the input file	Characters
Dam Text	field with an optional text string from the input file	Characters

Output from EVA_inbred

All output files are in ASCII format and can be imported into Excel.

USER SPECIFIED OUTPUT FILE
Gives information on data and average inbreeding by birth_time.

F_COEFF.LST
A file with one record for each animal in the same format as input file with each record extended with the inbreeding coefficient and the generation cooefficient of the animal.
At the end of the file summary statistics of the pedigree and its coeffecients of inbreeding is written.
Output :
Animal id (integer)
Parent id (integer, missing=0)
Other parent id (integer, missing=0)
Sex (1:males, 2:females)
Birth_time (Integer Birth year code or equivalent from input file)
Inbreeding coefficient (real between 0 and 1, real)
Generation coefficient (real >1)
Pedigree Completeness Index with 3, 5 and 10 generations, PEC3, PEC5, PEC10

MAX_GC.LST
A list of the 50 largest genetic contributions.

GEN_CONT.LST
A list of genetic contributions and additive genetic relationships as specified in the parameter file.

References

Michalewicz, Z. 1996. Genetic Algorithms + Data Structures = Evolution Programs. Springer Verlag.

Appendix A. Controlling rate of inbreeding with overlapping generations

Appendix B. Computational strategy used in EVA v1.4

Appendix A
Controlling rate of inbreeding with overlapping generations

The contribution to average relationships in year t (t->∞) as a function of current genetic contributions c, equals in the case of discrete generations

$$c^T \cdot A \cdot c$$

With overlapping generations, animals contributions accumulates over time.
We need to describe the cumulated genetic contribution of animals over a generation.

Following the gene-flow theory of Hill (1974), we can define a transition matrix, P, with elements in row i and column j representing the proportion of genes from sex-age class j at time t contributed to sex-age class i at time t+1. The asymptotic contributions of sex-age classes is

$$\lim_{t \to \infty} P^t := 1 \cdot v^T \cdot \frac{1}{2L}$$

where the i'th element of v represents the expected contribution of sex-age class i, as the proportion of the cohorts contributions that are still to be realised.
Then the total contribution over a generation equals (see Grundy et al (2000) for details)

$$\left(c + \sum_{i=1}^{Na} v_i \cdot P^i \right) \cdot \frac{1}{2L}$$

where c is current contributions (by definition having a weight of 1) and P^i represents the contributions of anials in the i'th previous time period. Na is the maximum number of age classes.

This derivation assumes
1. constant age distribution of parents
2. asymptotic genetic contributions equals first generation genetic contributions (see Bijma & Wolliams 2000), such that selective advantage is not inherited.

Appendix B
Computational strategy used in EVA v1.5

The function of average relationships to be minimised can be written

$$\frac{1}{(2\cdot L)^2}\cdot(c + P\cdot w)^T\cdot A\cdot(c + P\cdot w)$$

where
c is a vector of contributions to the next generation
P is a matrix of contributions to previous generations, one period per column
w is the lifetime breeding profile (Grundy et al 2000) weighing previous contributions and L is generation interval.

More specific, with two sexes P and w are defined as

$$P := \begin{pmatrix} P_m & 0 \\ 0 & P_f \end{pmatrix} \qquad w := \begin{pmatrix} w_m \\ w_f \end{pmatrix}$$

This can be rewritten as

$$\frac{1}{(2\cdot L)^2}\cdot\left(c^T\cdot A\cdot c + 2\cdot c^T\cdot A\cdot P\cdot w + w^T\cdot P^T\cdot A\cdot P\cdot w\right)$$

To evaluate a mating set a new c and w are computed conditional on the proposed matings and the mating set evaluated given formulas above. To simplify computations the following quantitties are computed once and stored

$A\cdot P\cdot 2$	with dimension number of candidates*number of age classes*2
$P^T\cdot A\cdot P$	square matrix with dimension 2*number of age classes

To further speed up computations, A and A*P are reduced to a dimension of number of candidates, i.e. animals that can have non-zero elements in c.

Including genetic merit (a) and inbreeding (F) in the function to be maximise results in

$$v_a \cdot c^T \cdot a + \frac{v_{rel}}{(2 \cdot L)^2} \cdot \left(c^T \cdot A \cdot c + 2 \cdot c^T \cdot A \cdot P \cdot w + w^T \cdot P^T \cdot A \cdot P \cdot w \right) + v_F \cdot F$$

where

a	is a vector of predicted breeding values
F	is the average coefficient of inbreeding of offspring
v_a	is the weight on average merit of offspring
v_{rel}	is the weight on the long-term rate of inbreeding
v_F	is the weight on inbreeding in the next generation

Appendix A
Controlling rate of inbreeding with overlapping generations

The contribution to average relationships in year t (t->∞) as a function of current genetic contributions c, equals in the case of discrete generations

$$c^T \cdot A \cdot c$$

With overlapping generations, animals contributions accumulates over time.
We need to describe the cumulated genetic contribution of animals over a generation.

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where the i'th element of v represents the expected contribution of sex-age class i, as the proportion of the cohorts contributions that are still to be realised.
Then the total contribution over a generation equals (see Grundy et al (2000) for details)

$$\left(c + \sum_{i=1}^{Na} v_i \cdot P^i \right) \cdot \frac{1}{2L}$$

where c is current contributions (by definition having a weight of 1) and P^i represents the contributions of anials in the i'th previous time period. Na is the maximum number of age classes.

This derivation assumes
1. constant age distribution of parents
2. asymptotic genetic contributions equals first generation genetic contributions (see Bijma & Wolliams 2000), such that selective advantage is not inherited.

Appendix B
Computational strategy used in EVA v1.5

The function of average relationships to be minimised can be written

$$\frac{1}{(2\cdot L)^2}\cdot(c + P\cdot w)^T\cdot A\cdot(c + P\cdot w)$$

where
c is a vector of contributions to the next generation
P is a matrix of contributions to previous generations, one period per column
w is the lifetime breeding profile (Grundy et al 2000) weighing previous contributions and L is generation interval.

More specific, with two sexes P and w are defined as

$$P := \begin{pmatrix} P_m & 0 \\ 0 & P_f \end{pmatrix} \qquad w := \begin{pmatrix} w_m \\ w_f \end{pmatrix}$$

This can be rewritten as

$$\frac{1}{(2\cdot L)^2}\cdot\left(c^T\cdot A\cdot c + 2\cdot c^T\cdot A\cdot P\cdot w + w^T\cdot P^T\cdot A\cdot P\cdot w\right)$$

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$A\cdot P\cdot 2$	with dimension number of candidates*number of age classes*2
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Including genetic merit (a) and inbreeding (F) in the function to be maximise results in

$$v_a \cdot c^T \cdot a + \frac{v_{rel}}{(2 \cdot L)^2} \cdot \left(c^T \cdot A \cdot c + 2 \cdot c^T \cdot A \cdot P \cdot w + w^T \cdot P^T \cdot A \cdot P \cdot w \right) + v_F \cdot F$$

where

a	is a vector of predicted breeding values
F	is the average coefficient of inbreeding of offspring
v_a	is the weight on average merit of offspring
v_{rel}	is the weight on the long-term rate of inbreeding
v_F	is the weight on inbreeding in the next generation

BLUP-model anvendt til at beregne avlsværdier hos regnbueørred

Modellen anvendt til opnåelse af BLUP avlsværdier er:

$$\mathbf{y = Xb + Z_k k + Z_m m + Z_a a + e}$$

hvor **y** er vektor af registreringer taget fra individuelle fisk eller helsøskende-familier, **b** er en vektor af systematiske effekter (f.eks. årseffekter, køn, dato og alder på hunnen ved strygning, **k** er en vektor af tilfældige tank/akvarium effekt ~ $N(0, \mathbf{I}_k\sigma_k^2)$, **m** er en vektor af tilfældige maternelle effekter $N(0, \mathbf{I}_m\sigma_m^2)$, **a** er vektor for tilfældig avlsværdier $N(0, \mathbf{A}\sigma_a^2)$, **e** er vektor for tilfældig residuelle fejl $N(0, \mathbf{I}_e\sigma_e^2)$, $\mathbf{X}$, $\mathbf{Z}_k$, $\mathbf{Z}_m$ og $\mathbf{Z}_a$ er kendte design matrix der associerer observationerne fundet i hver fisk eller helsøskende-familie med systematiske og tilfældige effekter. $\mathbf{I}_k$, $\mathbf{I}_m$ og $\mathbf{I}_e$ er identitetsmatricer og **A** er den additive genetiske slægtskabsmatrix.

I BLUP løses for **b, k, m, a** og **e** under forudsætning at **y,** $\mathbf{X}$, $\mathbf{Z}_k$, $\mathbf{Z}_m$, $\mathbf{Z}_a$, σ_k^2, σ_m^2, σ_a^2 og σ_e^2 er kendte. Elementerne i **a** er avlsværdier af den individuelle fisk eller helsøskende- familier. Beregninger af varianskomponenterne σ_k^2, σ_m^2, σ_a^2 og σ_e^2 kommer fra litteraturen.

DFU-rapporter – index

Denne liste dækker rapporter udgivet i indeværende år samt de foregående to kalenderår. Hele listen kan ses på DFU's hjemmeside www.dfu.min.dk, hvor de fleste nyere rapporter også findes som PDF-filer.

Nr. 130-04 Bestanden af blåmuslinger i Limfjorden 1993 til 2003. Per Sand Kristensen og Erik Hoffmann.

Nr. 131-04 Udsætningsforsøg med ørred (*Salmo trutta*) i Gudenåen og Randers Fjord, gennemført i 1982-83, 1987-89 og 1994-96. Stig Pedersen og Gorm Rasmussen

Nr. 132-04 En undersøgelse af muligheder for etablering af måleprogram på såkaldte modeldambrug. Lars M. Svendsen og Per Bovbjerg Pedersen

Nr. 133-04 Udnyttelse af strandkrabber. Knud Fischer, Ole S. Rasmussen, Ulrik Cold og Erling P. Larsen

Nr. 134-04 Skjern Å's lampretter. Nicolaj Ørskov Olsen og Anders Koed

Nr. 135-04 Undersøgelse af biologiske halveringstider, sedimentation og omdannelse af hjælpestoffer og medicin i dam- og havbrug, samt parameterfastsættelse og verifikation af udviklet dambrugsmodel. Lars-Flemming Pedersen, Ole Sortkjær, Morten Sichlau Bruun, Inger Dalsgaard & Per Bovbjerg Pedersen

Nr. 135a-04 Supplerende teknisk rapport (Anneks 1 – 8) til DFU-rapport nr. 135-04. Undersøgelse af biologiske halveringstider, sedimentation og omdannelse af hjælpestoffer og medicin i dam- og havbrug, samt parameterfastsættelse og verifikation af udviklet dambrugsmodel. Lars-Flemming Pedersen, Ole Sortkjær, Morten Sichlau Bruun, Inger Dalsgaard og Per Bovbjerg Pedersen

Nr. 136-04 Østersfiskeri i Limfjorden – sammenligning af redskaber. Per Dolmer og Erik Hoffmann

Nr. 137-04 Hjertemuslinger (*Cerastoderma edule*) på fiskebankerne omkring Grådyb i Vadehavet, 2004. Per Sand Kristensen og Niels Jørgen Pihl

Nr. 138-04 Blåmuslinger (*Mytilus edulis* L.) og molboøsters (*Arctica islandica* L.) i det nordlige Lillebælt i 2004 (fiskerizone 37 og 39). Forekomster og fiskeri. Per Sand Kristensen

Nr. 139-05 Smoltdødeligheder i Årslev Engsø, en nydannet Vandmiljøplan II-sø, og Brabrand Sø i foråret 2004. Kasper Rasmussen og Anders Koed

Nr. 140-05 Omplantede blåmuslinger fra Horns Rev på bankerne i Jørgens Lo og Ribe Strøm 2002-2004. Per Sand Kristensen og Niels Jørgen Pihl

Nr. 141-05 Blåmuslingebestanden i det danske Vadehav efteråret 2004. Per Sand Kristensen, Niels Jørgen Pihl og Rasmus Borgstrøm

Nr. 142-05 Fiskebestande og fiskeri i 2005. Sten Munch-Petersen

Nr. 143-05 Opdræt af torskeyngel til udsætning i Østersøen (forprojekt). Josianne G. Støttrup, Julia L. Overton, Christian Möllmann, Helge Paulsen, Per Bovbjerg Pedersen og Peter Lauesen

Nr. 144-05 Skrubbeundersøgelser i Limfjorden 1993-2004. Hanne Nicolajsen

Nr. 145-05 Overlevelsen af laksesmolt i Karlsgårde Sø i foråret 2004. Anders Koed, Michael Deacon, Kim Aarestrup og Gorm Rasmussen

Nr. 146-05 Introduktion af økologi og kvalitetsmærkning på danske pionerdambrug. Lars-Flemming Pedersen, Villy J. Larsen og Niels Henrik Henriksen

Nr. 147-05 Fisk, Fiskeri og Epifauna. Limfjorden 1984 – 2004. Erik Hoffmann

Nr. 148-05 Rødspætter og Isinger i Århus Bugt. Christian A. Jensen, Else Nielsen og Anne Margrethe Wegeberg

Nr. 149-05 Udvikling af opdræt af aborre (*Perca fluviatilis*), en mulig alternativ art i ferskvandsopdræt. Helge Paulsen, Julia L. Overton og Lars Brünner

Nr. 150-05 First feeding of Perch (*Perca fluviatilis*) larvae. Julia L. Overton og Helge Paulsen. (Kun udgivet elektronisk)

Nr. 151-05 Ongrowing of Perch (*Perca fluviatilis*) juveniles. Julia L. Overton og Helge Paulsen. (Kun udgivet elektronisk)

Nr. 152-05 Vurdering af ernæringstilstand for aborre. Helge Paulsen, Julia L. Overton, Dorthe Frandsen, Mia G.G. Larsen og Kathrine B. Hansen. (Kun udgivet elektronisk)

Nr. 153-05 Myndighedssamarbejdet om fiskeriet i Ringkøbing og Nissum fjorde. Redaktion: Henrik Baktoft og Anders Koed

Nr. 154-05 Undersøgelse af umodne havørreders (grønlændere) optræk i ferskvand om vinteren. Anders Koed og Dennis Søndergård Thomsen

Nr. 155-05 Registreringer af fangster i indre danske farvande 2002, 2003 og 2004. Slutrapport. Søren Anker Pedersen, Josianne Støttrup, Claus R. Sparrevohn og Hanne Nicolajsen

Nr. 156-05 Kystfodring og godt fiskeri. Josianne Støttrup, Per Dolmer, Maria Røjbek, Else Nielsen, Signe Ingvardsen, Christian Laustrup og Sune Riis Sørensen

Nr. 157-05 Nordatlantiske havøkosystemer under forandring – effekter af klima, havstrømme og fiskeri. Søren Anker Pedersen

Nr. 158-06 Østers (*Ostrea edulis*) i Limfjorden. Per Sand Kristensen og Erik Hoffmann

Nr. 159-06 Optimering af fangstværdien for jomfruhummere (*Nephrops norvegicus*) – forsøg med fangst og opbevaring af levende jomfruhummere. Lars-Flemming Pedersen

Nr. 160-06 Undersøgelse af smoltudtrækket fra Skjern Å samt smoltdødelighed ved passage af Ringkøbing Fjord 2005. Anders Koed

Nr. 161-06 Udsætning af geddeyngel i danske søer: Effektvurdering og perspektivering. Christian Skov, Lene Jacobsen, Søren Berg, Jimmi Olsen og Dorte Bekkevold

Nr. 162-06 Avlsprogram for regnbueørred i Danmark. Alfred Jokumsen, Ivar Lund, Mark Henryon, Peer Berg, Torben Nielsen, Simon B. Madsen, Torben Filt Jensen og Peter Faber

Nr. 162a-06 Avlsprogram for regnbueørred i Danmark. Bilagsrapport. Alfred Jokumsen, Ivar Lund, Mark Henryon, Peer Berg, Torben Nielsen, Simon B. Madsen, Torben Filt Jensen og Peter Faber